Parallel Sessions 31–41

Session 31. Kidney: Humoral rejection

O239 LONG-TERM RESULTS OF ABO INCOMPATIBLE KIDNEY TRANSPLANTATION USING ANTIGEN-SPECIFIC IMMUNOADSORPTION AND RITUXIMAB

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In 2001 a protocol for ABOi kidney transplantation based on antigen-specific immunoadsorption and rituximab in combination with tacrolimus, mycophenolate mofetil and prednisolone was introduced at our centre, short-term results being comparable to those of ABO compatible (ABOc) living donor kidney transplantation. Of more importance however, is long-term graft function, so far not evaluated. The aim of this study was to therefore to asses the long-term results of this protocol.
All adult cross-match negative ABOi kidney recipients with >12 month follow-up (n=15) were included in the study and compared to all adult ABOc cross-match negative living donor kidney recipients maintained on the same basic immunosuppressive therapy and transplanted during the same period, (n=27). Patient and graft survival and rejections were analyzed. Kidney function was assessed by calculated GFR (Cockroft-Gault). In the ABOi recipients A/B antibody titers were determined.
Mean follow-up was 3 years. There was no significant difference in patient survival (100% (ABOi) and 97% (ABOc), nor in graft survival (87% (ABOi) and 92% (ABOc)) or in the incidence of acute rejection (13% (ABOi) and 22% (ABOc) of kidney recipients). Kidney function was equivalent at all time points with a mean-GFR of 82, 80 and 79 mL/min at 1, 2 and 3 years in the ABOi kidney recipients compared to 79, 82 and 83 mL/min in the ABOc group. There was a significant reduction ($p<0.05$) without rebound in A/B antibody titer after transplantation (median IgG and IgM 1:1 >1year post-transplant) compared to pre-transplants levels (median IgG 1:32 and IgM 1:16).
We conclude that ABOi kidney transplantation using antigen-specific immunoadsorption and rituximab in combination with triple immunosuppressive therapy is equivalent to standard ABOc living donor kidney transplantation. ABOi transplantation following this protocol does not have a negative impact on graft function long-term.

O240 HISTOLOGICAL EVALUATION OF 6-MONTH AND 1-YEAR PROTOCOL BIOPSY IN ABO-INCOMPATIBLE RENAL TRANSPLANTATION

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Introduction: No data have been shown for histological evaluation of pre-discharge and/or 6-month and/or 1-year protocol biopsy in ABO-incompatible renal transplantation (ABOi-RT). In our center, the follow-up graft renal protocol biopsy (PBx) (pre-discharge, 6-month, 1-year) was started from March 2002. We assessed the significance of PBx and whether the histological findings suggest the therapeutic management for the long-termed graft survival.
Methods: In 33 cases of ABOi-RT between Jan. 2002 and Feb. 2006, the follow-up graft renal PBx was performed in 29 cases. We assessed the histology of the PBx and episode Bx within 1-year post-transplant period in 29 cases.
Results: Arteriosclerosis derived from the donor has been reported in 9 cases in 1-hour and/or pre-discarge PBx. Ten cases of chronic allograft nephropathy (CAN), 8 acute cellular rejection (borderline to Ia) (ACR) and 2 post-thrombotic glomerulopathy have been reported in 23 cases of the 6-month and/or 1-year PBx without episode Bx. All of 3 antibody-mediated rejection (AMR) was reversible in 6 episode Bx, however, one of these led to graft loss 5 month after RT. Overall, the conversion of immunosuppressants have not been needed without the cases of severe calcineurin inhibitor toxicity. No significant difference in the serum creatinine level was found between with and without histological findings. Histological evaluation was also needed because of the mixed type of ACR, arteriopathy and CAN, which will affect the therapeutic management. Strong C4d deposition seems to be a non-specific but sensitive indicator for AMR. Especially, the finding of the post-thrombotic glomerulpathy seems to be risk factor for graft survival in ABOi-RT.
Conclusion: PBx is significant follow-up method in early detection of the borderline to mild ACR or CAN.

O241 ENDOTHELIAL CELL-ASSOCIATED TRANSCRIPTS ARE SELECTIVELY INCREASED IN ANTIBODY-MEDIATED REJECTION IN HUMAN RENAL ALLOGRAFTS, AND CORRELATE WITH PATHOLOGIC FEATURES

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The pathogenesis of antibody-mediated rejection (ABMR), and the effects of ABMR on the transcriptome are currently unknown. Using Affymetrix microarrays, we analyzed gene expression in 135 renal allograft biopsies for clinical indications. We previously identified pathogenesis based transcript sets (PBTs) associated with cytotoxic T cells (CATs), macrophage activation (IMATs), and gamma interferon effects (GRITs). Geometric means of CATs, GRITs, and IMATs were higher in C4d diffuse+ biopsies compared to C4d- and C4d focal+ biopsies (Table 1). Both ABMR and T cell-mediated rejection (TCMR) biopsies showed increased CATs, GRITs, and IMATs compared to biopsies without rejection ($p<.05$).
We hypothesized that alloantibody acting on the microcirculation alters endothelial genes. We identified a literature based endothelial gene set (n=109 probe sets). Twelve endothelial cell-associated transcripts (ENDATs) were differentially increased in ABMR vs.TCMR ($p<.05$). ENDATs included established markers such as VWF, PECAM1, ICAM2, and endothelin 1. ENDAT geomeans significantly correlated with pathologic features of ABMR: C4d deposition, peritubular capillaritis, glomerulitis (g), glomerular double contours (cg), and peritubular capillary basement membrane multilayering (PTCBMML) ($p<.05$).
Thus ABMR creates extensive inflammation in the allograft, as measured by the PBTs, which is quantitatively similar to TCMR. However, increased expression of endothelial genes provides a diagnostic feature that distinguishes ABMR from TCMR.

O242 VARIABILITY IN DONOR-SPECIFIC ALLOANTIBODY PRODUCTION AFTER TRANSPLANTATION

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The role of donor-specific alloantibodies(DSAs) produced *de novo* after transplantation in renal allograft injury is still unclear. The aims of this study were to: 1) assess the development of DSA during the first year after transplanta-

Abstract O241 – Table 1. Endothelial cell-associated transcripts (ENDATs) differentiates ABMR from TCMR

	C4d diffuse+	C4d focal+	C4d -	ABMR	TCMR
n	15	5	115	13 [a]	27
ENDATs	1.1±0.4**	0.2±0.5	0.4±0.4	1.0±0.3**	0.4±0.4
CTL associated-transcripts	1.4±0.2*	1.1±0.2	1.2±0.3	1.4±0.2	1.4±0.4
Ifng induced transcripts	2.1±0.4**	1.3±0.4	1.5±0.5	1.9±0.4	1.9±0.6
Macrophage activation-associated transcripts	2.0±0.4*	1.3±0.4	1.4±0.5	1.9±0.4	1.9±0.7

Correlation coefficients between ENDATs and pathologic features

	C4d deposition	Peritubular capillaritis	g	cg	PTCBMML	t
ENDATs	.406**	.323*	.288**	.249*	.388**	.055

For gene sets, numbers indicate geometric mean SD. [a]mixed ABMR and TCMR cases were excluded. **$p<0.001$, *$p<0.05$

tion, 2) determine the cause of DSA production, 3) evaluate the association of DSA with allograft function.
The study included 78 consecutive renal transplant recipients with negative cross-match before transplantation. The recipients' serum were assayed for DSA at 2 weeks and 1, 3, 6, 9, 12 months. DSAs were assayed by a complement-dependent lymphocytotoxic (CDC) cross-match with donor lymphocytes. The cells were suspended in RPMI1640 (supplemented with 10%DMSO, 40%FBS) stored in vapours of liquid nitrogen until used.
Results: There were 545 cross-match tests performed after transplantation and 79 positive results were found. DSA appeared *de novo* in 35(44.8%)recipients. DSA was found in 20 patients at 2 weeks, in 23 at 1 month, in 14 at 3 months, in 9 at 6 months, in 5 at 9 months, and in 8 at 12 months. Between the 3rd and 9th month after transplantation, DSA disappeared in 22 patients and appeared in another 11. In 20 patients (57.1%) the appearance of DSA was associated with acute rejection. In 11 of these, C4d-deposition was found. The cause of DSA production in the remaining patients may be associated with bacterial and viral infections. The results of the patients with DSA were compared with those of 43 patients who did not develop DSA at any point. Demographic data, HLA missmatches, PRA% before transplantation did not differ between the groups. Serum creatinine level during the first year after transplantation was significantly lower in patients without DSA. In 13 of these, acute rejection occurred, but without C4d-staining.
Conclusions: Renal transplant patients produce antidonor alloantibodies, with the highest rate during the first month and the incidence rate starting to diminish three months after transplantation. The development of DSA in more than half of the patients was associated with rejection episodes. Patients with antidonor alloreactivity had worse renal function.

O243 POSITIVE HLA CLASS II AS COMPARED WITH HLA CLASS I DSA AFTER DESENSITIZATION IS ASSOCIATED WITH HIGHER RATE OF POST TRANSPLANT IMMUNOLOGIC INJURY

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We performed 47 kidney transplants (45 LD and 2 DD) following desensitization of highly sensitized patients who had positive CXM with their respective donors. Desensitization regimen consisted of immunologic risk stratification-based individualization of immunoadsorption, plasmapheresis, high dose IVIG, and anti-CD20 antibody. Low titer DSA at the time of transplantation is defined as the presence of HLA class I or II DSA alone or in association with either a positive AHG CDC CXM at a titer of $\leq$1:1, or a positive flow CXM (FCXM), or both. 20/47 (42%) patients were transplanted with low titer DSA (Group I: 10 with DSA against DR or DQ epitopes, Group II: 7 with DSA against A or B epitopes and Group III: 3 with DSA against A or B, and DR or DQ epitopes). Post transplant outcomes were compared between Group I and II (GI and GII). Over an average follow up of 13 months, patient and graft survival were 100% in both groups. The incidence of AMR in GI was 90% (9/10) as compared with 28% (2/7) in GII (p: $<$0.05). All episodes of AMR except 2 in Group I were completely reversed with plasmapheresis, IVIG, rATG, and anti-CD20 antibody. The incidence of a composite end point of AMR, ACR and TG was higher in GI as compared with GII ($p < 0.05$). Average serum creatinine in GI was 145 mmol/l (range: 75 – 400) and in GII 81 mmol/l (range: 45 – 111) (p: $<$0.05).
We conclude that in the context of desensitization: Low titer HLA class II DSA at transplantation is associated with higher rate of early and late immunologic injury, as compared with class I DSA.

O244 C4D AND DONOR SPECIFIC ANTIBODIES IN SEVERE REJECTIONS AFTER RENAL TRANSPLANTATION FROM DECEASED DONORS

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Backgroud: Antibody mediated rejection is a significant cause of early renal allograft loss. Our aim was to investigate crossmatch conversion, C4d positivity, development of donor specific antibodies (DSA) and MHC class I chain-related gene A (MIC) antibodies in severe acute rejections after kidney transplantation (TX) and their effect on graft function and survival.
Patients and Methods: Between 1994-2005, 1905 adult cadaveric kidney TXs were performed at our centre. Among these 101 steroid resistant rejections had been diagnosed. In 49 of them data on post TX crossmatch conversion was available and biopsy material and sera available for C4d staining by immunohistochemistry and HLA I, II and MIC-antibody determination by Luminex®. Induction immunosuppression was CNI, AZA/MMF and steroids. Rejections were verified by core biopsy and treated with bolus steroids, OKT-3 and/or plasma exhange (PE).
Results: Mean age at time of TX was 44.2 (26-69) years. Ten were re-TXs. The findings are summarised in the table. The grafts that were lost without attaining acceptable function are grouped as 'Poor prognosis'. One-year graft and patient survival in the 'Good prognosis'-group were 97% and their mean creatinine at 1 year was 141umol/L. As expected, high PRA and poor onset of graft function were associated with poor prognosis. At time of rejection, C4d positivity and the prevalence of DSA were significantly more common among grafts with poor outcome than those surviving rejection. Crossmatch conversion and MICA did not differentiate rejections in this respect.

	Pre TX PRA	DGF or NF	Crossmatch Conversion	PE	C4d Positivity	DSA at Rejection	MICA at Rejection
Good Prognosis (n=38)	7.8%	14 (37%)	20 (53%)	19 (50%)	3 (8%)	9 (24%)	5 (13%)
Poor Prognosis (n=11)	29.6%	9 (82%)	7 (64%)	8 (73%)	5 (46%)	7 (64%)	3 (27%)
	p=0.005	p=0.01	n.s	n.s	p=0.009	p=0.019	n.s

Conclusions: We conclude that development of DSA and C4d immunoperoxidase positivity in severe rejections are indicative of kidney grafts with poor prognosis.

O245 VALIDATION OF SOLID-PHASE ASSAY AND SCREENING FOR NON-HLA, AT1 RECEPTOR-AGONISTIC ANTIBODIES IN RENAL TRANSPLANT CANDIDATES

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We recently described a subset of humoral rejection due to non-HLA antibodies against angiotensin type 1 receptor (AT1R-AA) (N Engl J Med 2005; 352: 558-69). Renal allografts of patients with AT1R-AA pretransplant positivity treated with AT1R blockers and apheresis were rescued, emphasizing a need for AT1R-AA detection while wait-listed. Detection of AT1R-AA relied on a bioassay that precluded screening of larger cohorts. We developed and according to Guidance for Bioanalytical Method Validation (US FDA May 2001) validated a cell based ELISA in collaboration with CellTrend, Germany for detection of AT1R-AA. We tested sera of 845 patients on waiting-list. Repeated tests from 613 patients showed high reproducibility (Wilcoxon-Z=-0.021; p=0.983). ELISA measurements of 14 patients with non-HLA humoral rejection and positive for AT1R-AA in the bioassay were ROC-analyzed against measurements of bioassay negative sera (AUC= 0.9330$\pm$053, p$<$0.001). Setting cut-off at 2.04U sensitivity was 93% and specificity 75%. 192 (22.7%) of 845 patients were positive for AT1R-AA. There was no correlation with patients sex, age, causes for ESRD, time on dialysis, CMV, HBV or HCV serology with AT1R-AA positivity. AT1R-AA positivity showed no prediction of arterial hypertension in 514 patients with available data. The effect was undetectable as 82.3% of all patients have hypertension. Among 192 AT1R-AA positive patients, 18 patients had more frequently PRA than AT1R-AA negative patients (28/652), (p=0.006). AT1R-AA positivity was more frequent in patients with previous transplants (p=0.006). In 17 patients with AT1R-AA higher than +3STD ($>$4.8U) frequency and level of PRA was similar compared to remaining AT1R-AA positive patients. Whether or not AT1R-AA act as a risk factor for early graft-loss or for cardiovascular co-morbidity remains to be determined. Pretransplantation testing for AT1R-AA may help to improve risk assessment and offer patients adequate treatment.

O246 BLOOD GROUP LEWIS ANTIBODIES THE CAUSE OF ANTIBODY-MEDIATED REJECTION IN RENAL TRANSPLANT RECIPIENTS

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Matching the Lewis blood group system is not routinely performed because only 9% of the population is Lewis-negative and the relationship of graft rejection with Lewis incompatibility is not evident. We present three subjects with negative Lewis phenotype who had anti-Lewis antibodies and suffered from severe kidney allograft rejection.
Material and methods: One woman and two men (22-44 years) underwent kidney transplantation (between June 2004 and August 2006) from deceased donors. Donor-recipient cross-match tests before transplantation were negative. The patients were ABO blood group compatible. Two patients, Nos.1 and 2, had phenotype Le(a-b-) and developed anti-Le(a) and anti-Le(b) antibod-

ies. Patient No.3, with phenotype Le(a+b-), developed anti-Lewis(b) antibody a few months after transplantation. The immunosuppressive protocol included calcineurin inhibitor, MMF, and prednisone. The patients had immediate graft function after the surgical procedure.
Results: Patient No. 1 developed acute graft dysfunction five days after transplantation and did not fully recover despite treatment. Patient No.2 had recurrences of acute graft dysfunction at 6 and 12 months after transplantation. Patient No.3 had progressive graft dysfunction from the 9th month after the transplant procedure.
The biopsies showed histological changes of antibody-mediated rejection. Studies showed IgG and IgM in glomerular capillaries. C4d and C3 were found on endothelial cells of peritubular capillaries. Posttransplant cross-match tests with the donor's lymphocytes were negative.
All patients were treated with boluses of methylprednisolone, one additionally with ATG, and one with plasmapheresis. Two patients had moderate renal dysfunction after 5-15 months of follow-up. Patient No.3 lost her graft 12 months after transplantation.
Conclusions: The Lewis antibodies may injure a renal allograft by mechanism of antibody-mediated rejection.
Compatibility in Lewis blood antigens should be considered in renal transplantation.
C4d deposition and failure to show donor-specific anti-HLA antibody suggest the participation of other antibodies in allograft injuries.

O247 EVALUATION OF A FLOW CYTOMETRY-BASED CROSSMATCH FOR SIMULTANEOUS DETECTION OF ANTIBODIES REACTIVE WITH DONOR ENDOTHELIAL CELLS AND LYMPHOCYTES

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The deleterious effect of the presence of donor-specific HLA Abs detected either pre- and/or post-transplant is well established. Conventional complement-dependent cytotoxicity or flow cytometry-based cross-matching using donor T- and/or B-lymphocytes, are adequate to detect class I and II specific HLA Abs. However, despite negative lymphocyte crossmatches (LXM), approximately 15-20% of kidney transplants undergo early rejections some of which are humoral. Evidence from several studies demonstrates that these Abs are directed against endothelial cells (AECA) of the donor organ and therefore not detected in the conventional LXM. We have developed a flow cytometry-based endothelial crossmatch (ECXM) test (XM-ONE®; AbSorber AB, Stockholm) to circumvent this problem. This assay is based on the isolation of endothelial precursor cells from donor peripheral blood using magnetic beads carrying Tie-2 Abs.
Sera from 27 pats undergoing evaluation for LD kidney transplantation were analyzed by ECXM and LXM. In the ECXM, 15/27 sera were positive for IgG (>40 channel shifts on a 1024 scale) or IgM (>80 channel shifts), 8/27 were positive for only IgM, 3/27 for only IgG and 4/27 for IgG + IgM. In the FACS T-LXM, 2/27 patients had donor-reactive IgG. Of these two patient sera, one had donor-reactive AECA of IgG and IgM class, whereas one had AECA only of IgM class. When a lymphocyte gate was used in the ECXM, one of the patient sera with donor-reactive IgG in the conventional FACS T-LXM was positive whereas the other was negative. Further evaluation of the clinical significance of donor lymphocyte- and EC-reactive Abs detected using this one-tube assay is warranted.

Session 32. Experimental immunosuppression: Molecular

O248 CHANGES IN PERIPHERAL BLOOD LYMPHOCYTE SUBSETS, IFN-γ PRODUCTION CAPACITY AND GENE EXPRESSION FOLLOWING TREATMENT WITH JAK-3 INHIBITOR CP-690,550 IN RENAL ALLOGRAFT RECIPIENTS

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CP-690,550 is an inhibitor of Janus kinase 3 associated with the common gamma chain (γ_c) of cytokine receptors of the IL-2 family. Members of this family, including IL-2,-4,-7,-9,-15 and-21, play pivotal roles in lymphocyte activation, differentiation, and death. Based on these features, inhibition of JAK3, expressed by immunocompetent cells only, is expected to be immunosuppressive. In this study, lymphocyte subsets, gene expression levels of T-cell activation molecules and IFNγ-production by PBMC were analyzed in stable renal allograft recipients.
Methods: As part of a Phase1 dose-escalation trial, 8 stable kidney transplant recipients on mycophenolatemofetil 500-1000mg bid and prednisolone 5-7.5mg daily were treated with CP-690,550 30mg bid orally for 29 days. On Day1, Day15, Day29 and Day57, we measured lymphocyte subsets and gene expression levels in peripheral blood samples by flow cytometry and RT-PCR, respectively. IFNγ-production capacity was ascertained in mitogen activated PBMC.
Results: Significant changes in the cell counts of circulating lymphocytes were observed following CP-690,550 treatment. The absolute numbers of $CD19^+$B-lymphocytes increased (twofold, p=0.006), while those of NK-cells ($CD3\text{-}CD16^+CD56^+$) and of regulatory $CD4^+CD25^{bright+}$T-cells decreased during exposure to CP-690,550 (mean: 54%, $p<0.001$ and mean: 38%, p=0.04, respectively). In addition, in the presence of CP-690,550, the IFNγ-production capacity of PBMC was reduced by 41% (mean) compared to pre-dose baseline (p=0.02).All studied immune parameters recovered to pretreatment levels. No significant changes in IL-2, IL-15, IL-21, CD25 (IL-2Rα-chain), CD132(γ_c), SOCS3, FOXP3 and granzyme B mRNA expression levels were measured.
Conclusion: Numbers of circulating $CD19^+$B-cells increased and those of $CD3\text{-}CD16^+CD56^+$NK-cells and regulatory $CD4^+CD^{25bright+}$T-cells decreased during treatment with JAK3 inhibitor CP-690,550. In addition, the IFNγ-production capacity of PBMC was inhibited. These findings are consistent with a role of JAK3 in the homeostasis and function of lymphocyte subpopulations.

O249 MOLECULAR AND FUNCTIONAL EVIDENCE FOR THE INHIBITORY EFFECT OF CP-690,550 ON JAK/STAT-SIGNALLING IN STABLE KIDNEY TRANSPLANT PATIENTS

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Cytokines signalling via the common γ-chain (γ_c, CD132), such as IL-2 and IL-15, play pivotal roles in the expansion and survival of antigen activated lymphocytes. Upon receptor binding these cytokines trigger activation of the JAK/STAT-pathway, which involves recruitment and activation of JAK3 and subsequent phosphorylation and activation of a specific class of transcription factors called STATs. The aim of this study was to analyze the effect of the immunosuppressive drug CP-690,550, which is a JAK3 inhibitor, in kidney transplant (KTx) patients on JAK/STAT-signalling both at the molecular level as well as at the level of downstream biological effects.
As part of a Phase 1 trial, 8 stable KTx-patients on MMF/steroids were treated with CP-690,550 for 29 days. Serum and peripheral blood was collected before 1st dose (day1) and 12 hours after 1st dose (day 1/t=12h), at day 15, day 29 and day 57.
By quantitative fluorescent Western blotting we determined the effect of CP-690,550 on IL-2-induced phosphorylation of STAT5 in a human NK/T cell line. STAT5 phosphorylation was increasingly reduced in the presence of patient serum from day 1/t=12h, day 15 to day 29 (maximal reduction 74%, mean) compared to pre-dose baseline (day1) and recovery to baseline was observed at day 57. Reduction and recovery to pre-treatment levels was also observed for the IFNγ production capacity of activated PBMCs in the presence of patient serum (maximal reduction 41%, day 29). Furthermore, by flow cytometry we detected, already at day 1/t=12h, decreased expression of the IL-2 receptor subunits, CD25 (α-chain), CD122 (β-chain) and CD132 on CD3+CD4+ T-cells. These findings show that in KTx-patients blockade of JAK3 results in inhibition of the signalling cascade triggered by cytokines signalling via γ_c and of its downstream effects, such as IFNγ production.

O250 12-MONTH FOLLOW UP OF A PHASE 2A TRIAL OF CP-690,550, A JAK3 INHIBITOR, IN DE NOVO KIDNEY TRANSPLANT (KT) RECIPIENTS

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Purpose: This interim analysis extends the safety and efficacy evaluation of 2 CP-690,550-based regimens through Month 12 posttransplant in KT recipients.
Methods: 61 primary KT recipients were previously randomized to CP-690,550 15 mg BID, CP-690,550 30 mg BID or tacrolimus (CP15, CP30, Tac) in a Phase 2A, multicenter trial. All subjects initially received concomitant MMF and corticosteroids. Trial results through Month 6 have been reported. 45 subjects (14 in CP15, 13 in CP30, 18 in Tac) continued beyond Month 6 in an extension trial. In the extension trial, CP15 subjects reduced CP-690,550 dosage

to 10 mg BID and continued MMF and steroids. CP30 subjects reduced CP-690,660 dosage to 15 mg BID and continued steroids without MMF. One additional subject, who discontinued before Month 6, re-enrolled via a patient-specific amendment and subsequently died, was excluded from this analysis.
Results: The cumulative 12-month rates of biopsy-proven acute rejection (BPAR), clinically significant infections and polyomavirus-associated nephropathy (PVAN) are shown in Table 1. Notably, none of the CP-690,550-treated subjects developed BPAR or PVAN after Month 6. 44 subjects completed Month 12 evaluations. There was no patient death, graft loss or post-transplant lymphoproliferative disease. 3 neoplasms were observed (2 in CP30 and 1 in Tac). The least squares means of estimated GFR at Month 12 were 83.6, 77.6 and 73.3 mL/min for CP15, CP30 and Tac, respectively, with statistically significant difference between CP15 and Tac (P=0.02). WBC and Hgb were comparable among the treatment groups at Month 12.

Table 1. Cumulative rates of BPAR, clinically significant infections and PVAN at Month 12

	CP15	CP30	Tac
BPAR	5.3%	21.1%	9.8%
Clinically significant infections	50%	68.8%	38.9%
PVAN	0%	20%	0%

Conclusion: Despite dose reduction beyond Month 6, the CP-690,550 arms remained rejection-free with acceptable safety profile. These findings suggest that long-term treatment with CP-690,550 $\leq$10-15 mg BID is effective for prevention of acute rejection in KT recipients.

O251 EFFECTS OF RAPAMYCIN AND MMF ON UV-INDUCED SKIN CARCINOGENESIS

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Background: Immunosuppressive drugs, like cyclosporine A, have been shown to inhibit DNA repair, which is thought to permit or even enhance UV carcinogenesis. Indeed, transplant recipients are at a very high risk for developing skin cancer. However, new generations of immune suppressive drugs, e.g., rapamycin (Rapa) and mycophenolate mofetil (MMF), can impair tumor growth, and thus may be useful for reducing skin cancer in transplantation.
Methods: To ascertain how these newer immunosuppressive drugs would affect the long-term overall process of UV carcinogenesis, we exposed 4 groups of hairless SKH1 mice daily to 250 J/m^2 UV from TL12 lamps: group 1 was fed Rapa, group 2 MMF, group 3 Rapa+MMF, and group 4 (control) received normal food pellets without drugs. We have recently published the method of drug incorporation into mouse food pellets, and typical immunosuppressive drug levels were verified in the blood of treated mice.
Results: The 4 groups showed no significant difference in the development of tumors $<$2 mm in diameter. But larger tumors took significantly longer to develop in the Rapa (n=10) and Rapa+MMF (n=12) groups (median latency times of 190 and 165 days, respectively, for tumors $>$4 mm) than in the MMF (n=10) and control (n=10) groups (140 and 125 days, respectively). Moreover, the average multiplicity of tumors $>$4 mm was significantly lower in Rapa-treated mice (1.5 at 200 days, versus 4.0 in controls). MMF did not significantly affect tumor size or multiplicity.
Conclusions: These experimental results show that Rapa's antitumor properties, at immunosuppressive doses, may help to reduce the development of UV-induced skin carcinogenesis in immunosuppressed transplant recipients, while MMF does not appear to have a significant inhibitory effect on skin tumor formation.

O252 B-CELL TOLERANCE TO BLOOD-GROUP ANTIGENS POST PEDIATRIC ABO-INCOMPATIBLE LIVER TRANSPLANTATION

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A difference in the outcomes of adult and pediatric ABO-incompatible liver transplantations has been reported; pediatric transplantations are more successful. There are reports that long-term survivors post pediatric ABO-incompatible liver transplantation exhibit low or even undetectable levels of anti bodies (Abs) to donor AB antigens. The absence of anti-AB Abs is possibly due to tolerization of the host B cells responding to the donor-type blood antigens or due to the adsorption of Abs to AB antigens expressed on the *endothelium* of the transplanted liver graft. Previously our studies revealed that B cells bearing surface IgM (sIgM) receptors that recognize the blood group A-carbohydrate determinant were present exclusively in a small B-cell subpopulation*Ù sIgM$^+$ CD11b$^+$ CD5$^+$ B1*. This was observed in peripheral blood mononuclear cells (PBMCs) obtained from a human volunteer with blood group O but not in those obtained from a volunteer with blood group A or a liver-transplant patient with blood group O who had received a partial liver graft at 2 years of age from donor of A blood group. Despite the production of human immunoglobulins specific to antigens other than A antigens, anti-A Abs were not detected in the sera of the mice that received PBMCs from a blood group-A volunteer or a blood group-O recipient of a group-A liver allograft. This indicated profound B-cell tolerance to group-A antigens. Immunohistochemistry revealed that *liver sinusoidal endothelial cells (LSECs)* expressed self blood group-carbohydrate determinants. Flow cytometry revealed that human LSECs constitutively expressed PD-L1, and CD5$^+$ B1 cells expressed PD-1, suggesting that B1 cells recognizing blood-group carbohydrates are susceptible to specific tolerance. *The PD-1/PD-L1 pathway* possibly plays a role in LSEC-induced B-cell tolerance to blood-group antigens post ABO-incompatible liver transplantation.

O253 INTERIM REPORT OF PHASE 2 LONG-TERM SAFETY OF BELATACEPT

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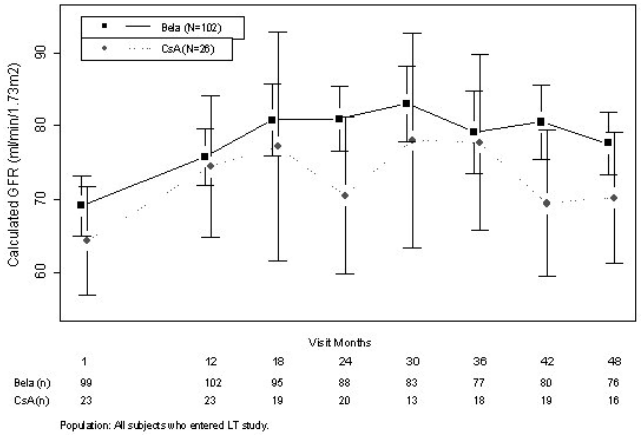
Belatacept (LEA29Y) is a first-in-class costimulation blocker, rationally designed as a high-affinity variant of CTLA4Ig, and is in Phase 3 trials as CNI-free immunosuppression in renal transplant. An interim report of patients who elected to enter an open-label 5 year long-term extension (LTE) of the phase 2 trial is presented.
Methods: The Phase 2 trial design and results have been described previously. For the LTE, Belatacept arms were dosed identically, as either q4week or q8week maintenance infusions of 5mg/kg; control patients received Cyclosporine (CsA), dosed to target C_0 levels. (Fig.1) All patients continued to receive MMF and steroids per protocol. Renal function was calculated via the MDRD (Levey) formula. Results for adverse events (AEs) are presented as incidence rates/100 patient-years of drug exposure. No formal statistical testing was applied, as this was a self-selected non-randomized population.
Results: 128 of 218 original patients elected to participate in the LTE; 102 received Belatacept, 26 received CsA. Among LTE patients enrolled, 98 (77.7%) patients remain (81/102, 79.4% Belatacept; 19/26, 73.1% CsA). Median follow-up from original randomization was 48 months. Safety data are shown (Table 1). Safety data was comparable across both belatacept dosing regimens, and are presented as the aggregate of both belatacept arms. GFR data are presented in Figure 1. Months 1-12 reflect data from the randomized double-blind phase of the study, whereas Months 18 -48 reflect data obtained during the LTE.

Table 1. Adverse Events (SAE) for Belatacept and Cyclopsorine (CsA) treated patients

	Bela Rate/100 pt-yr (95%CI)	CsA Rate/100 pt-yr (95% CI)
Serious Infections	4.2 (2.2-7.1)	8.9 (3.6-18.4)
Neoplasms	2.6 (1.1-5.0)	2.5 (0.3-9.2)
Treated Acute Rejection	3.2 (1.5-5.9)	2.5 (0.3-9.2)
Serious Cardiovascular	0.3 (0.0-1.8)	3.8 (0.8-11.2)
Death or Graft Loss	0.96 (0.2-2.8)	1.28 (0.03-7.12)



Plot of mean calculated GFR (Levey) over time

Summary: This report demonstrates continued safety of using Belatacept long-term as CNI-free immunosuppression in renal transplant. Definitive conclusions regarding long term safety and efficacy will await the completion of the ongoing Phase 3 trials.

O254 EARLY SHORT-TERM IMATINIB-TREATMENT PREVENTS CHRONIC ALLOGRAFT NEPHROPATHY AND FIBROGENIC GROWTH FACTOR EXPRESSION IN AN EXPERIMENTAL RAT KIDNEY TRANSPLANTATION

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Chronic allograft nephropathy (CAN) still remains the primary reason for late allograft loss in kidney transplantation. It is an irreversible fibrotizing process leading eventually to the loss of the kidney graft. Currently there is no treatment available for preventing CAN. Platelet-derived growth factor (PDGF) and transforming growth factor –β (TGF- β) are fibrogenic growth factors and major mitogens mediating mesenchymal cell proliferation in CAN. Here we investigated the effect of short-term imatinib treatment on the development of CAN and expression of fibrogenic growth factors.
Kidney transplantations were performed from DA to WF rats and syngenic controls were done between DA rats. Allograft recipients were immunosuppressed with CsA 1.5 mg/kg/d s.c. One group of allografts was also treated with imatinib (a selective PDGF receptor tyrosine kinase inhibitor) 10mg/kg/d p.o. 0-30 days after transplantation. Serum creatinine levels were measured once a week. Grafts were harvested 90 days after transplantation for histology and immunohistochemistry (PDGF-AA, -BB, PDGFR-a, -b, TGF-b, TGF-bR). Histological changes were scored according to Chronic Allograft Damage Index (CADI).
In syngenic grafts no signs of CAN were seen, CADI 0.8±0.2 (mean±SEM). In control allografts moderate to intense chronic changes were seen, CADI 6.5±1.3. The early short-term imatinib-treatment prevented the development of CAN significantly compared to control allografts. Only few histological changes were seen, CADI 3.3±1.4. PDGF and TGF-b ligand and receptor induction was significantly inhibited by imatinib compared to control allografts, the expression was nearly at the same level as in syngenic grafts. Creatinine values of imatinib-treated allografts were also lower compared to control allografts.
Our results demonstrate that early short-term imatinib-treatment prevents CAN significantly. This indicates that early PDGF induction has an important role in the pathogenesis of CAN.

O255 THE EFFECT OF CsA, FK506 AND MMF ON THE PLASMINOGEN ACTIVATOR SYSTEM AND TRANSPLANT VASCULOPATHY (TVP) IN EXPERIMENTAL TRANSPLANTATION

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Myointimal hyperplasia in TVP consists primarily of vascular smooth muscle cells (VSMC) having migrated from the tunica media to the subintimal area and proliferated. Proliferating VSMC are expriming u-PA whereas migrating VSMC are expriming t-PA. We evaluated the effects of CSA, FK-506 and MMF on u-PA or t-PA expression of VSMC after heterotopic cardiac transplantation in rats (Lewis to Fisher).
Methods: 340 animals were randomized after grafting into four groups of therapy: CSA 3mg/kg/d (n=74), MMF 40mg/kg/d (n=96), FK-506 0.3mg/kg/d (n=96) and a control group receiving no immunosuppressive therapy (n=74). 3-4 animals of each group were sacrified in intervals of 1-4 days up to day 60. Immunohistochemistry was used for analysis of u-PA and t-PA positive arteries and the intensity of the staining was semiquantitatively scored. The extent of neointimal proliferation of large (L) and small (S) arteries was assessed by digitizing morphometry.
Results: FK-506, CSA and MMF therapy is leading to a reduction of u-PA positive small and large arteries for the first 20 (CSA and FK-506) respectively 30 days (MMF). An increase of u-PA followed up to 80% 60 days after transplantation. MMF and FK-506 have no influence on the expression of t-PA compared with control. In CSA treated animals there was a decrease in t-PA positive small and large arteries compared with control, FK-506 and MMF treated groups.
Conclusions: The used drugs prevent proliferation of VSMC for a short time. Migration of VSMC into neointima was only reduced by CSA. A combination of u-PA and t-PA reducing agents may therefore be a useful strategy in the prevention of chronic rejection.

O256 MYCOPHENOLATE AND FK506 HAVE DIFFERENT EFFECTS ON EXPRESSION OF CTGF IN RATS KIDNEY WITH CHRONIC ALLOGRAFT NEPHROPATHY

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Aim: Connective Tissue Growth Factor(CTGF) is a mediator of fibrosis and is capable of inducing epithelial-mesenchymal transformation(EMT). FK506 is associated with renal fibrosis in long term using. It has been proved that MMF could attenuate fibrosis process. This study was designed to determine the differences of these two drugs on the expression of CTGF and the fibrosis-associated genes in rat kidney underwent chronic allograft nephropathy (CAN).
Methods: F344 rat renal grafts were orthotopically transplanted into Lewis rats following the procedure of Kamada with our modification. All the recipient rats were given CsA 10mg/kg^{-1}×d^{-1}10d and then divided into three oral treatment groups (each group n=9): (1)Vehicle: given vehicle orally, (2)FK506: 0.15mg/kg^{-1}.d^{-1}, (3)MMF: 20mg/kg^{-1}.d^{-1}. At 4w, 8w, 12w post-OP, SCr and pathologic changes were measured. The expression of CTGF mRNA(ΔCt) and protein (Densitometric quantification, DQ) were determined by real-time RT-PCR and Western blot. The expression of type **I, IV** collagen, *a*-SMA and E-cadherin were assessed simultaneously.
Results: The levels of SCr and Banff score among 3 groups obviously increased since 8w, but MMF group was significantly lower than that in Vehicle and FK506 group($p<0.05$); Comparing with vehicle and FK506 group, the expression of CTGF in MMF group at all the time points were significantly down regulated($p<0.05$); and the expression of type *I, IV* collagen and a-SMA were down regulated and was up regulated for E-cadherin in MMF group. However, the expression of 4 factors was in opposite manner in vehicle and FK506 groups.
Conclusion: We demonstrate that MMF can ameliorate fibrogenesis in transplant kidney by inhibiting the expression of CTGF, down-regulating the expression of the fibrosis-associated genes and inhibiting the EMT. FK506 has no these effects.

Session 33. Lung

O257 A DONOR HISTORY OF 3 PACK YEARS OR MORE OF CIGARETTE SMOKING AND ITS EFFECT ON OUTCOMES FOLLOWING LUNG TRANSPLANTATION

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Objective: To review outcomes of lung transplant recipients who received lungs from donors with a cigarette smoking history of 3 pack years or more.
Methods: We conducted a retrospective chart review of lung transplant recipients from 1998 to 2003. Donor charts provided demographics, cause of death, and smoking history. Recipient charts provided demographics and pulmonary function tests (PFT) as well as outcomes. Univariate analysis was performed to determine differences between recipients of smoker lungs and non-smoker lungs.
Results: Among 211 lung transplantation patients (83 double, 128 single), 99 (47%) patients received lungs from smokers. Demographics were similar between groups. Smoking donors had a lower PaO2/FiO2 (4.08 vs. 4.38, $p<0.05$) at time of procurement than in non-smokers. Pre-discharge PFT were not different between groups. However, follow-up FEV1 and FEF25-75 were reduced in smokers (1.46 vs. 1.71, $p<0.05$ and 0.89 vs. 1.24, $p<0.05$ respectively) when compared to non-smokers. 30-day survival was 98% for both groups. However, 1-year survival was 76% for smokers compared to 90% for non-smokers ($p<0.01$). Overall survival at follow-up was also less for smokers at 39% at 3.7 years compared to 56% at 4.21 years for non-smokers.
Conclusion: There are differences in outcomes when using lung donors with a smoking history of 3 pack years or greater compared to non-smoking donors, which are related to pulmonary function and survival.

O258 A SURVEY ON (HEART-)LUNG TRANSPLANTATIONS IN BELGIUM 1983 – 2006

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Objective: Belgium played a pioneering role with a unique 10-month survivor in the early days of lung transplantation. The goal of this study was to sur-

vey all (heart-)lung transplantations [(H)LTx] performed in Belgium since the successful start in 1983.
Methods: A simple questionnaire was sent annually since 2004 to all active Belgian LTx centers asking for the numbers, types and indications of first and redo (H)LTx as well as number of listed patients.
Results: Between 1983-2006, 701 first and 16 redo (10 single-6 double) (H)LTx have been performed. The number of transplants have constantly increased over the years (1990:8; 1995:35, 2000:43, 2005:63, and 2006:87). As a result, Belgium has now become by far the nation worldwide with the highest number of (H)LTx performed per year pmp (8.7 versus 3.9 for Eurotransplant and 4.7 for UNOS). The most common indication for transplantation was emphysema (36%) followed by cystic fibrosis (19%), pulmonary fibrosis (14%), PPH (10%), Eisenmenger's syndrome (6%), and other lung diseases (15%). Indications, however, differed amongst the 4 active LTx centers: emphysema: 21%, 49%, 43%, and 33%; cystic fibrosis: 29%, 10%, 14%, 11%; pulmonary fibrosis: 8%, 15%, 17%, 44%; PPH: 16%, 10%, 7%, 0%; Eisenmenger: 8%, 2%, 6%, 0%; other indications: 18%, 14%, 13%, 11% for ULB, UCL, KUL, UA, respectively; $p < 0.001$. On 01-01-2007, 68 patients across the 4 Belgian centers were actively waiting for a suitable lung donor (Belgium 6.8 versus Eurotransplant 6.2 and UNOS 10.0 pmp).

	ULB	ULC	ULg	KUL	UA	Total
Single lung	72	50	2	130	5	259
Double lung	87	42	0	192	4	325
Heart-Lung	94	0	0	39	0	133
TOTAL	253	92	2	361	9	717

Conclusions: The annual number of (H)LTx performed in Belgium is still raising probably as a result of relaxed donor criteria. Referral pattern of potential lung transplant candidates differs amongst the centers. Belgian patients on the (H)LTx waiting list have the highest chance of receiving an organ compared to Eurotransplant and UNOS listed patients.

O259 PULMONARY HEMODYNAMICS AS PREDICTORS OF MORTALITY IN PATIENTS AWAITING LUNG TRANSPLANTATION

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Background: Lung transplantation (LTx) is a therapeutic option for patients with end-stage lung disease. However, the mortality rate on the waiting list is high. The purpose of this study was to examine the prognostic value of cardiopulmonary hemodynamics for death among patients awaiting LTx.
Methods: Retrospectively, 177 patients with advanced lung disease accepted for lung transplantation at Sahlgrenska University Hospital from January 1990 through December 2003 were studied. Patient demographics, pulmonary function tests, gas exchange and hemodynamic variables were included in the analysis. Death while awaiting LTx was the primary endpoint for all analyses. Univariate and multivariate survival analysis were performed using Cox regression.
Results: Mean age was 49±9 years. Main diagnoses were alpha 1 antitrypsin deficiency (α1ATD, n=56), chronic obstructive pulmonary disease (COPD, n=61), cystic fibrosis (n=14) and interstitial lung disease (ILD, n=46). Thirty patients died (17%). LTx was performed in 143 cases and, 4 patients were alive and still waiting at the end of the study. By univariate analyses forced vital capacity (FVC) % of predicted (hazard ratio [HR], 0.96; 95% confidence interval [CI], 0.94-0.99; p=0.003), pulmonary vascular resistance (PVR) (HR, 1.23; 95% CI, 1.07-1.40; p=0.002), systemic vascular resistance (SVR) (HR,1.08, 95% CI, 1.02-1.15, p=0.011) and diagnosis (HR,2.26, 95% CI, 1.07-4.75, p=0.03) were associated with risk for death. In multivariate analysis PVR (HR, 1.23; 95% CI, 1.06-1.42; p=0.005) and FVC% of predicted (HR, 0.96; 95% CI, 0.94-0.99; p=0.004) were independently associated with death.
Conclusions: Patients with increased PVR and a lower FVC % of predicted awaiting LTx should be considered for a higher organ allocation priority. Assessment of pulmonary hemodynamics needs to be considered during evaluation for LTx particularly in patients with ILD.

O260 OUTCOME OF PATIENTS WITH PULMONARY HYPERTENSION (PPH) REMAINS INFERIOR TO OTHER INDICATIONS FOR LUNG TRANSPLANTATION

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Objective: Recent advances in medical treatment of PPH have changed the treatment algorithm and postponed referral to LuTX. We have taken an aggressive approach to offer LuTX even to very advanced PPH-patients (pts). This paper reviews our experience.
Methods and Results: All transplanted PPH-patients at our institution were retrospectively analysed. Results were compared to those of the total LuTX cohort. Possible influencing factors for perioperative death were analysed.
Results: :From 01/1999 to 11/2006 42 pts with PPH (23 f/55%, 19 m/45%; 32±13 yrs) underwent LuTX at our institution. 40pts (95%) were in NYHA III/IV, and only 2 pts (5%) were in NYHA < III. 15 pts (36%) had severe right heart failure and 28 pts (67%) had continous intravenous medication.
The immediate postoperative course in PPH pts was associated with a high rate of complications (pneumonia 17%, wound infection 7%, hemofiltration 45%, cardial 21%, bleeding 29%, neurological 26%). Analysis of potential factors influencing the outcome did not result in significant differences between survivors and non survivors.
30 day, 1 and 5 year survival of the PPH pts was 80%, 66% and 63% compared to all non PPH pts (n = 460) 93%, 78%, 61% (p = 0.7, ns).
Freedom from BOS in PPH pts after 1, 3 and 5 years was 97%, 87%, 63% compared to 93%, 81%, 71% in all other pts (p = 0.9, ns).
Conclusions: Although no parameters can be defined as predictor for the outcome in PPH pts, the early postoperative survival is significant inferior compared to all LuTX pts. We conclude that an earlier referral of PPH pts to LuTX in a better physical condition results in a lower perioperative mortality and long-time survival.

O261 A MISSING LINK BETWEEN RESPIRATORY VIRAL INFECTIONS (RVI) AND CHRONIC HUMAN LUNG ALLOGRAFT REJECTION (BOS)

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RVI predispose to BOS: We recently found that BOS is associated with loss of $CD4^+CD25^+foxp3^+$ regulatory T-cells (Tregs). Using the well-established murine orthotopic tracheal transplant model of BOS we tested the hypothesis that RVI predisposed to BOS by inducing loss of Tregs.
Methods: Tracheal transplants were performed between BALB/c and C57BL/6 mice. Murine parainfluenza Sendai Virus (SdV) was used for infection. Allograft rejection was expressed as percent intramural fibrosis on day 60.
Results: Tracheal allografts revealed increased fibrosis compared to isografts (20.77% vs 10.33%, p=0.02). Allograft recipients revealed loss of Tregs in the draining lymph nodes (DLN) from baseline 3.8% to 1.1% by day 7. Recipient epithelium in wild type recipients, but not MMP-7 KO (that have deficient epithelial regeneration), started re-populatating allografts by day 7 that increased frequency of Tregs to 4.5% by day 14. SdV infection at day 14 lead to at least 50% loss of Tregs in DLN secondary to apoptosis (65% annexin V^+ Tregs) and increased allograft fibrosis (32.5%, p=0.006). SdV infected, but not control, AEC upregulated CD95L (FasL) and induced apoptosis in syngeneic Tregs, but not $CD25^-$ T-cells, *in vitro,* that was prevented by anti-CD95L mAbs. Vaccination with inactivated SdV pre-transplant prevented loss of Tregs (decline <10%) and decreased allograft fibrosis (21.77%). RVI strongly correlated with decline in Tregs during human BOS with concomitant increase (>10 fold) in IFN-γ producing donor-HLA class-I and class-II specific allo-reactive, and collagen type-V specific autoreactive Th1-cells. Tregs isolated before BOS suppressed (>50 fold) both allo- and auto-reactive Th1-cells.
Conclusions: While syngeneic AEC induce Tregs, virus-infected AEC lead to Treg apoptosis with consequent expansion of allo- and auto-reactive T-cells. This represents a novel mechanism by which RVI promote BOS. Importantly, vaccination against RVI may prolong lung allograft survival.

O262 PRETRANSPLANT SOLUBLE CD30 IS A STRONGER PREDICTOR OF BOS THAN ANTI-HLA ANTIBODIES OCCURRING AFTER LUNG TRANSPLANTATION

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Background: The Bronchiolitis obliterans syndrome (BOS) represents chronic

allograft rejection after lung transplantation and limits long term survival. The aim of our study was to investigate the predictive value of pre- and posttransplant soluble CD30 (sCD30) for the development of BOS, compared to the development of HLA antibodies after transplantation.
Methods: In 45 patients (23 female, mean age 44 years; range 16-61 years) having a median follow-up of 29 months (range 9 - 63 months); we measured sCD30 and anti-HLA antibodies MHC Class I and II before and after transplantation.
Results: Eighty-two percent of the patients received a donor lung with 4 or more HLA mismatches. None of the patients had anti-HLA antibodies prior transplantation whereas in only 1 patient antibodies could be detected post transplant. BOS occurred in 40% of the 25 patients with high (>18U/ml) pre transplant sCD30 levels, compared to 10% of the 20 recipients with low sCD30 levels (P=0.02). Although no significant relation could be found between pre- and posttransplant sCD30 levels, pre-transplant levels of patients developing BOS dropped significantly after LOTx (P=0.03), whereas this could not be observed in closely matched BOS-free patients BOS-free. sCD30 levels in patients at the onset of BOS did not differ from those in BOS free patients which may be caused by the type of immunosuppression.
Conclusions: Measurement of pretransplant sCD30 is a more powerful tool in predicting BOS than that of anti-HLA antibody responses after lung transplantation. Furthermore, post transplant sCD30 does not discriminate BOS from BOS-free patients.

O263 LYMPHOCYTES PLAY AN ESSENTIAL ROLE IN LUNG ISCHEMIA REPERFUSION INJURY

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Introduction: Ischemia-Reperfusion Injury (IRI) remains a significant clinical problem following lung transplantation. We have previously demonstrated in a murine model of lung IRI that lymphocytes invade the alveolar space during the ischemic period. We questioned the role of these lymphocytes for neutrophil attraction at reperfusion.
Methods: Two groups of mice (n=6) were compared: control animals and SCID (Severe Combined Immunodeficiency) mice, the latter lacking lymphocytes. Cells (10^4/ml) and IL-1 β protein levels (pg/ml) were measured in BAL of the left lung after 90 minutes of in situ warm ischemia with hilar clamping followed by 4 hours of reperfusion.

Mean±SD (n=6)	Control	SCID	P value
Leukocytes	6.8±3.2	3.3±1.1	0.0295
Macrophages	3.7±0.3	3.0±1.1	0.2138
Lymphocytes	1.55±0.3	0.00±0.00	x
Neutrophils	1.16±0.62	0.21±0.22	0.0055
IL-1β	190.2±20.4	116.3±29.8	0.0005

Results: The number of neutrophils and IL-1β protein levels at the end of reperfusion were significantly lower in SCID mice compared to control animals.
Conclusion: The presence of lymphocytes correlates with the concentration of neutrophils (Pearson r: 0.7825; p= 0.0026) and IL-1β cytokine production (Pearson r: 0.7753; p= 0.0030) after reperfusion. Strategies that inhibit lymphocytes in the donor lung prior to ischemia should be further investigated to attenuate lung injury upon reperfusion.

O264 KERATINOCYTE GROWTH FACTOR PREVENTS ISCHEMIA/REPERFUSION INJURY IN ISOLATED MOUSE LUNGS

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Introduction: Primary graft dysfunction, characterized by intraalveolar oedema, is a major obstacle in human pulmonary transplantation. We evaluated the potential of keratinocyte growth factor (palifermin, DN23-KGF) to prevent oedema in experimental ischemia/reperfusion injury in the isolated mouse lung.
Methods: Intratracheal instillation of 10 mg/kg DN23-KGF (Amgen) was performed in a volume of 200 μl in mice (C57BL/6N) on day 3 before removal of the lung. In control animals, an equivalent volume of PBS was instilled. At day 3, isolated ventilated and buffer-perfused lung experiments were performed involving an ischemic time of 90 min and a reperfusion period of 1 h. Pulmonary artery pressure and lung weight gain were assessed for quantification of lung injury. Thereafter, the wet to dry weight ratio was determined and isoosmolar mannitol lavage was performed.
Results: In lungs from sham treated donors, pulmonary artery pressure after 1h of reperfusion amounted to 10.78±0.80 cm H_2O versus 9.28±0.87 cm H_2O in the DN23-KGF-treated group ($p \leq 0.05$). The absolute amount of Na^+ in the mannitol lavage increased significantly ($p \leq 0.01$) from 6.7±2.6 μmol in the control group compared to 15.6±2.8 μmol in the treated group. The wet to dry ratio weight was significantly ($p \leq 0.01$) higher in the control group (11.6±0.8) compared to the group pre-treated with DN23-KGF (8.3±1.6). An epithelial lining fluid volume of 0.16±0.04 ml was calculated in control lungs versus 0.09±0.03 ml in the treated group ($p \leq 0.05$).
Conclusion: Treatment of donor lungs with palifermin protects against intraalveolar oedema formation upon ischemia/reperfusion injury which is of particular interest for lung transplantation and other surgical procedures involving lung ischemia.

O265 CYCLOSPORINE A (CSA) BASED IMMUNOSUPPRESSIVE REGIMENS IN RAT TRACHEA TRANSPLANTATION: ROLE OF TRANSFORMING GROWTH FACTOR-BETA1 (TGF-β1) AND BONE MORPHOGENIC PROTEIN-7 (BMP-7) IN CSA INDUCED NEPHROTOXICITY

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Nephrotoxic side effect of CSA is well known problem following organ transplantation. Bronchiolitis obliterans (BO) is the major cause of graft failure after lung transplantation. We investigated the effectiveness of two different immunosuppressive protocols and the involvement of growth factors in nephrotoxicity and BO in both genders in a rat model of trachea transplantation.
Female (F) or male (M) Brown Norway tracheas were heterotopically transplanted into Lewis recipients of the respective gender and treated for 21 days with either high dose CSA, reduced dose CSA plus Sirolimus (CSA-SRL) or vehicle. Histology, as well as TGF-β1 (profibrogenic) and BMP-7 (anti-fibrogenic) mRNA expression in the grafts and kidney was analyzed, and nephrotoxicity assessed.
CSA and CSA-SRL treatment decreased histological signs of BO and associated with increased TGF-β1 and BMP-7 mRNA expression in both genders. Vehicle treatment induced severe BO and significantly decreased expression of TGF-β1 and BMP-7 without gender differences. CSA-SRL treatment resulted in increased proteinuria and blood urea nitrogen levels. In kidney cortex in M CSA-SRL treatment increased TGF-β1 expression, while no differences were seen in M kidney medulla and in F both in cortex and medulla. In contrary, in M in cortex BMP-7 expression significantly decreased in CSA-SRL group and no significant changes were detected in any other treatment group.
In summary, CSA and reduced dose CSA-SRL are effectively suppressing BO by increasing the expression of BMP-7 and TGF-β1 in both genders. Despite reduction of CSA dose, CSA-SRL combination increases the risk of nephrotoxicity.
Supported by: ETT 184 - 243/2003, OTKA F042563,T042609, OM and Bolyai grants.

Session 34. Pancreas

O266 REVASCULARIZATION OF THE RIGHT GASTROEPIPLOIC ARTERY IN PANCREAS TRANSPLANTATION

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The dorsal pancreatic artery constitutes the major collateral between the head and the body-tail of the pancreas. Its division during organ procurement jeopardizes the blood supply of the duodenum already diminished by the ligation of the gastroduodenal artery (GDA) and may lead to duodenal leak. A technique to improve the blood supply of the head of the pancreas is described.
Methods: The operative notes and angiograms of 110 consecutive pancreas transplants were reviewed. Of these, five pancreata were found deprived of blood supply to the head and the neck of the pancreas on indigocarmine-renografin table angiograms. All had the GDA ligated proximal to the right gastroepiploic artery. During back table reconstruction a distal branch of the SMA was dissected and anastomosed end to end to a widely spatulated gastroepiploic artery using 8-0 monofilament suture. Subsequent table angiogram

showed excellent blood supply to the head of the pancreas and the duodenum. The pancreas transplantation proceeded with iliac artery graft inflow, portal venous outflow and enteric drainage. Simultaneous quadruple therapy with thymoglobulin, CNI, MMF and recently a 4-day course of steroids was used. All patients became insulin independent and euglycemic (BS under 120 mg/dl), 53% within 6 hours, 26% between 6-12 hours and 21% between 12-24 hours. No duodenal leak was observed in the entire series.
In Summary, 1 - ligation of the GDA is not a safe procedure, especially when arterial collaterals to the graft duodenum from the inferior pancreaticoduodenal artery are poor in the absence of the dorsal pancreatic vessels, 2 - preservation of the dorsal pancreatic artery provides good blood supply to the head of the pancreas, 3 - preservation of the right gastroepiploic artery and the distal SMA branches allows a safe and easy revascularization of the pancreatic graft.

O267 NEW PANCREATIC TRANSPLANTATION BENCHWORK TECHNIQUE – REPORT AT SINGLE CENTRE

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Introduction: The aim of this technique is to decrease operative time, increase safety and simplify this complex procedure.
Method: The pancreas benchwork preparation consists of splenectomy, overrunning the duodenal staple lines, identification and ligation of peripancreatic lymphphatic tissue and small vessels and vascular reconstruction using an arterial extension iliac Y graft for the donor. In the classical benchwork, individual vessels are identified and double ligated. This technique is time consuming, require extensive graft manipulation and has the disadvantage that complete haemostasis is difficult to achieve. We have used the ETS-FLEX (articulating) endoscopic vascular cutter for dividing vessels in the pancreas benchwork. The vascular stapler applies three staple lines proximally and three distal and divides the vessels between them. There are three steps where the ELVC is applied: (a) the splenic hilar vessels are stapled first, then (b) the mesenteric root and finally (c) the peripancreatic lymphatic tissue.
Result: We have used this technique in 55 pancreatic grafts. The time for the benchwork preparation including the Y-graft anastomosis was 48±20 minutes. Following revascularization, there was excellent reperfusion of all grafts with minimal bleeding.
Conclusion: We believe that our modification makes a complex and time consuming procedure simple and fast, minimizing the chances for postoperative complications and resulting in excellent patient and graft survival.

O268 THROMBOELASTOGRAPHY DIRECTED ANTICOAGULATION IN PANCREAS TRANSPLANTATION

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Purpose: Graft thrombosis is one of the leading causes of early graft loss following pancreas transplantation. Unmonitored anticoagulation in these patients carries a risk of severe bleeding. This study reports the outcome following thromboelastography (TEG) monitored anticoagulation in these patients.
Materials and methods: From April 2004 to February 2007, 97 pancreas transplants (82 simultaneous kidney-pancreas (SPK), 12 pancreas after kidney (PAK), 3 pancreas transplant alone (PTA)) were performed. All patients had TEG pre-operatively, and anticoagulation post transplant was directed by daily TEG results. The aim of anticoagulation was to maintain the Coagulation Index within the normal range (CI, normal range -3 to +3). The incidence of hypercoagulability (CI>5) as well as thrombosis free graft survival was analysed.
Results: 26 patients (27%) had a CI of more than +5 resulting in therapeutic anticoagulation. Of these 24 patients (92%) were in the SPK group and 2 patients (8%) in the PAK group. Gender distribution in hypercoagulable patients in the SPK group was 3male: 1 female whereas in the PAK group all of them were female. 6 patients with CI >5 also had radiological evidence of partial thrombosis of splenic vein (successfully managed by heparin) compared to none in patients with normal CI. One patient (1.3%) had an intra-abdominal bleed that required a laparotomy for evacuation of hematoma. Two patients in the PAK group who had CI >5 and required anticoagulation, presented with graft thrombosis (at 3 and 12 months respectively) after anticoagulation was stopped.
Discussion: 27% of patients undergoing pancreas transplantation become hypercoagulable requiring therapeutic anticoagulation. Although pancreas graft thrombosis is mainly an early phenomenon, these patients seem to be at higher risk of delayed graft thrombosis after anticoagulation is withdrawn. Identification of this high-risk cohort as well as the underlying mechanism enables targeted therapy.

O269 A META-ANALYSIS OF LONG TERM OUTCOME FOLLOWING PANCREAS TRANSPLANTATION

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Introduction: Simultaneous pancreas-kidney transplantation (SPK) is an established treatment for patients with type 1 diabetes and end stage renal failure. However, there is controversy regarding the long-term outcome of pancreas after kidney transplants (PAK) and pancreas transplants alone (PTA).
Objective: To compare patient and graft survival for SPK, PAK and PTA using meta-analytical techniques.
Materials and Methods: Eighteen studies were identified by literature search in the last 20 years. Only studies with continuous pancreas or patient survival data for at least three years were selected. Care was taken not to include patients twice, where several studies were published by the same group. Seven studies matched the criteria for meta-analysis (1862 patients of whom 662 (35.6%) had an SPK, 706 (37.9%) had a PAK and 491 (26.4%) had a PTA, survival data from 1978 to 2002). The three groups were analysed in pairs, using REVMAN® software ($p \leq 0.05$ considered significant).
Results: At three years, there was no difference in patient survival between SPK and PAK (p=0.47), or PAK and PTA (p=0.44). Patient survival favoured the PTA compared to the SPK group but at a border line level (p=0.05).
The three year pancreas graft survival favoured the SPK compared to the PAK (p=0.005) and PTA (p=0.02) groups as well as the PTA compared the PAK group but at a border line level (p=0.05).
Conclusions: Recipients of SPK, PAK and PTA transplants have similar survival rates. The borderline difference in favour of the PTA compared to the SPK group possibly reflects the lack of the effect of long-term renal failure in the PTA group.

Summary of Results

	Outcome	Surgical Techniques	No. of Studies	Total No. of patients	Result	p value
1.	Patient Survival	SPK vs PAK	3	889	Not Significant	p=0.47
2.	Patient Survival	SPK vs PTA	2	676	Favours PTA	p=0.05
3.	Patient Survival	PTA vs PAK	3	661	Not Significant	p=0.44
4.	Pancreas Graft Survival	SPK vs PAK	4	1303	Favours SPK	p=0.005
5.	Pancreas Graft Survival	SPK vs PTA	3	1045	Favours SPK	p=0.02
6.	Pancreas Graft Survival	PTA vs PAK	4	1150	Favours PTA	p=0.05

Pancreatic graft survival clearly favoured the SPK compared to the PAK and PTA groups. For solitary pancreas transplant, the worst long-tem outcome (although borderline) is for the PAK group possibly due to sensitisation of the recipient.

O270 SIROLIMUS VERSUS MYCOPHENOLATE MOFETIL IN TACROLIMUS BASED PRIMARY SIMULTANEOUS PANCREAS-KIDNEY (SPK) TRANSPLANTATION: 1 YEAR RESULTS OF A MULTICENTRE TRIAL

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This open, prospective, randomized trial compares 2 parallel groups of patients who underwent simultaneous kidney and pancreas transplantation.
Methods: We present the 1 year analysis of the 241 primary SPK recipients from 13 centers throughout Europe and Israel. Following induction with antithymocyte globulin, patients were either given mycophenolate mofetil (MMF n=118) or sirolimus (Siro n=123) concomitant with tacrolimus and short-term steroids. RESULTS: Baseline data are equivalent in both groups. At 1 year, patient, kidney and pancreas graft survival rates were 97%, 95%, 86% in the MMF group and 96%, 94%, 76% in the Siro group. The 1-year rejection rate was 37% and 40%. Thirty-five % of the MMF patients and 46% of the Siro patients were withdrawn from study, mainly for graft loss (35% vs 34%) followed by immunosuppression toxicity (42% vs 45%). The most frequently reported adverse events were urinary tract infections (42%), CMV infections (27%), abdominal infections (14%). Wound problems and lymphoceles occurred more frequently in the Siro group (21%) as compared to the MMF group (13%, NS). Biochemistry results of the in-study patients at 1 year were for MMF and Siro group: serum creatinine: 1.3 and 1.4 mg/dl; fasting glucose: 92 and 91 mg/dl; HbA1C: 5 and 5%; total cholesterol: 171 and 188 mg/dl ($p<0.01$), triglycerides: 106 and 135 mg/dl ($p<0.01$), creatinine clearance: 77 vs 70 ml/min (NS), tacrolimus trough levels: 9.7 and 8.9 ng/ml ($p<0.05$).
Conclusion: At 1 year, good kidney and pancreas function are achieved in both groups despite a slightly lower creatinine clearance in the Siro group.

More patients are still in their study group if MMF is associated with tacrolimus. Wound problems and hyperlipidemia are more likely to occur in the Siro group.

O271 EARLY FAILURE OF PANCREAS GRAFT IN SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANTATION AND IMPACT ON SUBSEQUENT KIDNEY GRAFT FUNCTION. UK EXPERIENCE 2001-2005

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Background: Early loss (<90 days) of the pancreas graft may affect the functions of the kidney graft in Simultaneous Pancreas and Kidney Transplantation (SPK). The aim of this study is to evaluate kidney graft survival when the pancreas graft is lost early.
Methods: All SPK performed in the UK were retrospectively reviewed from data prospectively collected by UK Transplant between January 2001 and December 2005.
Results: In the study period 307 SPK were performed in the UK. Patient survival was 92.5% (23 deaths), pancreata and kidney graft survivals were 81% (n.58) and 92.2% (n.24) at 1 year.
In the first 90 days post SPK 19 deaths were observed (82% of total deaths) 51 pancreata graft failed (88% of total pancreata loss), 15 kidney grafts failed (62.5% of total kidney graft).The causes of death identified were: septicaemia (49%), myocardial infarction (10%), sudden unexplained death (15%), bleeding (15%), not recorded 15%. The causes of pancreata graft failure were rejection/thrombosis (50%), non viable organ (4%), operative problem (4%), not recorded (32%).
The causes of kidney graft failure were rejection (26%), non viable organ (14%), surgical complications (27%), not recorded (33%).
Nine kidneys were lost after 90 days and it was statistically significant when compared with the number of kidney grafts loss (15 out of 51) associated with early (<90 days) pancreata graft loss ($p<0.001$).
Conclusions: In this series most of the deaths and pancreata graft failure are observed in the first 90 days. There is a consistent association between early failure of the pancreas and kidney graft loss, and if no pancreas loss is observed the kidney graft survival is higher. This can be caused directly by systemic illness following failure of the transplant.

O272 EFFECTS ON ACUTE STRESSED FAT AND CARBOHYDRATE METABOLISM AFTER PORTAL OR SYSTEMIC VENOUS DRAINAGE IN SIMULTANEOUS PANCREAS-KIDNEY RECIPIENTS

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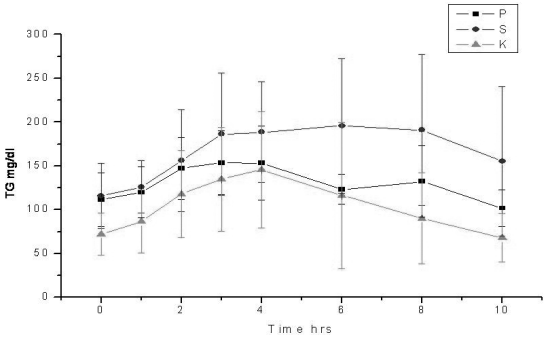
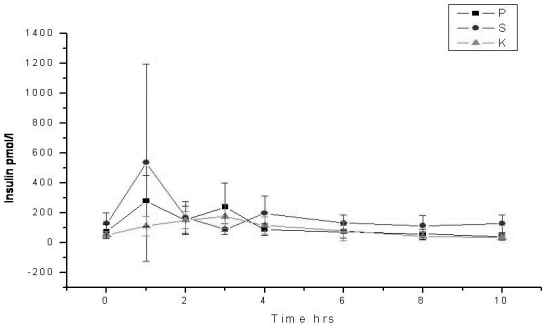
Purpose: It is still controversial, whether there are further advantages for portal drained pancreas recipients in carbohydrate and fat metabolism.
Methods: Prospectively 5 SPK recipients with portal venous drainage, 9 SPK recipients with systemic venous drainage and 7 healthy volunteers (control group) were subjected to a standardized liquid fatty meal (1362 Kcal/2 m^2). Before and after 1, 2, 3, 4, 6, 8 and 10hrs glucose metabolism parameters (blood glucose, C-peptide and insulin), blood lipids (total cholesterol, LDL, HDL, ApoA1, ApoB and triglycerides) and pancreatic enzymes (lipase, alpha-amylase and pancreatic-amylase) were measured. HbA1c, Adiponectin levels, hsCRP, PLTP-activity, GOT/GPT-ratio and creatinine were determined at baseline.
Results: HbA1c levels were within normal range in all three groups (portal: 5,1±0,3%, systemic: 5,3±0,3%, control: 5,3±0,1%). Additionally we calculated HOMA-IR which was significantly higher in the systemic group (portal: 2,0±1,2, systemic: 3,7±2,4, control: 1,5±0,8). No relevant difference in





HDL and LDL concentrations were observed. Triglycerides levels at baseline were the lowest in the control group (portal: 111±30 mg/dl, systemic: 115±37 mg/dl, control: 72±24 mg/dl, $P < 0.05$). In all groups triglycerides increased to 166±58 mg/dl after 4 hrs. In the portal and control group triglycerides decreased after 6 hrs (portal: 123±17 mg/dl, control: 116±83 mg/dl). However systemic-drained patients showed a further increase to 196±77 mg/dl. 10 hours postprandial triglycerides were slightly below baseline level, in the portal (91,5%) and in the control group (94%) but in the systemic group the levels still remained at 134% of the preprandial levels.
Conclusions: Fat metabolism in portal-drained SPK recipients is close to physiological values but pathological in systemic-drained recipients. There is a clear benefit of portal-drained SPK patients regarding fat metabolism.

O273 CORNEAL CONFOCAL MICROSCOPY DETECTS EARLY NEURAL REGENERATION IN TYPE 1 DIABETICS AFTER PANCREAS TRANSPLANTATION

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Aim: Pancreas transplantation (PTx) in Type 1 diabetes has been shown to prevent progression of retinopathy and nephropathy but to a lesser extent neuropathy. Various methods deployed have looked at large nerve fibres.Small nerve fibers may be the earliest to repair and have not been evaluated. Corneal confocal microscopy is a rapid, non invasive in vivo clinical examination technique which quantifies corneal small nerve fiber damage and repair.
Method: 20 Type 1 diabetic patients (pt) aged 41±8 yrs within 1 month of PTx and 14 non diabetic control subjects (ct) aged (45±13 yrs) underwent assessment of corneal sensitivity using non contact corneal aesthesiometry (NCCA) and corneal confocal microscopy to quantify corneal nerve fiber density (NFD), nerve fiber length (NFL), nerve branch density (NBD) and nerve fiber tortuosity (NFT).
Results: Corneal NFD (13.4±8.9 pt v 51.9±10.3 ct, P =0.0001), NBD (3.7±6.4 v 28.9±14.3, P=0.0001) and NFL (2.2±1.2 v 9.1 ±6.0, P=0.001) were significantly reduced and NFT (15.1±3.1 v 24.4 ±10.5, P=0.08) was increased in diabetic patients at transplantation.
6 months after PTx 15 patients underwent repeat assessment and showed improvement in NFD (18.04±5.75 pt v 9.25±7.26 ct, p= 0.001) NFL (3.60±1.16 v 1.84±1.44, p=0.002) with trends for improvement in NFT (15.58±3.98 v 16.30±3.76, p=0.67)and corneal sensitivity (NCCA), (1.23±1.18 v 1.54±1.19, p=0.59). To assess uremic neuropathy,non diabetic patients with renal transplant have been assessed but doesn't show any significant nerve damage.
Conclusion: Small nerve fibers regenerates within 6 months of pancreas transplantation. Corneal confocal microscopy is a novel, non-invasive, in vivo clinical examination technique which may be used to assess the benefits of therapeutic intervention in human diabetic neuropathy.

O273A SIX MONTH CONTROL OF DIABETES BY TRANSPLANTATION OF ENCAPSULATED PIG ISLETS IN DIABETIC PRIMATES WITHOUT IMMUNOSUPPRESSION

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Purpose: This study assessed the capacity of alginate encapsulated islets to reverse diabetes in "pig to primate" model.
Methods: Adult pig islets were encapsulated in microcapsules and implanted under the kidney capsula of 4 STZ-treated primates or in subcutaneous macrodevice in 4 additional diabetic animals. As controls, primates received non-encapsulated pig islets (n=2) or empty capsules (n=2). Body weight, gly-

cosuria, fasting blood glucose (FBG), insulin, porcine C-peptide, HbA1C and anti-pig antibodies were evaluated in sera. Immunostaining for CD3, CD68, C3, C9 were performed on explanted grafts.
Results: Diabetes was confirmed by a significant elevation of FBG and HbA1C ($\geq$13%), as well as glycosuria (1000mg/dl), polydypsia ($>$1100ml), polyuria ($>$600ml) and body weight lost (-28%). Non-encapsulated pig islets were rejected within 7 days as evidenced by lost of function and cellular/humoral responses. Although a significant reduction of FBG ($<$200 mg/dl) and a transient increase of insulin and porcine C-peptide levels were observed during 2 weeks after "microencapsulated pig islets" implantation, a gradual decline of function was observed after 6 weeks. After subcutaneous transplantation of a macrodevice, diabetes was corrected up to a maximum of 6 months: FBG ranged between 52-107 mg/dl, glycosuria was undetectable and HbA1C (after 16 weeks) reached 8$\pm$1.4%. Among the four animals, two were retransplanted with a new macrodevice between 25-35 weeks after the first graft whereas the latter clearly dysfunctioned (FBG $>$153 mg/dl, glycosuria, HbA1C $\geq$13). Diabetes was completely controlled again as evidenced by HbA1C (7.0-9.8%) ten weeks after retransplantation. Immunohistology demonstrated no sign of graft rejection and $>$95% of beta cell mass destruction in the native primate pancreas.
Conclusions: Adult pig islets encapsulated in a *subcutaneous* Macrodevice can completely reverse STZ-induced diabetes up to 6 months without immunosuppression.

Session 35. Pediatric transplantation

O274 RECONSTITUTION OF BKV-SPECIFIC IMMUNITY THROUGH IMMUNOSUPPRESSION REDUCTION PREVENTS BKV NEPHROPATHY IN PEDIATRIC KIDNEY RECIPIENTS MONITORED PROSPECTIVELY

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Polyomavirus BK (BKV)-associated nephritis (PVAN) is a relevant cause of reduced renal allograft survival. In adults, preemptive reduction of immunosuppression (IS) was associated with resolution of BK viremia and absence of PVAN development. However, caution against a preemptive strategy for PVAN is prompted by the notion that re-establishment of BKV-specific immunity by IS reduction may cause direct damage to the infected kidney. We evaluated the impact of prospective BKV monitoring and IS reduction on BKV-specific immunity, viral load kinetics, and PVAN development in pediatric kidney transplantation recipients. From 01/2002 to 08/2006, we monitored BK viruria and viremia by quantitative PCR, at months +1, +2, +3, +6, +9, +12, +18, +24 after transplantation, and yearly thereafter. Viremic patients were evaluated for BKV large T (LT) and VP1 protein-specific immunity by measuring IFN-g-producing cells and specific cytotoxicity in elispot and chromium-release assays. BKV viruria and viremia were observed in 39 and 13 of the 62 patients enrolled. Compared to recipients without viremia, patients with plasma BKV replication had higher peak urine levels ($2.6x10^6$ vs $2.1x10^9$), and a longer duration of viruria. BKV load in plasma reached a median peak level of $2.2x10^4$. Protocol (n=7) or preemptive (n=6) IS reduction was associated with significant increase in the frequency of BKV LT and VP1-specific IFN-g-secreting cells and cytotoxicity (VP1 pre-post: 0 vs 8% lysis; $p<0.05$; LT pre-post: 5 vs 26% lysis; $p<0.05$), and concomitant clearance of viremia in all patients. No PVAN had developed at the last observation date (median follow-up: 24 months). None of the patients experienced rejection as a result of IS reduction. Our data suggest that prospective BKV monitoring and IS reduction in kidney recipients with BK viremia restores BKV-specific immunity, thus preventing progression to PVAN.

O275 IMPROVED CONTROL OF HYPERTENSION IN CHILDREN AFTER RENAL TRANSPLANTATION

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Objective of the study: arterial hypertension is a known risk factor for impaired graft as well as patient survival in patients after renal transplantation (RTx). However, control of hypertension in transplanted children is unsatisfactorily low, 50-80% of children treated with antihypertensive drugs still have hypertension (i.e. have uncontrolled hypertension). The aim of this interventional study was to improve BP control in children after RTx.
Methods: in 36 of 45 children after RTx managed in our transplantation center ambulatory blood pressure monitoring (ABPM) and GFR evaluation (Schwartz formula) were performed. In children with uncontrolled hypertension, the number of antihypertensive drugs was increased to reach the goal ambulatory BP $<$95th centile. ABPM was repeated after 12 and 24 months.
Results: after 24 months night-time BP decreased significantly (from +1.57 to +0.88 SDS for night-time systolic BP and from +1.10 to +0.35 SDS for night-time diastolic BP, $p<0.05$), also daytime BP decreased (from +0.60 to +045 SDS for daytime systolic BP and from –0.09 to –0.65 SDS for daytime diastolic BP), however the decrease of daytime BP was not statistically significant. The prevalence of uncontrolled hypertension decreased from 42% to 25% (NS). Number of antihypertensive drugs increased from 2.1 to 2.7 drugs per patient ($p<0.01$), namely that of ACE-inhibitors and diuretics ($p<0.01$). GFR decreased from 75.5 to 71.9 ml/min/1.73m^2 after 12 months ($p<0.05$) but remained stable during the second year. Proteinuria decreased from 256 to 134 mg/m^2/day ($p<0.01$).
Conclusions: this is the first prospective interventional trial on the treatment of hypertension in children after RTx. We demonstrated that the control of post-transplant hypertension can be improved by increasing the number of antihypertensive drugs, namely ACE-inhibitors and diuretics. Graft function stabilized during the second year and proteinuria improved.

O276 NUTRITIONAL STATUS IN A PAEDIATRIC KIDNEY TRANSPLANTATION COHORT

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Kidney transplantation (KTx) represents for children with End Stage Renal Disease a good opportunity to correct the growth gap and improve their quality of life. In recent years the majority of the studies focused their attention on the role of immunosuppressive drugs on graft survival and incidence of rejection, infection and PTLD. Our goal was to study the prevalence of malnutrition in a pediatric population with KTx and its relations with clinical parameters (renal function and anemia). We performed an observational study analyzing the nutritional status of 36 pediatric patients (3.9-17.6 years) with different age of kidney transplantation (3-15,9 years).
The nutritional status was evaluated by means of the Anthropometry-BIA Nutrition score (ABNscore), an objective system derived from anthropometry and bioimpedance analysis: height, weight, BMI, MAMC, AMA, AFA, reactance, phase angle and distance. All the parameters were given scores of 1 to 5 according to SDS value. An average score was calculated and summed to obtain the ABNscore, which could therefore vary from 3 to 15. Patients with ABNscore $<$10.33 (3° percentile) were classified as malnourished. For each patient the data of ABNscore, age, primary renal disease, standard blood and urinary tests and estimated GFR were collected.
In our population 19: 4% (7/36) of patients were malnourished according to the ABNscore. Patients with malnutrition had lower values of haemoglobin (10.1$\pm$1.8vs11.9$\pm$1.5 gr/dl, p 0.01) and serum albumin (3.7vs4.4 gr/dl, p 0.03), and higher levels of proteinuria (uProtein/uCreatinine 2.9 vs 0.4, p 0.04) and ferritine (356.5 vs 65.4 mg/dl, p 0.001) than those with normal nutritional status. No differences were found between the two groups for eGFR and the other parameters analyzed.
In conclusion, the prevalence of malnutrition in children with KTx is not negligible. Anemia and hypoalbuminemia are associated with impaired nutritional status in this population.

O277 COMBINED LIVER-KIDNEY TRANSPLANTATION – SINGLE CENTER EXPERIENCE

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The aim of our study was the retrospective analysis of indications and results of cLKTx in children.
Materials and Methods: Between 1990 and 2006 we performed in our center 317 liver transplants and 503 renal transplants in children. Among them 16 were cLKTx. Primary diagnosis was: hepatorenal polycystic disease in 12 pts (one of them with HBV hepatitis), oxalosis in 3 pts, and HUS related renal failure associated with cirrhosis after HBV/HCV infection in 1 child. Age at cLKTx was between 3,7-23,3 yrs (mean 14,86 yrs). All pts received whole liver, kid-

ney graft was transplanted after liver reperfusion before biliary anastomosis. Cold ischemia time was 6,75-12,5 hrs (mean 9,54 hrs) for liver and 8-13,9 hrs (mean 10,97) for kidney. In 5 pts X-match was positive and in 5 pts no HLA compatibility was found. We analyzed posttransplant course, and late results in this group of pediatric recipients of combined grafts.
Results: Post Tx follow up range is 0,2-6,9 yrs (mean 3,33). Six pts were dialysed after cLKTx due to ATN. Time of kidney function recovery was 1-20 days. In 3 pts with oxalosis hemodialyses were performed for 1 month after Tx with the aim to remove accumulated oxalate. Primary immunossupression consisted of daclizumab, tacrolimus, mycofenolate mofetil and steroids. Acute rejection occurred in 3 livers and 1 kidney. One pt need reLTx due to HCV reccurrence. One pt with oxalosis lost renal graft and died 2,6 yrs after Tx due to complications of long term dialysis
Conclusions: cLKTx in children is followed by very low rate of rejection, and results in very good patients and graft survival. In patients with oxalosis combined transplantation should be considered early in the course of renal failure to prevent early damage of graft from oxalates accumulated in the body.

O278 LESSONS LEARNED FROM 200 CONSECUTIVE PRIMARY ISOLATED LIVER TRANSPLANTATIONS WITH LEFT LATERAL SEGMENT SPLIT GRAFTS IN CHILDREN

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Introduction: Orthotopic liver transplantation (LTx) is an established procedure for the treatment of children with end stage liver disease. Use of split liver grafts (SLG) have reduced mortality on the waiting list to near 0%.
Methods: From October 1997 to November 2006 we performed 332 LTx in 294 children. A total of 262 (79%) SLG were used. We analyzed 200 consecutive children (median age 0,93, weight median 8,) who received a left lateral segment graft as a primary isolated LTx (188 in situ split, 10 reduced size, 2 ex situ split). Indications for transplantation were biliary atresia in 129 (64,5%) children, Alagille syndrome in 17 (8,5%), Byler's disease in 9 (4,5%), cancer in 9 (4,5%), cryptogeneic cirrhosis in 6 (3%), fulminant or acute liver failure in 11 (5,5%), metabolic diseases in 6 (3%) and others in 13 (6,5%) cases.
Results: Overall patient/graft survival at 3 months, 1 year and 5 years was 93/88%, 90/85% and 88/82% respectively. Considering separately the periods of the years 1997-2003 and 2004-2006, 1 year patient/graft survival were 88/83% and 96/91% respectively. Incidence of hepatic artery thrombosis was 4,5% (9 cases) ReLTx was performed in 8 children and was successful in 6. Overall the incidence of biliary complications was 30% (stenosis of the anastomosis 16,5%, anastomotic fistula 5,5%, leakage from the cut surface 4,5%, bile collection 3,5%). A surgical re-intervention was required in 15 (7,5%) patients.
Conclusion: An aggressive approach allows to safely performed a large numbers of split procedures. The results are similar to those reported by other centers with living donor. In spite of large volume, the learning curve seems to be still present, even in the long term, as expressed by continuous improvement in the results.

O279 OUTFLOW RECONSTRUCTION IN PEDIATRIC LIVING DONOR LIVER TRANSPLANTATION: LESSONS LEARNED FROM 126 CONSECUTIVE RECIPIENTS

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Objective: Hepatic outflow reconstruction is one key in successful living donor liver transplantation (LDLT) because its obstruction leads to graft dysfunction and eventual loss. We review our experience in outflow reconstruction in pediatric LDLT.
Methods: From June 1994-September 2005, 126 children (63 male, 63 female) underwent LDLT. Seven underwent recipient venoplasty of middle and left hepatic veins (HV) prior to anastomosis; whereas 119 underwent recipient venoplasty comprising of right, middle, and left HV. Twenty-eight grafts underwent graft HV venoplasty prior to anastomosis with the recipient HV or vena cava.
Results: There were 76 (60%) left lateral segment, 36 (29%) extended left lateral segment, 4 (3%) left lobe without middle HV, 4 (3%) left lobe with middle HV, 5 (4%) right lobe without middle HV, and 1 (1%) right lobe with middle HV grafts. HV flow problems occurred in 8 (6%). Three of 7 who underwent recipient venoplasty of middle and left HV developed outflow narrowing where 2 had mild narrowing, and 1 severe narrowing which required balloon dilatation and stenting. Of 119 who underwent venoplasty of right, middle and left HV, 5 developed outflow problems including 4 moderate-severe narrowing which required balloon dilatation and 1 graft vessel malpositioning due to a tight abdominal wall closure. Two of the 4 moderate-severe HV narrowing (implanted into recipients who underwent right, middle, and left HV venoplasty) underwent graft venoplasty. There was no recipient or graft loss due to HV complications.
Conclusion: Hepatic outflow in pediatric LDLT must be constructed preferentially with wide recipient and graft orifices to avoid narrowing. Graft venoplasty is an acceptable method of augmenting this orifice size; it is easily performed at back table, and simplify graft-to-recipient cava anastomosis. In cases of stenosis, endovascular procedures are preferred management of choice followed by stent placement.

O280 RENAL DYSFUNCTION IN PEDIATRIC LIVER TRANSPLANTATION RECIPIENTS: RISK FACTORS

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Main concerns are expressed about the significant risk of immunosuppressive drug-related renal dysfunction following liver transplantation (LT). Factors responsible for the worsening of renal dysfunction following pediatric LT, despite reduced cyclosporine dosages remains uncertain.The purpose of this study was to identify risk factors for the development of chronic renal failure following pediatric LT.
Patients and Methods: Medical records of 123 children who underwent renal evaluation at 10 years post-LT were reviewed. All children received CsA primary immunosuppression associated with steroids. The following data were obtained at 10 years after LT : blood pressure, need for antihypertensive drugs, serum creatinine levels, proteinuria, glomerular filtration rate (GFR) evaluated by 51 Cr-EDTA clearance, renal US and CT imaging. Renal dysfunction was defined by a decrease in GFR <80 ml/mn/1,73m^2 Different variables were compared between children without (group 1) and with (group 2) renal dysfunction at 10 years post-LT.
Results: 45% (n=56) of the children presented with mild renal dysfunction at 10 years post-LT (table 1).

Table 1

Renal evaluation	Group 1 (N=67)	Group 2 (N=56)	p
Mean creatinine levels (μmol/l)	60.9	91.3	<0.001
Mean GFR (ml/min/1.73m^2)	94	65	<0.001
Need for antihypertensive medication	6%	18%	<0.001
Proteinuria $>$ 0,5g/24hrs	1.5%	3.6%	ns

Main differences between children with (n=56) and without (n=67) long term renal dysfunction are summarized in table 2.

Table 2

Variables	Group 1 (N=67)	Group 2 (N=56)	p*
Mean age at LT	6.56yrs	7.44 yrs	ns
Pre-LT renal dysfunction	15%	59%	<0.005
Indication for LT: cholestatic disease	87%	78%	ns
Mean pre-operative GFR	124.7 ml/min/1,73m^2	59.7 ml/min/1,73m^2	<0.001
Liver failure at LT	80%	71%	ns
Cold ischemia time (min)	412.5	414	ns
Mean blood masses (during LT)	2.8	2.4	ns
Post-LT acute renal failure	3%	21%	0.005
Acute rejection	28%	21%	ns
Chronic rejection	36%	30%	ns
Cumulative CsA doses(mg / kg / day)	7.72	6.10	<0.005
Acquired hepatitis C infection 9%)1.5% 16	1.5%	16%	<0.001
Acquired renal cysts at CT	19%	39%	<0.01

*Non-parametric tests (Pearson test, Fisher test and Wilcoxon t test) chi square tests as appropriate. P <0.05 was considered as significant.

Conclusion: Long term renal dysfunction is common in pediatric liver transplant recipients. Renal impairment prior to LT and acute renal failure in the immediate post-operative course, acquired hepatitis C infection and acquired renal cystic disease were significantly associated with renal dysfunction despite reducing cyclosporine doses.

O281 LONGITUDINAL SURVEILLANCE OF EPSTEIN-BARR VIRUS IN CHILDREN FOLLOWING CARDIOTHORACIC TRANSPLANTATION

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Elevation in Epstein-Barr virus (EBV) load measured in peripheral blood has been shown to be a weak predictor of early post-transplant lympho-proliferative

disease (PTLD) in children following cardiothoracic transplantation. We report the longitudinal surveillance of EBV for all cardiothoracic transplant recipients at our centre in the longer term.
Methods: Study population included all cardiothoracic transplant recipients between January 2003 and January 2006. EBV load was serially measured in peripheral blood by real time PCR. Results were correlated with recipient pre-transplant EBV status and subsequent development of PTLD. Patients were followed up until January 2007.
Results: 82 transplant operations were performed: 60 heart, 12 double-lung, 9 heart-lung, 1 single lung transplant. 61 (74%) patients were EBV sero-positive pre-transplant. Forty (49%) developed EB viremia post-transplant. Twenty-nine (73%) of whom were EBV sero-positive pre-transplant. EBV was first detected at a median of 20.5 days (range 2-81) post-transplant. Two patients developed PTLD, giving an incidence of 2.4% in the study cohort (1 heart-lung transplant, EBV sero-positive pre-transplant, EBV load 28,084 copies/ml whole blood at time of PTLD diagnosis, and 1 lung transplant, EBV sero-negative pre-transplant, EBV load 4,310 copies/ml). Since establishment of our transplant program in 1988, we encountered 8 cases of PTLD in 331 cardiothoracic transplants in children (overall incidence of PTLD 2.4%). The prevalence of EB viremia in the study cohort was high (median peak load 55,658 copies/ml whole blood). A positive pre-transplant EBV status did not predict post-transplant EB viremia (positive predictive value 0.03).
Conclusions: Our data show that high EB viral load does not inevitably lead to PTLD, even in the medium term. Substantial pre-emptive reduction of immunosuppression in patients with raised EBV load seems not justified.

O282 SURVEY OF THE USE OF NON-HEART BEATING ORGANS IN PAEDIATRIC PRACTICE

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Introduction: With the global shortage of donated kidneys for transplantation UK Transplant invested in programmes to retrieve organs from non-heart beating (NHB) donors. Whilst this has increased the numbers of organs available for transplant into adult recipients it has not had a significant effect on paediatric practice. The aim of this survey was to look at current paediatric practice.
Method: In June 2006 an online questionnaire was sent to research leads at all 13 paediatric nephrology units in the UK.
Results: Replies were received from 12 units giving a 92% response rate. 10 units were aware of local NHB programmes. Only one unit had actually transplanted a NHB organ into a paediatric recipient. Another unit was offering them to paediatric patients and had three children on the waiting list for them. Both these units had local programmes that retrieved organs of Maastricht category 3 and 4. None of the units whose local programmes involved retrieval of category 2 organs were offering organs to paediatric recipients.
Of the units not using these organs the main reason given was insufficient data regarding the use of these organs in children. In units where a local programme had been running for more than three years lack of data in adults to extrapolate to the paediatric population and poorer outcome compared to organs from other sources were also quoted.
Conclusions: Whilst most paediatric nephrology units have local NHB retrieval programmes these organs are not being transplanted into the paediatric population. Despite a lack of data regarding the use of organs from NHB donors into paediatric recipients there is good evidence that, in adults, they have equivalent survival to those from heart beating donors. Therefore, they should be strongly considered as an option for paediatric patients.

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O283 ALLOCHIMERIC THERAPY GENERATES CD4$^+$ T REGULATORY CELLS THAT INHIBIT THE DEVELOPMENT OF TRANSPLANT VASCULAR SCLEROSIS

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We have shown that peri- or post-operative peripheral delivery of allochimeric [a_{1h} ^{u}]-RT1.A^a class I MHC molecules (AlloP) induce tolerance and inhibit chronic rejection (CR) in ACI (RT1^a) recipients of WF (RT1^u) hearts. This study investigated the role of AlloP T-reg in the development of CR.
Methods: Irradiated (130R) ACI recipients of WF hearts were injected with singeneic CD4$^+$; CD8$^+$; CD4$^+$CD25$^+$ cells (10^7 cells $>$95% purity; iv) from long-term AlloP or high-dose CsA (CsA) treated cardiac hosts.
Results: *In vitro*, T-reg from AlloP and CsA rats inhibited proliferation of ACI cells against WF (750$\pm$100 &1600$\pm$200 ^{3}H-TdR cpm/min), but not against third-party BN (3200$\pm$500; $P<0.001$). *In vivo*, T-reg activity was confirmed by the ability of such cells to prolong WF graft survival ($>$120 d) in ACI hosts (n=17 & 8). However, only AlloP induced T-regs abrogated CR, as evidenced by intact architecture and absence of neointimal proliferation, as assessed by neointimal index (NI=15$\pm$5) with only 26$\pm$6% of vessels affected. In contrast, allografts of secondary ACI hosts treated with CsA-induced T-reg, exhibited severe CR, strong neointimal proliferation and increased NI (NI=50$\pm$16 with 79$\pm$1% vessels affected). Surprisingly, both CD4 and CD8 subsets induced long-term survival of secondary test grafts ($>$100 days). However, only the CD4$^+$ T-reg prevented CR (NI=19$\pm$5, 21$\pm$6% of vessels), as compared to CD8$^+$ T-reg (NI=40$\pm$9 in 50$\pm$15% of vessels). The cytokine profile indicated a dominant IL-10 response. Allo-antibody analysis showed up-regulation IL-4/IL-12 dependent IgG1 and IgG2c.
Conclusion: Allochimeric protein-therapy and CsA treatment generate T-reg that prolong graft survival, but only CD4$^+$ T-reg generated from allochimeric protein-therapy prevent CR. This CD4$^+$ T-reg may be responsible for long-term graft maintenance through its unique ability to control anti-inflammatory and alloantibody responses.

O284 MACROPHAGES DRIVEN TO A NOVEL STATE OF ACTIVATION HAVE TOLEROGENIC PROPERTIES IN MICE

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Background: The use of immunomodulatory cells in solid organ transplantation is considered a promising approach for tolerance induction. Here, we describe a novel population of macrophage-derived cells with tolerogenic properties.
Methods: Murine bone marrow, spleen and blood cells were cultured with macrophage-colony-stimulating-factor (M-CSF), and later with interferon-γ (IFN-γ), to produce what we refer to as "interferon-γ-stimulated monocyte-derived cells" (IFNγ-MdCs). IFNγ-MdCs were characterized by flow cytometry and immunomodulation was examined by assays for cell death and T cell regulation. Therapeutic effects were assessed in a murine heterotopic heart transplantation model and models of autoimmune colitis.
Results: IFNγ-MdCs express markers including CD11b/c, CD14, CD86, CD123, and programmed death ligand-1; they are negative for CD4, CD8, CD19, CD80, CD205, CD207 and CD209. IFNγ-MdCs only arise when macrophages are cultivated in the presence of CD4$^+$ T cells, M-CSF and IFN-γ. In vitro, IFNγ-MdCs profoundly delete lymphocytes (by $>$60%) derived from mice via a cell activation and caspase -dependent mechanisms. Intriguingly, lymphocytes surviving in IFNγ-MdC co-cultures are highly enriched for CD4$^+$CD25$^+$Foxp3$^+$ cells, which show up-regulation of IL-10, and actively suppress T cell proliferation. CD11b$^+$ cells within the IFNγ-MdC population are responsible for lymphocyte depletion and CD4$^+$CD25$^+$Foxp3$^+$ cell induction. Additionally, using corresponding knock-out mouse strains, we show that signaling via the IFN-γ receptor and CD40 on IFNγ-MdCs are necessary, but IDO is not, for the generation of T regulatory cells by IFNγ-MdCs. Regarding potential therapeutic activity, a single intravenous injection of donor IFNγ-MdCs prolonged C3H heart transplant survival by nearly 2 weeks in Balb/c recipients; furthermore, syngenic IFNγ-MdCs reversed established chronic autoimmune inflammation in two mouse models of chronic colitis.
Conclusions: We conclude that IFNγ-MdCs represent macrophages in a novel state of activation, possessing multiple T cell-suppressive effects with tolerogenic therapeutic potential.

O285 THE PLACE OF LIVER TRANSPLANTATION (LT) IN THE TREATMENT OF HEPATIC EPITHELOID HAEMANGIOENDOTHELIOMA (HEHE): REPORT OF THE EUROPEAN LIVER TRANSPLANT REGISTRY

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Background: HEHE is a rare low-grade tumour with an unclear treatment algorithm.
Material and methods: 59 recipients were analyzed.Eighteen (31%) pats had pre-LT surgical and/or systemic(10 pat) medical therapy. Ten (17%) pts had extra-hepatic disease(EHD) before or at moment of LT. Follow-up was complete(mean of 104±72 mo from moment of diagnosis and 83±55 mo from moment of LT
Results: HEHE was bi-lobar in 96% of pts.Macro-and microvascular invasion (MaVI; MiVI) was present in 9% and 44% of pts and lymph node invasion (LNI)in33% pts. Early (<3 mo) and late (>3 mo) mortality was 2% and 22%. Fourteen(24%) pts developed disease recurrence(DR) after a mean time of 45±35 mo. One, 5 and 10 yr patient survival from moment of diagnosis and of LT are 97;83,74% and 93;83,72%. Pre-LT tumor treatment (89;89 and 68% 1, 5 and 10 yr survival from momentn of LT vs. 95;80 and 73% in no pre-LT treatment), LNInv (96;81 and 71% 1, 5 and 10 yr survival vs. 83;78 and 67% in node negative patients) and EHD(90;80 and 80% 1,5 and 10 yr survival vs. 94,83 and 70% in no EHD) didn't influence survival whereas MiVI (96;75,52% 1,5 and 10 yr survival vs 96;92,85% in no MiVI) and combined MiVI and MaVI (90;72 and 54% 1,5 and 10 yr survival vs. 96;92 and 85% in noVI p 0.03) did. Disease free survival(DFS) at 1, 5 and 10 years post LT are 90;82 and 64%.DFS is not significantly influenced by pre-LT treatment, LNInv, EHD and VInv.
Conclusion: The results of largest reported LT series in the treatment of HEHE are excellent.EHD and LNI are not contraindications to LT.LT should be offered as a therapy early in the disease course.

O286 NON HEART BEATING LUNG DONATION: HOW BIG IS THE POOL?

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Introduction: In 2005 a Non Heart Beating (NHB) donor single center program for lung transplantation was started in the Netherlands, using category III donors. Although the NHBIII pool is increasing, the number of transplanted NHBIII lungs remains unexpectedly low.
Methods: We assessed all NHBIII and Heart Beating (HB) donor procedures between January 2005 and September 2006 in Dutch donor files. Information on which organ was used, age, reasons for not procuring the lungs, and settings of mechanical ventilation combined with arterial blood gas were collected. Internationally, lungs with a PO_2 >40 kPa after 100% oxygen and 5 cm H_2O PEEP are considered suitable for transplantation.
Results: From a total of 162 NHBIII procedures 29 lungs (18%) were offered for transplantation. Of these 29 procedures, 8 (5%) lungs were procured and transplanted successfully, 7 (4%) were lost for logistic reasons, 11 (7%) refused for medical reasons, and 3 (2%) started but failed.
In comparison, from 207 HB donations 88 (43%) lungs were procured and transplanted, 4 (2%) were not procured for logistic reasons, 7 (3%) for no consent, and 119 (57%) because of medical reasons, including 29 (14%) because of age.
In total 154 (95%) NHBIII lungs were not procured because of no consent in 16 (10%), age in 29 (18%), logistics in 7 (4%) and medical reasons in 102 (63%). Of these 102 medically rejected lungs, 30 fulfilled oxygenation and age criteria for donation. These 30 were potential donors, but were not offered. In 23 of these 30 no adequate lung function testing was performed despite good oxygenation.
Conclusions: The potential of NHBIII donor lungs is four times larger than the NHBIII donor lungs actually used. These data suggest that the amount of NHBIII donor lungs could be expanded with education, training, and transplant capacity.

O287 HOW TO RECOGNIZE A SUITABLE PANCREAS DONOR?

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Because of the increasing demand for pancreas transplantation, more marginal donors are offered to Eurotransplant. The aim of this study was to compose a donor quality score, based on donor factors, enabling the recognition of a suitable pancreas donor among all reported donors.
All 3310 consecutively reported pancreas donors and all 920 transplanted pancreata between 01.01.02 and 30.06.05 were analyzed. The influence of the pre-procurement pancreas suitability score (P-PASS) on the acceptance of a pancreas, and its influence on post-transplant outcome was studied. For each variable a range and point weight were defined based on clinical expertise and known literature. In a spin off study these ranges and weights were estimated based on the available data and allowed the construction of a second score, P-PASSm.
Multiple regression analysis using pancreas acceptance as outcome variable identified P-PASS $\geq$ 17 as a significant cut-off point ($p < 0.001$). Pancreas donors with P-PASS $\geq$ 17 were 3 times more likely to be rejected. Graft survival rate at 1-year was 82% and 64% for the P-PASS < 17 group *vs.* P-PASS $\geq$ 17 group, respectively ($p = 0.02$).
The P-PASSm was also significantly associated with pancreas acceptance.
Both scores, when assessed by the c-index were modest predictors for pancreas acceptance, 0.68 and 0.69 for the P-PASS and the P-PASSm, respectively.
The donor score can help in screening a potential pancreas donor, where an ideal donor has a P-PASS < 17 or a P-PASSm < 10.5. Furthermore, in case of a high score, 1-year graft survival is significantly jeopardized. Our data demonstrates that a combination of pre-procurement factors can help recognizing a suitable pancreas donor. We therefore recommend that upon organ donor reporting a pancreas donor score should be calculated where all low score donors should be considered for pancreas donation.

O288 FIRST FACE ALLOGRAFT ONE YEAR LATER

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The first human face allotransplantation was successfully performed on November 27, 2005. The recipient was a 38 year-old woman who was traumatically mutilated and disfigured by a dog bite on May 31th, 2005. She received a facial allograft (nose-lips-chin) from a brain dead female donor. A vascularized sentinel donor skin graft was performed under the recipient breast. Donor bone marrow was infused on days 4 and 11 post transplantation to improve graft acceptance. Immunosuppression included tacrolimus, prednisone and mycophenolate mofetil while antithymocyte globulins were added for induction.
The initial postoperative course was uneventful.
Two episodes of acute rejection occurred on days 18 and 214 post-transplant. Macroscopically the patients developed erythema and oedema on both skin of face and sentinel flap and oral mucosa. Biopsies revealed dense mononuclear cell infiltrate, vacuolisation of basal cells, occasional apoptotic keratinocytes in the mucosa biopsies. The two rejection episodes were resolved with 3 boluses of 1 g prednisolone and increasing tacrolimus and mycophenolate mofetil oral doses. In both cases topical immunosuppressants were used. In order to prevent another rejection episode since August 2006 extracorporeal photochemotherapy was performed twice a week for the 4 initial weeks, once a week for 8 weeks, and then decreased progressively.
The main side effect of immunosuppressive regimen was progressive renal failure; Sirolimus was introduced at 11 months post-transplant in order to avoid the nephrotoxicity of calcineurin inhibitors.
At present immunosuppressive regimen includes sirolimus, mycophenolate mofetil and steroids with an improvement of renal function and without other episodes of rejection.

One year later the excellent sensorimotor recovery allowed the patient to face the outside world and returned progressively to a normal social life.

Session 37. Kidney: Short & long-term complications

O289 WHICH FACTORS INFLUENCE THE NUMBER OF HOSPITALIZATION DAYS IN THE FIRST THREE MONTHS AFTER KIDNEY TRANSPLANTATION?

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Background: The Dutch organ transplant registry (NOTR) contains information about the hospitalization of recipients after organ transplantation. We analyzed the parameters that influence the number of hospitalization days in the first period after kidney transplantation.
Methods: In this study we analyzed the 3 month follow-up for kidney transplants between 1980 and 2006. Patients were included if they were alive with a functioning graft at three months after transplantation. Cases were assumed "missing" if less than 5 hospitalization days were reported. In approximately 70% of the cases (4267/6188 records) information about hospitalization was available.
Results: The mean number of hospitalization days during the first three months after transplantation was 26.6 days (range 5-92). A significant decrease in hospitalization days for all transplants over the years was found. Within the period 2000-2006 a significant difference was shown in the mean number of hospitalization days for the donor type (deceased donors versus living donors). For the deceased donors, significantly more hospitalization days were found for recipients who received kidneys from older donors. For living donors significantly more hospitalization days were found for the recipiens who received a kidney from an unrelated donor. The differences found for the cold ischemic period and the deceased donor type (non heartbeating versus heartbeating) were not significant.

Parameter		Hospitalization days*	N	
Transplant period	1980-1989	32.8	445	p<0.001
	1990-1999	29.9	1095	
	2000-2006	24.3	2727	
Transplant period 2000-2006				
Donor type	deceased donor	27.2	1680	p<0.001
	living donor	19.8	1047	
Deceased donors - donor age	0-19	23.9	165	p<0.001
	20-39	25.6	599	
	40-59	27.3	1480	
	60+	30.6	483	
Living donors - type living donor	unrelated donor	20.9	391	p=0.028
	related donor	19.1	656	

*Average number of hospitalization days in 3 months after transplantation

Conclusion: The NOTR information shows that the transplant period, the donor type, the donor age of deceased donors and the relation of living donors, have a significant effect on the number of hospitalization days for recipients in the first three months after transplantation.

O290 ELDERLY KIDNEY TRANSPLANT RECIPIENTS ARE A HIGH-RISK GROUP FOR DEATH, INFECTIONS AND POST-TRANSPLANT DIABETES: EVIDENCE FROM THE SYMPHONY STUDY

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While recipient age in renal transplantation steadily increases and elderly transplant patients are considered to have specific problems, no standard therapy exists for this group of patients.
Methods: We made a subgroup analysis of the Symphony data collected from 1645 patients randomised to receive standard-dose cyclosporine (Stand-CsA), or daclizumab and either low-dose CsA (Low-CsA), tacrolimus (Low-TAC) or sirolimus (Low SRL), all in addition to MMF and steroids. We compared the subgroup of 296 recipients aged ≥60 years to the 1292 patients aged <60 years in terms of GFR, BPAR, patient/graft survival, and safety at 12 months post-transplant.

	Stand-CsA	Low-CsA	Low-TAC	Low-SRL
Age groups		<60y/ ≥60y		
N (ITT)	319/71	319/80	323/78	331/67
GFR [ml/min]	60.0/44.0**	62.7/46.2**	68.9/50.9**	59.4/42.7**
BPAR (%)	26.1/24.4	24.7/21.0	12.1/13.6	38.2/31.5
Patient survival (%)	97.7/91.2*	99.0/94.7*	98.1/93.3*	97.1/95.3
Graft survival (uncensored, %)	90.8/82.6	94.0/89.7	94.7/92.1	89.0/90.6
N (Safety)	314/70	326/82	325/78	315/64
Infections (%)	60.2/74.3*	54.2/62.2	53.9/69.2*	57.1/70.3
Pneumonia (%)	4.5/14.3*	2.5/4.9	3.4/6.4	5.7/12.5
Urinary tract infection (%)	31.2/52.9*	27.0/41.5*	26.8/42.3*	29.2/32.8
Sepsis (%)	4.8/15.7*	2.2/8.5*	6.2/3.9	4.1/14.1*
Post-transplant diabetes (%)	4.5/18.4*	3.8/9.1	7.9/23.0*	5.8/16.6

*p<0.05, **p<0.0001

Results: There was no significant difference in graft survival, BPAR rate or DGF between the subgroups <60 and ≥60 years, whereas significantly lower GFR and patient survival were found in the ≥60 year old population (table). Significantly increased incidences of overall infections, pneumonia, sepsis, UTI and post-transplant diabetes, but not cytomegalovirus infections, characterise the high-risk profile of this elderly group. Within the group of ≥60 year old patients, no significant differences could be found in the efficacy and safety parameters, however, the relative efficacy of each regimen was similar to the overall study results.
Conclusions: Elderly renal transplant recipients are a high-risk group for low GFR, death, infections and post-transplant diabetes. While many safety parameters in the Stand-CsA group were worsened in elderly patients, low-dose immunosuppression regimens could prevent some of these complications.

O291 LATENT CYTOMEGALOVIRUS INFECTION IS ASSOCIATED WITH INCREASED RISK FOR LATE GRAFT LOSS IN RENAL TRANSPLANT RECIPIENTS

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Latent cytomegalovirus (CMV) has been suggested to play a role in late graft loss (GL) in renal transplant recipients (RTR). Most studies investigated symptomatic CMV disease as a risk factor and found only moderate increased risk for GL. Investigation of the association of CMV disease with GL may negate the possibility that absence of symptoms is still accompanied by ongoing CMV-related inflammation in the transplanted kidney. Therefore, we prospectively investigated the association of CMV serostatus in RTR >1 year after transplantation with death-censored graft loss (DCGL).
Outpatient-clinic RTR were invited to participate between August 2001 and July 2003. DCGL was recorded continuously. GL was defined as returning to dialysis or retransplantation. CMV IgG was assessed by ELISA. RTR were divided into CMV seropositive, CMV seroconverted, and CMV seronegative groups. DCGL was analysed with Cox-regression analyses and Log-rank test. RTR (n=606, aged 51±12 years, 55% male) were at a median time [interquartile range] of 6.0 [2.6-11.4] years post-transplant. During follow-up 37 RTR experienced DCGL. DCGL and CMV IgG levels according to CMV serostatus are shown in the table. After adjustment for recipient age and gender, time between transplantation and baseline, and serum creatinine and proteinuria at baseline, both CMV seropositive (HR=7.8, $P<0.01$) and CMV seroconverted (HR=5.1, $P<0.05$) RTR were at higher risk for DCGL (Model 2, table). Graft survival was significantly better in CMV seronegative RTR compared to CMV seropositive and seroconverted RTR (figure, $P<0.005$).
Our study shows that RTR who are CMV seropositive or seroconverted >1 year after transplantation are at higher risk for DCGL than CMV seronegative

Table 1. Cox-proportional hazards analysis of the effect of CMV serostatus on DCGL in RTR and characteristics according to CMV serostatus

	CMV seronegative		CMV seropositive		CMV seroconverted	
	RR (95% CI)	P	RR (95% CI)	P	RR (95% CI)	P
Model 1	1.0	<0.05	8.3 (2.0-35.0)	<0.005	7.0 (1.5-31.5)	0.01
Model 2	1.0	<0.05	7.8 (1.8-34.2)	<0.01	5.1 (1.1-24.7)	<0.05
RTR, n (%)	174 (28.7)		280 (46.2)		152 (25.2)	
DCGL, n (%)*	2 (1.1)		24 (8.6)		11 (7.2)	
CMV IgG level (U/mL)#	0 [0-0]		110 [62-198]		110 [62-191]	

Model 1: crude model, model 2: recipient age and gender, time between transplantation and baseline and serum creatinine concentration and proteinuria at baseline adjusted. RR: relative risk, CI: confidence interval. *P<0.05, #P<0.001.

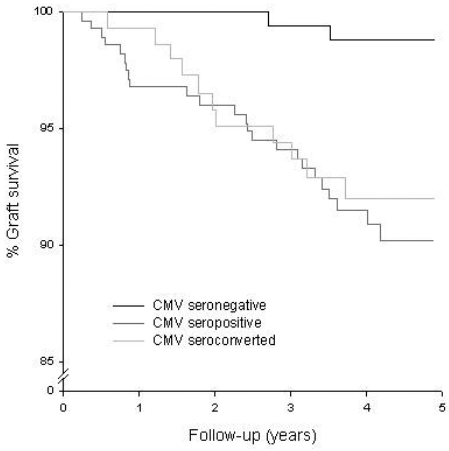

Fig. 1. Kaplan-Meier curve for DCGL for CMV seronegative, CMV seropositive, and CMV seroconverted RTR. The difference is significant (P<0.005).

RTR. Further studies are required to determine the role of CMV in late GL, and whether antiviral therapy and/or partial withdrawal of immunosuppression may improve long-term renal outcome in RTR.

O292 ANALYSIS OF CELLULAR IMMUNITY TO POLYOMAVIRUS BK LARGE T AND VP1 ANTIGENS AFTER KIDNEY TRANSPLANTATION

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Polyomavirus BK (BKV)-associated interstitial nephritis (PVAN) has emerged as the a relevant complication after kidney transplantation (KTx). We evaluated prospectively the cell-mediated immune response to the BKV antigens large T (LT) and VP1 in a cohort of 36 pediatric kidney recipients undergoing monitoring for BK viruria and viremia by quantitative PCR at months +1, +3, +6, +9, +12, +18, +24, +36 after KTx. Interferon-g (IFNg) secreting cells were measured in PBMC by flow cytometry and by ELISPOT assay in T-cell cultures after 9-day stimulation with overlapping peptides spanning the BKV VP-1 and large T proteins. We found that samples from virus-seropositive patients with BKV urinary shedding (n=26), obtained at the nearest time before BK positivity, had significantly lower responses to LT, but not to VP1, compared to samples from BKV-seropositive patients (n=7) who never reactivate the virus [mean IFN-g+ spots forming cells, SFC/10^5 cultured cells: 26±33 vs 73±65; median: 16 vs 71, p<0.05]. Among the patients with BKV viruria, the inability to maintain or mount a cellular immune response to both LT and VP1 was significantly associated to development of viremia (3, range 1-18 months post-KTx). The clearance of BKV DNA in plasma, observed in all viremic patients and secondary to reduction of immunosuppression, coincided with a significant increase in both LT and VP1 responses, with increase of both CD8+ and CD4+/IFNg+ T cells. Viremia duration was associated to the kinetics of reconstitution of BKV-specific cellular response, the longest replications being observed in patients persisting with low numbers of LT- and VP1-specific T cells. These latter patients may be candidates to a preemptive reduction of immunosuppression. Our study suggest that low levels of BKV-directed T cells predispose KTx recipients to BKV replication, and progression to PVAN.

O293 EVOLUTION OF PARATHYROID FUNCTION IN RENAL TRANSPLANT RECIPIENTS. A PROSPECTIVE STUDY

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Secondary hyperparathyroidism (sHPT), a common complication in patients with ESRD, may be partially reversed following successful renal transplantation (RTx). Purpose of this prospective study is to evaluate the evolution of sHPT in de novo RTx recipients. Patients and methods: Since August 2004 till December 2006, 42 patients (32 male) of median age 49.9, range 21-72 years old, who underwent successful RTx (32 cadaveric, 10 living-related) were assessed before RTx and every three months following RTx up to 12 months with biochemical and endocrinological parameters to evaluate renal graft (using MDRD equation) and parathyroid function. Median time of dialysis prior to RTx was 43.6 (range 4-131) months and median time of follow-up was 9 (range 3-12) months.

Results: Statistical analysis revealed significant decrease of parathyroid hormone (iPTH) both in the immediate and the long term period post-RTx (mean±SD, table 1). Recipients, who had remained on haemodialysis >5 years, had significantly higher values of iPTH before RTx and three months post-RTx (Pearson correlation: 0.71, p=0.01). According to K/DOQI guidelines, recipients of this study (table 1) should be considered equivalent to CKD stage 3 and iPTH values should be <70ng/ml. Even though iPTH levels fell, they did not reach the target values. Persistent hypercalcaemia (sCa>10.5mg/dl) was seen in 6/42 (14.3%) recipients. Screening before RTx showed 25(OH)VitD insufficiency [<30ng/dl] in 36/42, 85.7% RTx recipients of whom 4 displayed deficiency [25(OH)VitD <7ng/dl]. Three months post-RTx, 25(OH)VitD levels were further reduced but consequently increased to pre-RTx levels.

Table 1

Month	0	3	6	12	p
MDRD GFR (ml/min)	N/A	50.4±20.8	47.7±20.9	50.8±20.06	
iPTH (10-55 pg/ml)	411.1±351.7	166.1±104.3	174.9±97.3	143.6±112	<0.001
Serum Ca (8.5-10.5 mg/dl)	9.4±1	10.3±0.9	10.2±0.7	10±0.8	<0.001
Serum Phosphorus (2.5-5 mg/dl)	5.3±1.9	3.3±1.3	3.3±0.8	3.4±0.7	<0.001
25(OH)Vitamin D (ng/ml)	19.3±12.9	14.9±6.7	16.2±9.2	19.4±8.4	=0.151

Conclusions: Post successful RTx, good renal graft function contributes significantly in controlling sHPT. Total duration of dialysis pre-RTx determines progression of parathyroid function. Relative hypovitaminosis D, which may contribute to sHPT, should be identified and treated accordingly, since RTx, in the present study, does not correct 25(OH)Vit D levels.

O294 CARDIOVASCULAR EVENTS AND MORTALITY IN THE TWO YEARS AFTER RENAL TRANSPLANTATION. RESULTS OF A SPANISH MULTICENTRE DATABASE FOCUS IN CARDIOVASCULAR DISEASE

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With the aim to evaluate cardiovascular diseased after kidney transplantation (KTx) patients in Spain in the actual immunosuppressive era. We analyzed the results of 2600 KTx patients during years 2000-2002 in 14 Spanish units, most of them, from cadaveric donors. Donors and recipientent age were 46.9±17 and 49.7±13.7 years, 63% men, 16% were retransplanted and 12.5% hyperimmunized. The most frequent immunosuppressive protocol was Tac+MMF+St. Acute rejection rate at 2 years was 14%. Graft survival with death censored to 24 months was of 89.7%, being of 86% if the death were not censored. The first cause of graft loss was death with functioning graft (25%), followed by vascular (24%), acute rejection (16.5%) and chronic allograft nephropathy (13%). Patient survival at two years was 95%.The majority of the deaths (36%) had a cardiovascular origin (6% stroke and 30% cardiac cause). Also 5.5% patients had a cardiovascular event.

Cox regression analysis identified the donor age (RR1, 009; p=0.034); NTA (RR2.689; p=0.0001) and acute rejection (RR2, 086; p=0.0001) like independent risk factors for graft loss. Also, the independent risk factors for patient death were recipient age (RR1, 052; p=0.0001), pretransplant cardiovascular disease (RR 1,792; p=0.005) and the cause of end stage renal disease (diabetes mellitus and nephroangiosclerosis *vs* rest of causes) (RR=1,573; p=0.036). In conclusion, the death with functioning graft constitutes the main cause of graft loss in the present era in Spain. In addition, most important cause of death was cardiovascular disease. The age of our recipients, as their concomitants pathology at the moment of the transplant can be determinant of this situation. It is necessary to make a big effort to control cardiovascular risk factors, before and after transplantation.

O295 TEN YEARS FOLLOW-UP OF HYPERTENSIVE KIDNEY TRANSPLANT PATIENTS TREATED WITH CALCIUM-CHANNEL-BLOKKER (CCB) VERSUS ANGIOTENSIN-CONVERTING-ENZYME (ACE)-INHIBITOR

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Background: Hypertension is prevalent after renal transplantation and affects up to 60-90% of the recipients. Good blod-pressure (BP)-control is important for long-term graft and patient survival. We have previously shown that nifedipine, in contrast to lisinopril, improved glomerular-filtration-rate (GFR), measured by 99Tc-DTPA, during the first 2 years after transplantation(1). However, comparative long-term data for CCB versus ACE-inhibitors are scarce. We therefore performed an intention to treat analysis of these two antihypertensive regimens, ten years after the original study.
Methode: Hypertensive renal recipients were included in the original prospective double blind (nifedipine/lisinopril) trial between 1995 and 1997. Basic immunosuppression was cyclosporine A, prednisolone and azathioprine. The study was un-blinded in 1999 and patients continued the original allocated antihypertensive medication.
Results: Originally 154 patients were included (nifedipine=78, lisinopril=76). Ten years after randomization 103 patients (67%) still had a functioning graft (Group 1; original nifedipine=55; 70%/group 2; original lisinopril =48; 63%). Calculated GFR (MDRD-formula) was equal in the two groups (Group1; 52,8±19,5 ml/min, group 2; 52,1±21,1 ml/min). In group 1, 31(56%) patients still were treated with CCB and in group 2, 35(73%) patients had continued on ACE-inhibitor/Angiotensin-receptor-blokker. Calculated GFR only using patients *on treatment* for 10 years showed no difference (Group 1; 53,8±22,4 ml/min vs group 2; 53,1±21,9 ml/min). There was no difference in BP-control: Systolic-BP (Group 1; 132,7±12,7 mmHg vs. group 2; 135,2±14,2 mmHg), diastolic-BP (Group 1; 80,6±6,7 mmHg vs. group 2; 81,1±10,3 mmHg).
Conclusion: Both CCB and ACE-inhibitors are safe and effective in treatment of hypertension following renal transplantation. These data do not support the general belief that ACE-inhibitors are more nephroprotective than CCB in the kidney transplant population.
Reference: 1. Midtvedt et al, Transplantation.

O296 PREDICTION OF NEW ONSET DIABETES MELLITUS AFTER TRANSPLANTATION (NODAT): EARLY EVALUATION OF GLUCOSE METABOLISM

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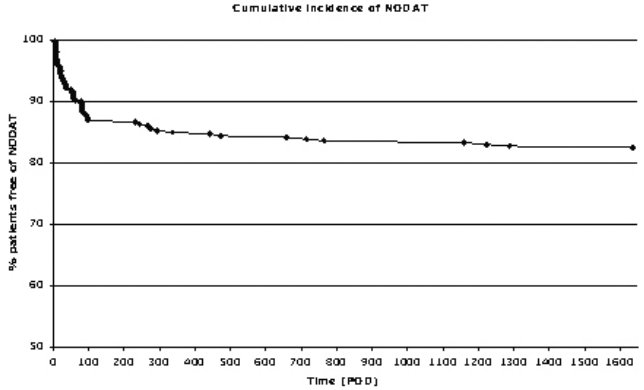
New onset diabetes mellitus after transplantation (NODAT) has an important impact on renal allograft survival, cardiovascular risk and patient survival. Therefore, the early diagnosis/prediction of NODAT is an important clinical tool for improving management of associated risk factors.
The predictive value of a fasting plasma glucose (FPG) level and oral glucose tolerance test (OGTT) on the fifth day posttransplantation for the development of subsequent NODAT were prospectively evaluated in 359 renal recipients.
Sixty-four patients (17.8%) developed NODAT (follow-up 42.8±16.9 months) with a median time to diagnosis of 54.5 days. Seventy-five percent of patients (n=48) developed NODAT within the first 100 days after grafting (Figure). Recipient age, BMI, biopsy-proven acute rejection (BPAR), early graft function and proteinuria, tacrolimus-based therapy, cumulative corticosteroid dose and thiazide diuretics were associated with NODAT (univariate analysis). Recipient gender, body weight, ethnicity, renal diagnosis, smoking, HLA-mm, PRA>20%, type of and time on renal replacement therapy, hepatitis C status, deceased versus living donor, and number of transplantations were not significantly associated with NODAT (univariate analysis). Multivariate logistic regression analysis identified age [OR: 1.05 (95% confidence interval: 1.019-1.083)], BMI [OR: 1.09 (1.013-1.189)], proteinuria on day 5 [OR: 1.51 (1.043-2.210)] and BPAR [OR: 2.74 (1.345-5.604)] as independent risk factors for NODAT while a normal OGTT on postoperative day 5 was independently associated with a strongly reduced risk for NODAT [OR: 0.03 (0.008-0.166)]. A similar risk reduction was conferred by a normal FPG [OR: 0.06 (0.012-0.338)]. OGTT had the best sensitivity (93.4%) and specificity (71.9%) with a high negative predictive value (97.6%).
Day five OGTT is an independent predictor of NODAT that can be used for identifying recipients at risk and could potentially be used to direct early adjustments of immunosuppressive therapy and management of associated risk factors.



O297 DIARRHEA POST KIDNEY TRANSPLANTATION: INCIDENCE, RISK FACTORS AND ASSOCIATED OUTCOMES

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Introduction: Gastrointestinal complications after kidney transplant are associated with inferior graft outcomes. Among gastrointestinal complications, diarrhea is very common. We examined the incidence, risk factors and the outcomes of post-transplant diarrhea using Medicare data.
Methods: Records for all adult renal-only transplant recipients were obtained from the United States Renal Data System (USRDS) between 1995 and 2002. Patients included were Medicare primary at transplant and followed until the end of Medicare eligibility, graft failure, death or three-years post-transplant. Diarrhea diagnoses were drawn from ICD-9-CM codes and subcategorized into 5 groups according to whether the specific causes of diarrhea were identified and whether it was infectious in origin. The risk of developing diarrhea, and the hazards of graft loss and patient death following diarrhea were estimated controlling for baseline recipient, donor and transplant related characteristics using multivariate Cox's proportional hazards analysis.
Results: We identified 32,724 eligible patients.The 3-year incidence of diarrhea was 22%, and the most common etiology was non-infectious diarrhea with unspecified cause (incidence: 18%). Factors associated with increased risk of non-infectious diarrhea were female gender (HR 1.41, 95% CI: 1.34-1.48), type 1 diabetes (1.25, 1.10-1.41), and regimens containing tacrolimus and mycophenolate mofetil (1.36, 1.28-1.45). The onset of noninfectious diarrhea doubled the hazard of graft failure (HR: 2.00, 1.87-2.15) and patient death (2.27, 2.00-2.57). Unspecified nonifectious diarrhea was associated to increased graft loss irrespective of diagnosis setting (hospital or outpatient visit), suggesting that even mild diarrhea cases can be followed by poor graft outcomes.
Conclusion: Diarrhea is commonly diagnosed following renal transplantation. Regimens containing tacrolimus and mycophenolate mofetil were associated with increased risk of developing noninfectious diarrhea. Unspecified noninfectious diarrhea is particularly concerning as it was diagnosed in nearly 20% of the patients and doubled the risk of graft failure and patient death.

Session 38. Liver: Immunosuppression & rejection

O298 COMPLETE CESSATION OF IMMUNOSUPPRESSION AFTER PEDIATRIC LIVING-DONOR LIVER TRANSPLANTATION ITS SAFETY AND FEASIBILITY

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Cases of operational tolerance (complete cessation of immunosuppressants:IS) after clinical transplantation (Tx) are still extremely rare in most organs. Despite such observations, operational tolerance is not 'Grail 'after immuno-privileged liver Tx in our pediatric patients (Transplantation 2001;72:449). Safety and feasibility of complete cessation of IS after pediatric living-donor liver Tx (LDLT) were re-evaluated.
Methods: From June 1990 to May 2005, 659 cases of LDLT were performed for 581 pediatric (<18years) patients. Patients were immunosuppressed with

FK506 and low-dose corticosteroids. Weaning from FK506 was begun by elective protocol, according to the following criteria: 1) normal liver function 2) >2 years after Tx 3) >1 year no episode of rejection. Even when these criteria were not met, IS were discontinued non-electively in case of severe IS related complications.
Results: 87 patients (15% of all the patients) have been completely weaned off IS by May 2006. IS was discontinued by elective protocol for 54 patients. The other 33 patients had to stop IS non-electively. 8 patients had biopsy-proven acute rejection during the weaning process, which was counteracted by increasing IS. 3 of these are again being weaned off IS; other 5 treated with FK506 monotherapy show normal liver function. In 4 patients, IS returned to the previous steps of weaning without histology due to an increase in transaminases. One patient exhibited early chronic rejection during the cessation of IS due to EBV infection, which was successfully treated with 3-drug IS.
Conclusion: Complete cessation of IS (operational tolerance) is feasible in no less than15% of patients after pediatric LDLT (significantly higher proportion, compared to other Tx centers) and yields no penalty in terms of immune graft loss. Both operating mechanisms and favorability of living-donor to tolerance are needed to be elucidated.

O299 IDENTIFICATION OF MOLECULAR MARKERS IN TOLERANT PEDIATRIC LIVER TRANSPLANT PATIENTS BY GENE EXPRESSION PROFILING

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The long-term use of immunosuppressive agents can lead to serious morbidity and enhanced rates of infectious diseases and cancer. Consequently, two of the major goals in transplant medicine are to reduce long-term maintenance immunosuppression in order to alleviate its associated complications, and to develop new, non-invasive diagnostic strategies to allow individualized therapy and optimize cost/benefit/risk ratios. In order to uncover biomarkers for successful drug withdrawal after liver transplantation, we used global gene expression monitoring by Affymetrix U133A chips to identify tolerance-associated genes in blood samples from operationally tolerant pediatric liver transplant patients (TOL; n=6), patients undergoing prospective uninterrupted drug withdrawal (PW; n=3) or patients requiring maintenance immunosuppression (MI; n=3). Statistical analysis was performed by applying Affymetrix GeneChip® Operating Software (GCOS) in combination with High Performance Chip Data Analysis (HPCDA®) from oligene. Hierarchical clustering of 137 significant genes revealed that four samples analyzed from TOL patients exhibited co-clustering closely related to one MI patient but were clearly distinct from PW patients which demonstrated a very close relationship based upon their gene expression profile. The remaining two TOL patients were grouped together with one MI patient. Interestingly, among the most significant regulated genes we could identify regulated transcripts including different chains of immunoglobulin isotypes, integrins, defensins or the thombospondin receptor CD36. Our data reveal novel insights in the development of operational liver transplant tolerance suggesting a possible functional role of these gene products to uncover patients for drug weaning protocols in the future.

O300 PROMINENT MIGRATION OF IL-10 PRODUCING DONOR DENDRITIC CELLS INTO THE RECIPIENT AFTER LIVER – BUT NOT AFTER KIDNEY TRANSPLANTATION: IMPLICATIONS FOR TOLERANCE INDUCTION?

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The role of chimerism in tolerance induction after organ transplantation is still under debate. We compared dendritic cell (DC) chimerism after liver transplantation (LTx) and kidney transplantation (RTx), and studied the functional properties of liver graft DC.
Methods: Donor $CD1c^+CD20^-$ DC were determined in blood of transplant recipients by flowcytometry using donor-recipient HLA-A2 mismatches. DC were immunomagnetically isolated from liver graft perfusates collected at the end of the cold storage period (n=7), and from blood of healthy individuals (n=7), and functionally compared.
Results: In liver graft recipients (n=11) donor DC made up 4.2% (range 0.0-18.1%) of total circulating DC on day 1, and 0.6% (range 0.0-1.3%) on day 5 post-LTx. In contrast, on day 1 post-RTx (n=6) only 0.3% (range 0.0-1.1%) of circulating DC were of donor origin (LTx versus RTx: p=0.015). During pre-transplant perfusion 0.9 $x10^6$ donor DC (range 0.1-4.5 $x10^6$) detached from liver grafts. Freshly isolated liver perfusate DC were able to stimulate allogeneic T-cell proliferation. Upon stimulation with LPS, liver DC produced higher amounts of IL-10 than blood DC (1.9±0.6 versus 0.15±0.05 ng/ml; p=0.006), but no IL-12. Likewise, upon stimulation with poly (I:C) and IFNγ liver DC produced 19 times more IL-10 (1.3±0.8 ng/ml) than IL-12 (0.07±0.03 ng/ml; p=0.029), while blood DC produced low amounts of both cytokines (0.1±0.07 and 0.09±0.04 ng/ml, respectively).
Conclusion: After LTx, but not after RTx, considerable numbers of donor-derived DC migrate from the donor graft via the blood circulation into the recipient. These liver DC strongly produce the immune-regulatory cytokine IL-10, but little of the T-helper1 driving cytokine IL-12, and may therefore contribute to lower immunogenicity of liver grafts in comparison with kidney grafts.

O301 TWO-YEAR FOLLOW-UP OF A ONCE DAILY MODIFIED RELEASE TACROLIMUS REGIMEN IN *DE NOVO* LIVER TRANSPLANT RECIPIENTS

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Prograf® (tacrolimus) is a twice daily (BID) formulation. Modified release (MR) tacrolimus was developed to enable once daily (QD) dosing. This study compared administration of MR QD and Prograf BID in patients following primary liver transplantation in an open-label, multi-centre, randomised comparative 6-week study, with a 2-year follow-up for MR patients.
Eligible patients (18 to 65 years) were recipients of a liver graft, received the first dose of tacrolimus within 12 to 18 hours following skin closure, and had normal renal function (serum creatinine < 175 mol/L). Patients received either Prograf BID or MR QD throughout the study at an initial total daily dose of 0.10 to 0.15 mg/kg, with target trough levels of 10 to 20 ng/mL. PK profiles (24-hour) were obtained following the first administration of tacrolimus (Day 1), and under steady-state conditions (14 days and 6 weeks after transplantation). 77/129 patients met pre-defined criteria for the 6-week PK evaluation (32 Prograf, 45 MR). The mean AUC of tacrolimus on Day 1 following administration of MR and Prograf were 145.97 and 263.82 ng.h/mL, respectively; however, values were comparable at steady-state (Day 14 and Week 6). There was good correlation between AUC and C_{min} for MR and Prograf, and similar trough levels could be targeted.
47/67 MR patients elected to continue follow-up; 36 completed 2 years of follow-up (4 patients died, 3 withdrew due to adverse events and 4 withdrew for other reasons). At 2 years post-transplantation, patient and graft survival in MR patients were 90.9%, and there were 2 acute rejection episodes.
Tacrolimus therapy following primary liver transplantation can be initiated with the MR formulation, with efficacy being maintained at 2 years post-transplant. The safety profile of MR was comparable to the established safety profile for Prograf.

O302 C4d DEPOSITS IN PERIOPERATIVE TRANSPLANTED LIVER BIOPSIES – THE IMPACT ON EARLY REJECTION

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Background: Ischemia-reperfusion injury was reported to induce activation of complement system in patients undergoing partial hepatectomy. This activation was proved to be local and restricted to the liver. Usefulness of C4d deposition as an adjunctive to histopathological assessment of liver allograft biopsy is still debated and to our knowledge no studies were done concerning C4d expression in perioperative biopsies of transplanted livers.
Aim of the study: to detect C4d expression in perioperative biopsies of transplanted livers and to assess its potential impact on rejection in early posttransplant period.
Methods: 30 "time zero" biopsies were consecutively chosen from our registry and C4d expression was detected using standard immunohistochemical method. C4d expression was assessed semiquantitively as 0 (no expression) to 3 (strong and diffuse expression in portal and central veins endothelium). The patients' history in early posttransplant period were investigated and biopsy confirmed acute rejection cases were selected.

Results: There were 15 cases with C4d deposits found mainly in portal veins endothelium (C4d(+) group) and in 15 cases C4d expression was negative. In 9 patients from C4d(+) group acute rejection was diagnosed histologicaly within first month after transplantation while only 3 patients from C4d(-) group rejected their livers within reported period (p=0.0335).
Conclusions: Activation of humoral branch of immunological response expressed by C4d complement split product deposition may help to differentiated liver allograft recipients who are specially endangered with early acute liver allograft rejection.

O303 PATIENTS WITH ALCOHOLIC CIRRHOSIS AWAITING FOR A LIVER TRANSPLANTATION ARE MORE IMMUNOSUPPRESSED THAN THOSE WITH HEPATITIS C VIRUS-RELATED CIRRHOSIS

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The incidence of acute rejection is significantly higher in liver-transplant patients grafted for hepatitis C virus (HCV)-related cirrhosis than in those grafted for alcoholic cirrhosis.
The purpose of this study was to assess T-cell function, activation and proliferation in patients awaiting for a liver transplantation.
31 patients were included in this study. Intra-lymphocyte cytokines IL-2 and TNF-a, lymphocyte-activation surface markers (CD25 and CD71), and T-cell proliferation (PCNA+/DNA+) were measured twice for each patient by FACS. The average time between the 2 analyses was 2 months. The mean value of the two analyses was used to compare different groups.
Different pharmacodynamic parameters did not differ significantly at both measurements. CD71 expression and T-cell proliferation were significantly higher in HCV (+) patients (n=9) than that in HCV (-) patients (n=22), respectively 41.6±3.9% vs. 28.1±2.45% for CD71 (p=0.02) and 23.9±2.7% vs. 16.9±1.3% for T-cell proliferation (p=0.015). T-cell activation and T-cell proliferation were lower in alcoholic patients (n=14) as compared to non-alcoholic patients (n=17), 39±3.8% vs. 52.5±2.9% for CD25 (p=0.001), 24.7±3.25% vs. 38±2.55% for CD71 (p=0.001), and 15.6±1.6% vs. 21.7±1.8% T-cell proliferation (p=0.004). In contrast, intra-lymphocyte IL-2 tended to be higher in alcoholic patients, 37±2.45% vs. 30.5±3.6% (p=0.07). Finally, T-cell activation (p= 0.04 for CD25 and p= 0.03 for CD71) and T-cell proliferation (p= 0.004) were higher in HCV (+) patients (n=9) than that in alcoholic patients (n=14).
Alcoholic patients awaiting for a liver transplantation are more imunosuppressed than those with HCV (+)-related cirrhosis. This might explain the lower incidence of acute rejection after liver transplantation in patients grafted for alcoholic cirrhosis.

O304 DACLIZUMAB REDUCES ISCHEMIA/REPERFUSION INJURY IN HUMAN LIVER TRANSPLANTATION

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Background: In experimental models, T-cell activation enhances neutrophil-mediated ischemia/reperfusion (I/R) injury. T-cell targeted immunosuppression may reduce I/R-injury in liver transplantation (LTX). We studied the effect of IL-2 receptor (IL-2R) antagonist daclizumab induction on inflammatory response during reperfusion and postoperative liver function in human LTX.
Materials and Methods: All patients received methylprednisolone before anhepatic period. Daclizumab group was treated with daclizumab during anhepatic period, and postoperative tacrolimus (n=7)., controls with postoperative tacrolimus and mycophenolate mofetil (n=6). Portal and hepatic venous blood was obtained at portal declamping, 10 min later, and 10 min after hepatic artery declamping. Soluble IL-2R (sIL-2R) was determined by ELISA and proinflammatory cytokine hypermobility group box 1 protein (HMGB1) by Western blotting. Peak alanine transferase (ALT) levels during the first 72 postoperative hours were recorded. Data are given as median (range).
Results: Plasma sIL-2R was undetectable in daclizumab group, while in controls sIL-2R efflux from the graft occurred at 10 min after portal declamping (P=0.027). In both groups, HMGB1 levels were significantly higher in hepatic venous than in portal blood during reperfusion (all time points $P<0.05$), and higher in controls than in daclizumab group (Table). Likewise, peak ALT was significantly higher in controls [361 (257-887) IU/L] than in daclizumab group [188 (105-305) IU/L, P=0.005]. Graft steatosis [control 0 (0-30) %; daclizumab 0 (0-5) %], cold ischemic time [control 305 (225-368) min; daclizumab 288 (239-363) min], and anhepatic time [control 58 (35-66) min; daclizumab 53 (36-69) min] were comparable.

HMGB1 levels during reperfusion

	Daclizumab	Control	*P*
Portal declamping*	89 (59-369)	363 (235-795)	0.035
10 min after portal declamping	68 (53-137)	307 (140-715)	0.022
10 min after artery declamping	42 (0-58)	172 (106-412)	0.002

HMGB1 ng/mL in hepatic venous blood, *caval effluent

Conclusions: sIL-2R is released upon T-cell activation, while HMGB1 is secreted by activated neutrophils. Undetectable sIL-2R and reduced HMGB1 levels during reperfusion, associated with lower postoperative ALT levels, suggest daclizumab limited both cytokine response and hepatocellular damage. Daclizumab may alleviate I/R-injury by reducing T-cell enhanced neutrophil activation in human LTX.

O305 VASCULAR DEPOSITION OF COMPLEMENT C4d IS INCREASED IN LIVER ALLOGRAFTS WITH CHRONIC REJECTION, COMMONLY PRECEDED BY CMV AND HHV-6 INFECTIONS

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Background: Complement protein C4d deposition on endothelium has long been used as a marker of antibody mediated rejection in kidney allografts. Recently it has been shown to be expressed in chronic kidney graft rejection, but also frequently in acute liver allograft rejection. In chronic liver allograft rejection this has not been studied.
methods: Seven liver allografts explanted at retransplantation due to chronic rejection were examined for expression of C4d protein. C4d was demonstrated from frozen sections of the liver biopsies by monoclonal anti-C4d antibody and immunoperoxidase staining and semiquantitatively scored from 1-3. The “zero” biopsies of the same livers obtained during the first transplantation served as controls. CMV and HHV-6 infections were diagnosed by the antigenemia tests. Previous CMV infections, HHV 6 infections and acute rejections were retrieved from the patient records and history of laboratory results.
results: Expression of C4d was significantly increased in portal and central veins as well as in the portal tract of the grafts with chronic rejection compared to the expression at transplantation of the graft. (the mean vessel intensity 2.1±0.4 vs 1.4±0.9, $p<0.05$). 5 out of 7 patients ending up with chronic rejection had experienced at least one CMV infection, 3 had HHV-6 infection, and 5 had at least one episode of acute rejection.
Conclusion: C4d deposition is common in the end stage of chronic liver allograft rejection. The complement system and alloantibodies may contribute to the process of chronic allograft rejection in the liver. CMV and HHV-6 infections could possibly stimulate the alloantibody mediated graft injury.

O306 INDIVIDUAL DRUG THERAPY ADJUSTED TO DRUG-METABOLIZING CAPACITY AFTER LIVER TRANSPLANTATION

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Background: Drug-metabolizing capacity of liver primarily depends on levels and activities of cytochrome P450 enzymes (CYP). Adverse drug-reactions and therapeutic failures are often caused by inter-individual differences in drug-metabolism [1,2]. Validated analytical system with metabolomic and transcriptomic tools has been developed for estimation of drug-metabolizing capacity of the liver.
Aim: The aim of our study was to classify donor livers on the basis of drug-metabolizing capacity (poor, intermediate, extensive).
Method: CYP phenotyping of donor liver covered measurements of CYP activities and mRNA levels of the liver and CYP expression in donor leukocytes. The expressionCYP2C9, CYP2C19 and CYP3A4 mRNA was normalized by glyceraldehyde-3-phosphate-dehydrogenase expression.
Results: Strong correlation (n=48, $r>0.9$) between CYP activities and mRNA levels in liver tissues was found for CYP2C9, CYP2C19 and CYP3A4. CYP mRNA levels in leukocytes also reflected (n=42; $r>0.9$) CYP activities of the liver. Testing drug-metabolizing status of 42 transplanted livers, the distribution of CYP gene expression measured from donor leukocytes are presented in Table 1.

Table 1

	Poor	Intermediate	Extensive
CYP3A4	40%	53%	7%
CYP2C9	20%	57%	23%
CYP2C19	23%	57%	20%

In the CYP3A4 poor-metabolizer group, blood levels of immunsuppressive drugs were significantly higher compared to intermediate-, or extensive-metabolizer group. The biopsy result proved drug toxicity in 4 cases within the poor metabolizer group. Reduced dose improved their liver function.
Conclusion: This transcriptomic analysis of donor leukocytes provides information on drug-metabolizing capacity of transplanted liver prospectively. This tool allows predicting potential poor or extensive metabolizer phenotypes of donors and facilitates improvement of individual drug therapy of recipient in early postoperative period reducing side effects and drug failures.
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2. Patonai et al.: Orv Hetil. 2001; 142(9):435-41.

Session 39. Humoral & cellular responses

O307 PANEL REACTIVE ANTIBODIES ARE PRODUCED BY $CD5^-/B220^+/IgM^+/IgD^+$ B CELLS IN ASSOCIATION WITH Th1 TRAIL

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Objective: Panel reactive antibodies (PRA) impose a daunting risk of acute humoral rejection to organ transplants. However, the nature of PRA-producing B cells and the mechanisms that regulate PRA production are poorly understood.
Methods: A unique murine model of allogenic sensitization has been developed and used for characterization of humoral responses to allograft.
Results: Immunization of C57BL/6 mice with transgenic HLA-A2 skin allograft induced an IgM response at day 7-14 post-transplantation (Ptx). IgG responses emerged at day 14 and peaked at day 28 Ptx. Low titers (1:10) of IgM antibodies reactive to HLA were detected in antisera harvested at days 7- 14 Ptx. High titers (1:100) of IgG2a antibodies were present in antisera at days 21 and 28 Ptx. Intriguingly, these antibodies not only bound to HLA-A2 but also reacted with a panel of class I (but not class II) HLA, thus, exhibiting a characteristic of PRA. IgG1 PRA occurred in less than 20% animals at day 14 post 2nd skin grafting, indicating a weak Th2 trail. Increases of serum PRA were synchronized with vigorous proliferation of $CD5^-/B220^+/IgM^+/IgD^+$B cells in the spleens. B cell depletion suppressed IgM and IgG PRA production. T cell depletion resulted in weakening of IgM and IgG PRA responses. Quantitative RT-PCR demonstrated significant increases in mRNA expression of INF-γ and IL-2 but not IL-4 and IL-10 in the spleens following primary immunization.
Conclusion: The data suggest that in this murine model the humoral response to the allograft consists of an IgM and an IgG phase which are both T cell-dependent. Th1 microenvironment may dictate the early phases of the antibody responses while a Th2-dependent IgG1 response may follow amid persisting alloantigen stimulation.

O308 DONOR MHC CLASS I HELICAL PEPTIDES PROLONG CARDIAC ALLOGRAFT SURVIVAL BUT FAIL TO AFFECT CHRONIC REJECTION

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Background: Chimeric class I MHC soluble protein that contains the donor-type sequence in α1-helical region (aa 50-90) flanked by recipient sequences induced donor-specific tolerance (Tx). Here, we tested the effect of 40 mer MHC class I peptides derived from donor a1-helix on Tx survival.
Methods: RT1.A^1 (LEW), RT1.A^u (WF), RT1.A^c (PVG), RT1.A^b (BUF) peptides were administered though portal vein (d0) into ACI (RT1.A^a) cardiac recipients. Hosts were analyzed for tolerance induction and evidence of chronic rejection (CR).
Results: Four doses of RT1.A^u peptide (1mg, 0.5, 0.125 and 0.05 mg) were tested. Only low-dose regimens (0.125; n=8 and 0.05 mg; n=3) consistently induced tolerance (MST >120d). Specificity was confirmed by the acceptance of donor-type (WF, MST >100d, n=5), but rejection of a third-party, BN (RT1^n) test Tx (MST=10d, n=5). Similarly, α1-helical peptides induced tolerance in ACI recipients of LEW or BUF or PVG cardiac Tx (MST>120d, n=6, n=5 and n=10, resp.). Specific tolerance to MHC peptides was confirmed by injection of irrelevant PVG peptide into ACI recipients of LEW Tx (MST=21, n=3). Graft histology showed marked fibrosis, smooth muscle and intimal proliferation in 58-75% of vessels that was not observed after allochimeric protein therapy (26-34% TVS, n=8). Unlike in allochimeric-treated hosts, strongly up-regulated circulating allo-Ab titers (IgG1, G2a, G2b and G2c) were detected in the peptide-treated rats. Real-time PCR showed 10-fold increase for IL-10 production and no detected IL-2, indication for non-healing chronic inflammatory process.
Conclusion: Donor MHC class I peptides derived from α-helical region can specifically prolong Tx survival. Due to development of non-healing inflammation they fail to inhibit CR. In contrast, allochimeric protein that contains donor and critical recipient-type epitopes readily abrogates CR. Processing of allochimeric protein may be critical for development of CR.

O309 LIPOCALIN-2, REGULATOR OR BYPRODUCT DURING ISCHEMIA AND REPERFUSION?

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Purpose: The main focus of this work was to analyze the possible implication of Lipocalin-2 (Lcn-2) upregulation for the course of ischemia/reperfusion (IR) during heart transplantation and effects on polymorphonuclear cells (PMN) as well as to investigate the nature of the Lcn-2 producing cell.
Material and Methods: Male inbred C57BL/6 and the Lcn-$2^{-/-}$ mouse were used in our transplantation experiments. PMN from wildtype and Lcn-$2^{-/-}$ mice as well were isolated and promyeloid cell lines (32D) used to demonstrate the effect of Lcn-2 on cell physiology. Western blot, RT-PCR, immunohistochemistry and TUNEL assay were performed to determine Lcn-2 expression and apoptosis in the graft. Cell viability and migration assays after various stimuli (e.g. IR) were applied to elucidate cell growth and viability.
Results: Infiltrating PMN were the major contributors to Lcn-2 expression during IR peaking 24h after reperfusion. The number of infiltrating PMN was significantly reduced in Lcn-$2^{-/-}$ recipients. No difference was observed in the apoptotic rate between wildtype and Lcn-$2^{-/-}$ donors and Lcn-2 expression also increased during acute graft rejection. Migration of PMN during reperfusion was negatively influenced by the absence of Lcn-2 or lack of Lcn-2 specific cell surface receptors in the Lcn-$2^{-/-}$ mice. The promyeloid cell lines responded to IR with increased Lcn-2 mRNA and protein levels.
Conclusion: Our data suggest a chemoattractant function of increased Lcn-2 expression in the transplanted heart due to infiltrating PMN. Lcn-2 is a novel inflammatory marker upregulated during IR and acute graft rejection. Our observations shed light on a possible function of Lcn-2 to the recruitment of PMN to the site of IR and identify possible targets for therapeutic intervention.

O310 PROOF OF MECHANISM: CIRCULATING ENDOTHELIAL PROGENITOR CELLS EFFECTVELY RECONSTITUTE ENDOTHELIUM AND REDUCE ALLO-REJECTION OF MOUSE AORTIC ALLOGRAFT

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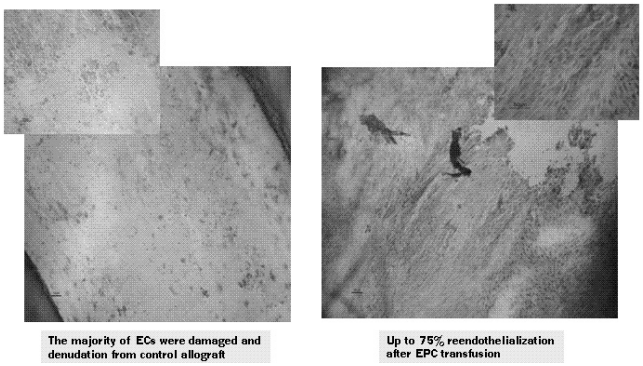
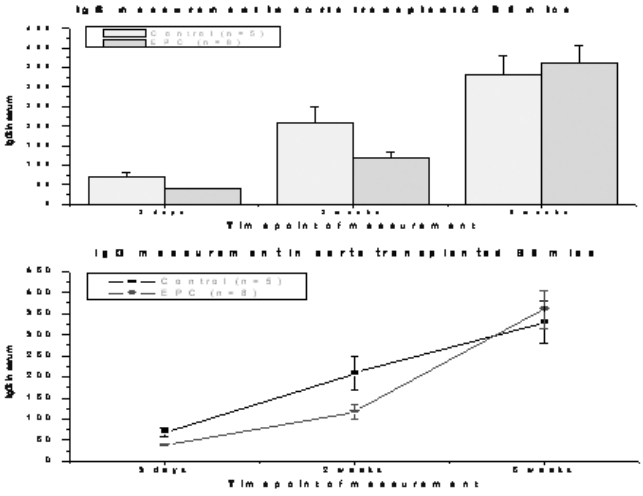
As vascular endothelium is a primary target of allo- immunity in organ transplantation, the reconstitution of the damaged endothelial monolayer at the early stage post transplantation may help to protect the allo-graft. Circulating endothelial progenitor cells (EPC) may allow faster endothelial regeneration. The key objective of this study is to demonstrate whether increased number of circulating EPCs lead to accelerated reconstitution of endothelium and diminish allogeneic rejection of mouse aortic allograft.
Methods: 1.5x10^6 EPCs expressing sca-1+VEGFR2+c-kit+CD34+ were isolated from the spleen of C57Bl/6 -actb-EGFP knockin mice and transfused intravenously into C57Bl/6 recipients (total n=22) of Balb/c mice aorta on day0, 3 and 7 after transplantation. Allografts of aorta were harvested on day 3, 14 or 35, respectively for analysis of endothelial composition and intima. Homing of circulating EPCs, hematology and allo-antibody level in the recipient mice were also evaluated.
Results: EPC transfusions cause accelerated re-endothelialization in up to 75% of the allografts on day14 post-transplantation. This is associated with a 30% reduction of neointima formation compared to control animals.

Newly generated endothelial cells were confirmed to derive from transfused EPC^{EGFP} because these cells express EGFP and the endothelial cell marker CD31/PECAM-1. Allo-antibody levels (IgG and IgM) in serum were reduced significantly early after EPC transfusion up to 14 days post-transplantation ($p<0.05$). Lymphocyte and WBC counts in blood were also reduced. Transfused EPC distribution in tissues was determined as blood > LN > BM. Circulating EPC count in transplanted mice is higher than that in non-transplant controls.



EPC transfusions enhanced reendothelialization of allograft at day 14 after transplantation (EPC transfusion at day 0, day 3 and day 7; aortic allograft, Balb/c-to-B6).



EPC transfusions reduced alloantibody IgG significantly at day 3 and day 14 (EPC transfusion at day 0, day 3 and day 7; aortic allograft, Balb/c-to-B6).

Conclusion: It is for the first time to demonstrate that EPCs significantly reconstitute endothelium of allograft and diminish allogeneic rejection. These findings allow novel insights in EPC biology and may lead to new therapeutic approaches to reduce allo-immunity-induce injury.

O311 CD8+ T CELL EFFECTOR MECHANISMS INVOLVED IN ALLOGRFT VASCULOPATHY UNDER CYCLOSPORINE IMMUNOSUPPRESSION

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Objective: Loss of cardiac grafts due to allograft vasculopathy (AV) has emerged as the major limitation in clinical cardiac transplantation. Understanding the immune effector mechanisms against which current immunosuppressive agents such as Cyclosporine A (CyA) fail to abrogate AV is critical for the development of novel therapy. We hypothesized that the CD8+ T (T8) cells require both the direct and indirect effector compartments to mediate AV under CyA immunosuppression.
Methods: To assess AV in the absence of IFN-γ, aortic grafts were transplanted into IFN-γ deficient ($IFN\text{-}\gamma^{-/-}$) recipients (+/- CyA). To assess the role of IFN-γ produced by T8 cells in AV under CyA, alloprimed T8 cells from CyA treated $IFN\text{-}\gamma^{-/-}$ mice were transferred into $RAG\text{-}1^{-/-}$ recipients treated with CyA. AV was assessed at 8 weeks post transplantation. The role of IFN-γ was aslo examined through full blockade of direct CTL activity by the transfer of alloprimed T8 cells from perforin deficient mice into $RAG1^{-/-}$ recipient of a Fas deficient graft. Moreover the chronological RNA expression of IFN-γ and CTL components (e.g. Granzyme B, FasL) in the graft were examined by RT-PCR.
Results: Allografts in $IFN\text{-}\gamma^{-/-}$ recipients did not develop AV when treated with CyA.T8 cells from alloprimed CyA treated $IFN\text{-}\gamma^{-/-}$ mice did not mediate AV in CyA treated $RAG\text{-}1^{-/-}$ recipients. Fas deficient grafts in CyA treated $RAG\text{-}1^{-/-}$ mice reconstituted with alloprimed perforin deficient T8 cells also did not develop AV.
RT-PCR on grafts from CyA treated wild type recipients indicate an earlier expression of CTL components followed by the expression of IFN-γ at later time points.
Conclusion: The data is consistent with our hypothesis that in the presence of CyA both direct CTL effector mechanisms and the indirect IFN-γ dependent effector mechanisms are required for T8 cells to mediate AV.

O312 DIFFERENTIAL EXPRESSION OF CD127 ON CD4+FoxP3+ T CELLS BETWEEN HEALTHY CONTROLS AND HEART TRANSPLANT PATIENTS

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Introduction: Recently, it has been shown in healthy volunteers that CD4+FoxP3+ regulatory T cells with suppressive activity *in vitro* displaid a lower expression of the IL-7 receptor α-chain (i.e. CD127), irrespective of their level of CD25 expression. Here, we investigated in heart transplant patients whether the CD127low phenotype can be used to discriminate peripheral FoxP3+ expressing CD4+ T cells and, therefore, allows quantification of regulation.
Methods: Flow cytometry was used to analyze the FoxP3, CD25, and CD127 expression of peripheral CD4+ T cells from 14 cardiac allograft patients before transplantation and during an acute rejection episode after transplantation. Additionally, PBMC were examined from 9 healthy controls (HC).
Results: The percentage of FoxP3+ expressing cells within the CD4+ T-cell population was comparable between patients before and after transplantation and HC (before transplantation: 6.9% (median), before rejection: 6.5%, during rejection: 6.2% and HC: 6.9%). Before transplantation, however, a significantly lower proportion of these CD4+FoxP3+ T cells were CD127low compared with HC (patients: 54% vs. HC: 66%, p=0.03). Moreover, after transplantation the proportion of CD127low expressing FoxP3+ T cells even further decreased from 54% (pre-transplant, median) to 42% before rejection, with the lowest proportion, i.e. 38%, during rejection (p=0.001). No differences were measured in the percentage of the CD4+FoxP3+ T cells that expressed CD25high between the different blood samples.
Conclusion: In peripheral blood of patients with end-stage heart failure and after transplantation we found a lower proportion of CD127low expressing CD4+FoxP3+ T cells, the most potent subset of regulatory T cells. Moreover, the CD127low expression on the CD4+FoxP3+ T cells was inversely associated with anti-donor immune reactivity in heart transplant patients.

O313 PROSPECTIVE ANALYSIS OF FUNCTIONAL FOXP3+CD4+CD25BRIGHT+ REGULATORY T-CELLS IN ANTI-CD25 TREATED KIDNEY TRANSPLANTS

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Introduction: The regulatory mechanism controlled by FOXP3+CD4+ $CD25^{bright+}$ T-cells, by which tolerance is established toward self- and foreign allo-antigens, is largely dependent on IL-2. Therefore, induction treatment with anti-IL-2Rα (i.e. CD25) antibodies could influence the development and function of FOXP3+CD4+$CD25^{bright+}$ regulatory T-cells.
Material and Methods: In a controlled trial, kidney recipients were randomized to receive either anti-IL-2Rα induction (Daclizumab) and steroids for 3 days or steroids (tapered to 0 at week 16) in combination with tacrolimus and mycophenolate mofetil (MMF). We analyzed the presence and function of FOXP3+CD4+$CD25^{bright+}$ peripheral T-cells and determined their suppressive activity in samples obtained pre- and 4-6 months after transplantation.
Results: No difference between rejection incidence (daclizumab group: 15% vs control group: 14%) or graft survival (91% vs 90%) was observed between the two study groups. Anti-IL2Rα induction therapy affected the expression of FOXP3 of the CD4+$CD25^{bright+}$ T-cells as the proportion of FOXP3 positivity in the CD4+$CD25^{bright+}$ T-cells decreased after from $77\pm8\%$ to $65\pm7\%$ (mean$\pm$SD, p=0.01). Nevertheless, functional regulatory T-cells could still be demonstrated in the anti-IL-2Rα treated group. Before and 4-6 months after transplantation, the inhibition of the anti-donor proliferative responses was 88% and 83% (median) at a 1:10 ratio ($CD25^{bright+}$:$CD25^{-/dim}$), and 47% and 44% at a 1:20 ratio.
Conclusions: In contrast to previous reports in isolated cell system, we show

that anti-CD25 induction therapy does not negatively affect the regulatory activities of FOXP3+CD4$^+$CD25$^{bright+}$ T-cells in kidney transplant recipients.

O314 A NOVEL ROLE FOR THE CALCINEURIN-NFAT1 PATHWAY IN PERIPHERAL CD8 TOLERANCE

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Mixed hematopoietic chimerism and tolerance are achieved across full MHC barriers in mice using low dose total body irradiation (3Gy), anti-CD154 (MR1) and bone marrow transplantation (BMT). To assess a role for the calcineurin pathway in tolerance, recipient wild-type (wt) mice were treated with cyclosporine (CyA 20mg/kg daily) for 2-4 weeks post-BMT. Alternatively, in order to prevent NFAT1 signaling downstream of calcineurin, NFAT1-/- mice were used as recipients. Chimerism was followed by FACS analysis of peripheral blood. To distinguish effects on CD8 vs CD4 tolerance induction, some groups received CD8 depleting antibody. To distingtuish effects on peripheral versus central tolerance we compared wt, CyA-treated and NFAT1-/- mice with and without thymectomy. In all groups, mixed chimerism was observed for at least 12 weeks. However, in mice receiving CyA and in NFAT1-/- mice, chimerism gradually declined over time, first in the granulocyte and later also in the lymphocyte lineages. After chimerism disappeared, anti-donor CML responses were detected in CyA-treated mice, while control chimeras demonstrated donor-specific tolerance. In contrast, chimerism was durable in mice depleted of CD8 T cells at the time of BMT, even though CD8 cells later recovered. Similar results were obtained in mice thymectomized at 4-5 weeks of age that received BMT 4-5 weeks later. Thus, blockade of the calcineurin pathway after BMT led to a failure of peripheral CD8 T cell tolerance, but did not impair CD4 T cell tolerance. To our knowledge, this is the first demonstration of a role for NFAT1 in CD8 T cell tolerance.

O315 T-CELL REACTIVITY IN HLA-IDENTICAL LIVING-RELATED KIDNEY TRANSPLANT RECIPIENTS DURING STEROID MONOTHERAPY

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HLA-identical living-related (LR) kidney transplant recipients routinely receive azathioprine (AZA) or mycophenolate mofetil (MMF) in combination with low dose prednisone as maintenance immunosuppression from one year after transplantation. We questioned the necessity of long-term use of immunosuppression in this patient group. Therefore, we discontinued the AZA or MMF medication and studied the effect on T-cell reactivity.
We studied 19 stable HLA-identical LR kidney transplant recipients, more than 1 year after transplantation (median, 6.3 year; range, 2.3-17.3), who received low dose AZA (50 mg/day) or MMF (500 mg/day) in combination with 5 mg/day prednisone. All patients discontinued their AZA or MMF medication in 2 steps of 2 months. Follow-up was one year. We monitored T-cell reactivity by IFN-γ, IL-10, and granzyme B (GrB) Elispot-assays.
The donor-specific frequency of IFN-γ producing cells (pc) [median: 1 (range: 0-13)/2x10^5 PBMC], IL-10 pc [0 (0-13)/2x10^5 PBMC] and GrB pc [1 (0-40)/2x10^5 PBMC] was low during dual therapy, and remained low at steroid monotherapy [0 (0-16) IFN-γ pc/2x10^5 PBMC; 1 (0-51) IL-10 pc/2x10^5 PBMC; 0 (0-6) GrB pc/2x10^5 PBMC). Third-party reactivity remained also unchanged in IFN-γ and GrB Elispot-assays after withdrawal of immunosuppression. However, the number of IL-10 pc directed to 3rd-party antigens increased during withdrawal of AZA or MMF [dual therapy: 3 (0-11)/2x10^5 PBMC; steroid monotherapy: 13 (0-72)/2x10^5 PBMC; p=0.006). None of the patients developed acute rejection.
In conclusion, HLA-identical LR kidney transplant recipients can be withdrawn from AZA or MMF without affecting their donor-specific T-cell reactivity. The increasing numbers of 3rd-party reactive IL-10 pc found after discontinuing AZA or MMF suggest that immunosuppression interferes with general downregulatory processes.

Session 40. Donor viability: Storage, perfusion & resuscitation

O316 EFFECT OF PRESERVATION METHOD ON HISTOPATHOLOGICAL LESIONS OF KIDNEYS ALLOGRAFTS

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In majority of patients surviving several years after kidney transplantation transplantation histopathology shows changes characteristic for chronic rejection (CR), interstitial fibrosis (FI) and chronic vascular changes (CV). All these lesions contribute to the deterioration of renal allograft function.
Aim: The aim of the study was to evaluate the influence of kidney preservation method prior to transplantation on the characteristics of histopathological lesions of kidney allografts long-term post transplant.
Patients and method: Out of 415 renal transplant recipients operated on between 1994 and 1999, 274 patients underwent biopsies due to deterioration of kidney function post transplant. Two groups of patients were identified: CS-patients who received a kidney allograft stored in Cold Storage (n=114); MP-patients who received kidneys stored by Machine Perfusion (n=160). Average cold storage time in CS group was 25.5 hours, in the MP group-33.3 hours ($p<0{,}00001$). The immunosuppressive regimens, donors age, episodes of acute rejection after transplantation and delayed graft function did not differ between the groups. Kidney biopsies were graded according to Banff 2005 criteria.
Results: At mean 7 years follow-up graft survival of CS-stored kidneys was 50%, vs 68% in MP group ($p<0.0004$). FI was observed in 90% (20/22) of biopsies at 6 years post-transplant in CS group and 64% (16/25) in MP group (p=0.03). Chronic rejection was observed 2.8-times more often in CS group then in MP group ($p<0.04$).

	MP	CS	p
Graft survival	68% (109/160)	50% (57/114)	P=0.0004
Chronic rejection	3,1% (5/160)	8,7% (10/114)	P=0.04
Interstitial fibrosis(at 6 years post-transplant)	64% (16/25)	90% (20/22)	P=0.03

Conclusions: Kidneys stored CS are significantly more frequently affected by chronic rejection and interstitial fibrosis. Storage of kidneys by MP may improve graft survival by limiting chronic changes in renal allografts.

O317 REDUCTION OF ISCHEMIA/REPERFUSION INJURY IN KIDNEY TRANSPLANTATION USING A NOVEL HMP DEVICE

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Introduction: Optimization of organ viability is a key factor to successful transplantation. One way to maintain donor organ viability is hypothermic machine perfusion (HMP). The aim of this study was to evaluate a new developed system in porcine kidney transplantation comparing graft function and ischemia reperfusion injury after HMP with two different pressure settings to static cold storage (CS).
Materials and methods: Donor kidneys were retrieved from female landrace pigs ($\pm$30 kg). After kidney retrieval, grafts were preserved at 4°C with either UW-CS (n=5), HMP with UW-MP using 30/20 mmHg (n=5) or HMP with UW-MP using 60/40 mmHg (n=5). Autotransplantion was followed by direct contralateral nephrectomy. Daily venous blood samples were taken to evaluate kidney function. Urinary biomarkers were used to quantify the following components of ischemia reperfusion injury: oxidative stress, proximal tubule injury and proteinuria. Several vascular injury markers were used to detect HMP related vascular damage.
Results: CS grafts showed a peak serum creatinine of 940$\pm$90 μmol/l on post operative day 3.4. In contrast, animals that received HMP 30/20 kidneys had a significantly lower peak creatinine of 463$\pm$127 μmol/l on postoperative day 1.8 ($p<0.05$). In the HMP 60/40 group two animals died with a thrombosed graft. Analysis of biomarkers revealed less oxidative stress, less proximal tubule

damage and less proteinuria in HMP groups compared to CS ($p<0.05$). Vascular damage markers VWF and MCP-1 were elevated in HMP 60/40 kidneys compared to HMP 30/20 and CS ($p<0.05$).
Conclusion: We conclude that preservation with the Groningen machine perfusion system results in better early graft function and reduction of ischemia/reperfusion injury compared to CS. Perfusion pressures are, however, critically important. HMP at 30/20 mmHg resulted in the best post transplant results in this experiment, while 60/40 mmHg caused vascular damage.

O318 COMPARISON OF COLD STORAGE, MACHINE PERFUSION AND RETROGRADE OXYGEN PERSUFFLATION IN THE PORCINE KIDNEY AUTOTRANSPLANTATION MODEL

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Purpose: In marginal donor organs machine perfusion (MP) and retrograde oxygen persufflation (ROP) as alternative methods to cold storage (CS) have the potential to improve organ function after transplantation. In an experimental study in porcine kidney autotransplantation we compared the effects of CS, ROP and MP concerning lipid peroxidation and energy status.
Materials/Methods: Kidneys of 21 pigs were exposed to warm ischemia for 60 min. The kidneys were then subjected either to retrograde persufflation with gaseous oxygen for 4 hrs at 4°C, to hypothermic machine perfusion or were stored in cold UW-solution, followed by autotransplantation of these kidneys. Animals were sacrificed on postop. day 7 and in addition to clinical outcome biopsies of the kidneys taken during autopsy were examined for clinical outcome, Adenonucleotide values (HPLC), malondialdehyde(according to Ohkawa), and other parameters. Normal kidneys of 13 pigs from other experiments served as baseline controls (BL).
Results: 3 pigs had to be excluded from the study, one in the ROP group, two in the MP group. Only in the ROP group all animals survived. Two animals in the MP group and 3 in the CS group died because of anuria and resulting bad clinical status before the 7th postop. day. Kidney function was significantly better after ROP than after MP. Data for adenonucleotides showed no differences of the different groups compared to baseline values of normal kidneys. There was a significant difference in the values for malondialdehyde in the MP group compared to all other groups (Anova/LSD) (Table 1).

Table 1. Results for creatinine, sum of adenonucleotides (SAN) and malondialdehyde (MDA) on postop. day 7

Variable	ROP (n=6)	CS (n=4)	MP (n=3)	BL (n=13)	p≤0,05
	Group 1	Group 2	Group 3	Group 0	ANOVA/LSD
Serum Creatinine (mg/dl)	2,11/0,75	3,41/2,59	4,80/2,85	1,14/0,15	2,3 vs. 0 / 1vs. 3
SAN (µmol/g dry weight)	13,9/4,23	13,9/1,98	17,6/1,60	13,78/1,12	n.s
MDA (nmol/g dry weight)	99,9/62,7	65,5/23,8	542/129	79,5/28,5	0,1,2 vs. 3

variables in mean/SD

Conclusion: ROP showed superior initial function of the transplanted kidneys compared to MP and CS. In the MP group MDA as parameter for lipid peroxidation was significantly increased possibly related to endothelial damage.

O319 VIABILITY OF PIG LIVERS EXPOSED TO WARM ISCHEMIA CAN BE PREDICTED PRIOR TO TRANSPLANTATION BASED ON A MORPHOLOGICAL SCORE

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Background: Livers exposed to prolonged Warm Ischemia (WI) as from Non-Heart-Beating Donors (NHBD) are at higher risk of primary graft non-function (PNF). In a pig model of liver transplantation (LTx) from NHBD, hepatocellular vacuolation, focal hepatocyte drop-out, congestion and sinusoidal dilatation appeared on biopsies taken after exposure to WI. In functioning grafts, vacuolation and sinusoidal dilation were reversible after LTx, contrary to PNF grafts.
Aims: We questioned whether the extent of these morphological signs and in particular of vacuolation -present on pre-LTx biopsies- was associated with WI length and able to predict PNF.
Methods: Pre-Tx biopsies from pig livers exposed to incremental periods of WI were reviewed retrospectively. The extent of vacuolation was quantified by a pathologist's semi-quantitative score, validated by stereological point counting and digital image analysis, and then used to predict PNF and hepatocellular damage.
Results: On biopsies taken after WI, stereological point counting contributed significantly and digital analysis score in predicting PNF (p=0.027, and 0.043 respectively) versus the pathologist's semi-quantitative score (p=0.058). Stereological counting and digital image analysis predicted the extent of hepatocellular damage ($p<0.0001$, and p=0.001) versus pathologist's semi-quantitative score (p=0.085).
Conclusion: The extent of parenchymal vacuolation present on WI liver grafts reflects the severity of hepatocellular damage and allows predicting pig liver graft viability *before* LTx. Further studies are now warranted to evaluate whether these "anoxic changes" that are' associated with liver graft viability in pig also apply on human NHBD liver biopsies.

O320 MITOCHONDRIAL FUNCTIONAL CHANGES IN NON-HEART-BEATING-DONOR LIVERS: ADVERSE EFFECT OF COOLING

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Purpose: The use of livers from non-heart-beating-donors (NHBD) has been restricted because the liver does not tolerate prolonged warm ischaemia followed by cold preservation. We have investigated the kinetics of energy metabolism and mitochondrial function during ischaemia and the relationship with hepatocellular injury in NHBD livers.
Material & Methods: After 60 minutes of warm ischaemia, porcine livers (n= 5) were cold-preserved for 60 minutes in UW solution and then connected to an extracorporeal reperfusion circuit for 24 hours for functional assessment. Sequential liver biopsies were analysed for ATP content and mitochondrial function (respiratory control ratio, cytochrome *c* release and caspase activation). The perfusate was analysed for serum transaminases, bile production and base deficit. Apoptosis and necrosis were examined by TUNEL and haematoxylin staining.
Results: Although cellular ATP levels declined sharply during 60 minutes of warm ischaemia ($p<0.01$), mitochondrial function was maintained. However, subsequent cold preservation produced significant decline in mitochondrial function (RCR 3.83±0.16 vs. 2.04±0.11, $p<0.01$). Subsequent reperfusion with oxygenated blood at physiological temperature led to further loss of mitochondrial function ($p<0.01$), ATP energetics ($p<0.05$), initiation of apoptosis through cytochrome *c* release and the caspase activation. This was associated with increased hepatocellular ($p<0.05$) damage with apoptosis, necrosis and destruction of architecture on histology. These features were not seen in livers subjected to warm ischaemia without cold ischaemia.
Conclusion: The combination of warm ischaemia and cold preservation produces significant hepatocellular injury due to profound effects on cellular energetics and mitochondrial function. This may have important implications in developing novel therapeutic strategies for resuscitation of NHBD livers.

O321 LIVER HYPOTHERMIC MACHINE PERFUSION: AN EX VIVO PORCINE MODEL

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Data and setting of Hypothermic-Machine-Perfusion (HMP) of livers remains poorly defined. Superiority of liver HMP over Simple-Cold-Storage (SCS) must be proven before it's applied clinically. In this study, functional, and morphological data of HMP-livers were compared to SCS.
Methods: Livers were procured, flushed and preserved (SCS or HMP). 3 HMP-settings were tested: High Flow (HF); Low Flow (LF); Low Flow+0_2{300mmHg}(LFO_2). HMP-livers were perfused *via* Hepatic Artery (HA) and Portal Vein (PV) with UW-gluconate (4C, 24h){HF: PV: 3-5mmHg, 1 ml/gliver/min, HA: 25mmHg); LF: PV: 3-5mmHg, 0.5ml/g liver/min, HA: 20mmHg}. Control livers were without preservation. AST and lactate were measured in perfusate. After 24hr, livers were perfused (37C, O_2 Krebs-Henseleit,1hr). Sinusoidal endothelial cell (SEC), Kupffer cell (KC) and hepatocyte function were then assessed by Hyaluronic Acid (HA), TNFalpha and beta-galactosidase, and Indocyanine (IDC), respectively. HE staining were scored for apoptosis, necrosis, architectural destruction, enlarged space of Disse, vacuoles, and sinusoidal dilatation.
Results: *During HMP*, lactate remained stable in LFO_2 and was lower at 12hr (0,5mmol/L) *vs* HF (1,3 mmol/L; $p<.005$) and LF (1,5 mmol/L; $p<.07$). O_2 pressure was higher in LFO_2 *vs* HF and LF ($p<.05$). AST progressively increased in all HMP without inter-group difference($p<.05$). *After rewarming*, AST increased in all groups but reached lowest values in LF and LFO_2 and were comparable to control (both p=1). HA clearance was poor in HF ($p<.02$), LF ($p<.05$) and SCS ($p<.06$) *vs* control, but was similar to control in LFO_2 (p=1). TNF-alpha

and beta-galactosidase increased in SCS and HMP, without inter-group difference. IDC clearance was observed ($p<.001$) (SCS and HMP). Sinusoids became dilated: HF>SCS>LF> control and LFO_2 ($p<.05$). Morphological score showed best preservation in LFO_2 (1.251).
Conclusion: HMP with LFO_2 is superior to SCS for SEC and hepatocytes preservation. Preservation activates KC, independently of the preservation type.

O322 RENAL PRESERVATION BY NORMOTHERMIC RESUSCITATION PERFUSION WITH AUTOLOGOUS BLOOD: A COMPARISON WITH STATIC HYPOTHERMIC STORAGE AND HYPOTHERMIC MACHINE PERFUSION

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Objective: Normothermic preservation(NP) has the potential to improve metabolic support and maintain viability of ischaemically-damaged organs retrieved from non-heart beating- donors(NHBD) prior to transplantation. This study investigated the effects of Normothermic resuscitation preservation with oxygenated blood in a model of controlled NHBD kidneys versus standard methods of organ preservation in current day practice.
Methods: Porcine kidneys (n=6) were subjected to 10min warm ischaemia and preserved:
Group 1: 2hr cold storage (CS2) - minimal ischaemia controls
Group 2: 18hr CS (CS18)
Group 3: 18hr Cold machine perfusion (CP)
Group 4: 16hr CS + 2hr Normothermic Perfusion (NP)
NP was *ex-vivo* normothermic perfusion using the Isolated-organ-perfusion-system (IOPS) designed based on paediatric cardiopulmonary bypass technology.
Renal haemodynamics and function were then measured during 3hr reperfusion with autologous blood using IOPS.
Results: Increasing CS from 2hr to 18hr reduced renal blood flow(AUC 444 ± 57 vs. 325 ± 70; $P<0.01$), but this was restored by NP(563 ± 119; P=0.035 vs.18hr CS) with no difference seen compared to CP(600 ± 319; P= 0.79). Renal function was also better in Groups 1, 3 and 4 vs. Group 2(% serum creatinine fall 92 ± 6, 79 ± 9 and 64 ± 17 vs. 44 ± 13% respectively, P=0.001). AUC serum creatinine was significantly lower in Group1 compared to Group2(1102 ± 260 vs. 2156 ± 401; P = 0.001), though statistically both CP and NP were not significantly different to CS(1354 ± 300 and 1756 ± 280; P >0.05). Two hours of NP reduced the ADP:ATP ratio to a significantly lower level than the pre-perfusion values of all other groups(P=0.046).
Conclusion: Normothermic perfusion with oxygenated blood was able to restore depleted ATP levels and to reverse some of the deleterious effects of cold storage in porcine kidneys. This new method of organ preservation has potential in the field of NHBD kidney transplantation and can be succinctly described by the term normothermic resuscitation perfusion.

O323 OXYGENATED PERFUSION: MOLECULAR MECHANISMS RESPONSIBLE FOR THE REDUCED HEPATIC ISCHAEMIA REPERFUSION INJURY AND RESUSCITATION OF NHBD LIVERS

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Purpose: Livers from non-heart-beating-donors (NHBD) may be important in alleviating the donor organ shortage. However, the liver does not tolerate prolonged warm ischaemia followed by cold preservation and, as a result, the application of non-heart-beating-donors has had limited impact in clinical liver transplantation to date. We have investigated whether addition of normothermic recirculation prior to retrieval complements the already proven benefit of normothermic preservation.
Material & Methods: After 60 minutes of warm ischaemia, porcine livers were treated by normothermic recirculation (n=5, NR), by in situ oxygenated perfusion for 1 hour followed by normothermic preservation for 23 hours. Group C (Control, n=5) did not receive NR, but were otherwise treated in the same way. We assessed liver function during preservation and during 24 hours of subsequent reperfusion (a surrogate for transplantation. The apoptotic and necrotic changes after reperfusion were examined by TUNEL and haematoxylin staining.
Results: Cellular ATP levels declined sharply during 60 minutes of warm ischaemia (by 90% of the basal level, $p<0.01$). NR improved mitochondrial function (mitochondrial respiratory control ratio, $p<0.05$) and ATP levels significantly ($p< 0.01$). This effect was maintained throughout the period of warm preservation and associated with greater functional recovery of the NR livers with superior bile production ($p<0.05$), base deficit ($p<0.05$) and reduced hepatocellular ($p<0.01$) damage. Both apoptosis and necrosis were attenuated in the NR group with significantly greater destruction of architecture on histology in group C livers.
Conclusion: NHBD livers are resuscitated by a combination of normothermic recirculation and normothermic preservation. This may have important clinical implications.

O324 A MULTIFACTORIAL PROTECTIVE STRATEGY AGAINST WARM ISCHEMIC INJURY OF PIG LIVERS FROM NON-HEART-BEATING-DONORS

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The use of non-heart-beating-donors (NHBD) for Liver Transplantation (LTx) remains limited, in part because Warm Ischemia (WI) causes Primary Non-Function (PNF). We observed 50% PNF in porcine NHBD-LTx if grafts are exposed to 45'WI (*Tx 2005*). Based on previously identified mechanisms of WI and reperfusion injury *(LTx* 2007), a multifactorial "Biological Modulation (BM) cocktail" was designed trying to augment tolerance of livers to WI.
Methods: Our validated model of NHBD-LTx was used. Livers were exposed to 45'WI, cold stored (4hrs) and transplanted. Outcome was compared between recipients receiving BM (n=6) *vs* no BM (n=6). BM protocol: *in donors* (prior to 4°C HTK preservation), flush with *Warm Ringers* (avoiding cold induced vasoconstriction/improving flushing of microcirculation), *Streptokinase* (eliminating stagnating thrombi) and *Epoprostenol* (prostaglandine); *in recipients*, administration of *Glycine* (Kupffer cell stabilizer), α_1*-acid glycoprotein* (anti-inflammatory plasma protein), *MAPKinase inhibitor* (pro-inflammatory cytokines inhibitor), α*-tocopherol* and *Glutathion* (antioxidants), and *apotransferrin* (redox-iron chelator). End-points measured: survival, graft function, PNF, lactate, TNF-α production, and Sinusoidal Endothelial Cell (SEC) function (Hyaluronic acid clearance).
Results: No PNF was seen in BM pigs *vs* 50% in controls ($p<.01$). All recipients were alive at day 3 in BM *vs* 33% in controls ($p<.01$). Lactate was lower in BM *vs* controls 180' postreperfusion (5.3 ± 0.9 *vs* 9.3 ± 2.5mmol/L, p=.03). 180' postreperfusion TNF-α was lower in BM (127 ± 18pg/ml) *vs* controls (320 ± 168627pg/ml); p=.05. 60' postreperfusion Hyaluronic acid was lower in BM *vs* controls (765 ± 83 *vs* 1250 ± 356ng/ml); p=.03.
Conclusion: A "cocktail" containing several biological reagents targeting previously identified definite mechanisms of WI and reperfusion injury remarkably improves outcome of NHBD-LTx: elimination of PNF, reduced TNF-α and improved liver function, SEC function and survival. Translating this strategy into the clinics may lead to wider and safer use of NHBD and eventually to decrease waiting list mortality.

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O325 EVEROLIMUS AND REDUCED-EXPOSURE CYCLOSPORINE VS MMF AND STANDARD-EXPSOSURE CYCLOSPORINE: 12-MONTH RESULTS IN *DE NOVO* CARDIAC TRANSPLANT RECIPIENTS

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The proliferation signal inhibitor everolimus significantly reduces the incidence of ISHLT grade $\geq$3A acute rejection, and the severity/incidence of cardiac allograft vasculopathy, in *de novo* heart transplant recipients versus azathioprine when administered at a fixed dose with standard-exposure cyclosporine (CsA)

and corticosteroids. A 12-month, multicenter, randomized, open-label, non-inferiority study has been undertaken to compare renal function and efficacy (biopsy-proven acute rejection) in *de novo* heart transplant recipients receiving concentration-controlled everolimus with reduced-exposure CsA versus MMF with standard-exposure CsA.

Methods: 176 adult recipients of a primary heart transplant were randomized to receive (a) everolimus at an initial dose of 0.75mg BID, adjusted to target trough level 3-8ng/mL with reduced-exposure CsA (Table) or (b) MMF 3g/day with standard-exposure CsA (Table). All patients received corticosteroids. Patients were excluded if they had a donor >60 years and/or with donor heart disease, had panel reactive antibodies >20%, or cold ischemia time >6 hours. Endomyocardial biopsies were performed at all study visits to detect and grade severity of acute rejection. The primary endpoint was calculated creatinine clearance (Cockcroft-Gault) at six months.

Results: Key 12-month data from all 176 patients will be presented, including renal function (calculated creatinine clearance and estimated glomerular filtration rate), acute rejection, and type and incidence of adverse events.

CsA target trough levels (ng/mL)

	Month 1	Month 2	Months 3-4	Months 5-6	>6 months
Reduced-exposure CsA	200-350	150-250	100-200	75-150	50-100
Standard-exposure CsA	200-350		200-300	150-250	100-250

Conclusions: The 12-month results of this trial will represent the first data concerning the relative effects of concentration-controlled everolimus with reduced-exposure CsA exposure versus MMF with standard-exposure CsA on the preservation of renal function and prevention of acute rejection following heart transplantation.

O326 CYCLOSPORINE REDUCTION IN THE PRESENCE OF CONCENTRATION-CONTROLLED EVEROLIMUS IN *DE NOVO* CARDIAC TRANSPLANTATION: 6-MONTH STUDY RESULTS

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Everolimus reduces acute rejection and graft vasculopathy in heart transplant patients receiving cyclosporine (CsA). A 6-month, multicenter, open-label, randomized trial was undertaken to evaluate if concentration-controlled everolimus with reduced-exposure (RE) CsA improves renal function compared to concentration-controlled everolimus with standard-exposure (SE) CsA.

Methods: Adult recipients of a primary heart transplant with serum creatinine ≤250mol/L were recruited. Main exclusion criteria were donor >60 years, cold ischemia time >6 hours and PRA ≥25%. All patients received CsA (C_2 target 1000-1400ng/mL), everolimus (C_0 target 3-8ng/mL) and corticosteroids to day 60, then entered one of two randomized groups: SE-CsA (days 60-140, 800-1200ng/mL; days 150-180, 600-1000ng/mL) or RE-CsA (days 60-89, 600-800ng/mL; days 90-140, 400-600ng/mL; days 150-180, 300-500ng/mL).

Results: The ITT population comprised 100 SE-CsA and 99 RE-CsA patients. Mean CsA C_2 level in the RE-CsA group exceeded target range from day 60 to month 6 and overlapped the SE-CsA group. Biopsy-proven acute rejection (BPAR) ≥grade 3A ISHLT occurred in 21 and 16 patients, respectively (21.0% versus 16.2%, n.s.). Mean serum creatinine at month 6 (primary endpoint) was 141.0±53.1μmol/L with SE-CsA and 130.1±53.72μmol/L with RE-CsA (p=0.093). Creatinine clearance (Cockcroft-Gault) was 68.9±40.9mL/min vs 70.8±29.2mL/min in SE- and RE-CsA respectively. Calculated GFR (MDRD) was 59.0±48.2mL/min vs 59.5±23.2mL/min with SE-CsA and RE-CsA respectively. There were 3 deaths (3.0%) with SE-CsA and 6 (6.1%) with RE-CsA. The type and incidence of adverse events was similar between groups.

Conclusion: Concentration-controlled everolimus with CsA C_2 monitoring and corticosteroids offers excellent efficacy and safety at six months. Everolimus with RE-CsA showed a trend toward improved renal function vs SE-CsA but small differences in CsA exposure are likely to have limited the impact of the RE-CsA regimen on renal function.

O327 IMPROVED RENAL FUNCTION AFTER SWITCH FROM CICLOSPORIN TO MYCOPHENOLATE-SIROLIMUS IMMUNOSUPPRESSION: EXPERIENCE WITH 2 REGIMENS AFTER HEART TRANSPLANTATION

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Calcineurin inhibitors are associated with chronic nephrotoxicity. We compared 2 ciclosporin (CsA) elimination protocols in heart transplant recipients with renal impairment. CsA was stopped and sirolimus (SRL) commenced immediately. Protocol A (PA): 14 patients (13 male, mean age 56±12 years) were switched 07/02-08/03, SRL target level was 16(12-20)ng/ml, mycophenolate (MMF) continued; those on azathioprine (AZA) were transferred to MMF 1g bd. The transfer period was covered with 30mg prednisolone daily, subsequently tapered. Protocol B (PB): 23 patients (all male, mean age 54±15 years) were switched March 04-06. Patients receiving AZA were transferred to MMF two weeks prior to the switch; SRL target level was 7(5-10)ng/ml. The switch was covered with 10mg prednisolone daily. Graft function was assessed clinically, by echo and, when indicated, biopsy. Baseline eGFR (MDRD), 24-hour proteinuria and ciclosporin levels were similar. Those in PB were switched later after their transplant (mean of 37.2 vs 90.1 months, p=0.006). Improvement in eGFR was similar, mean increase in eGFR of 14.5±19 vs 15.8±20 ml/min/1.73m^2, p=0.96 at 1 month which was maintained. Proteinuria increased post-switch: 0.57±0.9 vs 0.27±0.6 g/24 hours p=0.67. Seven patients discontinued PA: 2 experienced grade-3A rejection, 1 fall in LVEF without histological rejection, 1 progressive renal failure and 3 had side-effects (1 leucopenia and thrombocytopenia, 1 pneumonitis and 1 acne). Seven discontinued PB: 1 heavy proteinuria, 2 diarrhoea and acne, 1 pulmonary thromboembolism and there were 3 deaths (1 PTLD, 2 graft vascular disease). There were no acute rejection episodes in PB. 13/14 discontinuations occurred within 6 months. MMF-SRL substitution resulted in a prompt improvement in renal function that was maintained. 50% remained on PA long term and 70% remained on PB.

O328 REDUCTION OF CYCLOSPORINE DOSE IN MAINTENANCE CARDIAC TRANSPLANT RECIPIENTS AFTER CONVERSION FROM MMF/AZA TO EVEROLIMUS: 12-MONTH RESULTS FROM A PILOT STUDY

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Everolimus reduces risk of acute rejection and graft vasculopathy versus azathioprine in *de novo* cardiac transplant patients receiving cyclosporine (CsA). Data from renal transplantation suggest efficacy is maintained using everolimus with reduced-exposure CsA.

Methods: CADENCE was a 12-month, multicenter, open-label, single-arm trial. Heart transplant recipients >12 months post-transplant were converted from MMF or azathioprine to everolimus with stepwise CsA dose reduction. Everolimus was initiated at 1.5mg/day, MMF or azathioprine discontinued and CsA dose reduced by 25%. Everolimus dose was adjusted to target C_0 level 5-10ng/mL after 6-8 days. A further reduction in CsA dose was made if GFR decreased to <75% of baseline at any point.

Results: 36 patients were enrolled (33 male), mean age of 57.2±8.6 years. 27 patients completed the study. At 12 months, everolimus dose was 1.4±0.4mg/day and C_0 level was mean 6.0±1.6ng/mL (range 5-10ng/mL). Mean CsA dose decreased from 2.2±0.7mg/kg/day at baseline to 1.1±0.5mg/kg/day at 12 months. Mean CsA C_0 level was 63±45ng/mL and mean CsA C_2 level was 343±159ng/mL at 12 months. At baseline and 12 months, GFR (Nankivell) was 70±15mL/min and 66±9mL/min, respectively (p=0.2). Peripheral edema (n=12), diarrhea (n=8), acne/headache (n=6 each) were the most frequently reported adverse events (AEs). AEs led to study discontinuation in 9 patients (3 renal function; 3 skin/mucosal lesions; 1 headache; 1 edema; 1 anemia). There were 2 Grade 3A rejection episodes, at 80 and 150 days after conversion, with no hemodynamic compromise or sequelae. One patient was withdrawn following heart failure, Grade 1B rejection with hemodynamic compromise.

Conclusions: Conversion from MMF or azathioprine to everolimus permits a reduction in CsA dose (46%) without loss of efficacy in heart transplant patients and preservation of renal function at 12 months.

O329 POST TRANSPLANT KIDNEY DISEASE IN HEART TRANSPLANT RECIPIENTS

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Renal dysfunction is a significant problem after heart transplantation (HTx).
Purpose: To determine the frequency and clinical impact of advanced chronic kidney disease (ACKD) in HTx recipients and its relationship to dose and blood level of CNIs.
Methods: 94 consecutive HTx patients (1999 to 2004) who survived at least 3 months were analyzed. 37 patients (39.4%) with a sustained increase in creatinine $\geq$200 mmol/L were considered to have ACKD and were compared to the remaining 57 control (C) patients (60.6%) whose creatinine did not reach 200 mmol/L. 26.3% of C patients had a creatinine between 150 and 200 mmol/L.
Results: There were no differences in the baseline demographic features between groups. Twelve patients in control and 5 patients in the ACKD group were off CsA at 3 year follow-up (p=NS). At 3 and 6 months, and 1, 2 and 3 years post-transplant, serum creatinine was significantly higher in ACKD patients as compared to controls ($p<0.001$ ANOVA). However, by linear regression analysis, the slope of creatinine versus time was the same in patients with ACKD versus controls (NS). The dose of CsA at 3 years after heart transplantation in the ACKD group was 2.4$\pm$1.2 mg/kg/day versus 3.8$\pm$1.6 in control ($p<0.001$). However CsA trough, and C2 levels respectively were similar in both groups (330.6$\pm$232.8 and 350.0$\pm$299.0; 647$\pm$316 vs. 728$\pm$503; p=NS for both). The 5-year actuarial patient survival was similar (89.8%) in controls and ACKD patients (89.2%). No difference in biopsy proven ISHLT $\geq$ 3A rejection rates were seen at 3 months and 3 years after HTx.
Conclusion: A significant number (39.4%) of recipients develop ACKD by 3 months after HTx. Aggressive renal protective strategies, using dose reduced CsA, prevents progression of renal dysfunction, without increasing rejection, and does not influence patient survival over a 5 year observation period.

O330 WOUND HEALING IN *DE NOVO* RECIPIENTS OF CARDIAC ALLOGRAFTS: EVEROLIMUS-BASED IMMUNOSUPPRESSION VERSUS MMF IN COMBINATION WITH CYCLOSPORINE

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The proliferation signal inhibitor (PSI) everolimus exerts an immunosuppressive effect by arresting G1- to S-phase cell cycle progression and significantly reduces acute rejection and chronic allograft vasculopathy in cardiac allografts. Data from a randomized clinical trial are presented comparing concentration-controlled everolimus to MMF in *de novo* recipients of cardiac allografts with regard to wound healing related events.
Methods: A pre-specified interim 6-month analysis was performed on data from a 12-month, multicenter, randomized, open-label, non-inferiority study in *de novo* heart transplant recipients receiving concentration-controlled everolimus with reduced-exposure cyclosporine (CsA) vs MMF with standard-exposure CsA. 176 adult recipients were randomized to receive either everolimus at an initial dose of 0.75mg BID (adjusted to target trough level 3-8ng/mL) with reduced-exposure CsA (n=91) or MMF 3g/day with standard-exposure CsA (MMF, n=83). All patients received corticosteroids.
Results: Incision-site associated adverse events (AEs) (e.g. dehiscence, secretion, lymphocele, infections) occurred in 7.3% of everolimus patients versus 8.4% in MMF-patients. AEs connected with the engraftment procedure were cardiac tamponade (everolimus 5.5% vs MMF 4.8%), pericardial effusion (everolimus 35.2% vs MMF 25.3%) and pleural effusion/hemorrhage (everolimus 22.0% vs MMF 25.3%). Serious AEs were cardiac tamponade (everolimus 4.4% vs MMF 1.1%), pericardial effusion/hemorrhage (everolimus 6.6% vs MMF 4.8%) and pleural effusion/hemorrhage (everolimus 2.2% vs MMF 1.2%).
Conclusion: *De novo* immunosuppression with concentration-controlled everolimus plus reduced dose CsA in cardiac allograft recipients does not significantly increase the risk of wound healing related events compared to MMF-based therapy.

O331 EVEROLIMUS AND CYCLOSPORINE REDUCTION IN *DE NOVO* HEART TRANSPLANT RECIPIENTS TRANSPLANTS: IMPACT ON WOUND HEALING

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De novo use of proliferation signal inhibitors (PSIs) has historically been associated with an effect on wound healing due to their marked antiproliferative potential. We report on the use of concentration-controlled everolimus with standard-exposure (SE) or reduced-exposure (RE) cyclosporine (CsA) and the incidence of wound healing related events.
Methods: Six-month data were analyzed from a multicenter, randomized, open-label study of the safety, tolerability and efficacy of SE or RE CsA (Table) with everolimus targeting trough levels of 3-8 ng/mL and corticosteroids in *de novo* heart transplant recipients.
Results: 199 patients were randomized within 72 hours of transplantation to receive SE (n=100) or RE (n=99) CsA from month 2 onwards. 70 patients (35.2%) experienced adverse events (AEs) associated with wound healing, 74.3% of which (n=52) were mild or moderate. Incision-site associated AEs (dehiscence, secretion, lymphocele, infection) occurred in 8 patients (8.0%) in the SE group versus 11 patients (11.1%) in the RE group. AEs linked with the engraftment procedure were pericardial effusion/hemorrhage (SE 25 [25%] vs RE 11 [11%]), cardiac tamponade (SE 8 [8%] vs RE 3 [3%]) and pleural effusion/hemorrhage (SE 13 [13%] vs RE 16 [16%]). Serious adverse events were as follows: pericardial effusion/hemorrhage (SE 14 [14%] vs RE 6 [6%]), cardiac tamponade (7 [7%] vs 3 [3%]), post-operative wound infection (2 [2%] vs 5 [5%]) and pleural effusion/hemorrhage (1 [1%] vs 2 [2%]).

CsA C_2 target ranges (ng/mL)

	Month 1-2	Month 3	Month 4-5	Month 6
Standard-exposure	1000-1400	800-1200	800-1200	600-1000
Reduced-exposure	1000-1400	600-800	400-600	300-500

Conclusion: *De novo* concentration-controlled, everolimus-based immunosuppression with either SE or RE CsA in cardiac allograft recipients is associated with a low risk of wound healing related events.

O332 INCIDENCE OF CMV INFECTIONS WITHIN THE FIRST 6 MONTHS AFTER CARDIAC TRANSPLANTATION: CONCENTRATION-CONTROLLED EVEROLIMUS VS MMF

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CMV disease is associated with the progression of cardiac allograft vasculopathy (CAV), and coronary endothelial function may be impaired during CMV infection. CMV-negative recipients of grafts from CMV-positive donors have a higher risk for developing CAV. The proliferation signal inhibitor (PSI) everolimus has been shown to reduce the risk of CMV infection at 12 months in *de novo* cardiac allograft recipients in a highly significant manner versus an azathioprine-based regimen. Here we report on the 6-month incidence

of CMV infections in *de novo* recipients of cardiac allografts randomized to concentration-controlled everolimus vs mycophenolate mofetil (MMF) immunosuppression in combination with cyclosporine (CsA) and corticosteroids.
Methods: 6-month data on CMV infections were analyzed from a 12-month, multicenter, randomized, open-label, non-inferiority study comparing renal function and efficacy in *de novo* heart transplant recipients receiving concentration-controlled everolimus with reduced-exposure CsA microemulsion vs MMF with standard-exposure CsA. 176 adult recipients were randomized to everolimus at an initial dose of 0.75mg BID (adjusted to target trough level 3-8ng/mL) with reduced-exposure CsA (everolimus, n=91) or MMF 3g/day with standard-exposure CsA (MMF, n=83). All patients received corticosteroids. CMV prophylactic therapy was given to 21% of everolimus patients and 24% of MMF patients.
Results: CMV infection occurred in 3.3% of the everolimus vs 15.7% of MMF patients (p=0.0071). Among patients who were CMV-positive at transplant baseline, everolimus patients had a significantly lower incidence of CMV infection reported as an adverse event versus MMF, irrespective of donor CMV status and CMV prophylaxis.
Conclusion: *De novo* immunosuppression with concentration-controlled everolimus plus reduced-exposure CsA in cardiac allograft recipients is associated with a significant reduction in the incidence of CMV recurrence/infection reported as an adverse event compared to MMF-based immunosuppression.

O333 DOSE REDUCTION OF MMF DUE TO GASTROINTESTINAL INTOLERANCE IS ASSOCIATED WITH INCREASED REJECTION IN HEART TRANSPLANT RECIPIENTS

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Gastrointestinal (GI) intolerance to Mycophenolate mofetil (MMF) is common.
Purpose:: To determine rejection risk with MMF dose reduction due to GI intolerance (GI), leucopenia, or other, in heart transplant (HTx) recipients.
Methods: Retrospective analysis of consecutive HTx patients (189) treated with MMF between1999 - 2006. MMF dosing history was examined for dose reductions and reason. The two subsequent consecutive biopsy results were recorded. Sustained significant rejection (SSR) was defined as ISHLT $\geq$ 2 on two successive biopsies. Students T-test was used to compare rejection rates between populations. A p-value of $\leq$ 0.05 was defined as significant.
Results: 80% of recipients were male, mean age at transplant was 48 years. 182/189 (98%) of patients received MMF, and 71% of these patients had MMF dose reduced at some point because of intolerance or toxicity. The most common reasons for dose reduction were leukopenia (46%), GI intolerance (33%), and infections (23%). The prevalence of SSR in the GI intolerant group versus patients with no MMF dose reductions was 66% vs 35%, (p=.002). The SSR rate was similar in patients with MMF dose reduction due to leukopenia and/or infection versus those patients who never had a dose reduction (Leukopenia: 35% v 36%, p=1.0; Infection: 35% v 43%, p=0.5). The rate of SSR was significantly higher in the GI intolerant group versus those with dose reductions for non-GI reasons (67% v s 35%, p=0.003).
Conclusion: MMF dose reduction due to GI intolerance was associated with a significantly increased rate of sustained rejection compared to patients maintained on target doses or who had dose reduction for non GI reasons. MMF dose reductions for GI intolerance require careful follow-up due to this increased risk of rejection.