Monday, 31 August 2009

Parallel Sessions 1–18

Session 1. Translational research in kidney transplantation

O-1 EXPRESSION OF REGULATORY T CELL-RELATED MOLECULE-GENES AND CLINICAL OUTCOME IN KIDNEY TRANSPLANT RECIPIENTS

Caner Süsal[1], Dorottya Nemeth[1,5], Cristiam Alvarez[1,4], Jörg Ovens[1], Claudia Sommerer[2], Markus Mieth[3], Luis Garcia[4], Robert Langer[5], Jan Schmidt[3], Martin Zeier[2], Gerhard Opelz[1]. [1]*Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany;* [2]*Department of Nephrology, University of Heidelberg, Heidelberg, Germany;* [3]*Department of Surgery, University of Heidelberg, Heidelberg, Germany;* [4]*Grupo de Inmunología Celular e Inmunogenética, Universidad de Antioquia, Medellin, Colombia;* [5]*Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary*

Purpose: Regulatory T cells have been associated with long-term allograft survival. We investigated Foxp3 and other related gene transcripts in more than 200 kidney transplant recipients with clinical outcomes in the early and late posttransplant phase.
Methods: Gene transcripts were measured by RT-PCR in 507 serial peripheral blood samples from 181 patients obtained during the first 20 days after transplantation and 22 patients with long-term ($\geq$9 years) stable renal function or chronic rejection.
Results: In the early posttransplant phase, the lowest Foxp3 transcripts were observed in patients with acute rejection (ARE) and the highest in patients with Borderline rejection (Bord-R) and patients without rejection (Non-R). On posttransplant days 11-13, ARE patients demonstrated a relative Foxp3 gene expression of 62%, strikingly lower than that observed in Bord-R, Non-R or ATN patients (Bord-R: 241%, Non-R: 176%, ATN: 137%; p=0.001, p$<$0.001, and p=0.005, respectively). Patients with Bord-R showed already on posttransplant days 5-7 an increased Foxp3 transcript level of 180%, which reached its highest level (344%) on posttransplant days 14-17. ATN patients demonstrated an intermediately high Foxp3 gene expression throughout the observation period, suggesting the existence of a mixed population of patients with and without Treg-mediated regulatory events. When the late posttransplant phase was analyzed, the level of FOXP3 transcripts was lower in patients with chronic rejection than in patients with long-term stable function (p$<$0.01). The highest TGF-β transcripts were observed in ChrRx and the highest CCR7 and CXCR4 transcripts in patients with stable function.
Conclusion: Our results suggest that the detection of Foxp3 transcripts might be useful for non-invasive monitoring of early and late graft outcomes and might serve as a marker for distinguishing patients who developed long-term allograft acceptance from patients who are prone to chronic rejection.

O-2 2D-DIGE ANALYSIS OF THE URINE PROTEOME IN CHRONIC ALLOGRAPH DYSFUNCTION

Elisenda Bañón-Maneus, Fritz K. Diekmann, Daniel Moya-Rull, Luis F. Quintana, María José Ramírez-Bajo, Jordi Rovira, Ignacio Revuelta, Alex Gutierrez-Dalmau, Amanda Sole-Gonzalez, Josep M. Campistol. *Laboratori Experimental de Nefrologia i Trasplantament Renal (LENIT), Departament de Nefrologia i Trasplantament Renal, Hospital Clínic, IDIBAPS, Universitat de Barcelona, Barcelona, Catalunya, Spain*

Urinary proteins may provide clues regarding pathogenesis of kidney disease as well as providing markers of disease activity. We employed two-dimensional differential in-gel electrophoretic analysis (2-D DIGE) to assess multiple urine samples in patients with chronic allograph dysfunction (CAD), with different IFTA degrees.
Morning spot urine of kidney transplant patients treated with Tac+MMF+Pred with a protocol biopsy two years after TX. All the patients had IFTA degree determined by biopsy. Control (normal histology) and patient urinary protein were analyzed by 2-D DIGE and DeCyder analysis, 21 different proteins could be identified of the 31 differential expressed spots, of which 14 felt increased in IFTA II-III group in comparison to IFTA I or normal histology group (IFTA 0) and 7 felt decreased. Of these 21 proteins just 9 were referred into the normal urine. Surface-enhanced laser desorption/ ionization-time of flight analysis of in-gel tryptic digest of these spots reveladed us that beta-2 microglobulin, MASP-2, alpha-1-B-glycoprotein, leucine-rich alpha-2-glycoprotein 1, alpha-1-antitrypsin, Gelsolin, apoptosis-inducing factor, heparan sulfate proteoglycan, anti TNF-alpha antibody light-chain Fab fragment, immunoglobulin lambda light chain, dimethylarginine dimethylaminohydrolase 2 and protein 1 (under intellectual protection process) are differentially expressed. Western Blot of these proteins confirmed the differential expression into the different groups. Immunostaining of human kidney biopsies shows differential expression of the protein 1 in the proximal or distal tubules and glomeruli.
In conclusion, we developed a method to analyze numerous urine samples from patients and allowed for detection and identification of possible IFTA urinary biomarkers that could have important significance into the understanding of CAD.

O-3 *TCF7L2* POLYMORPHISM IS AN INDEPENDENT RISK FACTOR FOR NEW ONSET DIABETES MELLITUS AFTER TRANSPLANTATION: A COHORT STUDY ON 1229 RENAL TRANSPLANT PATIENTS

Lidia Ghisdal[1], Christophe Baron[2], Yvon Lebranchu[2], Jean-Michel Halimi[2], Yann Lemeur[3], Jean-Philippe Rerolle[3], Mireille Drouet[3], Arnaud Lionet[4], François Glowacki[4], Christian Noël[4], Karl Martin Wissing[1], Daniel Abramowicz[1], Marc Abramowicz[5]. [1]*Renal Transplantation Clinic, ULB-Erasme Hospital, Brussels, Belgium;* [2]*Department of Nephrology and Renal Transplantation, Centre Hospitalier Universitaire de Tours, Tours, France;* [3]*Department of Nephrology and Renal Transplantation, Centre Hospitalier Universitaire Dupuytren, Limoges, Belgium;* [4]*Department of Nephrology and Renal Transplantation, Centre Hospitalier Regional Universitaire de Lille, Lille, France;* [5]*Medical Genetics Clinic, ULB- Erasme Hospital, Brussels, Belgium*

Objective: Whether New Onset Diabetes Mellitus After Transplantation (NODAT) shares the same susceptibility genes with type 2 diabetes mellitus has not been adequately assessed to date. The aim of our study was to investigate the association between 11 type 2 diabetes mellitus-associated polymorphisms and the risk of NODAT within the first 6 months after-renal transplantation.
Methods: A total of 1229 patients free of diabetes at transplantation were genotyped for the following polymorphisms: rs7903146 (*TCF7L2*), rs8050136 (*FTO*), rs7754840 (*CDKAL1*), rs5215 (*KCNJ11*), rs1801282 (*PPARG*), rs1111875 (*HHEX-IDE*), rs13266634 (*SLC30A8*), rs10811661 (*CDKN2A-CDKN2B*), rs4402960 (*IGF2BP2),* rs757210 (*HNF1B*), rs10010131 (*WFS1*). NODAT was defined by fasting plasma glucose $\geq$ 126 mg/dL on at least two occasions or *de novo* hypoglycaemic therapy.
Results: Patients who developed NODAT (N=145, incidence=11.8%) within the first 6-months post-transplantation were compared to patients free of NODAT (N=1084) for clinical and genetic factors. NODAT was significantly associated with the following characteristics by multivariate analysis: *TCF7L2* polymorphism (P=0.014), older age (P$<$0.0001), black African or north-African ethnicities (P=0.003), higher body mass index at transplantation (P=0.016), tacrolimus (P=0.01) and mTOR inhibitors (P=0.003). The risk to develop NODAT was 1.55 (OR; 95%CI: 1.06-2.25; P=0.02) for CT genotype and 1.79 (OR; 95%CI: 1.02-3.14; P=0.04) for TT genotype, in comparison with the CC genotype of rs7903146. No other polymorphism was significantly associated with NODAT.
Conclusions: Our results show the independent contribution of the *TCF7L2* polymorphism in NODAT, and suggest that NODAT and type 2 diabetes mellitus share a common insulin secretion defect pathway. Our results may help to tailor immunosuppression in order to prevent NODAT.

O-4 PRETREATMENT WITH MONOCYTE CHEMOTACTIC PROTEIN-1 PREVENTS THE DESTRUCTIVE EFFECT OF OVERDOSE MESENCHYMAL STEM CELL IN ACUTE KIDNEY TRANSPLANTATION

Bo Chen[1], Jessy Deshane[1], Lingling Guo[2], James F. George[2], Sifeng Chen[3,1]. [1]*Department of Medicine, Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, AL, USA;* [2]*Department of Surgery, University of Alabama at Birmingham, Birmingham, AL, USA;* [3]*Department of Physiology and Pathophysiology, Fudan University Shanghai Medical College, Shanghai, China*

Background: Intravascularly administered mesenchymal stem cells (MSC) ameliorate acute renal injury. However, undesirable number and disproportional distribution of adherent cells in microcirculation of injured organ may be harmful. We observed the effects of different doses and monocyte chemotactic protein-1 (MCP-1) pretreatment of MSC in acute kidney transplantation.
Methods: In DA to Wistar-Furth rat kidney allograft transplantation, 8×10^5, 4×10^6 and 2×10^7 MSC/kg body weight were administered via the tail vein

30 min after transplantation. Serum creatinine was determined using a Creatinine Analyzer. Semiquantitative analysis of renal histology was performed using a scale of 0–4. Serum and renal homogenate levels of cytokines were measured by multiplex ELISA. Phenotyping of infiltrating cells in renal allograft was performed by immunohistochemistry.
Results: Intravenously administered MSC selectively resided in the capillaries of the renal allograft. 8×10^5 MSC/kg had no effect on allograft function. 4×10^6 cells/kg improved allograft function. 2×10^7 MSC/kg resulted in 100% mortality. However, MCP-1 pretreatment of MSC (2×10^7cells/kg) decreased MSC aggregation in the allografts, eliminated the lethal effect and significantly improved allograft function. The effect of MSC administration was not related to the infiltration of immune/inflammatory such as CD8-, B220- and CD68-positive cells. Administration of MCP-1-pretreated MSC (2×10^7MSC/kg) decreased proimmune/proinflammatory cytokines in allograft homogenate.
Conclusions: While confirming MSC's therapeutic potential, we demonstrated a lethal adverse effect of overdosed MSC. MCP-1 pretreatment eliminated this side effect. Our study highlights the importance of dosing and cytokine modification in MSC therapy for kidney transplantation.

O-5 NAKED SIRNAS SILENCING CASPASE-3 GENE REDUCED APOPTOSIS AND IMPROVED CELL VIABILITY IN PORCINE PROXIMAL TUBULAR CELLS WITH OR WITHOUT HYDROGEN PEROXIDE TREATMENT

Bin Yang[1], Joshua E. Elias[2], Michael L. Nicholson[3]. *[1]Transplant Surgery, [2]Infection, [3]Immunity and Inflammation, University of Leicester, Leicester, United Kingdom*

Purpose: Proximal tubular cells (PTCs) are most vulnerable to ischaemia reperfusion injury (IRI) in renal transplantation. Caspase-3 (C3) is crucial in apoptotic cascades and up-regulated by IRI due to various pathogenic processes including oxidative damage.
Methods: The effect of silencing C3 gene by naked small interfering RNAs (siRNAs) on C3 activity and protein, apoptosis and cell viability was investigated in porcine PTCs (LLC-PK1) with or without the stimulation of an oxidiser, hydrogen peroxide (H_2O_2).
Results: The level of C3 mRNA was reduced by 20 nM siRNAs up to 50% at 24h. C3 activity was increased by transfection reagent (TR, 140±3% vs. 100±13%), decreased by 2 & 20 nM siRNAs (114±13%; 49±10%) at 72h in normal cells; and raised by H_2O_2 dose- and time-dependently, maximised at 100 μM, 24h. This was further increased by TR (252±46% vs. 100±51%); reduced by siRNAs at both dosages (113±39%; 110±13%). C3 active protein (39±12% vs. 100±19%) and precursor (72.3±8.3% vs. 100.0±7.79%) were also decreased by 20 nM siRNAs at 96h. The number of apoptotic cells (per high power field) was increased by TR (2.5±0.3 vs. 0.4±0.2); reduced by siRNAs (0.5±0.3; 1.3±0.6) in normal cells; and greatly induced by H_2O_2 (11.8±2.0); further increased by TR (15.6±2.0), but decreased by 2 & 20 nM C3 siRNAs (3.0±0.9; 4.8±3.9) at 72h. Cell viability was unchanged by TR alone or siRNA in normal cells (93±1%); decreased by H_2O_2 (85±3%); but improved by 20 nM siRNAs (81±4% vs. 89±3% TR only).
Conclusions: Silencing C3 gene by naked siRNAs decreased C3 activity and protein expression, subsequently reduced apoptosis and improved cell viability in LLC-PK1 cells. Therefore, C3 siRNAs could be used as an intervention to ameliorate IRI in transplantation.

O-6 THE RENAL LYMPHATIC SYSTEM IN HEALTH AND DISEASE

David G. Vass[1], John Haylor[2], Bardi Shrestha[3], Jeremy Hughes[1], Lorna Marson[1], Lorna Marson[1]. *[1]MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Nephrology, University of Sheffield, Sheffield, United Kingdom; [3]Department of Surgery, University of Sheffield, Sheffield, United Kingdom*

Purpose: In contrast to arterial and venous structures, no attempt is made to reconnect the lymphatic vessels of the kidney during transplantation. Recent data has demonstrated lymphangiogenesis in patients with acute allograft rejection and in failing allografts with interstitial fibrosis and tubular atrophy (IFTA). We examined (i) the main route of lymphatic drainage from the normal kidney, (ii) the time course of lymphatic reconnection following disruption and (iii) whether new lymphatic vessels are evident in a rat model of IFTA.
Methods: Carbon black was injected into control rat kidneys or kidneys that had undergone careful microsurgical disruption of hilar lymphatic vessels. Renal and parathymic lymph node accumulation of carbon black was assessed at various time points. Orthotropic renal transplants were carried out in rats; isografts (5) and allografts (4). Animals were immunosuppressed and sacrificed after one year. Lymph nodes were immunostained for ED1 (rat macrophage marker) and tissue was immunostained for podoplanin (lymphatic marker).
Results: Carbon black was evident in the renal hilar and parathymic lymph nodes 24hrs after injection into the renal parenchyma. Carbon black was evident within ED1+ macrophages within the renal lymph node at 24hrs. Disruption of the hilar lymphatic vessels resulted in renal retention of carbon black with no evidence of carbon black in lymph nodes at 24 hours. Macroscopic evidence of carbon black in renal lymph nodes was evident at day 7 following lymphatic disruption. Histological analysis of rat allografts demonstrated features of IFTA. There was a 6-fold increase in the number of intar-renal lymphatic vessels in allografts compared to isografts.
Conclusions: (i) A functional lymphatic drainage system of the kidney is re-established at day 7 following hilar lymphatic vessel disruption. (iii) A dramatic increase in the numbers of interstitial lymphatic vessels is evident in a rat model of IFTA.

Session 2. Large programs and registries in small bowel & liver transplantation

O-7 IS INTESTINAL TRANSPLANTATION A VALUABLE ALTERNATIVE FOR CHILDREN WITH TOTAL AND DEFINITIVE INTESTINAL FAILURE?

Florence Lacaille[1], Frederique Sauvat[2], Francisca Joly[3], Jean-Pierre Hugot[4], Bernard Messing[3], Frank Ruemmele[1], Olivier Goulet[1]. *[1]Department of Paediatric Hepato-Gastroenterology-Nutrition, Hôpital Necker-Enfants Malades, Paris, France; [2]Department of Paediatric Surgery, Hôpital Necker-Enfants Malades, Paris, France; [3]Department of Gastroenterology, Hôpital Beaujon, Clichy, France; [4]Department of Paediatric Gastroenterology-Nutrition, Hôpital Robert-Debré, Paris, France*

Aim: Long-term parenteral nutrition (PN) is an effective treatment for children with intestinal failure; however, even in experienced centers, it is not devoid of complications, and the impact on quality of life is important. Small bowel transplantion (SBTx) may be an alternative to PN, but the long term results, as published in the International Registry, are still unsatisfactory. We want to report here our 15-year experience, in order to better delineate the problems, and the areas of possible improvement.
Patients: From 1994 on, 78 children underwent 83 SBTx, 36 combined with the liver, at a median age of 5 years. Indications were: short bowel syndrome (26), motility disorders (24), congenital enteropathies (25), retransplantation (6), no diagnosis (2).
Results: With a median follow-up of 7 years (6 m-15 y), 31 (37%) are PN-free, two are partly PN-dependent. 26 children died (80% patient survival after isolated SBTx, 46% after liver-SBTx), 22 of early infectious or surgical complications, 4 (5%) of late rejection, infection or lymphoma. Early removal of the graft was necessary in 17 SBTx, mainly for acute rejection. Late graft loss occurred in 7 cases (8%), also mainly due to rejection.
Conclusion: SBTx remains a difficult procedure with high early morbidity and mortality. Late severe complications are also a concern for the long term. Protocols of early care and long term follow up have to be continuously improved, especially immunosuppression and control of infections. SBTx should also be included early in the discussion of treatment for children with intestinal failure, in order to minimize the complications due to long term PN, and optimize the prognosis of these patients.

O-8 EVALUATING SURVIVAL BENEFIT-BASED DECEASED-DONOR LIVER ALLOCATION THROUGH MICROSIMULATION

Douglas E. Schaubel[1,2], Mary K. Guidinger[2], Robert M. Merion[2,3]. *[1]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA; [2]Scientific Registry of Transplant Recipients, Arbor Research Collaborative for Health, Ann Arbor, MI, USA; [3]Department of Surgery, University of Michigan, Ann Arbor, MI, USA*

Background: A survival benefit based system for the allocation of deceased-donor livers is currently being considered in the United States. The proposed survival benefit score is the difference in predicted future lifetimes (capped at 5 years): with versus without a liver transplant. The proposed score would replace the Model for End-stage Liver Disease (MELD) score as the basis for sequencing chronic end-stage liver disease patients on the wait list.
Methods: Using data obtained from the Scientific Registry of Transplant Recipients (SRTR) and the Liver Simulated Allocation Model (LSAM; the liver component of a set of SAMs developed by the SRTR), we evaluate the impact of changing from MELD- to benefit-based allocation. Inputs to each LSAM run include patients already on the wait list on 01/01/2006, as well as new wait listings and organ arrivals throughout 2006. Organs are allocated according to user-prescribed rules; in this case, MELD, or benefit. Patient follow-up is simulated until 12/31/2006. Results represent the mean across 10 LSAM runs.
Results: In the first year after implementation of survival benefit-based allocation, it is predicted that there would be 83 fewer wait list deaths and 102 fewer deaths overall. The average benefit score (i.e., 5-year lifetime gained) would increase by 0.38 years per liver transplant. Overall, based on 1 year's worth of

transplants, and five years of follow-up per patient, it is estimated that 2,223 additional life-years would be saved by a switch from MELD- to benefit-based allocation.
Conclusion: The great shortfall in the availability of donor livers increases the need to maximize the life-saving capacity of procured livers. A substantial number of life-years would be saved by the adoption of an allocation system based on liver transplant survival benefit.

O-9 CURRENT STATUS OF LIVER TRANSPLANTATION IN EUROPE: AN ANALYSIS OF ELTR DATA

Rene Adam[1], Vincent Karam[1], Valerie Delvart[1], John Buckels[2], John O'Grady[3], Denis Castaing[1], Jurgen Klempnauer[4], Neville Jamieson[5], Peter Neuhaus[6], Jan Lerut[7], Stephen Pollard[8], Mauro Salizzoni[9], Lutz Fischer[10], Juan Garcia-Valdecasas[11], Ferdinand Mulhbacher[12], Jose Mir[13]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Queen Elisabeth Medical Center, The Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Liver Transplantation–9th Floor Surgery, Royal Free Hospital, London, United Kingdom; [4]Klinik für Viszeral-und Transplantationschirurgie, Medizinische Hochschule Hannover, Hannover, Germany; [5]Department of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom; [6]Klinik für Allgemein-Viszeral-und Transplantationschirurgie, Charité Campus - Virchow Klinikum, Berlin, Germany; [7]Cliniques Universitaires Saint Luc, Catholic University of Louvain, Brussels, Belgium; [8]Department of Surgery, St Jame's & Seacroft University Hospital, Leeds, United Kingdom; [9]Centro de Trapianti di Fegato, Azienda Ospedaliera S. Giovanni Battista, Torino, Italy; [10]Dept. of Hepatobiliary Surgery, Universitatskrankenhaus Eppendorf - Transplantation Center, Hamburg, Germany; [11]Gastrointestinal Surgery Department, Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; [12]Klinishe Abteilung für Transplantation, Transplantationszentrum Allgemeines Krankenhaus, Wien, Austria; [13]Servicio de Medicina Digestiva, Hospital Universitario La Fe, Valencia, Spain*

From May 1968 to December 2007 the ELTR has cumulated data on 79044 Liver Transplantations (LT) at 138 centers. Analysis of this data gives a comprehensive overview of the evolution of LT in Europe.
The annual number of LT has increased reaching a peak of 5369 in 2005. Five-yr survival is currently 71% for patients and 63% for grafts. Twenty-six percent of post-LT mortality occurred within 1 month and 48% within 6 months of LT. Cirrhosis was the most frequent indication (58%) followed by cancer (13%), cholestatic diseases (11%), and acute hepatic failure (ACHF) (9%). Patient survivals at 5 yrs were 72% for cirrhosis, 63% for ACHF and 57% for cancer ($p<0.001$). In cirrhotic patients, 5-yr survival was better for primary biliary cirrhosis (79%) than for alcoholic (74%) or virus-related cirrhosis (71%)($p<0.001$), and survival with Hep B was better than with Hep C (74% vs 66% at 5 yrs, $p<0.01$). The 5-yr survival in pediatric recipients was 79% and was independent of exact patient age. In contrast, age influenced 5-yr survival in adults (74%: 16-45 yrs, 70%: 46-60 yrs, 64%: >60 yrs)($p<0.001$). Alternative procedures (AP) to full size cadaveric transplant (FSCT) were increasingly used accounting for 17% of all LT in 2007. Among AP, split liver grafts (SL) represent 46%, living donor grafts (LD, 37%), reduced liver grafts (RL, 12%), and domino transplants (DT, 6%). In rank order of survivals, 5-yr graft survivals were 57% for RL, 62% for SL, 64% for FSCT, 70% for LD and, 55% for DT.
Refined indications, improved surgical technique, and more effective immunosuppression have lead to significant improvements in outcome following LT. To address organ shortage, alternatives to conventional LT are increasingly used with LDLT being the most prevalent.

O-10 LIVER TRANSPLANTATION IN PATIENTS IN UNOS STATUS 1: OUTCOME OF 419 TRANSPLANTATIONS IN THE NORTH ITALY TRANSPLANT PROGRAM

Tullia M. De Feo[1], Nicola De Fazio[1], Enzo Andorno[2], Umberto Baccarani[3], Umberto Cillo[4], Michele Colledan[5], Luciano De Carlis[6], Matteo Donataccio[7], Enrico Regalia[8], Andrea Risaliti[9], Giorgio Rossi[10], Francesco Procaccio[11], Mario Scalamogna[1]. *[1]Organ and Tissues Transplant Immunology, Fond. IRCCS OMP, Ma.Re, Milan, Italy; [2]Transplant Center, Az. Osp. Univ. S. Martino, Genova, Italy; [3]Dept. of Transplantation, University Hospital, Udine, Italy; [4]Hepatobiliary Surgery and Liver Transplantation, Az. Osp. di Padova, Padova, Italy; [5]Liver and Lung Transplant Center, Az. Osp. "Ospedali Riuniti", Bergamo, Italy; [6]Hepatobiliary Surgery and Liver Transplantation, Niguarda Hospital, Milan, Italy; [7]II Surgery Division, Az Osp. "Ospedali Riuniti", Verona, Italy; [8]Surgery and Liver Transplantation, Fond. IRCCS Ist. Nazionale Tumori, Milan, Italy; [9]Digestive Diseases and General Surgery, Az Osp. Univ. "Ospedali Riuniti", Ancona, Italy; [10]Liver Transplant Center, Fond. IRCCS OMP, Ma.Re, Milan, Italy; [11]Neuro ICU, Az. Osp. Univ., Verona, Italy*

Background: In the North Italy Transplant program (NITp) patients with fulminant hepatitis liver failure (FHF) or graft failure (GF) within the first 10 days after transplantation are prioritized and considered in "Status 1" according to the UNOS classification. Since the beginning of liver transplant activity in 1983, the first available liver from deceased donors in NITp area is allocated to the patient in status 1 and, since 2003, status 1 is also considered a national emergency. Here we report the outcome of 419 transplants performed in patients in status 1 during our 20-year experience.
Patients and methods: In the NITp, from the beginning of the activity to December 2008, 5166 liver transplants were performed, of them 419 were emergencies: 170/419 (40.5%) FHF and 249/419 (59.5%) GF. We compared the patient and graft survival in the periods: 1983-1999 and 2000-2008. Donor age, recipient age, and waiting time before transplantation were the variables considered. One and 5-year patient and graft survival rates were calculated.
Results: In the NITp, in 1983-1999, 1922 liver transplants were performed: 86 (5%) FHF, 114 (6%) GF and 1722 in patients in non urgent status. In 2000-2008 the transplants were 3244: 84 (2.5%) FHF, 135 (4%) GF and 3025 in patients in non urgent status.
Results of FHF and GF groups are reported in the following table:

Years (n of tx)	Recipient age	Donor age	Waiting time	1-yr pts. survival	5-yr pts. survival	1-yr graft survival	5-yr graft survival
FHF group							
83-99 (86)	31±15	36±16*	2±2	49%*	43%*	43%*	37%*
00-08 (84)	31±19	44±21*	3±4	81%*	78%*	75%*	72%*
GF group							
83-99 (114)	37±19	36±17*	2±2	40%*	34%*	37%*	33%*
00-08 (135)	35±23	44±18*	3±4	67%*	53%*	60%*	46%*

*$p<0.05$.

In the period 2000-2008, 1- and 5-year patient and graft survival rates in non urgent recipients were 88%, 81% and 84%, 77%, respectively.
Conclusions: In the NITp area, in the last few years, short and medium-term survival rates of patients transplanted in status 1 have greatly improved. Despite this, survival rates are significantly lower in GF group than in FHF group, suggesting a negative effect of re-transplantation, per se, on the outcome.

O-11 LIVING DONOR LIVER TRANSPLANTATION: A EUROPEAN LIVER TRANSPLANT REGISTRY (ELTR) REPORT ON 2634 CASES

R. Adam[1], V. Karam[1], V. Delvart[1], L. Fischer[2], M. Kilic[3], J. Lerut[4], P. Neuhaus[5], P. Kalicinski[6], D. Castaing[1], B. de Hemptinne[7], O. Boillot[8], Y. Tokat[9], G. Klampnauer[10], J. O'Grady[11], Y. Revillon[12], J. Belghiti[13]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Dept. of Hepatobiliary Surgery, Universitatskrankenhaus Eppendorf, Transplantation Center, Hamburg, Germany; [3]Ege University Medical School, Department of Surgery - Division of Hepatobiliary and Liver Transplantation, Izmir, Turkey; [4]Cliniques Universitaires Saint Luc, Catholic University of Louvain, Brussels, Belgium; [5]Klinik für Allgemein-Viszeral- und Transplantationschirurgie, Charité- Campus - Virchow Klinikum, Berlin, Germany; [6]Department of Pediatric Surgery and Organ Transplantation, Children's Memorial Health Institute, Warsaw, Poland; [7]Dienst voor Levertransplantatie en Digestieve Heelkunde, Universitair Ziekenhuis, Ghent, Belgium; [8]Unité de Transplantation Hépatique, Hôpital Edouard Herriot, Lyon, France; [9]Department of Liver Surgery, Florence Naghtingate Hospital, Istanbul, Turkey; [10]Klinik für Viszeral-und Transplantationschirurgie, Medizinische Hochschule Hannover, Hannover, Germany; [11]Liver Transplantation–9th Floor Surgery, Royal Free Hospital, London, United Kingdom; [12]Service de Chirurgie Infantile, AP-HP Hopital Necker Enfants Malades, Paris, France; [13]Service de Chirurgie Digestive, AP-HP Hopital Beaujon, Clichy, France*

Living Donor Liver Transplantation (LDLT) is increasingly used in Europe but results concerning donor and recipient outcome still need to be extensively evaluated.
Material & methods: From October 1991 to December 2007, LDLT accounted for 2634 of the 71,422 (3.7%) transplants performed in 74 centers. We compared the outcome of LDLT to that of Full Size Cadaveric Liver Transplantation (FSCLT).
Results: LDLT that was initially reserved for pediatrics is now used mainly in adults (73% of cases in 2007). The right liver was donated to 92% of adults and the left lobe was donated to 86% of children. Overall 5-yr graft and patient survivals were 70% and 76%, and were better in children (78% and 84%) than in adults (64% and 70% – $p<0.01$). At a range from 2-56 days following donation, mortality occurred in 6 of the 2634 donors (0.2%) due to pulmonary embolism (1), sepsis (2), multiple organ failure (2) and cardiac failure (1). Early donor morbidity (3 months) was 20%. As compared to FSCLT performed during the same period, LDLT was used more often for cancers (20% vs 13%, $p<0.01$) and less often for fulminant hepatitis (3% vs 7%, $p<0.01$) and retransplantation (2% vs 10%, $p<0.01$). In children, 5-yr graft and patient survivals were better with LDLT than with FSCLT, (78 and 84% vs 71% and 82% – $p<0.01$). In adults, survivals were similar to those of the FSCLT (64% and 70% vs 64% and 70% – p=NS).
Conclusion: Although LDLT represents only 3.7% of all transplants, it has significantly improved over time. Compared to FSCLT, LDLT provide better results

in children and similar in adults. However, donor mortality is 0.2% with an early morbidity of 20%.

O-12 CURRENT STATUS OF LIVER TRANSPLANTATION (LTx) FOR HEREDITARY AMYLOIDOSES AND DOMINO LIVER TRANSPLANTATION (DLT): THE FAMILIAL AMYLOIDOTIC POLYNEUROPATHY WORLD TRANSPLANT REGISTRY (FAPWTR) AND THE DOMINO LIVER TRANSPLANT REGISTRY (DLTR) PERSPECTIVE

Henryk E. Wilczek, Marie Larsson, Bo-Göran Ericzon. *Department of Transplantation Surgery, Karolinska University Hospital Huddinge, Karolinska Institute, Stockholm, Sweden*

Material: Several hereditary amyloidosis diseases can be treated with Ltx. The FAPWTR registers such patients, and the DLTR has data on amyloidotic livers re-used for transplantation in patients with non-amyloidotic end stage liver disease.
Results: By Dec. 31, 2007, 1441 LTx patients from 68 Centres in 17 countries and 648 DLT from 49 centres were reported. 1199 of the patients had the Val30Met transthyretin (TTR) mutation, 175 patients had a nonVal30Met variant, 11 patients had a non-TTR variant and in 55 patients the information was missing or unknown. 44 different non-Val30Met mutations have been reported. In the Val30Met group, age at disease onset and type of initial symptoms differ between patients from different areas. Males with late disease onset had significantly inferior survival than females ($p<0.05$). 10-year survival in Val30Met patients was better than in patients with other TTR mutations (75% and 46% respectively $p<0.0001$). Main causes of death were infectious and cardiovascular (50% of deaths). DLT recipient mean age was 54.5±9.5 years, male/female proportion 75%/25%. 46% of the DLT recipients had a hepatic malignancy. The overall 5-year graft survival in DLT recipients was 66%. The longest DLT follow-up is now >12 years. Two DLT recipients have been retransplanted because they developed TTR amyloidotic disease. A few DLT recipients have been reported to have received non-amyloidotic liver grafts.
Conclusion: LTx in patients with amyloidotic disease saves lives. Val30Met and nonVal30Met TTR mutations differ clinically. NonVal30Met patients more often need combined liver-heart transplantation. Results in DLT recipients are excellent and the indications have expanded. However, the procedure carries a risk of transmitting TTR amyloidotic disease with the graft.

Session 3. Pancreas transplantation: technical aspects

O-13 TECHNICAL ASPECTS OF PANCREAS RETRANSPLANTATION – A SINGLE CENTER EXPERIENCE

Matthias Biebl, Michael Lienegger, Manuel Maglione, Schneeberger Stefan, Robert Öllinger, Christine Holzknecht, Ricardo-Pupo Yamila, Sabine Brandstätter, Aigner Felix, Mark Walter, Margreiter Raimund. *Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Tyrol, Austria*

Introduction: Pancreas transplantation is an established treatment for diabetes mellitus with end-stage renal disease, however graft loss occurs more frequently than in other organs.
Methods: Thirty-nine pancreas retransplantations (28 (71.7%) PAK, 7 (17.9%) SPK, 4 (10.2%) PA, 3 re-retransplantations) were included in this retrospective study. Before 1996, immunosuppression comprised steroids, cyclosporine and azathioprine, thereafter induction therapy (anti-thymocyte globuline 78%, alemtuzumab 16%, OKT3 3%, IL 2 antagonist 3%), followed by a triple regimen of steroids, calcineurin inhibitos and MMF. Data are reported as mean±standard deviation, or total numbers (%).
Results: Of 39 patients (51.3% female, mean age 43.9±9 years), 37 (94.9%) were type I diabetics. Retransplant was performed 67.0±67.3 months after the first transplant with the non-functioning pancreas being removed simultaneously (71.8%), previously (18.7%) or left in situ (9.3%). Operation time was 258.9±92.8 min, packed red blood cells were used in 36.1%. Severe adhesions were noted in 17.9%. Aterial anastomosis was performed to the common (77.1%) or external iliac artery (11.4%), the previous pancreas' conduit (8.5%), or the artery of an old renal transplant (2.8%). Venous anastomosis was performed using the inferior vena cava (68.5%), the iliac vein (19.9%), the first pancreas' portal vein (8.5%), the superior mesenteric vein (2.8%), or the stump of a previous renal transplant (2.8%). Early in this series, bladder (13.1%) or external (5.2%) drainage was used, later on only enteric drainage (81.5%). Length of stay was 34.4±22.8 days, ICU stay 3.2±3.9 days. Of 21 infectious episodes (56.7%), 10 (27.8%) were intraperitoneal. Morbidity, rejection and reoperation rate were 59.0%, 33.3% and 43.6%, respectively. Time to full endocrine function was 7±8.2 days. After a follow-up of 37.2±39.0 months, graft loss occurred in 34.3%.
Conclusion: While complication rates are higher after pancreas retransplantation compared to first-time transplantation, mid-term results are satisfactory.

O-14 PANCREAS RETRANSPLANTATION: CHALLENGING BUT JUSTIFIED

Deep J. Malde, Afshin Tavakoli, Ravi Pararajasingam, Babatunde Campbell, Hany Riad, Neil Parrott, Titus Augustine. *The Renal and Pancreas Transplant Unit, Manchester Royal Infirmary, Manchester, United Kingdom*

Introduction: There have been 31 pancreas retransplants in the U.K., forming a mere 3.7% of overall pancreases transplanted. The outcome of pancreas retransplantation continues to improve, with results comparable to primary pancreas transplants. As long-term survival rates for pancreas transplant recipients continue to improve, an increasing number are presenting for re-transplantation after loss of the initial graft.
Methods: We present our experience of re-transplantation in eight diabetic recipients after loss of their first grafts from thrombosis (N=4) and rejection (N=4). From 2001–2008 eight deceased donor pancreas re-transplants were carried out (bladder drained in 2 and enteric drained in 6). During this time period, 26 primary pancreas after kidney (PAK), 128 simultaneous kidney and pancreas (SPK) and 7 pancreas transplant alone (PTA) were carried out. The results of pancreas retransplants were compared with primary SPK, PAK and PTA outcomes.
Results: Mean waiting time for retransplants was 32 months (range 4-61). 4 were after primary PAK, 2 after primary SPK and two after primary PTA. 5 patients (62.5%) underwent reoperation due to complications, compared to 76 patients (47.2%) in primary transplants. 1 year graft survival rate was 62.5% for retransplants compared to 71% and 81% for primary pancreas only and SPK transplants respectively. Patient survival at one year was 100% for retransplants and 97% and 90% for primary pancreas only and SPK respectively. Graft thrombosis rate was 25% for retransplants (2/8) compared to 10.9%, 23% and 14.3% for primary SPK, PAK and PTA. Acute rejection rate was 12.5% for retransplants (1/8) compared to 23% and 27% for primary pancreas only and SPK.
Conclusion: Retransplantation remains a technically formidable procedure with a higher incidence of graft thrombosis and acute rejection. Although our numbers are small, we believe that in selected patients, the therapeutic effects on secondary complications of diabetes justify pancreas retransplantation.

O-15 INDICATIONS, SAFETY PROFILE, AND SUCCESS RATE OF PANCREAS GRAFT BIOPSY FOLLOWING PANCREAS TRANSPLANTATION

Ugo Boggi[1], Fabio Vistoli[1], Luca Morelli[2], Alessandro Campatelli[2], Marco Del Chiaro[1], Chiara Croce[1], Carlo Moretto[1], Stefano Signori[1], Gabriella Amorese[3], Sabrina Gabbriellini[1], Vittorio Perrone[2], Simone D'Imporzano[2], Piero Marchetti[4], Franco Mosca[2]. *[1]U.O. Chirurgia Generale e Trapianti nell'Uremico e nel Diabetico, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [2]U.O. Chirurgia Generale Universitaria 1, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [3]U.O. Anestesia e Terapia Intensiva, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [4]S.V.D. Endocrinologia e Metabolismo dei Trapianti d'Organo e Cellulari, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy*

Background: Percutaneous pancreas allograft biopsy (PPAB), either ultrasound (US) or computed tomography (CT) guided, is the technique of choice for evaluation of pancreatic allograft rejection or dysfunction. Timing, safety and success rate of PPAB have not been fully established yet.
Material and methods: Between May 1996 and February 2009 288 pancreas transplants (PTx) were performed. Forty-five PPAB were performed in 33 recipients. PPABs were performed using a 16-gauge automated needle under US (n=39) or CT (n=6) guidance. PPAB was performed in the presence of hyperamilasemia (HA), hyperlipasemia (HL) and/or hyperglicemia (HG) of unknown origin.
Results: Twenty-three recipients had 1 PPAB, 8 patients had 2 PPAB, and 2 patients had 3 PPAB. Forty-one PPABs were performed in 30 recipients with retroperitoneal PTx with enteric drainage; 4 PPABs were performed in 3 recipients with systemic-bladder drained PTx. Ninety-one percent of PPAB specimens (n=41) were adequate for histologic diagnosis. There was 1 PPAB-related extra-graft exocrine leak (2.2%) successfully treated by percutaneous drainage. No other abdominal organs were inadvertently biopsied or injured. Histological diagnosis concluded for acute rejection in 27 PPABs (60.0%). Indications to PPAB were: HA+HL in 21, HA+HL+HG in 8 and HG isolated in 12. Rate of biopsy proven acute rejection was similar irrespective of biochemical abnormalities leading to PPAB: 76.2%, 62.5% and 50.0%, respectively (p=NS). However, the rate treatment failure with loss of graft function differed among the 3 groups: 25.0% vs 71.4% (p=0.04) vs 77.8% (p=0.01), respectively.

Conclusion: PPAB has a low rate of complication and a high success rate. Our data suggest that a more aggressive policy, based on early PPAB in the presence of otherwise unexplained clinical or laboratory abnormalities, could enhance a timely detection of pancreas rejection before its progression to irreversible stages.

O-16 INTRAPYLORIC INJECTION OF BOTULINUM TOXIN A: AN ALTERNATIVE TREATMENT IN PANCREAS TRANSPLANT RECIPIENTS WITH SEVERE DIABETIC GASTROPARESIS

Manuel Maglione, Gerald Brandacher, Robert Öllinger, Matthias Biebl, Walter Mark, Helmut Weiss, Raimund Margreiter. *Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria*

Diabetic gastroparesis (DGP) is defined by chronic delayed gastric emptying in the absence of mechanical obstruction. Following successful pancreas transplantation DGP remains a major problem in one third of the patients. Here we report on the application of intrapyloric injection of botulinum toxin A (BoTx) in pancreas recipients refractory to prokinetic and anti-emetic medication.
All six patients (four males) with stable graft function suffered from severe gastroparesis. Symptoms were quantified by the Patient-Assessment-of-Gastrointestinal-Symptom (PAGI-Sym©) Severity-Index before injection and during follow-up. Likewise, Quality-of-life (QoL) was assessed by the Patient-Assessment-of-Gastrointestinal-Disorders-Quality-of-Life (PAGI-QoL©) Index. Total score varies from 0 to 5. To exclude other possible underlying causes gastric emptying was determined by X-ray and scintigraphic examination prior to treatment. BoTx-therapy consisted of 100U injected over the four quadrants of the pylorus. Control X-ray was performed 24 hours later.
Substantial therapeutic effects were evident within two weeks following BoTx-injection. While the mean symptom score before BoTx-injection was 3.5 (range 2.9 4.5) after the treatment it decreased to 0.7 (range 0.3 2). Similarly, the PAGI-QoL© Index decreased from 2.5 (range 2.6 3.2) to 1.1 (range 0 2.8). Two patients required a second injection due to recurrent symptoms after two and ten months, respectively, however, one of these two patients did not experience any symptom amelioration. In one patient there was no symptom worsening despite loss of pancreatic graft function. No adverse events were reported. After a mean follow up of 457.7 (65-694) days all treated patients are doing well. Five patients do experience considerable improvements of all symptoms and four do report conspicuous amelioration of their QoL.
Intrapyloric BoTx-injection should be considered in pancreas transplant recipients suffering from severe DGF if they are refractory to commonly used medication.

O-17 TREATMENT WITH A CXCR4 ANTAGONIST CURES DIABETES IN NOD MICE

Alice Schwarznau[1], Anja Barwig[1], Ulrike Bourquain[2], Veronika Auer[2], Nikolaus Schantz[2], Manfred J. Stangl[1]. *[1]Department of Surgery, Division of Transplantation, Klinikum Rechts der Isar, Munich, Germany; [2]IMETUM, Technical University of Munich, Garching, Germany*

Purpose: Typ 1 Diabetes is affecting a growing number of patients worldwide. The disease starts with a cellular infiltrate around the pancreatic islets and shows signs of an autoimmune reaction. There is bulk evidence that progenitor cells within the pancreas itself could start a self renewal of pancreatic islets and cure diabetes in its early stage. We have investigated the influence of a CXCR4 antagonist that leads to liberation of stem cells from the bone marrow. We hypothesize that the increased number of stem cells helps to regenerate pancreatic islets.
Methods/Materials: In our laboratory 80% of female NOD mice develop diabetes around day 140 to 160. Stem cell liberation was tested in control animals and a significant increase of CD 133 positive cells was seen as early as 2 h after administration of 5ug of a CXCR4 antagonist given by an i.v. injection. 2 Groups were tested: Both groups received 5ug CXCR4 antagonist i.v for 20 days. Group1 (pre-diabetes, n=12) from day 109 till day 139, group 2 (diabetes, n=8) from day 139 to 159.
Results: 83% of the animals in group 1 and 50% of the animals in group 2 showed a normal blood glucose on day 210 compared to the control group (untreated NOD mice, n=17) where only 17% of the animals showed no diabetes ($p<0.01$, group 1 vs. control, group 2 vs. control). Administration of a CXCR4 antagonist resulted in a significant cure of diabetes in NOD mice.
Conclusion: Stem cell liberation leads to a lasting cure of diabetes in NOD mice. The effect is greater if the animals are treated in a pre-diabetic state, but even after diabetes has occurred reversal of the disease is possible. Our data suggest that autologous stem cells are capable to cure diabetes typ I.

O-18 PORTAL-ENTERIC OR SYSTEMIC-ENTERIC DRAINAGE FOR RETROPERITONEAL PANCREAS TRANSPLANTATION

Ugo Boggi[1], Fabio Vistoli[1], Chiara Croce[1], Marco Del Chiaro[1], Carlo Moretto[1], Stefano Signori[1], Gabriella Amorese[2], Sabrina Gabbriellini[1], Vittorio Perrone[3], Simone D'Imporzano[3], Nelide De Lio[3], Piero Marchetti[4], Franco Mosca[3]. *[1]U.O. Chirurgia Generale e Trapianti nell'Uremico e nel Diabetico, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [2]U.O. Anestesia e Terapia Intensiva, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [3]U.O. Chirurgia Generale Universitaria 1, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [4]S.V.D.Endocrinologia e Metabolismo dei Trapianti d'Organo e Cellulari, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy*

Background: Pancreas transplantation with graft placement in the retroperitoneum (RPTx), described in the setting of portal enteric drainage (PED) (Transplantation 2005), is feasible also in case of systemic-enteric drainage (SED) and has surgical advantages compared to classical intraperitoneal method.
Material and methods: Between April 2001 and February 2009 240 RPTx were performed: 117 SPKTx and 123 solitary PTx. Venous effluent was drained in the portal circulation in 160 recipients and in the inferior vena cava in the remaining 80. All pancreata were drained enterically. Irrespective venous drainage site pancreas allografts were always placed in the right retroperitoneal space, covered by the ascending colon and its mesentery.
Results: Overall, 21 grafts were lost in first 3 months after PTx because of venous thrombosis (n=9; 3 PED vs 6 SED – p=0.03), recipient death (n=5; PED), acute rejection (n=5; PED), duodenum graft ischemia (n=1; PED), mycotic pseudoaneurism (n=1; SED). The 3-month relaparotomy rate was 13.8% (16.0% in PED and 15.0% in SED). One patient had a negative relaparotomy (PED) and 4 underwent two relaparotomies (3 PED vs 1 SED). Nonocclusive venous thrombosis was diagnosed in 17 (7.1%) recipients (14 PED vs 3 SED). During the mean follow-up period of 46.6±30.0 months, 29 recipients (12.1%) were diagnosed with peripancreatic fluid collections, all successfully treated by observation (n=12) or percutaneous drainage (n=17); 38 percutaneous pancreas biopsies (22 PED vs 16 SED) were performed in 29 recipients (18 PED vs 11 SED). One-year patient and pancreas survival rates were 95.4% and 87.1% in PED vs 97.1% and 83.3% in SED, respectively. Five-year figures were 95.4% and 80.5% in PED vs 97.1% and 78.8% in SED.
Conclusion: These data confirm that RPTx, with either portal or venous effluent, is a safe and effective method for PTx.

Session 4. Experimental immunosuppression

O-19 RECOMBINANT HUMAN C1-INHIBITOR PREVENTS ACUTE ANTIBODY-MEDIATED REJECTION IN ALLOIMMUNIZED BABOONS

Xavier Tillou[1], Nicolas Poirier[1], Stephanie LeBas-Bernardet[1], Jeremy Hervouet[1], David Minault[1], Karine Renaudin[2], Fabio Vistoli[1], Georges Karam[1], Mohamed Daha[3], Gilles Blancho[1]. *[1]UMR643, INSERM, Nantes, France; [2]Anapathology, CHU, Nantes, France; [3]Nepfrology, University Medical Center, Leinden, Netherlands*

Acute antibody-mediated rejection (AAMR) remains an unsolved issue in transplantation, especially in the context of pre-transplant immunization. The deleterious effect of preformed cytotoxic anti-HLA antibodies (Ab) through complement activation is well proven, but very little has been reported concerning complement blockade to prevent/cure AAMR. Here we used a baboon model of preimmunization to explore the prevention of AAMR by early inhibition of the classical complement pathway using human recombinant C1-inhibitor (rhC1INH).
Baboons were immunized against peripheral blood mononuclear cells from allogeneic donors and subsequently received a kidney from the same donor, once a specific and stable immunization had been established. Rejection occurred at day 2 post-transplant in untreated pre-sensitized recipients, with characteristic histological lesions of AAMR together with intragraft Ab and complement deposition. Since rhC1INH blocks the in vitro cytotoxicity induced by donor-specific Ab, other alloimmunized baboons received the drug thrice daily by i.v. injection during the first five post-transplant days. Rejection could be prevented during the treatment, but occurred 1-2 days after treatment discontinuation.
These results demonstrate that early blockade of complement activation by rhC1INH can prevent AAMR in presensitized recipients suggesting that this treatment could also be useful in other forms of AAMR due to induced Abs.

O-20 ENDOTOXIN CHALLENGE INDUCES MYELOID-DERIVED SUPPRESSOR CELLS CONTROLLING ALLOGRAFT REJECTION THROUGH HEME OXYGENASE-1

Nicolas Van Rompaey[1], Virginie De Wilde[1], Marcelo Hill[2], Maria-Cristina Cuturi[2], Michel Goldman[1], Alain Le Moine[1]. *[1]Transplantation Immunology, IMI, Gosselies, Belgium; [2]U643, ITERT-INSERM, Nantes, France*

Purpose: Inflammation and cancer are associated with impairment of T-cell responses by a heterogeneous population of myeloid-derived suppressor cells (MDSCs) co-expressing CD11b and GR-1 antigens. MDSCs have been recently implicated in co-stimulation blockade-induced transplantation tolerance in rat, which was under the control of inducible NO synthase (iNOS). Herein, we describe another method for generating MDSC-compatible cells using a unique mechanism of suppression.
Material and method: C57BL/6 mice were challenged with LPS (E.Coli 0111:B4) injected intraperitoneally before harvesting of spleen cells for in vitro or in vivo assays. In vitro suppression assays were performed using irradiated allogeneic BALB/c spleen cells or anti-CD3/CD28 coated microbeads as stimulators, purified C57BL/6 T cells as responders, and purified C57BL/6 CD11b+ cells as suppressors. Cell contact was investigated using a semipermeable transwell membrane. In vivo assays were performed using C57BL/6 male to female skin transplantation.
Results: Repetitive injections of lipopolysaccharide (LPS) triggered a dramatic increase in CD11b+GR-1+ myeloid cells. These cells suppressed T-cell proliferation, Th1 and Th2 cytokine productions in mixed lymphocyte reaction or polyclonal stimulation assays in a cell-cell contact dependent manner. Transfer of CD11b+ cells from LPS-treated mice in untreated recipients significantly prolonged skin allograft survival. They produced large amounts of IL-10 and expressed heme oxygenase-1 (HO-1), a stress-responsive enzyme endowed with immunoregulatory and cytoprotective properties not previously associated with MDSC activity. HO-1 inhibition by the specific inhibitor, tin-protoporphyrin IX (SnPP), completely abolished T-cell suppression and IL-10 production. By contrast, neither iNOS nor arginase 1 inhibition did affect suppression. Importantly, HO-1 inhibition before CD11b+ cell transfer prevented the delay of allograft rejection revealing a new MDSC-associated suppressor mechanism.
Conclusion: Altogether, these results suggest that MDSCs with HO-1 activity could become the subject of new cell therapy for transplantation tolerance.

O-21 PLASMACYTOID DENDRITIC CELLS: A PROMISING TOOL FOR IMMUNE-CONDITIONING THERAPY OF ORGAN TRANSPLANT RECIPIENTS

Patrick Boor[1], Herold J. Metselaar[1], Sarina de Jonge[1], Luc J. van der Laan[2], Jaap Kwekkeboom[1]. *[1]Gastroenterology and Hepatology, Erasmus MC, Rotterdam, Netherlands; [2]Surgery, Erasmus MC, Rotterdam, Netherlands*

The major barrier for induction of immunological tolerance to allogeneic organ grafts in humans is their large repertoire of pre-existing alloreactive memory T-cells. Here we show that TLR-stimulated human plasmacytoid dendritic cells (PDC) inhibit allogeneic memory T-cell responses by induction of profound anergy in conventional T-cells and *de novo* generation of $CD8^+$ regulatory T-cells (Treg).
PDC were purified by immunomagnetic selection with anti-BDCA4 mAb from human blood, and stimulated with TLR-7 agonist loxoribine (LOX) or TLR-9 ligand CpG-A DNA. After 20 hours, unfractionated allogeneic T-cells, containing both naïve and memory cells, were added. Cytokine production, responsiveness to re-stimulation and suppressive capacity of PDC-primed T-cells were determined after 7 days of culture.
Allogeneic T-cells primed by TLR-stimulated PDC produced IL-10 (LOX-PDC: 715±369; CpG-PDC: 668±260 pg/ml) and became hyporesponsive to restimulation with monocyte-derived DC (MoDC) from the same donor as PDC (90% reduction of proliferation compared to non-primed T-cells). T-cell hyporesponsiveness was due to anergy, since proliferation was restored by addition of IL-2. Moreover, PDC-primed T-cells suppressed allogeneic responses of conventional T cells and of $CD45RO^+$ memory T-cells to MoDC in a donor-specific manner (60% inhibition of memory T-cell proliferation at ratio PDC-primed T-cells: memory T-cells = 1:2). Depletion of $CD25^+$ T-cells from allogeneic T-cells did not prevent induction of anergy or suppressive capacity by PDC, showing that natural Treg were not required. Instead, PDC induced regulatory capacity in $CD8^+$ T-cells, which was abrogated by anti-IL-10 receptor antibody.
In conclusion, TLR-stimulated human PDC induce profound anergy in allogeneic (memory) T-cells, and induce *de novo* differentiation of IL-10 producing $CD8^+$ Treg that are able to inhibit memory T-cell responses. Donor-derived PDC are therefore a promising immunotherapeutic tool to silence the alloreactive T-cell repertoire of organ transplant recipients.

O-22 EPIDERMAL GROWTH FACTOR INHIBITION, A NOVEL PATHWAY TO PREVENT CHRONIC RENAL ALLOGRAFT REJECTION

Johanna Savikko[1], Jukka Rintala[1], Niina Malmström[1], Sini Rintala[1], Petri Koskinen[2]. *[1]Transplantation Laboratory, University of Helsinki, and Helsinki University Central Hospital, Helsinki, Finland; [2]Department of Medicine, Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland*

Chronic rejection (CR) still remains the major unsolved problem in clinical kidney transplantation. It is an irreversible fibrotizing process leading eventually to the graft loss. Currently there is no treatment available for preventing it. Several fibrogenic growth factors like PDGF and TGF-b have been demonstrated to be major mitogens mediating mesenchymal cell proliferation in CR. The role of epidermal growth factor (EGF) is unknown during the development of CR. Here we investigated the effect of erlotinib, a selective EGF receptor inhibitor, on the development of CR.
Kidney transplantations were performed from DA to WF rats and syngenic controls were done between DA rats. Allografts were immunosuppressed with CsA 1.5 mg/kg/d s.c. One group of allografts was also treated with erlotinib 10mg/kg/d perorally. 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 CR were seen, CADI 0.8±0.2 (mean±SEM). In control allografts intense chronic changes were seen, CADI 8.8±1.0. Erlotinib-treatment prevented the development of CR significantly compared to control allografts. Only few histological changes were seen, CADI 3.3±0.4. Especially arterial neointimal proliferation and glomerular mesangial matrix increase were decreased by erlotinib compared to controls. Fibrogenic growth factor ligand and receptor induction was significantly inhibited by erlotinib compared to control allografts. Creatinine values of erlotinib-treated allografts were also lower compared to control allografts.
Our results demonstrate that erlotinib-treatment prevents the development of chronic changes in renal allografts. This indicates that EGF inhibition provides a novel pathway to prevent CR.

O-23 THE IMMUNOSUPPRESSANT RAPAMYCIN REDUCES NEOPLASIA IN $APC^{min/+}$ MICE AND NORMALISES ION CHANNELS

Gudrun E. Koehl[1], Melanie Spitzner[2], Jiraporn Ousingsawat[2], Rainer Schreiber[2], Karl Kunzelmann[2], Edward K. Geissler[1]. *[1]Department of Surgery, Medical Center, University of Regensburg, Regensburg, Germany; [2]Department of Physiology, University of Regensburg, Regensburg, Germany*

Background: The immunosuppressant rapamycin has been shown to inhibit K^+-channel function, which is important for lymphocyte activation. Another already well known therapeutic property of mTOR-inhibitors is reduction of tumor growth. Since for transplant patients tumor prevention is of high importance, we have looked at long-term tumorigenesis in a mouse strain carrying an oncogenic mutation that causes intestinal cancer in humans and is associated with ion channel dysregulation. Early in colorectal tumorigenesis, $APC^{Min/+}$ mice show enhancement of Akt/mTOR signaling and upregulation of oncogenic K^+- and Na^+-channels.
Methods: $APC^{Min/+}$ mice and their wildtype siblings were fed a high fat diet with or without rapamycin starting at age 5 weeks. For long-term survival (up to 1 year), weight loss >20% or clinical symptoms (rectal prolapse, anemia, apathy) were experimental endpoints. Additionally, in 20 week-old animals polyp formation was analysed and ion-channel (EnaC, BK, Elk1, Erg1) expression (RT-RCR) and function (Ussig chamber) were measured.
Results: Untreated $APC^{Min/+}$ mice succumbed to multiple neoplasia by 22.3±1.4 weeks of age, whereas mice treated with rapamycin maintained stable weight and hematocrit, and survived long-term (39.6±3.4 weeks, with over 30% surviving more than 1 year). Abnormalities in colonic electrolyte transport typical for $APC^{Min/+}$ mice were abolished by rapamycin treatment. Epithelial Na^+-channels (ENaC) and oncogenic K^+- channels (BK, Elk1 and Erg1) were suppressed close to wildtype levels, both at mRNA and functional levels.
Conclusions: Our results demonstrate that continuous prophylaxis with rapamycin markedly inhibits APC tumorigenesis and suggests that a novel mechanism of rapamycin action is via modulation of oncogenic ion channels. Therefore rapamycin may reduce the tumor risk for transplant patients by compensating for functional abnormalities and mutations not immediately related to mTOR-pathway.

O-24 LIVER SINUSOIDAL ENDOTHELIAL CELLS TOLERIZE ALLOREACTIVE T CELLS IN A LIVER ENDOTHELIUM REPOPULATION *IN VIVO* MODEL

Masataka Banshodani, Yuka Igarashi, Yuka Tanaka, Hideki Ohdan.
Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Allogeneic liver transplants are often accepted by a recipient, leading to the development of tolerance to further organ transplants from the same donor but not from a third party. We have previously demonstrated that LSECs selectively tolerize allospecific T cells across MHC barriers in mice and the FasL expressed on LSECs can impart tolerogenic potential upon alloantigen recognition. In addition to this *in vitro* demonstration, we have established an *in vivo* model for evaluating the immunomodulatory effects of LSECs. LSECs isolated from BALB/c mice were injected into recombinase-activating gene-2/gamma-chain double knockout (DKO) B6 mice, lacking T, B, and NK cells, via the portal vein. At seven days after the adoptive transfer, when orthotopic implantation of the LSECs into the liver was observed, splenocytes from wild-type B6 mice were intravenously injected into the DKO B6 mice. At seven days after splenocyte inoculation, a mixed lymphocyte reaction (MLR) assay using splenocytes from these recipients revealed specific inhibition of $CD4^+$ T-cell proliferation in response to stimulation with irradiated BALB/c mouse splenocytes (n = 5). In order to facilitate the engraftment of the LSECs, 2 days before the intraportal adoptive transfer of LSECs from the BALB/c mice, the DKO B6 mice were intraperitoneally injected with monocrotaline, which impaired the host-LSECs, conferring a proliferative advantage to the transplanted LSECs. Six weeks after the adoptive transfer, splenocytes from wild-type B6 mice were intravenously injected into the DKO B6 mice. An MLR assay performed 7 days after the inoculation revealed specific inhibition of both $CD8^+$ and $CD4^+$ T-cell proliferation (n = 4). Further these recipients indefinitely accepted heart allografts that were subsequently transplanted from BALB/c mice. We therefore conclude that LSECs suppress the alloimmune responses of T cells during allogeneic liver transplantation.

Session 5. Optimisation of current immunosuppressive strategies in kidney transplantation

O-25 OPTIMISATION OF MYCOPHENOLIC ACID (MPA) EXPOSURE FOR EARLY STEROID WITHDRAWAL IN KIDNEY GRAFT RECIPIENTS: THE OPERA TRIAL

Y. Le Meur[1], G. Touchard[2], F. Glowacki[3], J.P. Rerolle[4], V. Garrigue[5], N. Ouali[6], A.E. Heng[7], M. Delahousse[8], L. Albano[9], L. Couzi[10], P. Lang[11], P.F. Westeel[12], F. Allano[13], N. Tagieva[13], L. Rostaing[14]. *[1]Nephrology, CHU, Brest, France; [2]Nephrology, CHU, Poitiers, France; [3]Nephrology, CHU, Lille, France; [4]Nephrology, CHU, Limoges, France; [5]Nephrology, CHU, Montpellier, France; [6]Nephrology, CHU, Paris, France; [7]Nephrology, CHU, Clermont Ferrand, France; [8]Nephrology, CHU, Suresnes, France; [9]Nephrology, CHU, Nice, France; [10]Transplantation, CHU, Bordeaux, France; [11]Nephrology, CHU, Creteil, France; [12]Nephrology, CHU, Amiens, France; [13]Transplantation, Roche, Neuilly, France; [14]Transplantation, CHU, Toulouse, France*

Early steroid withdrawal may expose patients to a higher risk of clinical or subclinical rejections. We conducted the randomized multicenter OPERA trial to assess the benefit of the combination of early corticosteroids (CS) withdrawal and MMF therapeutic drug monitoring (TDM) in kidney transplant recipients under cyclosporine (CsA).

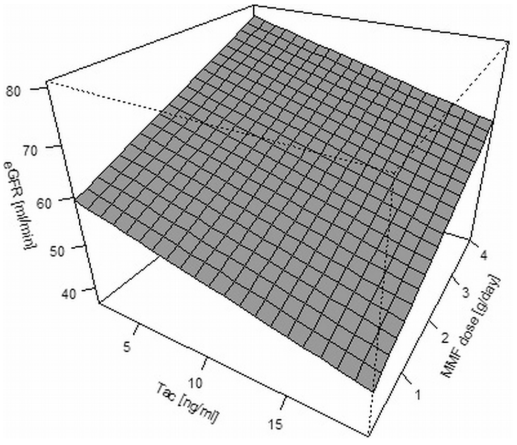
In this 12-months, 258 adult kidney transplant patients with low immunological risk were randomized to a concentration-controlled (A, n=130) or a fixed-dose of 2 g per day (B, n=128) of MMF. In the arm A MMF was introduced at 3 g per day and MMF doses were adjusted according to the calculated MPA exposure with a therapeutic target of 40mg.h/L. All patients received anti-IL2R induction therapy, CsA and 7 days of CS. The primary endpoint was number of patients experienced either biopsy-proven acute rejection [BPAR] within 3 months after transplantation or subclinical acute rejection [SCAR] on protocol biopsy at month 3. The intent-to-treat population comprised 247 patients (A: n=126; B: n=121). The number of biopsies performed during Day 0 and W12 visits was 176 among which 156 were evaluable. MPA exposures were significantly higher in arm A at W2 and W6 but similar in both arms at W12. Only 22 patients in arm A (17.5%) and 17 in arm B (14.1%) experienced a BPAR over the first 3 months or SCAR at M3 (p= 0.46). The incidence of BPAR at 12 months was not significantly different between arms: 21.4% in A and 13.2% in B (p=0.09).

In this study the rates of SCAR at 3 months and one year were unexpectedly low and not improved by TDM. In patients with low immunological risk, MMF associated with CsA allows early CS discontinuation with a good tolerability and safety outcome.

O-26 RELATIONSHIP OF TACROLIMUS EXPOSURE AND MMF (CellCept®) DOSE WITH RENAL FUNCTION AT ONE YEAR AFTER RENAL TRANSPLANTATION

Henrik Ekberg[1], Teun van Gelder[2], Bruce Kaplan[3], Corrado Bernasconi[4]. *[1]Department of Nephrology and Transplantation, University Hospital, Malmö, Sweden; [2]Deaprtment of Hospital Pharmacy, Erasmus Medical Center, Rotterdam, Netherlands; [3]School of Medicine, Univerity of Arizona, Tucson, AZ, USA; [4]Medical Affairs, F. Hoffmann-La Roche Ltd., Basel, Switzerland*

Purpose: The most common treatment in de-novo renal transplantation is a triple regimen including mycophenolate mofetil (MMF, CellCept®), tacrolimus and corticosteroids, with possible antibody induction in addition. The nephrotoxicity of tacrolimus at the currently employed dosages is an open question.
Methods: We pooled data from three large recent randomized de-novo studies (SYMPHONY, FDCC, OPTICEPT) using variations of the triple regimen with respect to tacrolimus target levels, MMF dosing and antibody induction. We used multivariate linear regression to explore the relationship of renal function at one year post-transplant (estimated GFR, 4-variable MDRD formula) with tacrolimus levels and MMF dose measured over the previous 6 months. The model included also a series of possible confounders.
Results: 976 patients were evaluable. On average, tacrolimus levels were in a range considered low (mean $\pm$ s.d.: 7.2 ± 2.54 ng/ml) and MMF dose was 1.5 ± 0.61 g/day. Lower tacrolimus levels and higher MMF doses were associated with significantly better renal function (figure 1, table 1). There were other variables associated with renal function, most notably acute rejection, donor age, delayed graft function. Subanalyses in the three studies gave a consistent picture, with the drug effects going in the same direction as in the pooled analysis, although results, with smaller sample sizes, were not always significant.



Modeling results

	Coefficient	p-value
Intercept	101.1	<0.001
Tacrolimus level [ng/ml]	-0.77	0.002
MMF dose [g/day]	4.84	<0.001
BPAR	-6.82	<0.001
Donor age [years]	-0.39	<0.001
Donor: living related (vs. deceased)	1.018	0.46
Donor: living unrelated (vs. deceased)	3.94	0.019
Delayed graft function	-6.88	<0.001
Recipient age [years]	-0.22	<0.001
Weight [kg]	-0.16	<0.001

There was no overt difference in the effect sizes when we split patients having Stage II (eGFR: 60-89 ml/min) and Stage III (30-59 ml/min) chronic kidney disease.
Conclusion: Tacrolimus appears to have a moderate but consistent nephrotoxic effect even in modern efficient immunosuppressive regimens where it is employed at lower doses than in previous years.

O-27 EARLY CORTICOSTEROID DISCONTINUATION AFTER KIDNEY TRANSPLANT DOES NOT DETERIORATE MIDDLE TERM GRAFT HISTOLOGY OF PROTOCOL BIOPSIES

Masayoshi Miura[1], Hiroshi Harada[2], Naohiko Shimoda[1], Kanako C. Kubota[3], Yayoi Ogawa[3], Nobuyuki Fukuzawa[1], Tatsu Tanabe[1], Tetsuo Hirano[2], Katsuya Nonomura[1]. *[1]Department of Urology, Hokkaido University Hospital, Sapporo, Japan; [2]Department of Urology and Renal Transplantation, Sapporo City General Hospital, Sapporo, Japan; [3]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan*

Objectives: Favorable clinical outcomes of steroid minimization in renal allograft recipients have been reported in terms of graft survival, acute rejection episodes and metabolic disorders. The aim of this study was to investigate the effect of early steroid discontinuation on subclinical changes in graft histology up to 3 years posttransplant.
Methods: One-hundred and thirty consecutive living-donor renal transplant recipients were included. The immunosuppressive regimen consisted of tacrolimus (TAC), mycophenolate mofetil (MMF) and 2 doses of basiliximab. Steroid was discontinued at day 3 posttransplant in 50 recipients (SED group), while it was given chronically in 80 recipients (CS group). The followings were compared between the groups at 1, 3, 6 months, 1, 2 and 3 years posttransplant: serum creatinine (sCr), biopsy-proven acute rejection (BPAR), graft survival (GS), 12 h area-under-the-curve of tacrolimus and mycophenolic acid blood levels (TAC-AUC, ng.hr/ml, MPA-AUC, μg.hr/ml), MMF dose (mg) and histopathological findings of protocol biopsy according to the Banff '07 classification.
Results: SCr was comparable up to 3 years except for 1 month (SED group>CS group). TAC-AUC was significantly higher in SED group at 1 month but was equivalent thereafter. MMF dose and MPA-AUC were comparable throughout the period. The incidence of BPAR until 12 months was equivalent (24% in SED group and 19% in CS group). 40% of the BPAR cases in SED group remained steroid-free after treatment. Graft survival was equivalent. The averages of both acute and chronic Banff histopathological scores up to 3 years were also equivalent between the groups both on intention-to-treat or per protocol analyses.
Conclusions: Favorable middle-term outcomes were achieved clinically as well as histologically after early steroid discontinuation compared to the conventional protocol. Early steroid discontinuation did not enhance chronic deterioration of renal allograft histology.

O-28 SWITCHING AZATHIOPRINE TO MYCOPHENOLATE MOFETIL REDUCES SKIN UVA PHOTOSENSITIVITY AND WHITE CELL DNA 6-THIOGUANINE LEVELS IN RENAL TRANSPLANT RECIPIENTS

Gunther F. Hofbauer[1], Gilles Straub[1], Robert Meyer[1], Peter Karran[2], Natalie Attard[2], Lars French[1], Rudolf P. Wuthrich[3], Andreas L. Serra[3]. *[1]Department of Dermatology, University Hospital, Zurich, Switzerland; [2]Cancer Research UK London Research Institute, Clare Hall Laboratories, Hertfordshire, United Kingdom; [3]Division of Nephrology, University Hospital, Zurich, Switzerland*

Background: UVA is an established risk factor for squamous cell carcinoma of the skin transplant recipients. Azathioprine (AZA) causes selective ultraviolet A (UVA) photosensitivity in patients. Its metabolism culminates in 6-thioguanine (6-TG) incorporation into DNA which are potentially DNA damaging. We evaluated skin photosensitivity and 6-TG DNA incorporation in renal transplant recipients immunosuppressed with CNI in combination with either AZA or mycophenolate mofetil (MMF).
Methods: Skin UVA and UVB photosensitivity was assessed by minimal erythema dose (MED) and the amount of DNA 6TG in peripheral blood mononuclear cells was measured using HPLC.
Results: MED values were obtained for 16 renal transplant recipients on AZA and 17 on MMF. The mean UVA MED was significantly lower in the AZA treated compared with MMF treated patients (21.3 vs. 34.0 J/cm^2, p=0.021). To investigate the relative contribution of AZA to skin photosensitivity, we switched 25 (18 male, 7 female, age 55.6$\pm$12.5yrs) Caucasian long-term renal transplant recipients from AZA to MMF. The MED was measured prior to switching from AZA to MMF and three months later. The average daily AZA dose was 57 mg/day and the subsequent dose of MMF was 1084 mg/day. After the switch from AZA to MMF, the UVA MED increased significantly from 16.0$\pm$7.2 to 25.4$\pm$11.5 J/cm^2 ($p<0.0001$). The average 6-thioguanine content in DNA in 7 patients taking azathioprine was 98.6$\pm$27.8 and declined to 43.3$\pm$15.0 pmol/mg total DNA three months after switching to MMF.
Conclusion: This study demonstrates that the skin of AZA treated patients exhibits selective UVA photosensitivity and switching from AZA to MMF significantly reduced UVA photosensitivity. Diminished UVA photosensitivity correlated positively with a reduced DNA 6-TG level. As DNA 6-TG and UVA interact to form DNA damaging ROS, elimination of DNA 6-TG by switching from AZA to MMF might reduce the risk of SCC in renal transplant recipients.

O-29 SOTRASTAURIN AND MYCOPHENOLIC ACID IN *DE NOVO* RENAL TRANSPLANT PATIENTS: EFFICACY AND SAFETY OF A CNI-FREE REGIMEN

Styrbjorn Friman[1], Wolfgang Arns[1], Bernhard Banas[1], Larry Chan[1], Shamkant Mulgaonkar[1], Bjorn Nashan[1], Marianne Soergel[2], Flavio Vincenti[1], Karl-Martin Wissing[1], Steffen Witte[2], E. Steve Woodle[1]. *[1]For the AEB071 A2207, Global Study Group, Göteborg, Sweden; [2]Global Development, Novartis Pharmaceuticals, Basel, Switzerland*

Sotrastaurin, a selective protein kinase C-inhibitor, prevents early T-cell activation via a calcineurin-independent pathway. Phase II study results for sotrastaurin+mycophenolate (MPA) in *de novo* renal transplant recipients are reported.
Methods: Recipients were randomized 2:1 to sotrastaurin 300mg bid+MPA (sotrastaurin, n=81), or tacrolimus+MPA (Tac, n=44). All received basiliximab and corticosteroids. Primary efficacy endpoint was composite of BPAR, graft loss, death or loss-to-follow-up at month 3 and primary safety endpoint was MDRD calculated glomerular filtration rate (GFR).
Results: Although both regimens showed equivalent efficacy up to one month, the 3 month Kaplan-Meier estimate of the primary efficacy endpoint was significantly higher in the sotrastaurin group (26% vs. 5% Tac; p=0.001). The three month estimate of BPAR was 24% in the sotrastaurin and 5% in the Tac group; P=0.003. Continued rejections beyond month 3 in the sotrastaurin arm resulted in premature study termination. Despite more acute rejection episodes, mean GFR was higher in the sotrastaurin group (10-17 mL/min/1.73m^2; $p<0.01$) from week 2 through month 3. Serious adverse events were reported in 47% of sotrastaurin and 30% of Tac patients, including infections (11% sotrastaurin, 7% Tac). Most frequent adverse events (AE) were gastrointestinal (89% sotrastaurin, 64% Tac) with nausea, vomiting and constipation occurring more frequently in the sotrastaurin group whereas both regimens had similar incidences of diarrhea. The sotrastaurin group showed an increase of 2-10 beats per minute in mean heart rate and more frequent tachycardia AEs (10% vs 5% Tac).
Conclusion: The sotrastaurin+MPA regimen showed similar efficacy to tacrolimus+MPA during the first month, but was inferior afterwards. Sotrastaurin+MPA was associated with better renal function and acceptable tolerability. Further studies are needed to determine the optimal regimen to benefit from sotrastaurin's novel mode of action.

O-30 NOVEL PROTEIN KINASE C-INHIBITOR SOTRASTAURIN PLUS TACROLIMUS IN RENAL TRANSPLANTION

Markus Weber[1], Claudia Sommerer[1], Thomas Becker[1], Argiris Asderakis[1], Frank Pietruck[1], Josep M. Grinyo[1], Paolo Rigotti[1], Jacques Dantal[1], Jennifer Ng[2], Markus J. Barten[2], Klemens Budde[1]. *[1]For the AEB071 A2203, Global Study Group, Zurich, Switzerland; [2]Global Development, Novartis Pharmaceuticals, Basel, Switzerland*

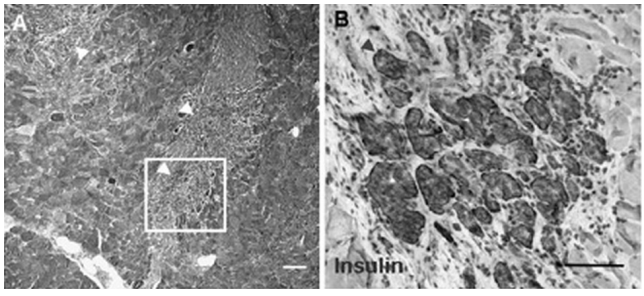
Sotrastaurin prevents early T-cell activation via a calcineurin-independent pathway. The first phase 2 *de novo* renal transplant study evaluated sotrastaurin+tacrolimus.
Methods: Recipients were randomized to sotrastaurin 200mg bid with standard (sotrastaurin-1,n=76) or reduced exposure of tacrolimus (sotrastaurin-2,n=66) for 3 months and subsequently switched to sotrastaurin 200mg bid/mycophenolic acid (MPA 720mg bid) till Month 12. Control group (control,n=74) received MPA with standard exposure tacrolimus. All recipients received basiliximab and steroids. The composite efficacy endpoint (BPAR, graft loss, death or loss to follow-up) and safety endpoint (GFR, MDRD) were calculated at Month 6.
Results: At Month 3, all arms showed equivalent efficacy failure with Kaplan-Meier estimates of 4%, 5% and 2% for control, sotrastaurin-1 and sotrastaurin-2, respectively. After conversion to sotrastaurin+MPA, the efficacy failure rates were 8%, 45%, and 34% for control, sotrastaurin-1 and sotrastaurin-2, and the study was prematurely terminated. GFR at Month 3 was: 57, 53, and 57mL/min; and at Month 6: 54, 57, and 64 mL/min for control, sotrastaurin-1 and sotrastaurin-2 (NS). Overall safety appeared comparable with study drug discontinuations due to adverse events (AE) in 16%, 18% and 12% for control, sotrastaurin-1 and sotrastaurin-2 respectively. Reported infections were 70%, 71% and 71%, including CMV in 4%, 5% and 12% for control, sotrastaurin-1 and sotrastaurin-2. The overall incidence of gastro-intestinal AEs was similar in the study arms (76-82%) with serious AEs in 10%, 8% and 3%. During the first 3 months, sotrastaurin recipients had a lower incidence of neutropenia (<1300/L): 3% sotrastaurin-1, 4% sotrastaurin-2 versus 11% control.
Conclusion: The first study of sotrastaurin in renal transplant recipients showed excellent efficacy of the sotrastaurin+tacrolimus regimen, unlike the post-conversion sotrastaurin+MPA regimen. Both sotrastaurin regimens were well tolerated. Further studies will determine the optimal regimen to benefit from sotrastaurin's novel mode of action.

Session 6. Cell therapies for regeneration and immunomodulation

O-31 EVIDENCE OF ISLET SURVIVAL AND REVASCULARIZATION FOLLOWING INTRAMUSCULAR AUTOTRANSPLANTATION IN THE MINIPIG

Adrien Sterkers, Thomas Hubert, Robert Caizzo, Valery Gmyr, François Pattou. *U859, Lille University Hospital, Lille, France*

Background: The intrahepatic environment does not seem favourable for long term islet survival and reduces alpha cell response to hypoglycemia. Initiated long ago in rodents and recently proposed for clinical transplantation, intra muscular islet transplantation (IMIT) offers attractive prospects for its simplicity and an easier access to the graft for non invasive imaging techniques and/or cell explantation. In this study we aimed to obtain unequivocal proof of islet survival and revascularization after IMIT in a relevant pre-clinical model.
Methods: Islets were isolated from adult minipigs (n=13, 20-40 kg) with standard automated technique following distal pancreatectomy. Standardized autologous islet grafts (unpurified/n=16 or purified/n=42) were implanted in the thigh gracile muscle (direct surgical acesss/n=14 or intramuscular injection/ n=44). Transplanted sites were explanted at 15 and 30 days after IMIT and analyzed by immumochemistry for cell composition (insulin and glucagon staining), revascularization (vWF staining) and hypoxia (pimonidazol accumulation). We studied the influence of graft technique and purity of the preparation islet survival (semi-quantitative score).
Results: Immunostaining confirmed the presence of alpha and beta cells 15 and 30 days after the grafts.

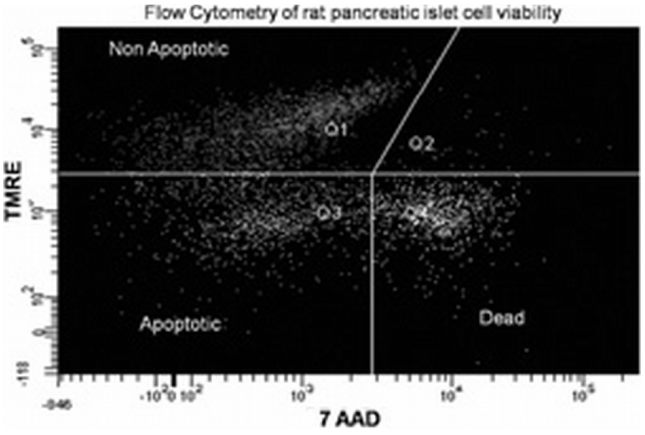


Injection route transplantation and purification significantly influenced islet survival ($p<0.001$ and $p<0.01$ respectively). Intramuscular injection of purified islets allowed the survival of large intact islets at 30 days in 7/9 cases. Islet revascularisation increased at day 30 days and was correlated to islet survival. Small beta cell clusters showed little sign of revascularisation and increased accumulation of hypoxia marker.
Conclusion: We confirmed the engraftment and revascularization of intact islets expressing both insulin and glucagon for a least one month in the minipig following intramuscular injection of purified islet preparations. When long term islet survival and function after IMIT is documented, this simple technique could be tested in clinical trials.

O-32 INCREASED PANCREATIC ISLET CELL VIABILITY AND INSULIN SECRETION AFTER ENZYMATIC DIGESTION USING EDTA AS A COLLAGENASE INHIBITOR

Soroush Sohrabi[1], Susan Stamp[2], Bob Peaston[3], Noel Carter[1], David Talbot[3]. *[1]Immunology, Sunderland University, Sunderland, United Kingdom; [2]Surgery, Newcastle University, Newcastle, United Kingdom; [3]Transplant Surgery, Freeman Hospital, Newcastle, United Kingdom*

Introduction: Although pancreatic islet cell transplantation has promising results in short term as a novel method for treatment of diabetes, after 5 years only 10% of initial successful transplant recipients remaining insulin independent after five years. It has been suggested that the current islet isolation techniques are not islet friendly and cause damage to the islets that is not entirely reversible. These pre-damaged islets when transplanted in the recipient undergo immunological and non-immunological destruction leading to graft failure.
Material and methods: Cultured rat beta cells were exposed to collagenase and washed either with EDTA+ Human Albumin (HA) and HA alone followed by washing with cold HBSS.Islet insulin secretion was assessed using ELISA. Beta cell apoptosis and death was analysed using a novel method of flow cytometry and TMRE (Tetramethylrhodamine Ethyl Ester) and 7-AAD (7-aminoactinomycin D) staining.
Results: Viable islet count (Non-apoptotic live islets) was significantly higher in the group of islets which were treated with EDTA + HA compared to HA alone



($p<0.05$). Insulin secretion in islets washed with EDTA+HA was significantly higher than islets washed with HA alone ($p<0.05$).
Discussion: Adding EDTA as a collagenase inhibitor to human albumin after digestion with collagenase increases islet cell viability and insulin secretion.

O-33 NEURAL PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS ARE TARGETED BY ALLOGENEIC T AND NATURAL KILLER CELLS

Casimir de Rham, Olivier Preynat-Seauve, Sylvie Ferrari-Lacraz, Diderik Tirefort, Karlheinz Krause, Jean Villard. *Immunology and Transplant Unit, Geneva University Hospital and Medical School, Geneva, Switzerland; Laboratory of Experimental Cell Therapy, Geneva University Hospital and Medical School, Geneva, Switzerland; Immunology and Transplant Unit, Geneva University Hospital and Medical School, Geneva, Switzerland; Laboratory of Experimental Cell Therapy, Geneva University Hospital and Medical School, Geneva, Switzerland; Laboratory of Experimental Cell Therapy, Geneva University Hospital and Medical School, Geneva, Switzerland; Immunology and Transplant Unit, Geneva University Hospital and Medical School, Geneva, Switzerland*

Purpose: Neural progenitor cells (NPC) of fetal origin or derived from human embryonic stem cells (HESC) have the potential to differentiate into mature neurons after transplantation into the central nervous system (CNS), opening the possibility of cell therapy for neurodegenerative disorders. In most cases, the transplanted NPC are genetically unrelated to the recipient, leading to potential rejection of the transplanted cells. Very few data provide reliable information as to the potential immune response of allogeneic neural progenitors derived from HESC.
Methods: We analyzed *in vitro* the allogeneic immune response of T lymphocytes by MLR and of natural killer (NK) cells by killing assay and cytokine production to NPC derived from HESC. Similar experiments were performed in presence of the immunosuppressive drugs ciclosporine/CSA and dexamethasone (Dexa).
Results: NPC stimulated by gamma-interferon induce T cell stimulation and also strong NK cytotoxic response. NK cell activity is unrelated to MHC-I expression but driven by the activating NKG2D receptor. Cyclosporine and dexamethasone previously used in clinical studies with fetal NPC did not only fail to prevent NK alloreactivity, but strongly inhibited the terminal maturation from NPC into mature neurons.
Conclusion: Allogenic transplantation of NPC in the CNS will most likely require an immunosuppressive regimen targeting allogenic T and NK cells while possible interference with the differentiation of NPC needs to be carefully evaluated.

O-34 PROLONGATION OF COMPOSITE TISSUE ALLOGRAFT SURVIVAL USING ALTERNATIVE ROUTES OF BONE MARROW TRANSPLANTATION

Arkadiusz Jundzill[1,2], Aleksandra Klimczak[1], Erhan Sonmez[1], Grzegorz Brzezicki[1], James Gatherwright[1], Maria Siemionow[1]. *[1]Department of Plastic Surgery, Cleveland Clinic, Cleveland, OH, USA; [2]Department of Vascular and General Surgery, Tissue Engineering Department, Collegium Medicum, Bydgoszcz, Poland*

Background: Allogenic bone marrow transplantation (BMT) is an option for the induction of donor specific chimerism and prevention of graft rejection. In vascularized skin allograft (VSA) model we compared the tolerognic effects of different routes of BMT under a immunosuppressive therapy.
Methods: Twenty one fully MHC mismatched VSA transplants were performed between ACI ($RT1^a$) donors and Lewis ($RT1^l$) recipients in 3 groups under

a 7-day protocol of alfa/beta TCRmAb/CsA. VSA were supported with donor BMT: Group-1: Control, without cell supportive therapy. Group-2:Intragonadal BMT (100×10^6 cells). Group-3:Intracapsular BMT (100×10^6 cells). Before BMT were stained with PKH-26 dye to evaluate migration and engraftment into lymphoid and non-lymphoid organs of recipients. In Group-2 BMT were into right testis between tunica albuginea and seminiferous tubules. In Group-3 BMT were under right kidney capsule. We performed: clinical skin examination for signs of grade of rejection and immunohistochemistry for donor cells engraftment into host compartments. Donor-specific chimerism for MHC class I ($RT1^a$) antigens and presence of regulatory T-cells CD4+/CD25+ were assessed in the peripheral blood of recipients.
Results: The controls, without cellular therapy, rejected between 33 to 52 days post transplantation. Allografts in Group-2 and Group-3 rejected between 35-68, and 35-66 days respectively. The level of T-cell-($RT1^a$/CD4, $RT1^a$/CD8), B-cell-($RT1^a$/CD45RA) and monocyte/granulocyte-($RT1^a/CD11_{b/c}$) donor chimerism was similar after intarcapsular and intragonadal BMT. The level of Regulatory T cells in the peripheral blood was higher in intragonadal group compared to intacapsular group at each time point post-transplant.
Conclusion: BMT significantly delayed the rejection in fully mismatched VSA and resulted in induction of robust donor specific chimerism. Increased level of regulatory T-cells was observed after intragonadal BMT and this was associated with longer VSA survival.

O-35 EFFECT OF CHIMERISM INDUCTION AFTER BONE MARROW TRANSPLANTATION INTO ALTERNATIVE ANATOMICAL COMPARTMENTS

Arkadiusz Jundzill[1,2], Erhan Sonmez[1], Aleksandra Klimczak[1], Grzegorz Brzezicki[1], James Gatherwright[1], Maria Siemionow[1]. *[1]Department of Plastic Surgery, Cleveland Clinic, Cleveland, OH, USA; [2]Department of Vascular and General Surgery, Tissue Engineering Department, Collegium Medicum, Bydgoszcz, Poland*

Purpose: Improved allograft survival following transplantation with the use of donor derived stem cells has been well documented. However the efficacy of bone marrow cell transplantation (BMCT), engrafment and connection between graft survival, route of delivery and chimerism is not well established. This study was designed to evaluate the efficacy of alternative routes of BMCT on the development of donor specific chimerism across MHC barrier under 7-day ab-TCR/CsA protocol.
Methods: 36 BMCT (100×10^6) were performed between ACI ($RT1^a$) donors and Lewis ($RT1^l$) recipients. Three alternative routes of BMCT were studied in six groups (n=6 per group) as follow: Intra-testicular (Groups 1 and 2), renal intra-capsular (Groups 3 and 4), intra-thecal (Groups 5 and 6). Groups 2, 4 and 6 served as controls and received BMCT without immunosuppression, whereas groups 1,3,5 received both BMC and 7-day of ab-TCR/CsA protocol. Immunodepletion and donor specific chimerism for MHC class I for T-cells ($RT1^a$/CD4, $RT1^a$/CD8) B-cells-($RT1^a$/CD45RA), monocyte/granulocyte-($RT1^a/CD11_{b/c}$) and Regulatory T cells (CD4+/CD25+) were assessed using flow cytometry.
Results: All animals survived without graft-versus-host disease up to 100 days and they are still under observation. In groups 1, 3 and 5 at 100 day post-transplant, chimerism was predominated by T-cells and monocyte/granulocyte populations.

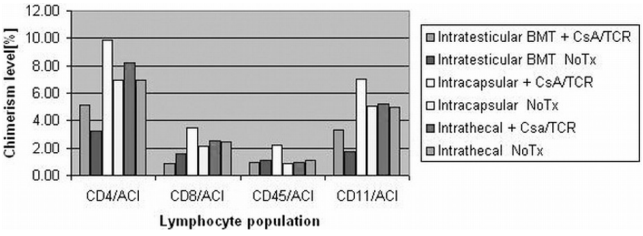


Figure 1. Donor-origin cells in the peripheral blood at 100 days post BMCT.

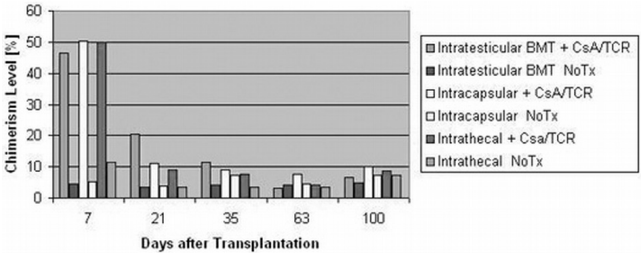


Figure 2. Total chimerism in peripheral blood.

At day 100 post-transplant, chimerism declined in all transplanted groups. The highest level of chimerism was maintained in ab-TCR/CsA treatment group after intra-capsular BMCT.
The level of Regulatory T-cells in the peripheral blood after 100 days was higher in intra-thecal group without treatment compared to both intra-capsular and intra-testicular groups.
Conclusion: Alternative routes of bone marrow delivery into different immunoprivileged compartments: intra-capsular, intra-thecal and intra-testicular to induce chimerism are encouraging and may provide a putative therapy for enhancing composite tissue allograft survival.

O-36 HUMAN MESENCHYMAL STEM CELLS STIMULATE T-CELL PROLIFERATION AND GENERATE REGULATORY LYMPHOCYTES THAT INHIBIT ALLOREACTIVITY

Meindert J. Crop, Carla C. Baan, Sander S. Korevaar, Willem Weimar, Martin J. Hoogduijn. *Internal Medicine-Transplantation, Erasmus University Medical Center, Rotterdam, Netherlands*

Purpose: Mesenchymal stem cells (MSC) have immunomodulatory properties and inhibit the proliferation of allo-activated lymphocytes. The underlying mechanisms are unclear, but partly depend on the secretion of anti-inflammatory factors, e.g. IL-10, IDO and PGE2, which are induced under inflammatory conditions. However, MSC also produce pro-inflammatory cytokines and it is unknown how these factors affect immune cells. This study examined the effects of MSC on PBMC under non-inflammatory conditions.
Methods/Materials: Human peripheral-blood-mononuclear-cells (PBMC) were cultured in direct-contact or transwell (TW)-systems with or without autologous or allogeneic human MSC. After 7 days, activation and proliferation of PBMC was measured by ^{3}H-thymidine incorporation and flow cytometry. Gene expression by MSC was analysed by real-time RT-PCR. After further culturing of the cells in the absence of MSC, PBMC were phenotyped by flow cytometry and RT-PCR, and functionality examined in mixed-lymphocyte-reactions (MLR).
Results: Seven days of co-culture with autologous or allogeneic MSC increased the proliferation of PBMC 5.4-fold ($p<0.0001$), both in direct and TW-systems. PCR-analyses showed increased expression of IL-6, IL-8, TNFα, bFGF and VEGF by MSC when co-cultured with PBMC. After further culture in the absence of MSC, CD4+ T-cells and to a lesser extent CD8+ T-cells showed accelerated proliferation compared to cells that had not been cultured with MSC with a maximum at day 7. The cells highly expressed CD25 and FoxP3. Upon stimulation with alloantigen, the generated cells were hyporesponsive. Strikingly, these cells inhibited proliferation in MLR, whereas PBMC pre-cultured without MSC did not.
Conclusion: Autologous and allogeneic MSC induce expansion of PBMC under non-inflammatory conditions. These PBMC demonstrated immunosuppressive capacity. MSC can thus transfer immunosuppressive functions either via the expansion of the regulatory lymphocyte compartment or via the generation of new regulatory cells. This suggests that MSC have short-term and long-term immunosuppressive effects.

Session 7. Liver transplantation & tumors

O-39 TRANSARTERIAL CHEMOEMBOLISATION BEFORE LIVER TRANSPLANTATION: WHO DROPS OUT?

Gerd Otto[1], Maria Hoppe-Lotichius[1], Dagmar Laubert-Reh[1], Anja Lautem[1], Fernandes Bittinger[2], Michael Pitton[3]. *[1]Transplantation and Hepatobiliopancreatic Surgery, University of Mainz, Mainz, Germany; [2]Insitute of Pathology, University of Mainz, Mainz, Germany; [3]Diagnostic and Interventional Radiology, University of Mainz, Mainz, Germany*

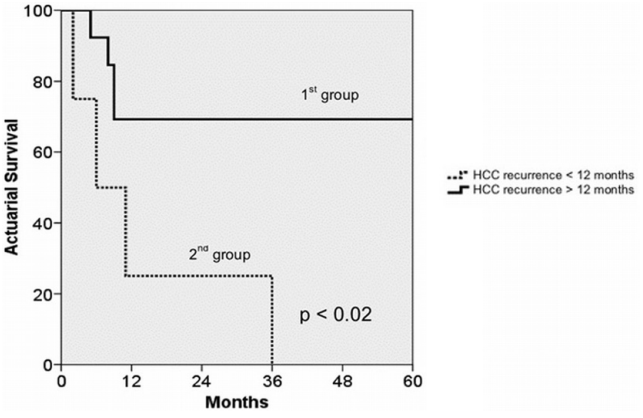
Background: Waiting for liver transplantation (LT) in patients with hepatocellular carcinoma (HCC) results in a considerable drop-out rate. In order to bridge the waiting time we perform transarterial chemoembolisation.
Patients and methods: One-hundred-seventy-six patients were included in a pretreatment protocol consisting of repeatedly performed TACE using Lipiodol and Mitomycin. LT was restricted to patients responding to this pre-treatment. Tumor characteristics, such as size and number of nodules, grading, AFP, VEGF and angiopoietin 2, were analysed with particular focus on response to TACE.
Results: Of 176 patients, 100 underwent LT after responding to TACE. Five-year survival (Kaplan Meier) calculated from the first TACE was 0 and 69% for patients without versus with consecutive LT (p=0.0001). Drop-out during waiting time reflecting tumor progress could not be predicted by size (p=0.176), number (p=0.185) and grading (p= 0.144) of tumor nodules. AFP was also incapable of predicting drop-out (p=0.232). Tumor progress correlated with elevation of angiopoietin 2 levels before initiation of TACE ($p < 0.01$) and with elevated VEGF levels during TACE ($p < 0.001$) as measured in serum.

Conclusion: Pathomorphological features of HCC are unreliable in predicting tumor response during TACE pre-treatment. Serum VEGF and angiopoietin 2 reflecting biological properties of HCC may help to identify patients amenable to LT.

O-40 OPTIMIZATION OF LIVER TRANSPLANTATION FOR THE TREATMENT OF LIVER HEPATOCELLULAR CARCINOMA RECURRENCE. EXPERIENCE OF A SINGLE EUROPEAN CENTER

Gonzalo Sapisochin[1], Bilbao Itxarone[1], Joaquin Balsells[1], Cristina Dopazo[1], Mireia Caralt[1], Laia Blanco[1], Amaia Gantxegi[1], Luis Castells[2], Jose Luis Lázaro[1], Hellena Allende[3], Ramón Charco[1]. *[1]HBP Surgery & Transplant Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain; [2]Hepatology Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain; [3]Pathology Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain*

Introduction: Liver transplantation outcome in patients transplanted for HCC who have undergone partial LR for a previous tumor is controversial.
Objective: To ascertain the outcome of patients with liver transplantation (LT) due to hepatocelullar carcinoma (HCC) who had undergone a partial LR for a previous tumor, and determine whether a group of patients exists in whom LT would be contraindicated after LR.
Material and methods: A case-control study (1:2) was designed to compare patients who underwent LT due to HCC recurrence with a previous LR for HCC (study group) with those who underwent LT for primary HCC,without previous LR (control group).
Results: From January 1990-December 2007,303 cirrhotic patients with primary HCC were evaluated for surgery. Primary LT was performed in 191 and LR in 100. Of these,33 were classified as non-transplantable and 67 as transplantable. When HCC recurrence was diagnosed after LR,17 underwent LT (study group). Median follow-up: 70 months (r: 12.7-203).
Disease-free survival at 1, 3 and 5 years in the study group vs control group was 86%, 68%, 58% vs 97%, 93%, 89%, respectively ($p<0.04$). One-, 3- and 5-year actuarial patient survival in the study group vs control group was 59%, 52%, 52% vs 85%, 76%, 65%, respectively, p=ns. Patients of the study group were divided into two groups according to time to recurrence after LR: group 1: before one year and group 2: after one year. Recurrence after LT was 75% in group 1 vs 15.4% in group 2 ($p<0.03$). One-, 3- and 5-year actuarial patient survival was 25%, 0%, 0% in group 1 and 69%, 69%, 69% in group 2, $p<0.02$).



Conclusions: LT can be safely performed after a previous LR for HCC. The fact that patients with recurrence during the first year post-hepatectomy have such poor prognosis postransplant should induce us to consider whether, given the shortage of donors, these patients should or should not be transplanted.

O-41 PREDICTING RECURRENCE AFTER LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA EXCEEDING THE UP-TO-SEVEN CRITERIA

Francesco D'Amico[1], Myron Schwartz[2], Alessandro Vitale[3], Sasan Roayaie[2], Swan Thung[2], Maria Guido[4], Juan del Rio Martin[2], Thomas Schiano[2], Umberto Cillo[1]. *[1]Azienda-Università di Padova, Unità di Chirurgia Epatobiliare e Trapianto Epatico, Padova, Italy; [2]Mount Sinai School of Medicine, Recanati-Miller Transplantation Institute, New York, USA; [3]Istituto Oncologico Veneto, IRCCS, Unità di Chirurgia Oncologica, Padova, Italy; [4]Università di Padova, Anatomia Patologica, Padova, Italy*

Background/Aim: The up-to-seven (Up-to-7) criteria (with seven being the result of the sum of size and number of tumours for any given HCC) have been recently proposed to identify potential candidates for liver transplantation (LT) among patients exceeding the Milan criteria. The aim of this study is to compare the ability in predicting recurrence of available pathologic staging systems (Milan vs. UCSF, vs. Up-to-7).
Methods: A study population of 479 HCC transplanted patients was identified from prospectively-collected databases at Mount Sinai Medical Center and the University of Padua. The best pathologic staging system was identified by using the Log-rank, the proportion of separation, and Cox analyses. Pathologic tumor characteristics (tumors number, size, sum of diameters, macroscopic and microscopic vascular invasion, grading) were then tested by uni- and multivariate Cox analyses in the prognostic subgroups within and beyond the calculated criteria.
Results: Up-to-7 criteria performed as the best pathologic staging system, being the calculated 1, 3, 5-year recurrence probabilities 4%, 8%, 14% within (n = 355) and 22%, 45%, 51% beyond (n = 124) the criteria ($p<.0001$), and the calculate PSEP = 0.27 (95% CI= 0.23-0.31).
At multivariate analysis, only biological variables (vascular invasion and tumor grade) significantly predicted recurrence beyond Up-to-7 criteria. A 3-stage pathologic staging system with a potential to be applied in the preoperative setting was thus created: within Up-to-7 (recurrence rate = 8%); beyond Up-to-7 without macrovascular invasion and poorly differentiated grade (recurrence rate = 24%); beyond Up-to-7 with macrovascular invasion and/or poorly differentiated grade (recurrence rate = 45%).
Conclusions: HCC patients within pathologic Up-to-7 criteria were associated to a low risk of recurrence after LT. Beyond these criteria, however, a significant proportion of patients with a good HCC biological profile had an acceptable risk of recurrence.

O-42 FROM MILAN-OUT TO MILAN-IN THROUGH AN AGGRESSIVE DOWNSTAGING PRIOR TO TRANSPLANTATION FOR HCC SEEMS TO BE AN EFFECTIVE STRATEGY

Alessandro Giacomoni, Andrea Lauterio, Abdallah Slim, Stefano Di Sandro, Paolo Aseni, Vincenzo Pirotta, Mohamed Al Kofahi, Luciano De Carlis. *Hepatobiliary Surgery and Liver Transplantation Unit, Niguarda Hospital, Milan, Italy*

Introduction: Transplanting patients with HCC outside the Milan criteria but treated with an aggressive downstaging able to bring back the tumor within them is still controversial.
Methods: From January 2000 to December 2007, 179 patients with HCC have been transplanted in our Unit. Among them 109 patients were "Milan in" while on waiting list and at time of transplantation (group-A). All of them but 16 underwent downstaging procedures such as liver resection, radiofrequency ablation (RF) and/or transarterial chemoembolization (TACE). Among the patients outside the Milan criteria, 12 became "Milan in" after the same kind of aggressive downstaging and were transplanted (group-B). Fifty eight out of 179 patients transplanted for HCC did not match any of these 2 groups and they are not considered in this analysis.
Results: With a median follow-up of 41.2 months the cumulative overall patients survival rate 3 years after transplantation is 82.8% for group-A and 91,7% for group-B (p-value=NS). Five years after transplantation the cumulative patients overall survival rate is 77.2% for group-A and 91,7% for group-B (p-value=NS). As far as hystologic grading on the explanted livers is concerned in group A we found: 19 (17,4%) G3, 49 (44.9%) G2, 16 (14,6%) G1, 15 (13.7%) complete necrosis; in group B: 4 (33.3%) G3, 6 (50%) G2, 0 (0%) G1, 2 (16,6%) complete necrosis. In group-A 10 histological grading are not available.
Conclusions: An aggressive tumor downstaging throughout liver resection, RF and/or TACE able to bring back within Milan criteria patients with HCC out of them prior to liver transplantation is an effective strategy. It offers the same long term survival rate showed by transplanted patients for HCC that have always been inside the Milan criteria while on waiting list for transplantation.

O-43 LIVER RESECTION VERSUS LIVER TRANSPLANTATION AS TREATMENT OF EARLY HEPATOCELLULAR CARCINOMA: A CASE-CONTROL STUDY

Roberto Montalti[1], Valentina Serra[1], Gianluca Rompianesi[1], Fabrizio Di Benedetto[1], Nicola De Ruvo[1], Nicola Cautero[1], Roberto Ballarin[1], Rosa M. Iemmolo[1], Stefano Busani[2], Gian Piero Guerrini[1], Giorgio E. Gerunda. *[1]Liver and Multivisceral Transplant Centre, Azienda Ospedaliero-Universitaria di Modena Policlinico, Modena, Italy; [2]Departement of Anesthesiology, Azienda Ospedaliero-Universitaria di Modena Policlinico, Modena, Italy*

Background and aim: The choice of the best surgical strategy for early hepatocellular carcinoma (HCC) on well-compensated cirrhosis is still controversial. We designed a retrospective, 1:1 ratio case-controlled study between patients affected by HCC on well compensated cirrhosis undergoing liver transplantation (LT) vs liver resection (LR). Aim of the study is to evaluate morbidity, mortality, patient survival and disease-free survival (DSF) of the two considered groups.

Materials and methods: Since 10-2000 to 01-2008, 129 LT and 105 LR were performed for histological confirmed HCC. Thirty-five LT recipients (Transplant Group, TG), without a previous LR, were paired with 35 patients that underwent a liver resection (Resection Group, RG), not afterwards been transplanted, matched by: MELD $\leq$15, HCC fulfilling Milan criteria, gender, age, surgical year and liver disease etiology (HBV/HCV/alcoholic). DFS events were defined as HCC recurrence or end-stage liver failure due to primary indication recurrence.
Results: Average follow-up was 52 months. The overall patient survival at 1, 3, 5 yrs was, respectively, 82.4%, 71.7% and 71.7% in TG vs 94.3%, 65.6% and 22.0% in RG (p=0,014). The DFS at the same time points was 96.7%, 83.9% and 83.9% in TG vs 65.7%, 32.4% and 9.2% in RG (p=0,000). Morbidity in terms of: infections, renal impairment, and also the complications occurring exclusively following LT, resulted statistically higher in TG. HCC recurrence occurred in 5,7% of patients of TG vs 51,4% of patients of RG. In RG the 61% of patients with HCC recurrence died because of the recurrence.
Discussion: In patients with HCC within Milan criteria on well compensated cirrhosis, LT and LR during early and middle term follow-up allow to obtain similar results in terms of patient survival and DFS, whereas in the long term the advantage of LT is manifest.

O-44 ESTIMATION OF THE HARM TO THE WAITING LIST AS CRUCIAL FACTOR IN THE SELECTION OF PATIENTS WITH HEPATOCELLULAR CARCINOMA FOR LIVER TRANSPLANTATION

Alessandro Vitale[1], Martina Gambato[2], Michael Volk[3], Alberto Brolese[4], Giacomo Zanus[4], Francesco D'Amico[4], Daniele Neri[4], Marco Senzolo[2], Francesco Russo[2], Umberto Cillo[4]. *[1]Istituto Oncologico Veneto, IRCCS, Unità di Chirurgia Oncologica, Padova, Italy; [2]Azienda-Università di Padova, Divisione di Gastroenterologia, Padova, Italy; [3]University of Michigan Health System, Division of Gastroenterology, Ann Arbor, MI, USA; [4]Azienda-Università di Padova, Unità di Chirurgia Epatobiliare e Trapianto Epatico, Padova, Italy*

Background: Long-term survival rates after liver transplantation (LT) for patients with hepatocellular carcinoma (HCC) may be calculated using the Metroticket website calculator (http://89.96.76.14/metroticket/calculator/). There are no studies, however, evaluating the post-LT survival threshold that would justify the selection of a patient with HCC for LT.
Methods: We hypothesized that a patient with HCC should receive a LT if his transplant benefit is greater than the cumulative harm to the rest of the waiting list (WL). We created, therefore, a Markov model in collaboration with the University of Michigan. The data sources to construct and validate the model were: the online UNOS web – site, and the University of Padua prospective database on a new LT allocation model.
Results: Although our Centre was characterized by a higher proportion of HCC patients in the WL (25% versus 10%) and a lower proportion of high MELD score ($>$ 20) non-HCC patients (17% versus 27%) than the average US centre, these proportion were similar among transplanted patients. The calculated harm to the WL was 434 quality-adjusted days of life in Padua, and 957 in US ($p<.01$). In a clinical scenario of a patient with HCC having a poor survival perspective without LT (5-year survival = 10%), the LT benefit outweighed the harm to the WL when 5-year post-LT survival was higher than 30% in Padua, and 61% in US.
Conclusions: In a decision model including the concepts of transplant benefit and harm to the WL, the 5-year post-LT survival threshold to achieve for a patient with HCC is strongly related to local WL characteristics and priority-allocation criteria.

O-45 SALVAGE TRANSPLANTATION AFTER LIVER RESECTION FOR HEPATOCELLULAR CARCINOMA WITH CIRRHOSIS (HCC-CIR)

Rene Adam, Eric Vibert, Daniel Azoulay, Jean-Charles Duclos-Vallee, Faouzi Saliba, Didier Samuel, Henri Bismuth, Denis Castaing. *Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France*

Liver Resection (LR) is considered as a reasonable first-line treatment of patients with small HCC-Cir and good liver function, with salvage LT as a perspective in case of recurrence. Among 619 consecutive patients with HCC+Cir treated by LR (n=267; 115 of whom were transplantable) or by LT (n=352) from 1984 to 2008, the feasibility and outcome of secondary LT was evaluated in a 2-step fashion. First, secondary LT for tumor recurrence after LR (n=27) was compared with primary LT (n=352), Second, primary LR in transplantable patients (n=115) was compared with that of primary LT (n=352) on an intention-to-treat basis. Transplantability of resected patients was retrospectively determined according to selection criteria of LT for HCC.
Intraoperative bleeding of secondary LT was higher than that of primary LT (mean transfused blood units, 14.1 versus 9.3; $P<0.05$). Tumor recurrence occurred more frequently after secondary LT (33% versus 16%; P=0.05). Post-transplant 5-year survival was 47% versus 68% (P=0.03), and disease-free survival (DFS) was 41% versus 65% (P=0.002), respectively. Of 115 patients treated by LR while initially eligible for LT, 30 (25.6%) were transplanted. Compared with primarily LT, transplantable resected patients had a decreased 5-year overall survival (59% versus 68%; $P<0.0001$) and DFS (19% versus 65%; $P<0.0001$). At multivariate analysis, LR with salvage LT (P=0.02; RR=1.91) emerged as a negative independent factor of DFS as compared with primary LT. A number of nodules $>$ 3 ($P<0.001$; RR=2.03) and a maximum tumor size exceeding 30 mm (P=0.002; RR=1.71) were also predictive of lower DFS.
In conclusion, salvage LT after liver resection is associated with a higher risk of recurrence than primary LT. Primary LT remains the ideal choice even when the tumor is resectable, to the condition of organ availability.

O-46 PROTEOMIC ANALYSIS OF NUCLEAR ENRICHED FRACTIONS FROM HUMAN HEPATOCELLULAR CARCINOMA TISSUES

Umberto Baccarani, Erika Codarin, Claudio Avellini, Lory Crocè, Claudio Tiribelli, Dario Lorenzin, Gian Luigi Adani, Franco Quadrifoglio, Andrea Scaloni, Gianluca Tell. *Surgery & Transplantation, University, Udine, Italy*

To discover new potential biomarkers of HCC we used two-dimensional (2-DE) gel electrophoresis separation and MALDI-TOF-MS analysis of nuclear-enriched liver biopsies from 20 different patients. We obtained a proteome map of the nuclear enriched liver samples including 83 proteins which correspond to 52 different species. A differential analysis of proteins from tumoral and control tissues revealed a significant change in the expression level of 18 proteins associated to cytoskeleton, stress response and metabolism. The identified proteins were classified into two groups according to their association to HCC. The first contained proteins already described as deregulated in HCC (Hsc71, Hsp70, Hsp60, a-enolase, cathepsin D, 3-ketoacyl-CoA thiolase, ATP synthase a, fructose-bisphosphate aldolase B and peptidyl-prolyl cis-trans isomerase A) while the second included proteins that have never been associated to this type of cancer (electrontransfer flavoprotein b, hnRNP A2/B1, UTP-glucose-1-phosphate uridyltransferase 2A, hCG2001950 scaffold protein, hydroxymethylglutaryl-CoA synthase, actin-related protein 2 and methylmalonate-semialdehyde dehydrogenase), thus representing possible novel biomarkers. The majority (9/18) of the changes observed consisted in a decreased protein expression level in tumoral tissue. A reduced abundance was observed for cathepsin D, ATP synthase a, electron transfer flavoprotein b, fructose-bisphosphate aldolase B, 3-ketoacyl-CoA thiolase, UTP-glucose-1-phosphate uridyltransferase 2A, hCG2001950 scaffold protein and methylmalonate-semialdehyde dehydrogenase. All the other proteins (8/18) were characterized by an increased abundance in the tumoral tissue. To check for the reliability of the quantitative data obtained, five differentially expressed proteins found by 2-DE (Hsc71, Hsp60, Hsp70, hnRNP A2/B1 and ATP synthase a) were also validated by Western blotting analysis obtaining data in agreement with 2-DE. The data may provide useful insights for understanding the mechanisms of HCC pathogenesis and progression as well as provide new candidate biomarkers for HCC.

O-47 INCREASED RISK OF EARLY *DE NOVO* CANCER IN LIVER GRAFT RECIPIENTS TRANSPLANTED IN RECENT YEARS

Angela S.W. Tjon[1], Jerome Sint Nicolaas[1], Jaap Kwekkeboom[1], Hugo W. Tilanus[2], Geert Kazemier[2], Rob A. de Man[1], Luc J.W. van der Laan[2], Bettina E. Hansen[1], Thanyalak Tha-In[1,2], Herold J. Metselaar[1]. *[1]Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Surgery, Erasmus University Medical Center, Rotterdam, Netherlands*

Patients after liver transplantation (LTx) are at higher risk for *de novo* malignancy. Whether changes in the immunosuppressive regimens over the years have influenced the incidence of post-transplant cancer is unknown.
We retrospectively reviewed 393 LTx-patients who were transplanted between 1986 and 2007 with at least 3 months follow-up post-LTx. Observed cancer risk was compared with the expected cancer risk in the Dutch population matched for age and gender. Forty-nine (12.5%) LTx-patients developed *de novo* malignancy. The cumulative incidence of *de novo* cancer at 1, 5, 10 and 15 years post-LTx was 1.4%, 6.8%, 18.5% and 44.6%, respectively. The standardized incidence risk (SIR) of malignancy in LTx-patients compared to the general population was 2.7 (95% CI: 2.0-3.5). The most common cancer types were skin cancer (49%) and post-transplant lymphoproliferative disorder (21%). Survival rate was lower in *de novo* cancer recipients as compared to cancer-free recipients ($P < 0.001$). Twelve of the 49 patients (25%) died due to *de novo* cancer. Strikingly, patients transplanted in the period 2002-2006 had 4.9-times (95% CI: 1.4-17.1) higher risk to develop early cancer as compared to the period 1997-2001 adjusted for age, gender and LTx indication ($P = 0.013$). We found no differences in rejection episodes between both time periods. However, the use of cyclosporine based on C2 monitoring was associated with a borderline significant higher risk ($P = 0.07$).
In this 21-year follow-up study we showed a high risk of *de novo* cancer after LTx. Interestingly, patients transplanted in more recent years experienced

a 4.9-times higher risk to develop early cancer as compared to earlier time period. This raises the question whether recent changes in immunosuppressive regimen may have caused over-immune suppression and attributed to an increased risk for cancer.

Session 8. Novel pathways of recipient sensitization

O-48 TARGETING THE NK CELL CYTOTOXICITY RECEPTOR NKp46 DELAYS ACUTE GRAFT REJECTION IN A MURINE MODEL OF HEART TRANSPLANTATION

Cam-Tien Le, Katrin Klippert, Diana Stauch, Katja Kotsch. *Institute of Medical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany*

Although it has been shown that Natural Killer (NK) cells promote the rejection of solid organ allografts, little is known about the involvement of their receptors in acute rejection (aRx). In order to assess the role of the NK cell specific cytotoxicity receptor NKp46 (Ncr1) in mediating aRx, we used Ncr1 knockout (Ncr ko) mice on a C57BL/6 background where a GFP cassette was knocked into the Ncr1 locus ($^{GFP/GFP}$), thereby disrupting Ncr1 functionality. In an established experimental model of acute rejection BALB/c hearts were transplanted into Ncr1$^{GFP/GFP}$ mice, whereby BALB/c to C57BL/6 transplantations served as controls. Graft survival revealed a significant delay of aRx in the Ncr1 deficient mice in comparison to the wildtype (p=0.034). Furthermore, in contrast to wildtype animals we identified lower numbers of graft infiltrating NK cells by targeting the GFP signal in the BALB/c donor hearts in the Ncr1 ko by real-time-PCR. Although both strains possess similar numbers of various lymphocyte subsets in the naive state, Ncr1$^{GFP/GFP}$ recipients showed significant higher levels of splenic CD4$^+$CD25$^+$FoxP3$^+$ T regulatory (Treg) cell numbers compared to C57BL/6 mice at the day of rejection. Among the T cell population cytotoxic CD3$^+$CD8$^+$ cells and CD3$^+$ T expressing MHC class II were upregulated in Ncr1 ko spleens guided by enhanced IFNg expression. NK cell subsets including NK1.1$^+$ NKG2D$^+$ cells were less expressed compared to wildtype littermates. Our studies demonstrate the involvement of Ncr1 in aRx of solid organs but also indicate that its deletion is not sufficient for preventing mice from aRx. An Ncr1 ko results in an induction of various T cell subsets, suggesting that in the absence of activating NK cell receptors the rejection process is mainly mediated by T cell activation.

O-49 THE FATE AND FUNCTIONS OF NK CELL SUBSETS ARE DIFFERENTIALLY REGULATED BY T-Bet, Eomes, AND IL-15

Alexander H.K. Kroemer, Xiang Xiao, Karoline Edtinger, Gulcin Demirci, Xian C. Li. *The Transplant Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*

We have previously shown that IL-15 activated NK cells mediate acute allograft rejection in the absence of adaptive immunity. As NK cells are functionally diverse, we hypothesized that different NK subsets may respond differentially to IL-15, based on their regulation by distinct transcription factors.
In mice, NK cells (NK1.1+CD3-) can be divided into two major subsets based on differential expression of CD27: CD27+ and CD27- NK cells. We used a polychromatic approach to study the biology of these subsets in vivo. To stimulate NK cells with IL-15 in vivo, we treated host mice with IL-15 precomplexed with IL-15Receptor-α (IL-15:Rα), which has been shown to increase half-life and effectiveness.
We observed that treatment of B6 mice with IL-15:Rα induced vigorous proliferation of NK cells as determined by BrdU-uptake-assay in vivo. Interestingly, we found that CD27+ NK cells preferentially responded to IL-15:Rα, suggesting that CD27+ NK cells contributed significantly to the expanded pool of NK cells after activation. To prove this, we used an adoptive transfer model and showed that CD27+ but not CD27- NK cells expanded extensively upon IL-15:Rα treatment. Importantly, the CD27+ subset expressed high levels of IFN-γ, GranzymeB, and Perforin, and was highly cytotoxic against allogeneic targets in vivo. Next, we studied the mechanisms that account for this differential responsiveness to IL-15. We found that expression of CD122 is strikingly different among the NK subsets, with CD27+ NK cells expressing the highest levels. This difference is due to reciprocal expression of T-Bet and Eomes among the subsets.
We show that NK cells consist of functionally diverse subsets with distinct responsiveness to IL-15, due to differential expression of CD122, T-Bet, and Eomes. Our studies may have important clinical implications in targeting innate immunity in transplantation.

O-50 ROLE OF ABC TRANSPORTERS (MDR1 AND MRPs) ON DENDRITIC CELL MATURATION AND LYMPHOCYTE PROLIFERATION AFTER HYPOXIA

Nuria Lloberas[1], Ines Rama[1], Linda Cassis[1], Ines Llaudó[1], Marcel-la Franquesa[1], Joan Torras[1], Josep M. Cruzado[1], Oriol Bestard[1], Esther Castaño[2], Josep M. Grinyó[1]. *[1]Nephrology Unit, Hospital Universitari Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain; [2]Technic Scientific Department, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain*

Introduction: The role of ATP-binding cassette (ABC) transporters: P-glycoprotein (Pgp, MDR1 gene product) and the multidrug resistance proteins (MRPs: MRP1 and MRP2) has been scarcely studied in dendritic cells (DCs) maturation. In a previous study we showed that hypoxia induces the maturation of DCs. The goal of this study is to evaluate whether these ABC proteins transporters are involved in DCs maturation by analysing the effect of specific inhibitors on the phenotypes and the allostimulatory capacity of DCs stimulated with hypoxia.
Methodology: Peripheral blood monocytes were transformed into DCs by adding IL-4/GM-CSF. Maturation of monocyte-derived DC under hypoxemic conditions was evaluated by assessing the expression of CD40, CD80, CD83, CD86 and CD54 by flow cytometry. The effect of ABC transporters on DC maturation was evaluated by using specific inhibitors (MK571 and Probenecid (PBN)) for MRPs and PSC833 for MDR1. The functional capacity of DCs depending on their maturation status to elicit T cell alloresponse was studied in 6 days MLR between DCs and allo T cells (CFSE-labelled).
Results: 1) Hypoxia induced DCs maturation. The up-regulation of maturation markers in stimulated cells was strongly abrogated when MDR1 inhibitor (MDR1i) or MRP inhibitors (MRPi) were added (See table). The expression of the markers for MRPi followed a similar pattern than MDR1i.

Dendritic cells phenotype

Mean fluorescence intensity	CD40	CD80	CD83	CD86	CD54
C	83,2±9,7^{a,b}	15,9±4,7^{a,c}	41,5±6,7^{a}	399,3±88,9^{a,b}	1052±126, 9^{a,b}
C + MDR1i	20,9±4.17	7,5±2,2	32,3±17,3	188,4±56,9	265±87,9
Hypoxia	189,4±66,5^{a,c}	43,4±22,4^{a,c}	57,1±15,4	715,7±330,7^{a,c}	1496,2±119,6^{a,c}
Hypoxia + MDR1i	44,9±13,9	3,7±1,5	43±36	153±54,2	163,2±18,8

$^{a}p<0.05$ C vs H; $^{b}p<0.05$ C vs C + PSC; $^{c}p<0.05$ H vs H + PSC.

2) Hypoxia induced higher T cell proliferation than control non stimulated DCs. The MDR1 and MRP inhibitors significantly reduced the T cell alloresponses in MLR (approximately 50%) compared with hypoxic DCs without inhibitors.
Conclusions: Our study shows that ABC transporters play a key role in DC maturation induced by hypoxia, which influences T-cell mediated alloresponses.

O-51 RECIPIENT DCs GENERATE CYTOTOXIC AND ALLOANTIBODY RESPONSES BY A NOVEL PATHWAY OF ALLOANTIGEN PRESENTATION

Sivasuriya Sivaganesh, Kourosh Saeb-Parsy, Reza Motalleb-Zadeh, Christopher J. Callaghan, Thomas M. Conlon, Margaret C. Negus, Eleanor M. Bolton, Andrew Bradley, Gavin J. Pettigrew. *Department of Surgery, University of Cambridge, Cambridge, United Kingdom*

Introduction: The ability of DCs to retain and re-present unprocessed antigen raises the possibility of a novel pathway of allorecognition, whereby recipient DCs present intact alloantigen to direct pathway T cells. This is advantageous over conventional pathways only if intact and processed alloAg are presented by the same DC, thereby providing a mechanism for the delivery of help from indirect pathway CD4$^+$ to direct-pathway cytotoxic CD8$^+$ T cells via interaction on a single cell.
Methods: Day 7 BMDCs from B6 (H-2^b) and (H-2^d) mice were co-cultured overnight. Acquisition of MHC I alloAg and presentation of processed MHC II alloAg on B6 DCs was assessed using mAbs. Co-cultured B6 DCs were sorted and injected into naive B6 mice. CD8$^+$ T cell, alloantibody and indirect pathway CD4$^+$ T cell responses were assayed 10 days later.
Results: B6 BMDCs present processed alloAg after co-culture with BALB/c DCs (mean 16.1%). Smaller numbers of B6 DCs expressed intact K^d alloantigen on their surface (mean 4.4%). Importantly, 2.3% of DCs presented both intact and processed alloantigen. B6 DCs, sorted after co-culture, provoked alloantibody, cytotoxic and indirect-pathway CD4$^+$ responses in naive B6 recipients. BALB/c DC contamination was excluded, because B6 DCs that expressed K^d alloAg provoked strong CD8$^+$ responses, whereas only minimal responses were generated by B6 DCs with undetectable surface K^d. MHC II deficient B6 DCs acquired similar levels of intact K^d, but provoked only minimal CD8$^+$ responses; thus highlighting that effective cytotoxic alloimmunity requires presentation of both processed and intact alloantigen by the same recipient DC.
Conclusions: DCs present simultaneously intact and processed alloantigen

for indirect pathway CD4$^+$ and direct-pathway CD8$^+$ responses. This may be particularly important for the generation of delayed anti-graft cytotoxicity, when donor DCs are no longer present.

O-52 CYTOTOXIC CD8 T CELLS RECEIVE HELP FROM INDIRECT PATHWAY CD4 T CELLS BY PRESENTING PROCESSED ALLOANTIGEN ON MHC II

K. Saeb-Parsy, T.M. Conlon, A.L. Taylor, M. Negus, S.L. Negus, E.M. Bolton, J.A. A. Bradley, G.J. Pettigrew. *Department of Surgery, University of Cambridge, Cambridge, United Kingdom*

We investigated how indirect pathway CD4 T cells that recognise processed alloantigen presented by recipient APCs provide 'unlinked' help for direct pathway cytotoxic CD8 T cells recognising allogeneic MHC class I on donor cells.
Female Mar (B6 RAG1$^{-/-}$) recipients, whose monoclonal CD4 T cells recognise self-restricted male H-Y peptide, rejected male BALB/c heart grafts acutely (MST 12d) if reconstituted with 10^6 effector female B6 CD8 T cells. Female BALB/c grafts survived $>$50 days, confirming rejection was dependent on help from Mar CD4 T cells. CD8-reconstituted Mar recipients that were additionally challenged with male B6 APCs mounted minimal cytotoxic T cell responses and did not reject female BALB/c grafts: Effective help was therefore generated only when H-Y and MHC I alloantigens were co-expressed on graft cells.
We hypothesised that the requirement for co-expression reflected acquisition of H-Y antigen from graft cells by allospecific CD8 T cells, with subsequent processing and presentation in the context of MHC II for Mar CD4 T cell recognition. In support, MHC II I-Aβ gene expression was detected in naive and activated (but not MHC II$^{-/-}$) CD8 T cells and flow cytometric analysis revealed surface MHC II expression, but only on activated CD8 T cells. Furthermore, reconstitution of Mar recipients with MHC II$^{-/-}$ CD8 T cells delayed rejection of male BALB/c grafts (MST 21d). In contrast, male B6 x BALB/c F1 grafts (which enable 'linked' help) were rejected at similar tempo, indicating that MHC II expression on CD8 T cells is only required for indirect CD4 T cell help.
During indirect allorecognition, help for allospecific CD8 T cells is potentiated through TCR-mediated internalisation of alloantigen and presentation of processed allopeptide on surface MHC II for recognition by indirect pathway CD4 T cells.

O-53 IMPAIRED VARICELLA ZOSTER VIRUS (VZV) SPECIFIC IMMUNE RESPONSE IN TRANSPLANT RECIPIENTS

Nicole M. van Besouw[1], George M.G.M. Verjans[2], Joke M. Zuijderwijk[1], Annemarie L. Geel[1], Willem Weimar[1]. *[1]Internal Medicine–Transplantation, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Virology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands*

Purpose: VZV causes varicella, becomes latent in sensory ganglia from which it may reactivate as herpes zoster. The frequency and severity of herpes zoster is increased in transplant recipients compared to healthy individuals. VZV-specific memory T-cells are considered to prevent VZV reactivation. DCs are essential to present the cognate VZV antigens to T-cells.
We questioned whether mature moDCs from renal transplant patients are more susceptible to VZV than those from healthy controls, and whether circulating VZV-reactive memory T-cells and VZV-specific IgG titres in transplant recipients are comparable with controls.
Methods: CD14$^+$ cells from patients and sex and aged-matched controls were differentiated into mature moDC, and infected by VZV-infected melanoma cells. The level of VZV infection was determined by staining with moAb specific for membrane bound VZV-glycoproteins. The CD3$^+$ cells were incubated with autologous VZV-infected moDC. The frequency of VZV-specific naïve (NA: CCR7$^+$CD45RO$^-$), central (CM: CCR7$^+$CD45RO$^+$) and effector (EM: CCR7$^-$CD45RO$^+$) memory CD4$^+$ and CD8$^+$ T-cells was determined by flow-cytometry.
Results: There was no difference in grade of VZV infection between moDC from transplant recipients and controls. The VZV-specific IgG titres were significantly lower after transplantation compared to before transplantation (p=0.002). No difference was found in the percentage of VZV-reactive CD4$^+$ NA, CM and EM T-cells between transplant recipients and controls. However, the percentage of IFN-γ producing VZV-specific CD8$^+$ memory T-cells was lower in patients compared to controls. This impairment (p=0.05) was mainly due to the CD8$^+$ EM T-cells.
Conclusion: Mature moDC from transplant recipients and healthy individuals can be similarly infected by VZV. The VZV-specific humoral and cellular immune response in transplant recipients is impaired compared to healthy individuals. Prophylactic VZV vaccination before transplantation might boost the patient's memory T and B-cell repertoire and thereby reduces the morbidity associated with herpes zoster after transplantation.

O-54 INTRA-GRAFT CD40 SILENCING SWITCHES IMMUNE RESPONSE FROM HUMORAL TOWARDS CELLULAR REJECTION

Èlia Ripoll Llagostera[1], August Vidal Bel[2], Marcel-la Franquesa Bartolomé[1], Joan Torras Ambrós[1], Josep Maria Grinyó Boira[1], Immaculada Herrero Fresneda[1]. *[1]EXP Renal Transplant, EXP Nephrology, IDIBELL-HUB, l'Hospitalet de Llobregat, Barcelona, Spain; [2]Pathology Serv, HUB, l'Hospitalet de Llobregat, Barcelona, Spain*

The co-stimulation pathway, necessary for the whole activation of the immune response, is activated in response to an antigenic stimulus and also by an inflammatory injury, both present in renal transplantation.
Tromboembolitic complications derived from systemic administration of antibodies against co-stimulatory molecules such as CD40L have been reported.
Intra-graft silencing of the CD40/CD40L pathway with a siRNA-antiCD40 in a model of humoral acute renal rejection (Ab-AR), would avoid or reduce rejection prolonging the graft survival.
Four groups were designed in a rat model of Ab-AR: Inespecific siRNA as control (NoTreat); sub-therapeutic Rapamycin (Rp, 0.5 mg/kg/d); siRNA-CD40 (siCD40, 2uM); and a group of combination therapies (siCD40-Rp). siRNA molecules were intra-arterially administered to donor kidneys followed by tissue electroporation.
Survival time, renal function, conventional histology, circulating DSA, Banff classification, percentage of CD40+-Bcells in spleen, and gene expression quantification (CD40 and other cytokines) were determined.
Survival time was nearly doubled with siCD40 and significantly increased when it was associated to Rp. Deposits of IgG and C4d were significantly decreased in siCD40-treated groups. Banff classification showed more AbAR cases among NoTreat kidneys than in both siCD40-treated ones with a clear switch towards cellular rejection in these last groups.
The CD40 mRNA was over-expressed in NoTreat group. Local silencing with siCD40 decreased its expression, especially when associated to Rp. Spleen B cells were significantly reduced in both siCD40-treated groups, similarly to the number of B cells expressing CD40 protein.
Local gene silencing of CD40 is effective in the blockade of the co-stimulatory signal, reduces the onset of acute rejection, prolongs survival and changes the type of rejection. Results points to a possible counteraction of siCD40 with B cell function and/or production.

O-55 AUTOANTIBODY AND CONVENTIONAL ALLOIMMUNITY TOGETHER CONTRIBUTE TO DEVELOPMENT OF ALLOGRAFT VASCULOPATHY

Ines Harper[1], Kourosh Saeb-Parsy[1], Thet Su Win[1], Martin Goddard[2], Eleanor Bolton[1], Andrew Bradley[1], Gavin Pettigrew[1]. *[1]Department of Surgery, University of Cambridge, Cambridge, United Kingdom; [2]Department of Pathology, Papworth Hospital, Papworth Everard, United Kingdom*

The development of autoimmunity is increasingly associated with poor outcomes after transplantation. An effector role for autoantibody in graft damage has been suggested, but it is unclear whether this is independent of, or whether it complements, conventional *allo*immune responses. Here we investigate synergy between humoral autoimmunity and alloimmunity in the development of allograft vasculopathy (AV).
We previously demonstrated that rejection of MHC II-disparate bm12 heart grafts involves progressive AV, complement endothelial deposition and development of anti-nuclear auto-, but not allo-, antibody. Autoantibody responses

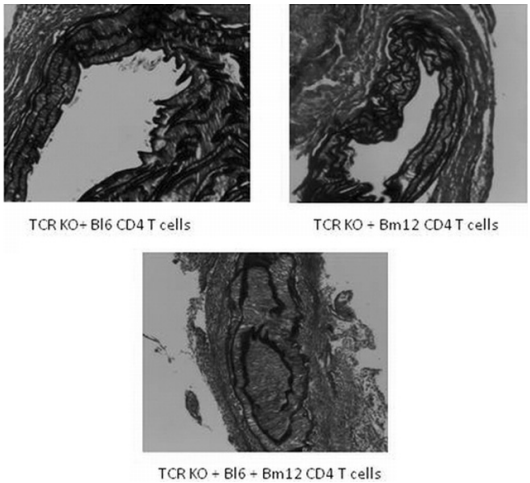


Figure 1. Bm12 Aortoc allograft histology at day 40 (EVG staining).

depend on help from passenger donor CD4 T cells within the graft. Thus the effector role of autoantibody can be examined by reconstituting T cell-deficient TCR KO B6 recipients of bm12 heart or aortic allografts with bm12 or B6 CD4T cells or a combination of both.
Injecting B6 mice with 10^6 bm12 CD4 T cells 2 weeks prior to bm12 heart grafting primed for autoantibody and resulted in severe AV and rapid rejection (MST 35 days vs. WT MST 95). In contrast, isografts in autoantibody-primed recipients survived indefinitely, suggesting that autoantibody only contributes to rejection by exacerbating alloimmune-mediated damage.
Potential synergy was further examined by aortic allograft transplantation. Bm12 aortic grafts neither developed AV in B6 recipients nor provoked autoantibody; thus, unlike heart grafts, aortic grafts do not contain significant numbers of CD4 T cells. In contrast, priming for autoantibody at time of transplantation resulted in severe AV. The essential role of recipient CD4 T cell responses was confirmed by the demonstration that TCR KO recipients only developed significant aortic AV when reconstituted with both B6 and bm12 CD4 T cells.
Autoantibody only effects AV in concert with conventional alloimmune responses, presumably because autoantibody binding is dependent on an initiating insult translocating autoantigen to the endothelial cell surface.

O-56 MYCOPHENOLIC ACID DISABLED HUMAN DENDRITIC CELLS TO INDUCE ALLOGENEIC CYTOTOXIC CD8+ T CELLS THROUGH INHIBITION OF INTERFERON GAMMA SYNTHESIS IN DENDRITIC CELLS

Roxane Lemoine[1], Florence Velge-Roussel[1], Hubert Nivet[1,2], Yvon Lebranchu[1,2], Christophe Baron[1,2]. *[1]UPRES EA 4245 "Cellules Dendritiques, Immunomodulation et Greffes", UFR de Médecine, Université François Rabelais, Tours, France; [2]Service de Néphrologie et Immunologie Clinique, CHRU Bretonneau de Tours, Tours, France*

Purpose: Several studies have highlighted that human dendritic cells (DC) can activate $CD8^+$ T cell cytotoxic activity independently of $CD4^+$ helper T cells and have reported that CD8-dependent rejection might be resistant to immunotherapeutic agents. Modulating CD4 independent-$CD8^+$ T cell cytotoxic alloresponse might be important for inducing organ acceptance. We have previously shown that DC pretreated with mycophenolic acid (MPA) could regulate $CD4^+$ T cell alloresponse. In this study, we found a great interest to know how MPA could affect the ability of DC to induce the differentiation of allogeneic $CD8^+$ T cells into effector cytotoxic cells independently of $CD4^+$ T cells.
Methods: The maturation of human monocyte-derived DC was induced by LPS in the presence or not of 100μM MPA. Allogeneic $CD8^+$ T cells were cultured for 6 days with LPS-DC or MPA-DC. T cell proliferation was analyzed by [^{3}H]-thymidine incorporation and cytokine productions were assessed by ELISA. The cytotoxic function was also analyzed by granzymes A and B, perforin and CD107 expression and killing of targets was measured by CFSE/7-AAD double staining.
Results: LPS-DC support the proliferation of allogeneic $CD8^+$ T cells independently of $CD4^+$ T cells whereas DC modified by MPA did not. MPA-DC induced also antigen-specific $CD8^+$ T cell anergy in both naive and memory $CD8^+$ T cells that secrete high levels of IL-4, IL-5, IL-10 and TGF-β. MPA strongly reduced the capacity of DC to induce cytotoxic activity in allogeneic $CD8^+$ T cells. Finally, we found that exogenous IFN-g completely restores IL-12 and TNF-α synthesis in MPA-DC and reconstitute their ability to activate $CD8^+$ T cells.
Conclusion: These results may provide a new approach to modulate CD8 cytotoxic alloresponse to promote allograft tolerance.

O-57 HUMAN MESENCHYMAL STEM CELLS ARE SUSCEPTIBLE FOR LYSIS BY CYTOTOXIC IMMUNE CELLS

Meindert J. Crop, Sander S. Korevaar, Carla C. Baan, Ronella de Kuiper, Nicole M. van Besouw, Willem Weimar, Martin J. Hoogduijn. *Internal Medicine-Transplantation, Erasmus University Medical Center, Rotterdam, Netherlands*

Purpose: Mesenchymal stem cells (MSC) have beneficial immunological properties for organ transplantation. They have immunosuppressive capacity and are considered to be low immunogenic due to low HLA class I and lack of HLA class II, CD80 and CD86 expression. There are, however, reports that MSC can trigger immune responses. Furthermore, under inflammatory conditions the expression of HLA class I and II molecules on MSC is increased, which may boost the immunogenicity of MSC. In the present study we investigated whether kidney donor-derived MSC, in the absence or presence of inflammatory cytokines, are susceptible for lysis by recipient cytotoxic immune cells before and after transplantation
Methods/Materials: MSC were isolated and expanded from adipose tissue of kidney donors and immunophenotyped by flow cytometry. MSC were cultured in the absence or presence of IFN-γ, TNF-α and IL-6. After 7 days of culture, the cytotoxic lysis of Europium-labelled donor T-cell blasts and donor MSC by recipient PBMC was determined. Europium release was measured in a time-resolved fluorometer.
Results: MSC isolated from adipose tissue showed multilineage differentiation capacity and expression of CD90, CD105 and CD166. After culture with IFN-γ, TNF-α and IL-6, HLA class I and II expression was increased. Despite a low immunophenotype, Europium-labelled donor MSC were lysed by recipient PBMC, although less efficiently than donor T-cell blasts (Figure 1). Remarkably, donor MSC cultured with IFN-γ, TNF-α and IL-6 were less susceptible for lysis. Autologous recipient PBMC were not lysed.

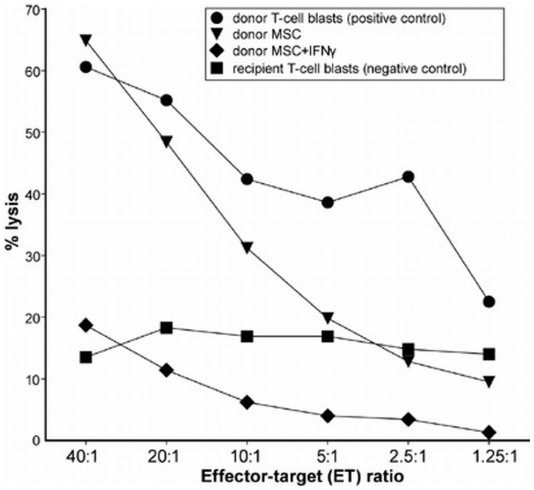


Figure 1. Percentage lysis of donor MSC and donor T-cell blasts by recipient's PBMC.

Conclusion: MSC are susceptible for lysis by allogeneic immune cells. Treatment of MSC under inflammatory conditions protects MSC from cytotoxic lysis. We are currently examining the lysis of donor MSC by post-transplantation recipient PBMC.

O-58 ANTI-CD3 THERAPY INDUCES RAPID RENAISSANCE OF $B220^{low}$ B LYMPHOCYTES FOLLOWING B-CELL DEPLETION BY ANTI-CD20

Gordon D. Wu, Andrew S. Klein. *Comprehensive Transplant Center, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Objective: Production of donor-specific antibodies (DSA) by B cells is T-dependent and T cell suppression is the core anti-rejection therapy in organ transplantation. The effects of T cell depletion on alloreactive B cells, however, are poorly understood.
Methods: A murine model of allogenic sensitization was used to investigate the impact of CD3+ T cell depletion on alloreactive B cell responses.
Results: Immunization of C57BL/6 mice with C57BL/6-tg-HLA.A2 skin allograft resulted in robust production of DSA featured by an early IgM, then a strong IgG2a and a weak IgG1 response. Anti-CD20 antibody eliminated >95% of $B220^{high}$ B cells from the blood, the spleen and the bone marrow of the recipients, resulting in significant suppression of serum DSA ($P<0.01$ vs. control). Anti-CD3 treatment decreased splenic T cells by 90% and moderately tapered DSA production ($P<0.05$ vs. control). Combined anti-CD20 and anti-CD3 treatment effectively removed the majority of CD3+ cells and $B220^{high}$ B cells in the spleens, resulting in profound suppression of DSA responses ($p<0.01$ vs. control). Interestingly, the skin allograft recipients treated with combined regimen exhibited a resurgence of splenic $B220^{low}$ B cells despite re-treatment with anti-CD3 and CD20 at day 14. This rebound of $B220^{low}$ B cells in the spleen was not in association with alloantibody production since serum DSA remained minimal. Quantitative RT-PCR showed that the $B220^{low}$ splenic lymphocytes isolated by FACS expressed immature B cell markers.
Conclusion: T cell depletion by anti-CD3 can taper DSA production by B cells which are T-cell dependent. However, pan T cell depletion could unintentionally eliminate T cell subsets, which have suppressor effects on B cells, as indicated by the data, leading to rebound of immature B cells. The pathogenic role of these resurging B cells in allogenic sensitization merits further investigation.

Session 9. Long-term kidney graft survival parameters

O-59 CLINICAL VALIDITY OF TIME-ZERO BIOPSIES IN RENAL ALLOGRAFT

Dong Jin Joo[1,2], Soo Jin Kim[1,2], Kyung Ock Jeon[2], Jong Hoon Lee[3], Kiil Park[3], Soon Il Kim[1,2], Gi Hong Choi[1,2], Jinsub Choi[1,2], Yu Seun Kim[1,2]. *[1]Surgery, Yonsei University College of Medicine, Seoul, Korea; [2]The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea; [3]Surgery, Kwandong University Myongji Hospital, Goyang, Korea*

Introduction: Time-zero renal allograft biopsy provides valuable diagnostic information for allograft outcome and the present donor condition. We retrospectively reviewed renal functions and pathologic reports of time-zero biopsies.
Methods and materials: Among 257 kidney transplantation from 1 May 2006 to 30 May 2008, 56 zero-time biopsies were done in the kidney upper pole with 16G needle gun.
Results: Mean age of donor and recipient was 40.3±12.6 year and 43.4±10.2 year respectively. There were 36 living donor and 20 deceased donor cases. Mean graft weight was 232±47 g. An average of 16±9 glomeruli were obtained in the biopsies. Mean follow-up period was 13.2±6.5 months. Histologic findings were categorized as Table 1. As a result of correlation analysis, serum creatinine of 1 year after transplantation correlated with donor age (r=0.416, *p=0.014*), graft weight (r=−0.383, *p=0.026*), interstitial fibrosis (IF; r=0.489, *p=0.003*) and tubular atrophy (TA; r=0.471, *p=0.005*). Glomerulosclerosis (GS) strongly correlated with donor age (r=0.419, *p=0.001*). Remarkably, interstitial inflammation (II) was correlated with donor BMI (r=0.381, *p=0.004*).
The mean eGFR of 1 week, 1, 3, 6, 9, 12 months after transplantation was evaluated. The group with pathologic features such as IF, TA, and GS showed statistically different mean eGFR from the group with normal histology. However, II, AH, AS, GIC and IgAN did not significantly differ in eGFR. Two donor kidney disease were detected in the time-zero biopsy, GN (focal endocapillary proliferative glomerulonephritis) and IgAN.

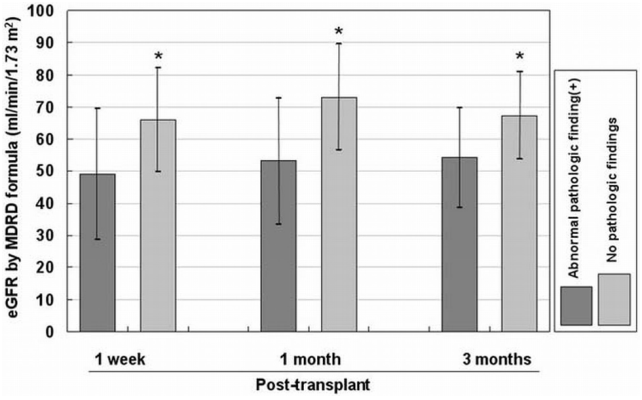


Figure 1. Mean eGFR of no pathologic finding group vs. abnormal pathologic finding group.

Zero-time biopsy (one or more abnormalities)

Histologic findings	N=56	%
No histologic abnormality (NHA)	14	25
Interstitial inflammation (II)	5	8.9
Interstitial fibrosis (IF)	19	33.9
Tubular atrophy (TA)	26	46.4
Arteriolar hyalinosis (AH)	7	12.5
Glomerulosclerosis (GS)	21	37.5
Arteriolar sclerosis (AS)	11	19.6
Glomerular ischemic change (GIC)	7	12.5
Glomerular nephritis (GN)	1	1.79
IgA nephropathy (IgAN)	2	3.57

Conclusion: It is still controversial about the efficacy of time-zero biopsy in renal allograft. However, zero-time biopsy can imply important information for donor kidney disease and prognostic factors for graft function. Further study is needed to examine zero-time biopsy in a prospective randomized fashion.

O-60 EARLY RENAL TRANSPLANT PROTOCOL BIOPSIES DIRECTLY INFLUENCE PATIENT MANAGEMENT

A.O. Mahendran[1], M. Elvey[1], M. Mahendran[2], A.J. Howie[3], P. Dupont[1], P.S. Veitch[1]. *[1]Department of Renal Transplantation, Royal Free Hospital & University College London Medical School, London, United Kingdom; [2]Faculty of Life Sciences, University College London Medical School, London, United Kingdom; [3]Department of Pathology, University College London, London, United Kingdom*

Introduction: In this prospective observational study, we explored whether early protocol biopsies directly influenced patient management and subsequent graft function.
Method: A protocol biopsy programme was instituted for patients transplanted consecutively from September 2006. All recipients received baseline immunosuppression; a calcineuirin inhibitor and mycophenolate mofetil with early steroid weaning for the majority. Protocol biopsies were performed at 2/3 and 12 months post-transplantation. Specimens were analysed for adequacy and conventional histology. An index of chronic damage (morphometric assessment of tubular atrophy and instititial fibrosis – ICD) was also assigned. ICD values at implantation and 2/3 months were compared. Biopsy results were reviewed at a weekly multi-disciplinary meeting. An independent observer recorded whether a change in patient management was instituted as a direct result of the biopsy findings.
Results: 154 patients were transplanted between Sep 2006 and July 2008. 35.2% received a live donor graft. 142/154 patients (92.2%) underwent biopsy at a median 2 months post-transplantation. No major complications were reported.
In 32 patients (23.2%) protocol biopsy findings directly altered patient management. 7 patients were treated for subclinical rejection, 5 for hitherto undiagnosed viral infection in the graft and 21 had evidence of CNI toxicity. Early protocol biopsy findings instituted a management change which was shown to improve graft function in patients with subclinical rejection (p= 0.011).
In 110 patients, negative biopsy findings did not directly alter management but engendered confidence to further reduce CNI dosage. This may be important for longterm graft survival as we noted the ICD increased significantly between implantation and biopsy suggesting that chronic allograft nephropathy develops early post-transplantation.
Conclusions: Early protocol biopsies are safe and directly influence patient management in >20% of cases. Longterm strategies are needed to mitigate the impact of chronic graft damage which develops as early as 2 months post-transplantation.

O-61 FOXP3 LEVELS PREDICT CLINICAL OUTCOME IN PATIENTS WITH BORDERLINE CHANGE AFTER RENAL TRANSPLANTATION

Sébastien Homs, Hicham Mansour, Dominique Desvaux, Philippe Lang, Philippe Grimbert. *Nephrology and Transplantation, CHU Henri Mondor, Créteil, France*

Purpose: The relationship between borderline infiltrates and acute rejection in renal allograft recipients is still unclear and the therapeutic management of these lesions is not well defined. To determine molecular factors involved in borderline change outcome, we studied levels of Foxp3, Granzyme B, Interferon g, IL-23 and RORgt messenger RNA in renal biopsies from 46 subjects with untreated borderline change infiltrate.
Results: Twenty-five patients were considered "non progressive", as judged by a reduction in serum creatinine to <110% of baseline. Twenty-one patients were considered "progressive" judged by an increase in serm creatinine > 110% from baseline and confirmed by histological examination performed within 40 days showing progression toward acute rejection. Only Foxp3 mRNA levels were significantly higher in the non-progressive group than in the progressive group (p=0.001). Analysis of receiver operating characteristic curves demonstrated that the outcome for patients with borderline change biopsies can be predicted with 90% sensitivity and 79.1% specificity using the optimal Foxp3 mRNA cut-off value of 1.05 (p<0.001).
Conclusion: Our findings suggest that the measurement of Foxp3 mRNA offers a means of improving predictions of borderline change outcome.

O-62 A MODEL BASED ON DONOR FACTORS FOR PREDICTING LONG TERM SURVIVAL OF LIVING RELATED RENAL TRANSPLANTS

Mirza Naqi Zafar, S.A. Anwar Naqvi, S. Adibul Hasan Rizvi. *Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Sindh, Pakistan*

Purpose: Donor factors age, sex, absolute GFR, donor recipient body weight ratio and HLA match have been used to develop a model to predict long-term graft survival in living related renal transplant. Each factor was assigned a range of scores 0-3 where 0 had the best 1 and 5 years graft survival.
Methods and materials: Outcome of 784 living related donor transplants were

analyzed from total donor scores. The following scores were given for each factor. Donor age (years) upto 40=0, 41–50=1 and >50 = 2. Donor sex, male=0 and female=1, uncorrected creatinine clearance (ml/min) >120=0, 101–120=1, 81–100=2 and ≤80=3. Donor recipient body weight ratio >1.5=0, 1.0–1.5=1 and <1=2. HLA match identical=0 and any other=1. The total score (TS) for each donor was arrived at by summation. One and 5 year graft survival was determined for different TS from 0 to 8. Immuosuppression was by triple drug regimen with cyclosporine

Results: Of the 784 recipients 68 (9%) had a TS of 0-1 (G1) where 1 and 5 year graft survival was 100% and 95%, 253 (32%) had TS of 2-3 (G2) with survival of 91% and 76%, 302 (38%) had TS of 4-5 (G3) and survival of 89% and 68% and 159 (20%) had TS of 6-8 (G4) with survival of 81% to 46% respectively (p=0.001). Acute rejection rates in G1 was (19%), G2 (21%), G3 (23%) and G4 (29%). One and 5 year graft survival for TS=0 was 100% and ITS=8 was 80% and 40% respectively.

Conclusion: Using this model one can assign scores to the donors available in the family and select the donor with the best long term outcome. TS can also help develop tailored immuosuppression.

O-63 LONG-TERM OUTCOME OF KIDNEY TRANSPLANTATION FROM LIVING DONORS OLDER THAN 65 YEARS: A SINGLE CENTER EXPERIENCE

Yasuo Ishii, Kazuhiro Iwado, Ichiro Nakajima, Shohei Fuchinoue, Satoshi Teraoka. *Surgery of Kidney Center, Tokyo Womens Medical University, Tokyo, Japan*

Background: The role of advanced age live donors remains controversial because of decline in glomerular filtration rate and perceived increased risks of perioperative complications. There is a paucity of data on long-term outcomes of older kidney recipients. Our aim was to compare the early and long-term outcomes of kidney transplantation from older living donors (>or=65 years) with outcomes in younger donors

Materials and methods: From 1989 to 1998, we performed 466 live donor kidney transplants. 284 (60.9%) donors were female and 182 (39.0%) were male. There were 69 (14.8%) older donors (>65 years old), 397 (85.1%) were younger donors (<65 years old). We performed open nephrectomy for all patients. Older candidates were carefully selected based on their physiologic, cardiac, and performance status. Demographic data, including clinical characteristics, mortality, and patient and graft survival rates, were collected and analyzed.

Results: Overall resipients and graft survival at 1 year are 100% and 100% in the older vs. young donors group. 3-year graft survival is 95.6% vs. 96.6%. 5-year graft survival is 89.4% vs. 88.8%. 7-year graft survival is 75.8% vs. 83.6%. And 10-year graft survival is 57.8% vs. 75.0%. There was a significant difference in long term graft survival (>5 years) between the two groups (p=0.012), but no significant difference in early term graft survival (<5 years). And, postoperative serum creatinine of recipients in the old donor group was higher than young donor group, 1.92±0.10 mg/dL vs. 1.58±0.07 mg/dL (p <0.001). The rates of acute rejection and major complication were also comparable between the two groups.

Conclusions: Transplants from older donor kidneys are associated with a higher risk of graft loss and patient death. The risk was highest when older donor kidneys were transplanted into old recipients.

O-64 PRE-TRANSPLANT CELLULAR ALLOREACTIVITY IS PREDICTIVE OF ACUTE GRAFT REJECTION AND LONG TERM GRAFT FUNCTION IN KIDNEY TRANSPLANT RECIPIENTS

Katarzyna Koscielska-Kasprzak[1], Dominika Drulis-Fajdasz[1], Dorota Kaminska[1], Oktawia Mazanowska[1], Magdalena Krajewska[1], Wojciech Bieniecki[2], Pawel Chudoba[3], Wojciech Polak[3], Dariusz Janczak[3], Dariusz Patrzalek[3], Marian Klinger[1]. *[1]Dept.of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland; [2]Computer Engineering Department, Technical University Lodz, Lodz, Poland; [3]Dept.of Vascular, General and Transplant Surgery, Wroclaw Medical University, Wroclaw, Poland*

In our study we investigated the cellular alloimmunity of kidney allograft recipients using direct interferon (IFN)-γ enzyme-linked immunospot assay (ELISPOT). Donor splenocytes were obtained along kidney recovery and PBMC of 53 recipients were collected before Tx. 11 recipients presented positive pretransplant PRA values. For ELISPOT data analysis we calculated the number, but also the size and intensity of the spots, which reflected the volume of cytokine secretion at the single cell level. The results were recalculated as the ratios of the values observed for donor stimulated to unstimulated recipient cells and corrected for residual donor activity. Significantly higher pretransplant donor stimulated activity was observed for the recipients who underwent AR episode within one year after Tx (p<0.05). The mean change in the spot numer, size and intensity observed for rejectors vs nonrejectors were 0.99 vs 3.33, 1.6 vs 6.05, and 1.4 vs 6.31, respectively. Based upon our data we were able to estimate the values of parameters that are prognostic of high AR risk – 1.5-fold increase in spot number (AR incidence 9% vs. 52%), 2.0-fold increase in size (AR incidence 11% vs. 44%), and 2.7-fold increase in intensity (AR incidence 9% vs. 52%) (p<0.05). Moreover, all the three parameters analyzed were corelated with the serum creatinine concentration observed one year after Tx (p<0.05). From 14 AR positive recipients, 11 could have been predicted with pretransplant ELISPOT measures, while only 2 based upon PRA values. Our data show that the ELISPOT determined volume of donor induced activity observed for the recipient cells obtained just before Tx is predictive of the risk of graft rejection and one-year graft function.

O-65 NATURAL HISTORY OF ISOLATED TRANSPLANT GLOMERULITIS IN RENAL TRANSPLANTATION

David Buob[1], François Glowacki[2], Myriam Labalette[3], Emmanuel Boleslawaski[4], Françoise Dufossé[3], Marie Christine Copin[1], Christian Noël[2], Marc Hazzan[2]. *[1]Pathology, CHRU de Lille, Lille, France; [2]Nephrology, CHRU de Lille, Lille, France; [3]Immunology, CHRU de Lille, Lille, France; [4]Transplant Surgery, CHRU de Lille, Lille, France*

Background: Transplant glomerulitis is a lesion scored in the Banff 97 classification scheme, which can lead to the diagnosis of acute humoral rejection when associated with C4d deposition in peritubular capillaries. However, the signification of isolated transplant glomerulitis (ITG) in protocol biopsies remains undetermined.

Methods: From 2005 to 2008, ITG, i.e. without C4d deposits or other morphological evidence of rejection, was diagnosed in 21 renal transplant patients who underwent 3-month protocol biopsies. No specific treatment was introduced after the discovery of ITG. We compared these patients to 44 selected recipients without ITG or any lesions of rejection in their protocol biopsies.

Results: ITG was found in 4.4% of the 3-month protocol biopsies (21/476). Baseline characteristics were similar in the 2 groups, especially for the HLA matching and the proportion of high immunological risk patients. Induction therapies were equivalent.

At the 3-month protocol biopsy, the 2 groups received the same immunosuppressive regimen. Serum creatinine (1.6±0.5 versus 1.5±0.4 mg/dl) and proteinuria/creatininuria ratio (0.26±0.23 versus 0.19±0.19 in the ITG and the control group, respectively) were not significantly different between the 2 groups.

After 1-year follow-up, serum creatinine (1.4±0.4 versus 1.4±0.4 mg/dl) and proteinuria/creatininuria ratio (0.35±0.63 versus 0.24±0.46 in the ITG and the control group, respectively) remained stable. Incidence of acute rejection was 4.8% versus 6.8% in the ITG and control group, respectively (NS). No patient death or graft loss was registered during the study period. Only 2 recipients developed de novo anti-HLA antibodies in the control group versus 0 in the ITG group (NS).

Conclusion: In the present study, no predictive factor of ITG was identified and ITG had no deleterious consequences on the 1-year graft outcome. These results could suggest that the presence of ITG in 3-month protocol biopsies does not indicate intensification of immunosuppression.

O-66 EARLY HIGH PULSE PRESSURE IS ASSOCIATED WITH GRAFT DYSFUNCTION AND PREDICTS POOR KIDNEY ALLOGRAFT SURVIVAL

Fernando Vetromile, Ilan Szwarc, Valérie Garrigue, Sylvie Delmas, Georges Mourad. *Nephrology and Transplantation, Hôpital Lapeyronie, University of Montpellier, Montpellier, France*

Background: Pulse pressure (PP), which reflects the pulsatile component of the blood pressure (BP), is known as a major predictor of cardio-vascular events and death. In the elderly and type 2 diabetic patients, PP is associated with low glomerular filtration rate (GFR) and albuminuria. As kidney allograft survival is closely related to BP levels, we investigated the impact of early high PP, systolic (SBP), diastolic (DBP) and mean arterial (MAP) BP on kidney allograft survival.

Methods: Renal hemodynamic and function studies using isotopic methods (GFR and effective renal plasma flow estimated by urinary clearances of technetium-labelled diethylene-triamino-pentaacetic acid (^{99m}Tc-DTPA) and ^{131}I ortho-iodohippurate respectively) were prospectively performed in 493 renal transplant patients at three months post-transplantation to determine the impact of the different BP components on allograft survival using a proportional Hazard model.

Results: After a median follow-up of 6.3 years, 91 allografts were lost. High PP was associated with high SBP, DBP and MAP, heart rate (HR), recipient age, glycemia and low GFR. Moreover, PP emerged as the strongest BP component influencing overall as well as death-censored kidney allograft survival.

Conclusion: High PP is an early marker of poor allograft outcome that could be corrected by therapeutic intervention.

O-67 SCREENING FOR GENETIC VARIATIONS IN THE KEY MOLECULE OF TOLL LIKE RECEPTOR SIGNALING – IMPLICATIONS FOR RENAL TRANSPLANT OUTCOMES

Andreas Walberer[1], Bernd Krüger[1,2], Michael Fischereder[3], Bernhard Banas[2], Bernhard K. Krämer[1,2]. *[1]Medizinische Klinik I, Marienhospital Herne, Klinikum der Ruhr-Universität Bochum, Herne, Germany; [2]Klinik und Poliklinik für Innere Medizin II, Universitätsklinikum Regensburg, Regensburg, Germany; [3]Medizinische Poliklinik Innenstadt, Nephrologisches Zentrum, Klinikum der Ludwig-Maximillians-Universität München, München, Germany*

Controversial results about the role of genetic variations in toll like receptors (TLRs) for renal transplant outcomes were described in recent studies. Currently there are >10 human TLRs known, with different postulated impacts on transplant outcome, that all use a key signal molecule called MAL. Genetic variations in this signaling molecule provide an elegant method to study the overall role of TLRs in kidney transplantation. Here, we screened a representative cohort for potential polymorphisms in the MAL gene, reconstructed relevant haplotypes, and associated them with renal transplant outcomes.
We screened a representative number of patients (n=48) for genetic variations in the exons, overlapping areas of the introns, and the 5'- and 3'-region comprising the MAL gene by SSCP (single strand conformation polymorphism). Conspicuous variations were verified by sequencing. In a cohort of 352 patients who received their first kidney transplant at the University of Regensburg, proven polymorphisms were analysed with subsequently reconstruction of their haplotypes.
We identified 9 different polymorphisms within the MAL-gene. For further analysis in our cohort of transplant recipients we excluded 4 polymorphisms because of significant linkage among them. After reconstructing we observed 6 different haplotypes, which we stratified into 3 diplotypes. When testing these diplotypes for various outcomes (delayed graft function, acute rejection, cardiovascular morbidity/mortality, all-cause mortality, graft survival) in our transplant cohort no associations were found.
Screening for genetic variations in a key signal protein of the TLR-system showed numerous, in some cases unknown, polymorphisms. However, no differences in renal transplant outcomes caused by any of the haplotypes/diplotypes were found, suggesting either a lack of change in function or the substitution of function by other signaling pathways. Further studies will be needed to clarify the alterations by these polymorphisms.

O-68 VASCULAR ENDOTHELIAL GROWTH FACTOR GENETIC POLYMORPHISM AND CARDIOVASCULAR RISK IN RENAL TRANSPLANT RECIPIENTS

Maria L. Cappuccilli, Gaetano La Manna, Elisa Persici, Laura Panicali, Diletta Conte, Nicole Lanci, Serena Corsini, Giuseppe Cianciolo, Maria P. Scolari, Sergio Stefoni. *Nephrology, Dialysis and Renal Transplant Unit, S. Orsola University Hospital, Bologna, BO, Italy*

Purpose: VEGF-mediated inflammation has been implicated in atherosclerotic cardiovascular disease in the general population, but poor data is currently available on kidney transplant recipients (KTR). Inter-individual production of VEGF is under genetic regulation: a common C to T substitution at position 936 in the 3' untranslated region of the VEGF gene has been associated with lower levels of circulating VEGF. This study was undertaken to investigate the effect of VEGF genotype on cardiovascular risk biomarkers in KTR.
Methods: VEGF/C936T polymorphism was analyzed by PCR-RFLP in 175 KTR transplanted between 1997 and 2006 (minimum follow-up: 12 months). On the basis of previous studies, VEGF producing genotypes were assigned as follows: high producers (VEGF/936CC) and low producers (VEGF/936CT+TT). Multiple cardiovascular risk biomarkers were compared between VEGF high producers and low producers: total and LDL-cholesterol, homocysteine, Lipoprotein(a), C-reactive protein (CRP), fibrinogen, tissue plasminogen activator (t-PA), monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (sVCAM-1), P-selectin (sP-selectin) and CD40 ligand (sCD40L). Total and LDL-cholesterol, CRP, fibrinogen, Lp(a) and homocysteine were assayed using routine methods. Detection of sP-selectin, sCD40L, st-PA, sVCAM-1 and sMCP-1 was performed by FlowCytomix assay.
Results: The two groups of patients carrying the VEGF high producer genotype (n=132) or the VEGF low producer genotype (n=42) were matched for sex distribution, donor age, recipient age, time on dialysis, cold ischemia time, HLA-mismatches and therapy. Significantly increased levels of circulating t-PA and MCP-1 were observed in VEGF high producers compared to the low producers (t-PA: 6419±820 ng/mL vs 4337±1729 ng/mL, p=0.04; MCP-1: 4805±223 ng/mL vs 1574±178 ng/mL, p=0.04).
Conclusions: VEGF high producing genotype seems to be associated with an increase in some circulating cardiovascular risk markers, suggesting a significant prognostic value for gene polymorphisms involved in inflammatory response in kidney transplant recipients.

O-69 PREDICTION OF TRANSPLANT SUCCESS WITH SERUM ANGIOPOIETIN-2 LEVELS MEASURED IN THE DONOR

Lyan G. Koudstaal[1], Cyril Moers[1], Hans Burgerhof[2], Andreas Paul[3], Jacques Pirenne[4], Harry van Goor[5], Rutger J. Ploeg[1], Henri G.D. Leuvenink[1]. *[1]Surgery, University Medical Center Groningen, Groningen, Netherlands; [2]Epidemiology, University Medical Center Groningen, Groningen, Netherlands; [3]General, Visceral and Transplant Surgery, University Hospital Essen, Essen, Germany; [4]Abdominal Transplant Surgery, University Hospital Leuven, Leuven, Belgium; [5]Pathology, University Medical Center Groningen, Groningen, Netherlands*

Introduction: Prediction of transplant outcome using donor material such as blood or biopsies will prevent unnecessary discard of donor organs and is useful for post-operative treatment. Attempts to use pre-transplant biopsies in the US show moderate predictability. Recently we found in a small group of donors that Angiopoietin levels are elevated in brain dead donors. In critical ill patients Angiopoietin-2 was recently described to be a prognostic survival marker. Here we investigate if Angiopoietins have a predictive value in renal transplantation.
Methods: From 297 deceased kidney donors included in an international prospective RCT (the Machine Preservation Trial), serum was analyzed for Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2). All samples were measured by ELISA in duplicate. Donors were either heart-beating, or controlled non-heart-beating. We tested with logistic and Cox regression models whether Angiopoietins were associated with delayed graft function (DGF), and graft survival (GS).
Results: Ang-2 concentration was significantly associated with GS: higher values in donor plasma were predictive of a lower risk of graft failure (Hazard Ratio (HR)=0.91, $p<0.05$). Other significant variables in the model were donor age (HR=1.05), number of mismatches (HR=1.25), and recipient age (HR=0.97). For neither Ang-1 levels nor the ratio Ang-1/Ang-2 any association with GS could be found. Angiopoietin levels in donor serum were not associated with DGF.
Conclusion: This study for the first time shows that Angiopoietin-2 concentration measured in donor serum samples prior to donation and transplantation is an independent predictor of graft survival.

Session 10. Ethical, legal and psychosocial aspects of transplantation

O-70 LEGAL PROHIBITION OF ORGAN COMMERCIALISM: DO WE TAKE IT SERIOUSLY?

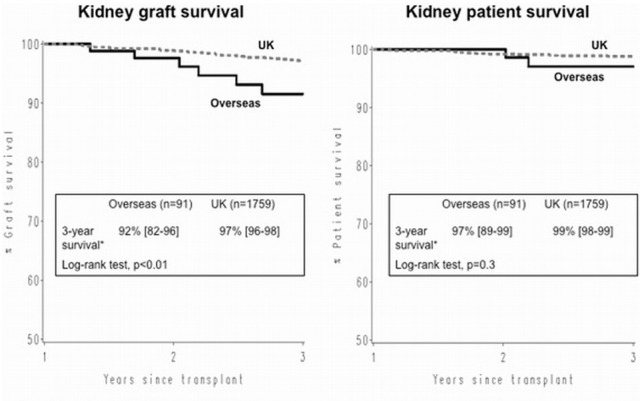
Michael Bos. *Medical Care Advisory Group, Health Council of the Netherlands, The Hague, Netherlands*

Practically all European countries have legislation to regulate donation of human organs. Although the Council of Europe, since 1970's, has made efforts to harmonize these laws with respect to scope, system of donation and application, reality today is that these laws are perplexingly heterogeneous across Europe. One issue addressed in all legislation, is the prohibition of material gain from donating and handling human organs for transplantation, and prevention of commercialism, including trafficking and tourism. During the last decades international treaties, conventions and guidelines have aiming to offer legal and ethical guidance to countries, concerning the handling of the human body and its parts (WHO Guidelines, Council of Europe Recommendations, UN Protocol on Human Trafficking, Declaration of Istanbul). These documents speak out against commercialization of human organs, and prohibit activities such as organ tourism, trafficking and soliciting of donors, urging governments to take legal and other measures. A recent survey of European countries shows that this appeal has been implemented in different ways, ranging from a simple ban on material gain from organs to explicit legal provisions to prevent and prohibit organ tourism, trafficking, brokering and soliciting of (paid) donors. Legal sanctions range from a (moderate) fine to several years imprisonment or losing one's license to practice. The survey shows that most countries only focus on situations where an organ donor gets monetary reward, or a doctor illegaly sells tissues to a company. Serious loopholes in the law exist concerning international organ tourism, cross-border trafficking, organized crime syndicates or insurance agencies facilitating tourism. Many countries fail to take legal action against these acts, and those involved in criminal activities are seldom brought to trial. This presentation gives an overview of European countries' legislation and its effectiveness to curb commercialization of organs.

O-71 INFERIOR GRAFT SURVIVAL IN PATIENTS TRANSPLANTED OVERSEAS: A SUMMARY OF UK DATA

Antonia J. Cronin, Rachel Johnson, Robert Lechler. *Institute of Medicine Law and Bioethics, School of Law, University of Manchester, Manchester, United Kingdom; Audit and Statistics Directorate, NHS Blood and Transplant, Bristol, United Kingdom; Department of Transplant Immunology, MRC Centre for Transplantation, King's College London, London, United Kingdom*

Background: The shortfall of organs available for transplantation has prompted individuals to source organs from overseas. We report a summary of the information held on the national transplant database about UK residents who have travelled overseas to receive a kidney transplant and returned to the UK for post-transplant follow-up.
Method: Follow-up data were obtained on 144 living donor and 15 deceased donor transplants undertaken overseas between 1 January 2000 and 12 March 2007. A further 11 transplants from donors of unspecified type were included.
Results: Overseas transplants took place predominantly in India (22%) and Pakistan (51%). Transplants were predominantly from living unrelated donors (57%). Of those transplants from living related donors (28%) the majority were recorded as cousins. Transplant recipients were predominantly male (48%) and of Asian ethnicity (68%). The mean age of kidney transplant recipients whose transplant was carried out overseas was 44.8 years (range 15-83 years, n=170) compared with 42.7 years (range 1-85 years, n=13101) for all UK transplant recipients over the same time period. The median follow-up time was 4 years and 5 months.
Three-year Kaplan-Meier survival curves for living donor kidney transplants performed overseas and in the UK between 1 January 2000 and 1 January 2006 are illustrated in Figure 1. For those patients who return and are reported, there was no significant difference in terms of three-year patient survival compared with patients transplanted in the UK. However graft survival of those patients transplanted overseas was significantly inferior ($p<0.01$).



* Conditional on survival to one-year

Figure 1. Survival after living donor kidney transplant overseas, conditional on survival to one year post-transplant, 2001–2006.

Discussion and conclusions: The data we have presented is limited, however preliminary outcome analyses demonstrate inferior graft outcome in those patients transplanted overseas. The paucity of information available makes meaningful analyses difficult. All transplant units should be encouraged to provide data on such transplants to assist future national analyses.

O-72 ATTITUDES OF HEALTHCARE PROFESSIONALS AND PATIENTS TOWARDS NON-DIRECTED DONATION (NDD) AND COMMERCIALISATION OF LIVE DONOR KIDNEY TRANSPLANTATION (LDKT)

Evangelos M. Mazaris, Anthony W. Warrens, Glenn Smith, Paris Tekkis, Vassilios E. Papalois. *Kidney and Transplant Institute, Imperial College, Hammersmith Hospital, London, United Kingdom*

Purpose: We surveyed the views of medical and nursing staff involved in the care of patients with end-stage renal failure and of patients on dialysis, kidney transplant (deceased or live donor) recipients and live kidney donors regarding the acceptability of NDD (between strangers) and commercialisation of LDKT.
Material & methods: The participants were recruited in a tertiary renal and transplant centre and were invited to complete an anonymous questionnaire. They were then involved into focus groups and semi-structured interviews were conducted.
Results: 464 participants completed the questionnaire (36% healthcare professionals and 64% patients). Overall, live related, unrelated and NDD were considered acceptable by 92% (78% for child to parent donation), 87% and 58% of participants respectively. 15% of participants were willing to donate a kidney to a stranger and 54% to accept a kidney from a stranger. For live related and unrelated LDKT, participants thought that the donor should: have no financial reward (29%), be compensated for expenses only (61%), receive a direct financial reward (10%). For NDD, 23%, 56% and 21% were in support of no reward, compensation only and direct financial reward respectively. In the structured interviews, most participants expressed concern regarding the motives and psychological status of non-directed donors. Most participants rejected the idea that direct financial rewards will boost LDKT but believed that donor's reimbursement for travel, accommodation and loss of work hours is paramount. The few participants who were prepared to accept direct financial rewards for the donors stated that this should not be done in the open market but under direct government control.
Conclusions: Healthcare professionals and patients become more supportive of the concept of NDD (albeit with some scepticism) and overall they reject commercialisation of LDKT.

O-73 TRANSPLANT TOURISM FROM THE NETHERLANDS

Frederike Ambagtsheer[1], Willem Weimar[1], Willij Zuidema[1], Mike Bos[2]. *[1]Internal Medicine–Kidney Transplant Unit, Erasmus Medical Centre, Rotterdam, Netherlands; [2]Health Council of the Netherlands, Ministry of Health, The Hague, Netherlands*

Purpose: Recently, increased attention is given to transplant tourism. Commercial transplant tourism is a phenomenon in which patients travel abroad to undergo paid organ transplants. This study focuses on transplant tourism from the Netherlands by drawing upon data gathered through an empirical research conducted for the Dutch National Rapporteur on Trafficking in Persons and under supervision of the programme 'Criminology' of a Dutch University.
Methods: For this research, twenty interviews were conducted with nephrologists, transplant coordinators, other medical professionals and kidney patients. The aim was to gather information about the scale, nature, and development of transplant tourism from the Netherlands. The scope was narrowed down to kidneys.
Results: The study found that all Dutch transplant centres are confronted with transplant tourism. The number of reported cases per centre ranges from two patients a year to less than five over three decades. The estimated total number for the Netherlands is four per year. This number may not be accurate as many cases of tourism will not be known to transplant centres.
An important finding is that ethical and legal considerations affect the way in which physicians deal with transplant tourism. Most physicians disapprove of commercial tourism and thus do not assist patients with obtaining transplants abroad. Other physicians assist patients with facilitating a transplant overseas by arguing that the country of origin does not prohibit paid donations.
Conclusion: Transplant tourism creates ethical and legal dilemmas for health professionals. The varying ways in which physicians deal with transplant tourism raises the need for a uniform approach. Also, the guarded attitude of physicians and patients leads to a lack of accurate information. Transplant tourism therefore warrants closer examination. It is recommended that transplant tourism cases be registered in order to establish a more reliable estimate of this phenomenon.

O-74 LIVE DONOR KIDNEY TRANSPLANTATION (LDKT): ATTITUDES TOWARDS DONOR APPROACH, MOTIVES AND FACTORS PROMOTING DONATION

Evangelos M. Mazaris, Anthony W. Warrens, Glenn Smith, Paris Tekkis, Vassilios E. Papalois. *Kidney and Transplant Institute, Imperial College, Hammersmith Hospital, London, United Kingdom*

Purpose: We surveyed the views of medical and nursing staff involved in the care of patients with end-stage renal failure and of patients on dialysis, kidney transplant (deceased or live donor) recipients and live kidney donors regarding the issues of the initial approach of the donor, motives for donation and factors promoting LDKT.
Material & methods: The participants were recruited in a tertiary renal and transplant centre and were invited to complete an anonymous questionnaire. They were then involved into focus groups and semi-structured interviews were conducted.
Results: 464 participants completed the questionnaire (36% healthcare professionals and 64% patients). Participants stated that the first approach to a potential donor should be made by the recipient (27%) or the transplant team (25%). Participants believed that the most important motives of a donor are relief from the recipient's improved health (83%) and altruism (80%). 89% of participants believed that proper long-term follow-up of the donor is the most important factor that can promote LDKT. In the semi-structured interviews, most healthcare professionals advocated that the recipient should make the initial approach to the donor since 'this is the most honest approach and he needs the kidney'. Patients believed that the transplant team should make this approach since they may provide better information regarding potential risks. Healthcare professionals stated that pressure towards the donor from the re-

cipient and his family plays a more important than usually admitted role in LDKT.
Conclusions: Participants put the patients and secondly the transplant teams in the centre of the process of approaching the donor and promoting LDKT. Altruism is always the main driving force for the donors who, however, frequently have a lot to gain from the recipient's improved health.

O-75 FOLLOW-UP STUDY AMONG SAMARITAN DONORS: MOTIVATION TO DONATE

Willij Zuidema[1], Emma K. Massey[1], Leonieke W. Kranenburg[2], Gert Hak[3], Ruud A.M. Erdman[2], Medard T. Hilhorst[4], Jan N.M. Ijzermans[3], Willem Weimar[1]. *[1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands; [2]Medical Psychology and Psychotherpy, Erasmus MC, Rotterdam, Netherlands; [3]General Surgery, Erasmus MC, Rotterdam, Netherlands; [4]Medical Ethics, Erasmus MC, Rotterdam, Netherlands*

Introduction: In Rotterdam we run a living donation programme with altruistic kidney donors.
We interviewed 24 Samaritan donors after donation in an attempt to evaluate the mainly anonymous donation procedure and look in more detail at the motivation of these Samaritans.
Methods: We invited 24 Samaritan donors to participate in a follow up study. An independent nurse asked in a face-to-face interview questions about anonymity, allocation of the kidney and motivation to donate. Twenty-four donors, 11 male, 13 female were interviewed, median follow-up time after donation 18 (range 3-97) months. 4/24 donated directed to a (known to them) not emotionally related patient, 12 donated anonymously in a domino-paired procedure and 8 to a patient on the wait list.
Results: Most donors indicated that helping others fitted in their way of life, as reflected by other altruistic deeds e.g. blood/bone marrow donation, and voluntary social work. Half of the donors had experience with serious ill patients in their direct environment. In some, donation functioned as a symbolic act in commemoration of deceased loved ones.
Media attention on organ shortage triggered 40% of the actual donor to contact the transplantation team. Uncertainty about their possibility of organ donation after death was mentioned by 8/24.
While our programme is based on anonymity, we nevertheless asked whether non-directed donors would be anxious to meet the recipient after the procedure. Only 4/20 answered yes. None regretted their decision to donate.
Conclusions: Samaritan donors appear to be driven by real altruistic motives and personal experience with illness. They considered kidney donation as a natural act of loyalty to this patient group, consistent with their social behavior in other domains.

O-76 WHAT THE CHILD THINKS; CHILDREN'S OPINIONS ON ORGAN DONATION

Marion J. Siebelink[1], H.B.M. van de Wiel[2], M.J.I.J. Albers[3], P.F. Roodbol[2]. *[1]Management Staff, UMC, Groningen, Netherlands; [2]Wenckebach Institute, UMC, Groningen, Netherlands; [3]Beatrix Children's Hospital, UMC, Groningen, Netherlands*

Introduction: Decisions of parents to donate their child's organs are influenced by many factors. One factors is the earlier expressed opinion of the child towards donation. Many parents do not have any idea what their children's wishes are in respect to donation.
The goal of this study is to gain insight into the opinions of children toward donation and how these develop with age. Main research questions: their familiarity with the topic, their willingness to donate and their involvement into the decisions. We also investigated their opinions on whether organ donation is a topic for family discussion and school education. We clarified the relations with demographic variables gender, age, religion and socio-economic status.
Methods: Explorative study into the opinions of children from 8 till 16 years old. An online survey was completed by 2637 children. This large survey is a representative sample of the Dutch population.
Results: Dutch children appear familiar with the topic organ donation, increasing with age. Of the respondents 62% want to become a donor. There is a significant positive correlation between age and willingness to donate. Also religion and socio-economic status influence the willingness. Relation between gender and willingness was not found.
The majority of the children prefer to make their own decisions towards donation. More than half of the children never or only once discussed the topic at home.
The children are interested in more information; they would like to have lessons devoted to donation in school.
Conclusion: Young children are able to think about donation. Children who refuse donation are able to express their considerations.
Most families do not communicate freely about donation. School based education programs can also contribute to the child's opinions. Both will be helpful to start a guided discussion about donation at home.

O-77 UK RELIGIOUS LEADERS UNITED IN THEIR SUPPORT FOR ORGAN DONATION – THE FINDINGS FROM THE ORGAN DONATION TASKFORCE

Gurch Randhawa. *Institute for Health Research, University of Bedfordshire, Luton, United Kingdom*

Background: UK transplant waiting lists and organ donor lists highlight significant disparities between ethnic groups. Empirical studies have shown that cultural issues are important influencing factors when making a decision about organ donation. The influence of belief and faith systems is less clear.
Methods: The Organ Donation Taskforce consequently commissioned the author to gather views on the issue of organ donation from the different leading religious leaders in the UK.
Results: From the 17 interviews, it is clear that, while the majority of religious leaders interviewed tend to allow organ donation, diverse views exist not only between but also within these groups. A potentially significant finding is that religion per se is not described as a key influence on people's decision to opt in. Many of those interviewed felt strongly that the decision to donate is a personal choice for the individual to make.
There was widespread recognition of the extent of work required at grass roots level within their communities to encourage donation and a willingness to engage with the Government in this work. There was little prior awareness among the interviewees of the leaflets published some years ago setting out the views of some prominent faiths on organ donation. This suggests that written leaflets alone may be ineffective and that other, more direct, methods of engagement need to be found.
Conclusion: There are a lot of misconceptions surrounding the views of religious groups in relation to organ donation. This study demonstrates that Organ Donation is universally supported by religious leaders in the UK. If this support is to become widespread, then a national and local level communications strategy is essential which promotes debate concerning organ donation in all religious communities.

O-78 THE "BLOOD GROUP 0 PROBLEM" IN RENAL TRANSPLANTATION – TIME TO CHANGE?

Petra Glander[1], Danilo Schmidt[1], Florian Füller[2], Markus Giessing[3], Klemens Budde[1], Hans H. Neumayer[1], Lutz Liefeldt[1]. *[1]Nephrology, Charite Universitätsmedizin, Berlin, Germany; [2]Urology, Charite Universitätsmedizin, Berlin, Germany; [3]Urology, Universitätsklinikum Düsseldorf, Düsseldorf, Germany*

Background: In recent years in renal transplantation the so called "blood group 0 problem" has emerged. This is presumably due to disadvantages of these patients in the allocation of deceased donor organs as well as to the lack of an AB0-compatible living donor. In order to assess the importance of this problem we have analysed data of our transplantation program.
Methods: We have performed a single centre analysis of median waiting times on a first deceased donor kidney graft between 1996 and 2007 and analysed the mechanisms of blood group dependant differences. In a second step we have assessed the consequences of these differences with respect to graft and recipient outcome.
Results: ESRD patients with blood group 0 have significantly longer waiting times on deceased donor kidney grafts as compared with non-0-recipients (0: 80.9 (2.6-239), A: 70.9 (0.1-140), B: 54.2 (2.9-173), AB: 36.9 (0-84.7) months; $p<0.00001$). The longer waiting times increase their risk of removal from the waiting list without transplantation (13.8% for 0-patients vs. 8.9% for non-0-patients; p=0.019) and lead to a worse survival of ESRD-patients with blood-type 0 after initiation of renal replacement therapy (p=0.13) despite a significantly lower HLA mismatch rate (p=0.001) and a full match allocation rate which is most frequently in 0-recipients. One mechanism for this imbalance is the fact that 0-recipients in our transplantation program developed a significant "deficit" by "loosing" 11.76% (95% CI, 5.97-17.55%) of all 0-kidneys from deceased donors to non-0-recipients.
Conclusion: Allocation practice in the future should take the "blood group 0 problem" into account and should not further aggravate this imbalance. Political decision making should focus on an increase in post mortem organ donation as well as the enabling of living donation kidney transplantation e.g. by living donor kidney exchange programs.

O-79 INTENSIVE PHYSICAL EXERCISE AFTER ORGAN TRANSPLANTATION

Diethard Monbaliu, Glenn Vanhelleputte, Pieter Dauwen, Pieter Gillard, Bert Bammens, Tom Cornelis, Sigmund Keuleers, Peter Hespel, Rik Gosselinck, Jacques Pirenne. *University Hospitals Leuven, Catholic University, Leuven, Belgium*

Background: Transplantation (Tx) now offers many patients suffering from end-stage-organ failure long-term survival. Therefore, quality of life, full rehabilitation to a (near-to) normal lifestyle and prevention of cardiovascular disease

become prominent concerns in Tx-recipients whose health status may benefit from regular physical activity. The short-term aim of this pilotproject was to determine whether stable Tx-recipients could safely and efficiently follow intensive exercise programs eventually allowing them to climb (as final fitness exercise test) one of the most challenging bicyclerides: MontVentoux (altitude 2000m, 7-10% slope). The long-term aims were (i) raising donor awareness by showing how well Tx-recipients are doing; (ii) raising awareness among Tx-recipients on the importance of physical exercise.
Methods: 12 Tx-recipients (2 females/10 males; median age 47yo; 3 liverTx, 6 kidneyTx, 1 heart/lungTx, 1 pancreasTx, 1 smallbowelTx) with stable graftfunction, >2 years post-Tx, without rejection or cardiovascular disease were selected. They participated in a rigorous individualized trainingscheme supervised by exercise physiotherapists and Tx-physicians during 6 months. Physical condition was assessed by measuring exercise capacity (aerobic/anaerobic threshold, maximal power output) and peak oxygenintake (VO_2max) before starting en ending training. 6 accompanying healthy volunteers (matched for sex/BMI) served as controls.
Results: 3 Tx-recipients didn't complete their training (reasons unrelated to the exercise program). In the remaining 9 recipients, exercise capacity had increased substantially (median15% IQR 3-28%), similar to the healthy volunteers (median 9%, IQR 6-24%). Eventually, these 9 Tx-recipients summited MontVentoux after 129' similar to controls (average 138').
Conclusion: This project demonstrates that *selected* Tx-recipients i) are capable of participating in intensive exercise programs, ii) respond to these programs similar to controls and iii) are capable -after completion of these programs- to perform strenuous physical activities. This type of project raises awareness on the benefit of organ donation and stimulates physical activity among Tx-recipients.

O-80 QUALITY OF LIFE AFTER LIVER TRANSPLANTATION IN ESTABLISHED AND INDIVIDUAL SCALES

Martin Schenk, Kerstin Landgrebe, Christian Thiel, Karolin Thiel, Wolfgang Steurer, Alfred Koenigsrainer. *Klinik fuer Allgemeine, Viszeral- und Transplantationschirurgie, Universitaetsklinikum Tuebingen, Tuebingen, Germany*

The individual improvement of life quality after liver transplantation is different. The fixed scales of an SF-36 questionnaire were compared to the results of the SEIQoL questionnaire with individual scales.
In an interview style 71 patients (77% male, median of age 54 years, 21 months from transplantation) were asked to complete the SF-36 and the SEIQoL questionnaire in a cross-section design after liver transplantation (Follow-up: 0.5-159 month, Median: 21 month). The scales were calculated and compared to the published results of a normal population. 26 scales were set up, 5 scales were mentioned more than any other (family, friends, sports, partner and job). People with a high priority for the job reported a significantly lower quality of life (65 SD19) than the others (79 SD12).
It became clear that in the SF-36 scores the total life quality of our transplant patients (sum score) was excellent an directly comparable to a normal population. The transplant recipients only were lower in two scales directly linked to physical strength. In the SEIQoL questionnaire resulted in 72 (SD17) points compared to 77 (SD10) in the normal population.
Comparing the age-dependency in three groups (A:≤ 40 y; B:40- 60 y; C:≥ 60 y) it became obvious that the results of the SF-36 resulted comparable in all three groups (75 SD15, 72 SD17, and 76 SD17), but raised in the more individual scores (A: 66 SD21, B: 71 SD17 and C: 80 SD13 points.)
Beside many more details the results of this study can be used for interviews with transplants candidates. The complete analysis of the former data can make this large change a little bit smaller and shows more or less helpful items in the personal transplant preparation.

Session 11. Viral infections & tuberculosis in kidney transplantation

O-81 IMPACT OF CONCURRENT HHV-6 AND HHV-7 INFECTION ON CHRONIC ALLOGRAFT NEPHROPATHY DEVELOPMENT

Inese Folkmane[1,2], Svetlana Chapenko[1], Ieva Ziedina[2], Maxim Chistyakovs[1], Rafail Rozental[2], Modra Murovska[1]. [1]*The Transplantology Laboratory, Riga Stradins University, Riga, Latvia;* [2]*August Kirchenstein Institute of Microbiology and Virology, Riga Stradins University, Riga, Latvia*

Background: The long-term effect of HHV-6 and HHV-7 infections on chronic allograft nephropathy (CAN) development after renal transplantation is still uncertain.
Objectives: To determine HHV-6 and HHV-7 infection alone and concurrent viral activation during the post-transplantation period and to evaluate the possible effects of the activation of these viruses on CAN development in renal transplant patients.
Study design: 81 renal allograft recipients (28 with CAN, 53 with – normal transplant function) were studied on frequency of HHV-6 and HHV-7 reactivation during 36.4±7.8 months after renal transplantation using nPCR. Maintenance therapy consisted of oral Cyclosporine A, steroids and mycophenolate mofetil. Almost all patients had received induction with monoclonal IL-2R antibodies. AR episodes were treated with methylprednisolone or with antithymocyte globulin. All patients with high and moderate risk for CMV disease had received ganciclovir. *Nested polymerase chain reaction* was used for the detection of viral sequences in DNA isolated from peripheral blood leukocytes and plasma (markers of latent/persistent and active infection, respectively). HHV-6 variants were identified using restriction endonuclease analysis. Time dependent co-varieties of viruses' activation and CAN development were analyzed.
Results: The frequency of HHV-6 and/or HHV-7 reactivation was significantly higher in CAN patients (25/28, 89.3%) in comparison with control group patients (13/48, 27.1%, p=0.0001) as well as the frequency of simultaneous both viruses activation (20/25, 80% and 2/13, 15.4%, p=0.0003, respectively). In all 50 HHV-6 positive cases, HHV-6B variant was identified. HHV-7 activation precedes HHV-6 activation. Shortened period of CAN development and grafts loss was detected only in patients with simultaneous HHV-6 and HHV-7 activation.
Conclusions: Reactivation of HHV-6 and HHV-7 in renal graft recipients is the risk factor for CAN development. The presence of concurrent HHV-6 and HHV-7 infection is an unfavourable prognostic factor for CAN development.

O-82 CMV MISMATCH DOES NOT AFFECT PATIENT AND ALLOGRAFT SURVIVAL IN UK RENAL TRANSPLANT RECIPIENTS

Menna R. Clatworthy[1], Rachel Johnson[2], Rhiannon Birch[2], Abdul Hammed[3], J. Andrew Bradley[1]. [1]*Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom;* [2]*Kidney Advisory Group, NHS Blood & Transplant, Bristol, United Kingdom;* [3]*Surgery, Royal Liverpool University Hospital, Liverpool, United Kingdom*

Background: Cytomegalovirus (CMV) is one of the major infections encountered post-transplantation. UK Guidelines (2003) recommend CMV prophylaxis or screening with pre-emptive treatment for all high risk recipients. Studies pre-dating the widespread use of CMV prophylaxis, have shown that CMV seronegative recipients (R-) receiving a renal allograft from a CMV seropositive donor (D+) have worse outcomes than those avoiding primary CMV infection. It has therefore been suggested that CMV matching should be part of the UK national deceased donor kidney allocation scheme.
Methods: We examined patient and allograft survival according to donor and recipient CMV serostatus in 10,190 UK adult and paediatric deceased donor renal transplant recipients transplanted between, 2000 and 2007. We also ascertained CMV prophylaxis strategies in all UK renal transplant units.
Results: 21 of the 22 UK renal transplant centres used prophylactic oral valganciclovir for 3 months post-transplant in the D+R- transplants, having done so for a median of 4 years. Unadjusted data showed that D+R+ rather than D+R- transplants had the lowest patient and allograft survival at 3 years post-transplant.

Unadjusted graft and patient survival after deceased donor kidney only transplants performed in the UK (2000 – 2007) according to CMV serostatus

CMV serostatus	% 5 year Patient Survival (95% CI)	% 5 year Graft survival (95% CI)
D-R-	91.6 (89.9-93.1)	84.7 (82.8-86.5)
D-R+	86.9 (84.5-88.9)	83.3 (81.0-85.3)
D+R-	89.0 (86.8-90.8)	82.3 (80.0-84.4)
D+R+	85.5 (83.3-87.5)	80.3 (78.2-82.3)
P value	<0.001	P=0.008

However, following adjustment for confounding variables there was no statistically significant difference in graft or patient survival between the CMV mismatch groups. Further analysis identified donor age as the major confounding factor explaining the significant effect seen in the unadjusted analysis.
Conclusions: This is the largest UK study to date examining the relationship between CMV seropairing and allograft and patient outcome in renal transplantation. The results suggest that in an era where CMV prophylaxis is routinely used in D+R- transplants, the previously noted adverse effects of primary CMV infection on allograft and patient survival can be avoided (perhaps through a reduction in the incidence and/or severity of primary CMV infection), without the use of a CMV- matching allocation scheme.

O-83 INTERACTION BETWEEN CMV/EBV VIRUSES AND DE NOVO COLONIC TUMOUR AFTER KIDNEY TRANSPLANTATION

Gian Luigi Adani[1], Umberto Baccarani[1], Dario Lorenzin[1], Patrizia Tulissi[2], Domenico Montanaro[2], Vittorio Bresadola[1]. *[1]Surgery & Transplantation, University Hospital, Udine, Italy; [2]Nephrology, Dialysis & Transplantation, University Hospital, Udine, Italy*

De novo post-transplant lymphoproliferative diseases and skin cancer has been shown to have an increased incidence in long-term surviving solid organ transplant recipients, however the association with colonic cancer is controversial. 55/553 renal transplant recipients developed 62 de novo tumours; 31 skin and 31 non-skin cancers. 7/31 patients with non-skin cancer developed de novo colonic tumour. Within 1530 days from the diagnosis, Calcineurin inhibitor was progressively stopped, and Rapamicine was introduced. The tumour was resected with curative intent in six cases, while one patient had only palliative surgery because of metastatic disease. All patients maintained normal graft function. Six colon cancers were with Dukes C, whereas in one case an intramucous adenocarcinoma was found. Only the patient with metastatic colon tumour underwent a cycle of post-operative chemotherapy. However, 3/7 patients died of progression of the neoplasm. Further, we investigated a possible correlations between de novo colonic cancer and viruses as HCV, HBV status, infections, cytomegalovirus (CMV) and EpsteinBarr virus (EBV) reactivation. We even considered pretransplant renal disease, episodes of acute/chronic rejection, and blood transfusions. All cases of de novo GI cancer had early CMV and EBV reactivation (within three months after kidney transplantation). Pre-transplant serology for EBV and CMV was IgG positive for both donors and recipients. Recently CMV was isolated from cell cultures derived from surgical specimens of adenocarcinoma of the colon. EBV play an oncogenic role in epithelial cancers and certain dysplasias preceding carcinomas. Although only seven cases of colonic de novo tumours have been analyzed, all patients had early CMV and EBV reactivation after KTX. Thereafter we suggest a possible role of CMV prophylaxis in high risk patients, and a closer follow-up for de novo GI cancer in renal transplants with early CMV and EBV reactivation.

O-84 TUBERCULOSIS AFTER KIDNEY TRANSPLANTATION; SINGLE CENTER EXPERIENCE IN ENDEMIC AREA

Dong Jin Joo[1,2], Soo Jin Kim[1,2], Kyung Ock Jeon[2], Jong Hoon Lee[3], Kiil Park[3], Jinsub Choi[1,2], Soon Il Kim[1,2], Yu Seun Kim[1,2]. *[1]Surgery, Yonsei University College of Medicine, Seoul, Korea; [2]The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea; [3]Surgery, Kwandong University Myongji Hospital, Goyang, Korea*

Purpose: Mycobacterium tuberculosis (TB) infection is a one of the common infections in transplant recipients. This report summarized the clinical characteristics, risk factors and effect on graft/patient survival of post-transplant tuberculosis infection of a single center in Korea.
Patients and methods: Two thousand and seven hundred ninety nine kidney recipients in Yonsei University Health System from April 1979 to August 2008 were analyzed retrospectively.
Results: TB was diagnosed in 144 (109 males and 35 females) among 2799 patients (5.1%). Newly developed TB was found in 116 (81%) cases and 28 (19%) cases had a history of pre-transplant TB. The mean interval between transplant and TB diagnosis was 55±47.9 months (0~225 months). There were 45 extrapulmonary TB (31.2%) cases, which is relatively high when compared with the general population (12.2%). In Cox regression analysis, previous history of TB was the strongest risk factor (OR=20.377) of post-transplant TB infection. TB affected the graft survival and patient survival after kidney transplantation. Overall cure rate was 76.5% (104/144). But there were 5 reactivation cases (4.8%) in 104 cured patients. Among the 15 graft losses (10.4%) and 16 mortalities (11.1%), 20 cases (64.5%, 20/31) were TB-related graft losses and mortalities; 4 cases of immunosuppressive agent modulation failure, 9 cases of sepsis due to primary TB, 3 cases of central nervous system TB and 4 cases of acute graft failure. But there was no significant difference between extrapulmonary TB and pulmonary TB group (Fig. 1).
Conclusion: Taking consideration that the pre-transplant TB history was the strongest risk factor for post-transplant TB, a strategy on the prophylaxis or treatment of TB before transplant should be planned.

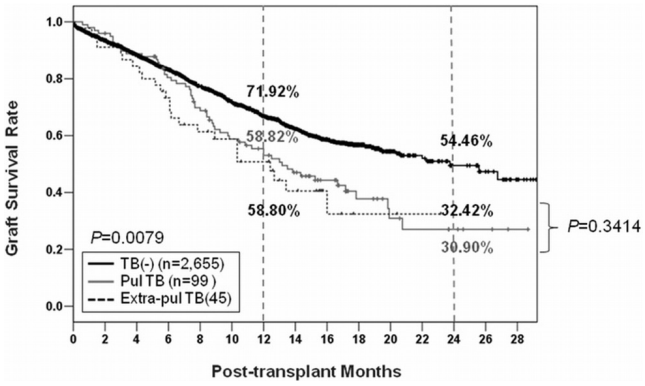


Figure 1. Graft survival rates of the non-tuberculosis group and the tuberculosis group (pulmonary type and extrapulmonary type). TB, tuberculosis; Pul, pulmonary; Extra-pul, extrapulmonary.

O-85 SEVERE NOSOCOMIAL TUBERCULOSIS IN A KIDNEY TRANSPLANT UNIT

Carlos Frangié[1], Cécile Thorette[2], Antonin Levy[1], François-Xavier Blanc[2], Nicolas Fortineau[3], Antoine Jacquet[1], Hélène François[1], Séverine Beaudreuil[1], Antoine Durrbach[1], Bernard Charpentier[1]. *[1]Néphrologie & Transplantation Rénale, CHU Bicêtre, Le Kremlin-Bicêtre, France; [2]Unité de Pneumologie–Médecine Interne, CHU Bicêtre, Le Kremlin-Bicêtre, France; [3]Service de Bactériologie, CHU Bicêtre, Le Kremlin-Bicêtre, France*

Background: Tuberculosis (TB) is a severe contagious disease in kidney transplant recipients. Symptoms could be non specific leading to a delayed diagnosis. Many risk factors have been described including immunosuppression, co-infections and chronic diseases. Clinical trials suggest that TB occurs frequently in the first year after transplantation, with a high prevalence and mortality rate. Morbidity is related to TB drugs interaction with immunosuppressive treatment.
Study: A kidney graft recipient was admitted 4 months after transplantation with cough, fever and inflammatory syndrome. Chest X-Ray was normal but CT-scan showed micronodules of the upper right lobe. Sputum was smear-negative but the first gastric aspiration showed acid fast bacilli 3+. The patient was isolated 4 days after his admission and started classic TB treatment. A classification of all contact subjects within the past 3 months was rapidly made, taking into account immunosuppression, duration of exposition and of transplantation. Screening included physical examination, chest X-Ray and PPD. INH chemoprophylaxis was given to all negative screened transplanted patients within six months. Overall, 516 patients had been exposed in the past 3 months. 278 patients had an insignificant risk. Among the remaining 238 patients, 11 patients died to an unrelated event, 36 patients were lost for follow up and 191 were screened: 8 patients revealed pulmonary TB within 3 to 6 months after exposition, with identical genotype profile of the TB strain in 6 of them; 183 patients were negative and 50 of them received INH chemoprophylaxis (none of them further developped TB). Death occurred in 2 TB and graft was lost in 2 others.
Conclusion: This nosocomial episode of pulmonary TB showed the extreme contagiousness of this disease when it occurs in a kidney transplant unit and illustrates the severity of the disease in such patients.

O-86 RENAL TRANSPLANTATION IN HIV INFECTED PATIENTS: THE FRENCH EXPERIENCE

Maxime Touzot[1], Evangeline Pillebout[1], Jean-Paul Viard[2], Eric Rondeau[3], Michal Delahousse[4], Christophe Legendre[5], Denis Glotz[1], Philippe Lang[6], Marie-Noelle Peraldi[1]. *[1]Service de Nephrologie et Transplantation, Hôpital Saint-Louis, Paris, France; [2]Service de Maladies Infectieuses, Hôpital Necker, Paris, France; [3]Service de Nephrologie, Hôpital Tenon, Paris, France; [4]Service de Nephrologie, Hôpital Foch, Suresnes, France; [5]Service de Transplantation, Hôpital Necker, Paris, France; [6]Service de Néphrologie, Hôpital Henri Mondor, Créteil, France*

Background: Previous study showed the safety of renal transplantation in HIV infected patients with however multiples complications such as high incidence of acute rejection, severe infection and interaction between immunosuppressive drug and antiretrovirale therapy HAART. We report here the first French multicentric retrospective study.
Methods: We undertook a retrospective analysis of 25 cases of renal transplantation in 5 teaching hospitals in Paris. Immunosuppressive therapy consisted of an induction by a anti-interleukine 2 receptor antibody or thymoglobuline and a maintenance therapy by steroid, mycophenolate mofetyl and a calcineurin inhibitor.
Results: After a median follow up of 19,3 months 6 -36 months), patient and renal survivals were 100%. Renal function defined by MDRD was 61,2 and 59,3ml/min/1,73m^2 at 12 and 24 months respectively, The incidence of acute rejection was 10%. The control group was defined by the patients who received the graft from the same donor. There was no statistical difference in GFR at 12 and 24 months and no difference in the incidence of acute rejection between the two groups. Excellent control of HIV replication by HAART was observed, Interaction between immunossuppresive drugs and HAART was the mainly complication in post-transplantation period.
Conclusion: Our results showed that under conventionnal immunosuppressive therapy, renal survival, graft function and incidence of acute rejection are

identical to those observed in the control group. Drug interacion can be managed by carefully pharmacological monitoring. Long term data are need to estimate the metabolic, cardiovascular and neoplasic complications.

O-87 INTRADERMAL INFLUENZA VACCINATION OF ADULT RENAL TRANSPLANT RECIPIENTS: RANDOMISED CONTROLLED PHASE 2 TRIAL

Emmanuel Morelon[1], Claire Pouteil-Noble[2], Sameh Daoud[1], Remi Cahen[2], Jean-Louis Touraine[1], Catherine Goujon-Henry[3], Françoise Weber[4], Pascal Laurent[5], Dominique Kaiserlian[6], Jean-Francois Nicolas[6]. *[1]Transplantation, Edouard Herriot Hospital, Lyon, France; [2]Service de Néphrologie-Transplantation, Lyon-Sud Hospital, Pierre Bénite, France; [3]URCI-LS, Hospices Civils de Lyon, Lyon, France; [4]Vaccination Department, Sanofi Pasteur, Marcy l'Etoile, France; [5]Beckton Dickinson, Beckton Dickinson, Lyon, France; [6]INSERM U 851, Université Claude Bernard Lyon1, Lyon, France*

Background: Influenza causes substantial morbidity and mortality, particularly in at-risk populations such as organ transplant recipients. Intradermal (ID) vaccination has been used as to overcome non-responsiveness to hepatitis B vaccine in transplant recipients. We investigated this strategy for the influenza immunisation of renal transplant recipients.
Methods: In Oct-Nov 2006, 201 renal transplant patients aged 18-60 from two transplantation units were vaccinated subcutaneously (SC) against influenza and screened to identify 80 who failed to develop seroprotective antibody titres against the 3 influenza vaccine strains. A year later (early 2007) selected patients were enrolled and randomised to receive trivalent inactivated influenza vaccine (Sanofi Pasteur, Lyon) either ID or SC. Vaccines contained 15g of haemagglutinin per strain per dose. ID injections were given using a microinjection system (BD Soluvia). Haemagglutination inhibiting antibody titres were assessed on day 0 and 21. The study was descriptive and not powered to detect statistically significant differences.
Results: The proportion of non-responders at screening was lower than expected (32 to 67/201 per strain). 62 non-responders to the most prevalent strain, influenza A (H3N2), were enrolled and randomised (31 per group). Seroprotection rates (% of vaccinees with titres 40) were 52% after ID and 36% after SC against A (H3N2). Against the 2 other strains, seroprotection rates were, respectively: 71% and 52% against A (H1N1), and 71% and 61% against influenza B.
Conclusion: This randomised, prospective study suggests that in renal transplant recipients on immunosuppressive therapy who are non-responders to classical vaccination, influenza vaccination using intradermal microinjection is more immunogenic than standard vaccination.

O-88 DETECTION OF VIRAL NUCLEIC ACIDS IN RENAL GRAFT PRESERVATION AND WASHING SOLUTIONS PREDICT THE OCCURRENCE OF POST-TRANSPLANT INFECTIONS

Luisa Murer[1], Elisa Benetti[1], Manuela Della Vella[1], Giovanni Franco Zanon[2], Piergiorgio Gamba[2], Giorgio Palù[3], Luisa Barzon[3]. *[1]Pediatric Nephrology, Dialysis and Transplant Unit, Department of Pediatrics, University of Padova, Padova, Italy; [2]Pediatric Surgery, Department of Pediatrics, University of Padova, Padova, Italy; [3]Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy*

Viral infections are serious complications in kidney transplant recipients and the graft may be a source of transmission. We investigated whether genome sequences of parvovirus B19, Epstein-Barr virus (EBV), cytomegalovirus (HCMV), or BK polyomavirus (BKV) could be detected in kidney graft biopsy, preservation and washing solutions (PS and WS) before implantation and whether they represented a risk factor for infections in the recipient. We analysed 75 renal unit samples from 74 cadaveric donors (mean age 14,8±8,2 years) by RT-PCR. Results were compared with pre-transplant D/R serological status and with the rate of occurence of post trasplant symptomatic or asymptomatic viral infections. 32% of the biopsies were B19 positive, whereas EBV, HCMV and BKV were detectable in about 1% of renal tissue samples. The prevalence of virus isolation from PSs and WSs was 26% and 30%, respectively, for B19 (concordance of about 65% with positive biopsies), 13% and 20% for EBV and 9% and 4% for HCMV. BKV was isolated in 3% of PSs (with negative biopsy). Viral DNA detection in the renal unit, especially in PSs and WSs, was more predictive of early post-transplant infections than D/R serological match. B19-DNA detection was a significant risk factor for infection in both seronegative and seropositive recipients, whereas EBV-DNA in donor grafts was associated with the risk of infection in seronegative recipients. Molecular testing for HCMV and BKV had less diagnostic utility. Overall, viral DNA was detected in 50/75 investigated grafts and sensitivity and specificity of the molecular test depended on viral tropism for resident and circulanting graft cells. The detection of viral sequences in renal unit solutions before implantation seems to be a useful non-invasive test to identify recipients at risk of post-transplant infections.

O-89 THE EFFECT OF PREOPERATIVE INTRAVESICAL AMIKACIN SOLUTION IN THE PROPHYLAXIS OF URINARY TRACT INFECTION AFTER RENAL TRANSPLANTATION

Mehdi Salehipour, Heshmatollah Salahi, Ali Bahador, Nikeghbalian Saman, Kourosh Kazemi, Farzad Kakaei, Ali FathiKaljahi, Seyed Ali Malek-hosseini. *Organ Transplantation Center, Shiraz University of Medical Sciences, Shiraz, Fars, Islamic Republic of Iran*

Urinary tract infection (UTI) is a major cause of morbidity and mortality in renal transplant recipients. The effect of intravesically applied antibiotic solution in the prevention of infectious complications of renal transplantation is still controversial. The aim of this study was to evaluate the efficacy of intravesical amikacin for the prophylaxis of the post transplant UTI within the first three months after kidney transplantation. In a prospective, randomized controlled trial, two hundred consecutive renal transplant recipients were randomly allocated to two 100 study groups. The bladder was filled preoperatively with saline solution containing amikacin (1 gr in adults or 30 mg/kg in children) in the test group, and with saline solution only in the controls. Patients were followed up for three months after the transplantation. Post operative urinary tract infection was defined based on a urine culture with a bacterial count of 100,000 CFU per mL of urine, or a positive nitrate test. Factors such as gender, age, the underlying kidney disease, receiving the first graft or subsequent retransplantation, and the source of graft (living-related, unrelated, or deceased donor) were analysed. The overall incidence of UTI was significantly lower in the test group (25% vs. 49%; p=0.0007). In addition, male patients, adults, first kidney graft recipients, patients with ESRD due to glomerulonephritis, having renal transplantation for the first time, or those patients from the test group receiving a living-related graft have significantly lower episodes of UTI than their control counterparts ($p<0.05$). Ecoli was the most common organism causing UTI (28.9%). In conclusion, the addition of amikacin to the bladder irrigation fluid could have significant effects on the overall incidence of UTI in the first three months after the kidney transplantation.

O-90 SCREENING AND EARLY TREATMENT OF BK VIREMIA IMPROVES THE OUTCOME AFTER KIDNEY TRANSPLANTATION

Amir Sedigh, Lars Wennberg, Gunnar Tydén, Jarmo Henriksson. *Dep of Transplantation Surgery, Karolinska University Hospital Huddinge, Stockholm, Sweden*

Purpose: Screening for BK viremia followed by early reduction of immunosuppression and, in selected cases, addition of leflunomid improves the outcome after kidney transplantation.
Methods/Materials: 47 patients (16 females and 31 males) transplantated (35 CD and 12 LD) in between November 2007 and September 2008. PCR analysed for BK virus in blood weekly in the first month, then monthly until 6 months and 9, 12 months post TX. In all patients with BK viremia (BKV PCR $>$ 10 000 copies/ml)immunosuppression was reduced by discontinuing AZA/MMF, reducing tacrolimus concentration to about 5 ng/ml and reducing prednisolon intake to 5-10 mg per day. If BKV was not eradicated (BKV PCR$<$10000) within four weeks leflunomid was added to the treatment regime by 20-40 mg daily (target blood concentration up to 40 ug/ml)
Results: Three patients (6%) died from non-transplant related causes. Seven patients (15%) were diagnosed with BK viremia (BKV PCR 50 000-1 000 000 copies/ml).Two of these patients were treated for acute cellular rejection prior to BK viremia. BKV was eradicated in all patients. In four of seven (57%) cases, this was by reduction of the immunosuppression only. In the remaining three, BK viremia was eradicated after the addition of leflunomid. Leflunomide was well tolerated in all patients. All patients diagnosed and treated for BK viremia still have well functioning kidney transplants (16 month). In one patient, acute rejection developed two weeks after the reduction of immunosuppression. However, this rejection could be treated successfully with Metylprednison.
Conclusion: Screening and early diagnosis of BK viremia followed by preemptive treatment prevents the development of BK virus associated nephropathy and graft loss. Addition of leflunomid with the studied regime was safe and efficient. After reduction of immunosuppression, patients should be monitored also for acute rejection.

O-91 POLYOMAVIRUS ASSOCIATED NEPHROPATHY TYPE B AND LATE ACUTE CELLULAR REJECTION GRADE 1: MORPHOLOGICALLY INDISTINGUISHABLE OR NOT?

Nadine Karremans[1], Maarten Naesens[2], Yves Vanrenterghem[2], Dirk R.J. Kuypers[2], Evelyne Lerut[1]. *[1]Morphology and Molecular Pathology, University Hospitals Leuven, Leuven, Belgium; [2]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium*

Purpose: Both polyomavirus associated nephropathy (PVAN) and late acute cellular rejection (LACR) are important contributors to renal allograft failure.

PVAN and LACR can have a very similar clinical presentations but require opposing therapies. As biopsies of both PVAN type B and LACR grade 1 show variable degrees of inflammation and tubulitis, we questioned whether composition/distribution of the inflammatory infiltrate are different in: 1) both entities, 2) in both entities when protocol and indication biopsies are compared (different pathogenesis in (sub)clinical events).
Methods/Materials: 24 patients underwent a late renal allograft biopsy (median 147 days, range 79-2710 days). Patient and transplantation characteristics were not different between PVAN and LACR groups.

Population

	n biopsies	n protocol biopsies	n indication biopsies	polyoma PCR blood	immunohistochemistry Large T Ag
PVAN	14	5	9	+	+
LACR	10	7	3	–	–

To characterize the infiltrates, immunohistochemistry was performed (CD3-CD4-CD8-CD27-FoxP3-CD20-CD79a-MUM1-CD56-TIA1-CD68). Additionally, HLA-DR stains were performed on all biopsies. The severity of the cell type was semiquantitatively scored (0-3) and distribution was scored as 'diffusely present' or 'present with formation of aggregates'. HLA-DR was scored as 'negative', 'focally positive' or 'positive' in the tubular epithelium.
Results: When all biopsies (protocol+indication) were compared, PVAN biopsies contained more CD79a+ (p=0.0415) and MUM1+ (p=0.0493) B cells. The infiltrate in LACR biopsies contained more CD8+ (p=0.0104) and CD4+ (p=0.0193) T cells.
When comparing only protocol biopsies between PVAN and LACR, a trend for more CD79a positivity in PVAN was seen (p=0.0549).
When comparing only indication biopsies, PVAN biopsies showed more aggregates of MUM1 (p=0.0455) and CD68+ (p=0.0182) inflammatory cells.
HLA-DR expression was not different between groups.
Conclusion: Besides PCR_{blood} and immunohistochemistry on the renal allograft biopsy, studying the severity/distribution of the inflammation might contribute in distinguishing PVAN and LACR. Higher numbers and a quantitative scoring system might reveal more pronounced differences between groups.

Session 12. How can we make the heart beating better?

O-92 RISK FACTORS FOR POST-TRANSPLANTATION MALIGNANCY IN HEART, LUNG AND HEART-LUNG TRANSPLANT RECIPIENTS REPORTED BY UNOS 1988-2006

Andrew Brett, Cara R. Baker, Mohammed Morsy, Iain MacPhee, Jiri Fronek, Nicos Kessaris. *Renal Unit, St George's Hospital, London, United Kingdom*

Aim: To investigate the risk factors for development of post-transplant malignancy in heart (H), lung (L) and heart-lung (HL) transplant recipients.
Methods: United Network for Organ Sharing and Organ Procurement and Transplantation Network data* as of 25/02/2008 was used. Age, gender, ethnicity, ABO match, history of pre-transplant malignancy, type of malignancy, years between transplant and cancer diagnosis and immunosuppressant used were analysed. Odds ratio (OR) or relative risk (RR) was calculated.
Results: Of 40903 heart (M=30839, F=10064, M/F=3.1), 15168 lung (M=7638, F=7530, M/F=1.0) and 934 heart-lung (M=397, F=537, M/F=0.7) transplant recipients, 17.1% H, 11.6% L and 10.6% HL developed malignancy post-transplantation. Malignancy was diagnosed within 3 years of transplantation in 20% H, 44% L and 32% HL recipients to develop cancer and after 6 years in 51% H, 24% L and 47% HL cases. Skin cancer was the most commonly diagnosed malignancy, in 31% H, 33% L, and 25% HL recipients to develop cancer.

Table 1. Odds Ratio or Relative Risk of post-transplant malignancy in heart, lung and heart-lung transplant recipients

Increased Risk Malignancy				Decreased Risk Malignancy			
Odds Ratio	Heart	Lung	Heart-Lung	Odds Ratio	Heart	Lung	Heart-Lung
Age ≥50y	2.7	1.8	1.9	Age ≥50y	0.4	0.6	0.5
Male	1.9	1.4	–	Male	–	-	0.7
Caucasian	3.4	2.5	1.6	Afrocarribean	0.3	0.4	0.1
Pre-Transplant Malignancy	1.3	1.3	–	ABO Compatible	0.8	0.7	0.3
Immunosuppressant Relative Risk				Immunosuppressant Relative Risk			
Cyclosporine	1.4	1.3	1.2	Tacrolimus	0.4	0.6	0.7
ATG	1.6	1.9	1.6	Sirolimus	0.6	0.4	–
OKT3	1.4	1.2	–	Mycophenolate	0.5	0.7	0.6
Daclizumab	1.1	1.1	–	Steroids	0.9	–	–
Steroids	–	1.9	1.3				
Azathioprine	1.8	1.6	1.3				

Age ≥50 years, Caucasian ethnicity, previous history of cancer, immunosuppression with cyclosporine, ATG, OKT3, dacluzimab, steroids (L, HL only) and azathioprine were associated with increased risk of cancer. Age < 50 years, Afrocarribean ethnicity, ABO compatible transplants, and immunosuppression with tacrolimus, sirolimus (H, L), and mycophenolate mofetil were associated with a reduced risk of cancer (Table 1).
Conclusion: Age ≥50 years, Caucasian ethnicity, certain immunosuppression medications and history of pre-transplant malignancy were risk factors for post-transplant malignancy in heart, lung and heart-lung transplant recipients from 1988-2006. The continuing development of malignancy greater than six years post transplantation has implications for the close follow up and screening of transplant recipients.
*Supported by Health Resources & Services Administration contract 234-2005-370011C. The content is the author's responsibility.

O-93 LONG-TERM RESULTS OF COMBINED HEART AND KIDNEY TRANSPLANTATION: A FRENCH MULTICENTER STUDY

Emmanuelle Vermes[1], Philippe Grimbert[2], Philippe Lang[2], Benoit Barrou[3], Jean François Obadia[4], Laurent Sebbag[4], Alain Pavie[5], Claire Pouteil-Noble[6], Mathias Kirsch[1]. *[1]Chirurgie Cardiaque, Chu Henri Mondor, Créteil, France, Metropolitan; [2]Néphrologie-Transplantation, Chu Henri Mondor, Créteil, France; [3]Urologie, CHU Pitié Salpétriere, Paris, France; [4]Chirurgie Cardiaque, Louis Pradel, Lyon, France; [5]Chirurgie Cardiaque, CHU Pitié Salpétriere, Paris, France; [6]Néphrologie, Hospice Civil de Lyon, Lyon, France*

Purpose: The purpose of the present analysis was to define long-term results of combined HK transplantation in a large French multicenter cohort.
Results: Between 1984 and 2007, 67 consecutive patients from 3 French centers underwent combined HK transplantation. There were 61 male and 6 female. All patients received immediate triple immunosuppression (anticalcineurine, steroids, azathioprine or mycophenolate), 38 patients (57%) were on dialysis at transplantation.Twelve patients (17.9%) showed evidence of significant cardiac allograft rejection needing treatment. Nine patients (13.4%) experienced kidney allograft rejection. Three patients (9.3%) developed an angiographic coronary artery vasculopathy (CAV). The actuarial survival rates at 1, 3, 5 and 10 years was at 62.0%; 60.3%; 53.3% and 46.5% respectively. Survival improved in the last 10 years experience: at 1, 3, and 5 years, survival rate was 71.1%, 67.5% and 60% respectively, very similar to those observed in the French isolated heart recipients during the same period (72.1%; 66% and 61% respectively). Kidney graft survival was 95.9% at 1, 3, 5 and 10 years.
Conclusion: Long-term survival in a large cohort of combined HK recipients is similar to those of isolated heart recipients in France. The rate of acute heart and kidney rejection and angiographic CAV seem low in this specific population.

O-94 DONOR CORONAROPATHY AND HEART TRANSPLANTATION: IS THERE ANY INFLUENCE ON OUTCOME?

Giorgio Guzzi[1], Massimo Maiani[1], Vincenzo Tursi[1], Fabio Ius[1], Enrico Spagna[1], Blanca Martinez[2], Maria Cecilia Albanese[1], Daniela Miani[1], Ugolino Livi[1]. *[1]CardioPulmonary Sciences, "S.Maria della Misericordia" University Hospital, Udine, Italy; [2]Anaesthesia and Intensive Care, "S.Maria della Misericordia" University Hospital, Udine, Italy*

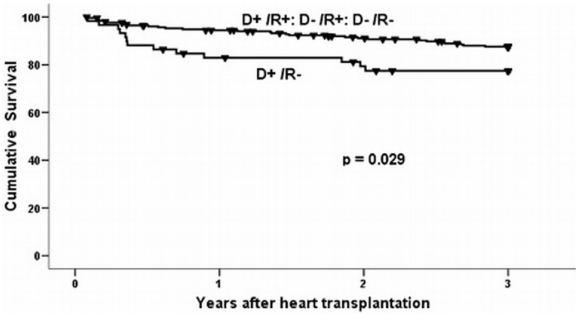
Objectives: Due to shortage of donors and their progressive aging, coronaropathy is a ever more common problem in donor heart and it may possibly affect outcome of heart transplantation (HTx). We investigate presence of coronaropathy and its influence on survival in our experience.
Materials and methods: Between January 1999 and February 2009, we performed 238 HTx in 237 patients (1 re-HTx for acute graft failure); among them, 73 donors hearts were investigated with cardiac catheterism before retrieval, being them >50 years or having risk factors for coronaropathy.
Fifty-three patients (group G0) were free from coronary pathology at time of harvesting, while 20 pts (group G1) were not; the two groups were similar in terms of recipient age [mean (median) 59 (60)±7 yrs vs 61 (61)±5 yrs] and sex (M/F 46/7 vs 18/2), donor age [53 (54)±8 yrs vs 55 (55)±7 yrs] and sex (30/23 vs 12/8), and ischemia time [196 (205)±67 min vs 184 (167)±59 min], G0 vs G1 respectively.
Routine cardiac catheterisms were performed at 1 and 3 years after HTx in search of coronary pathology stability/progression, if present at time of retrieval, or appearance, if not.
Results: No statistical significative difference was found between groups both in terms of survival at 1 year [G0 84.6% vs G1 92.9%] and 3 years [G0 77.2% vs G1 85.1%]; freedom from coronaropathy appearance in G0 group was 72.2% and 61.5%, while stability/progression of coronaropathy in G1 group was 92% and 92%, at the same time intervals, respectively.
Conclusions: In our experience, donor coronaropathy do not affect outcome of HTx, neither on survival nor on appearance and/or stability/progression of such a pathology, confirming our policy not to exclude these donors from HTx.

Further analysis and longer follow-up are needed to definitely validate these preliminary results

O-95 PRETRANSPLANT CYTOMEGALOVIRUS MISMATCH SEROLOGY (D+/R-) IS A RISK FACTOR FOR MORTALITY DURING THE FIRST THREE YEARS AFTER HEART TRANSPLANTATION

Entela Bollano[1], Bert Andersson[1], Bengt Rundqvist[1], Ulla Nyström[2], Vilborg Sigurdardottir[1], Folke Nilsson[2], Nedim Selimovic[1]. *[1]Dept. of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Transplant Center, Sahlgrenska University Hospital, Gothenburg, Sweden*

Purpose: The aim of this study was to evaluate association between cytomegalovirus (CMV) serology in donors and recipients regarding outcome after heart transplantation.
Methods: Retrospective analysis of all heart transplanted adult patients at Sahlgrenska University Hospital from January 1988 through December 2007. Risk factors tested were recipient age, sex, blood group, pretransplant CMV serology, allograft ischemic time, diagnosis, donor age, sex, blood group, and donor CMV serology. Primary outcome was mortality during the first 3 years after transplantation.
Results: During this period 362 adults underwent heart transplantation with a mean age at transplantation of 46±12 (mean ± SD), 79% were males. The diagnoses were: cardiomyopathy (n=226), coronary artery disease (n=110), retransplantation because of cardiac allograft vasculopathy (n=7), valvular disease (n=8) and others (n=11). The study population was divided into 2 groups according to donor and recipient serology at the time of transplantation [(D+/R+; D-/R+; D-/R- = low risk group) and D+/R- = high risk group]. During the first three years after transplantation died 84 patients (23%).
In multivariate analysis, including recipient age, donor age and CMV serology, only mismatch of pretranslant CMV serology (D+/R-) was independent predictor of mortality (HR 2.2; 95% CI 1.13-4.4; p = 0.02).



Conclusion: In adults, mismatch of pretransplant CMV serology is an independent predictor for early and midterm mortality after heart transplantation

O-96 NT-PROBNP ASSAY MAY PRECLUDE THE NEED FOR ROUTINE ENDOMYOCARDIAL BIOPSY TO DETECT CARDIAC ALLOGRAFT REJECTION, BUT NOT EARLY AFTER TRANSPLANTATION

Maria José Rebocho[1], Carlos Aguiar[2], Marta Marques[1], Miguel Abecasis[1], José Pedro Neves[1], João Figueira[3], Rosa Gouveia[4], João Melo[1]. *[1]Cardiothoracic Surgery, Hospital Santa Cruz, Carnaxide, Portugal; [2]Cardiology, Hospital Santa Cruz, Carnaxide, Portugal; [3]Biochemistry, Hospital Santa Cruz, Carnaxide, Portugal; [4]Pathology, Hospital Santa Cruz, Carnaxide, Portugal*

Background: Endomyocardial biopsy (EMB) remains the standard for diagnosis of rejection after heart transplantation (HTx). Very high levels of NT-proBNP are common in the first 3 months after HTx. The aim of this study was to determine whether the NT-proBNP assay may detect or exclude cardiac allograft rejection.
Methods: From Jan 2003 to Dec 2008, we performed 678 EMB in 81 HTx recipients. Immediately before EMB, venous blood was drawn to determine NT-proBNP. Clinically significant rejection was moderate-to-severe rejection or any rejection episode with accompanying hemodynamic compromise and requiring treatment. We related NT-proBNP levels with the presence or absence of clinically significant rejection, before and after 90 days post HTx, and identified the NT-proBNP level that more accurately predicted rejection.
Results: Due to insufficient biopsy specimen, histological diagnosis of rejection was impossible in 26 EMB. For the remaining 652 EMB, median time between HTx and EMB was 7 (IQR: 2.3; 21.0) months. We recorded 36 clinically significant rejections, of which 27 occurred >90 days after HTx. We found a strong interaction between time after HTx and the diagnostic accuracy of NT-proBNP (interaction test P<0.001): NT-proBNP predicted clinically significant rejection only after 90 days post-HTx. An NT-proBNP level > 1231 pg/mL (best discriminatory value) was associated with clinically significant rejection >90 days after HTx (area under ROC curve 0.79; 95% CI, 0.75-0.83). The criterion NT-proBNP > 1231 pg/mL, present in 92 of 446 EMB >90 days after HTx, showed 70% sensitivity, 83% specificity, 21% positive predictive value, and 98% negative predictive value for diagnosis of clinically significant rejection.
Conclusion: NT-proBNP levels relate with histological findings of EMB specimens and have a high negative predictive value for detection of clinically significant rejection after 90 days post-HTx.

O-97 STANDARD IN SURVEILLANCE ENDOMYOCARDIAL BIOSPY (EMB) PROTOCOL IN THE FIRST MONTH AFTER HEART TRANSPLANTATION: TIME FOR A REAPPRAISAL?

Antonio Gambino[1], Chiara D'Agostino[1], Gianluca Torregrossa[1], Giuseppe Toscano[1], Giusppe Feltrin[1], Francesco Tona[2], Alessandra Andreotti[3], Annalisa Angelini[4], Gaetano Thiene[4], Gino Gerosa[1]. *[1]Department of Cardiac, Thoracic and Vascular Sciences, Cardiac Surgery Unit, Padova, Italy; [2]Department of Cardiac, Thoracic and Vascular Sciences, Cardiology Unit, Padova, Italy; [3]CNR, Neuroscience, Padova, Italy; [4]Department of Medical DIagnostic Sciences and Special Therapies, Pathological Anatomy, Padova, Italy*

EMB is the gold standard for acute rejection diagnosis in Htx. We reviewed the rusults of weekly EMB's within the 1 month after HTx to validate effectivness in directing immunosoppressor therapy procedure related complications and costs per pts.We reviewed retrospectivly 500 consecutive adult pts who underwent HTx (1991- 2007), 87 female and 413 male,14 bridged to HTx on VAD's.Mean ischemic time was 160±50 min, 104 pts recieved heart from marginal donors. Induction therapy: steroid bolus alone in 38 pts (7,6%) or with: thymoglobuline (RATG) in 231 pts (46,2%) lymphoglobulines (ALG) in 208 pts (41,6%), OKT3 in 23 (4,6%).
Manteinance immunosuppresion: CSA+AZA± Steroids (STER) in 377 pts (83%); CSA or Tacrolimus and Everolimus or MMF± STER in the rest.
We plotted results of the first four EMB's according to ISHLT grading.EMB's greater than 3A were considered positive for acute rejection and treated with 3-day steroids bolus. Our data were analyzed with SAS statistical software.We have performed a Chi-squared (χ^2) test.
None of the positive presented hemodynamic failure.
EMB 1st week: 30 positive (6%); 6 inadequate (1,2%);
2nd week: 113 positive (24,5%); 19 inadequate (3,8%);
3rd week: 103 positive (20,6%); 32 inadequate (6,4%);
4th week: 101 positive (20,2%); 34 inadequate (6,8%).
EMB1 was negative in all RATG pts. Results of EMB1 are related to induction therapy (p=0.0043; α=0,05) independently from manteinance immunosoppression. There was a highly significant relation between usage of RATG and EMB1 (p<0.0001, α=0,05).
EMB1 is strongly dependent on type of induction therapy. EMB1 was negative in all pts treated with RATG. EMBs 2,3,4 are indeed influenced by maintenaince immunosuppression. We suggest that in RATG pts EMB1 can be safely avoided. We turned into a reduced surveillance protocol with EMBs performed weekly starting from the second week,implementing post operative managment of pts.

O98 PROGNOSTIC IMPACT OF NON-HLA ANTIBODIES TARGETING VASCULAR RECEPTORS FOR THE DEVELOPMENT OF MICROVASCULOPATHY IN BIOPSY AFTER HEART TRANSPLANTATION

Nicola E. Hiemann[1], Christiane Renner[1], Rudolf Meyer[1], Ernst Wellnhofer[2], Costanze Schoenemann[3], Heike Heidecke[4], Hans B. Lehmkuhl[1], Roland Hetzer[1], Duska Dragun[5]. *[1]Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Germany; [2]Cardiology, Deutsches Herzzentrum Berlin, Berlin, Germany; [3]Institute of Transfusion Medicine, Humboldt University Berlin Charité, Berlin, Germany; [4]Celltrend GmbH4, Celltrend GmbH4, Luckenwalde, Germany; [5]Nephrology and Intensive Care Medicine, Humboldt University Berlin Charité, Berlin, Germany*

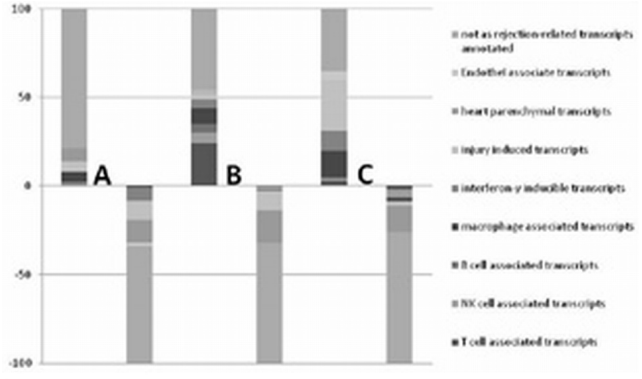
Aims: We tested the impact of non-HLA Abs and activating angiotensin II type 1 receptor (AT_1Rabs) on rejection and microvasculopathy (MVP) in heart transplant (HTx) recipients.
Methods: We studied prospectively 30 HTx pts (22 men, age 48 yrs) at 24 hrs, 2 weeks, 1 month, 6 months and 1 yr post HTx for presence of IgG directed against endothelin-1 type A (ET_AR-Abs) and angiotensin II type 1 (AT_1R-Abs) receptors (elevated level cut-off >10 U/L) by means of cell-ELISA. Endomyocardial biopsies were obtained at 1 month (Bx1=20) and 1 yr (Bx2=20). Conventional histology (H&E) served for diagnosis of acute cellular rejection [ISHLT] and MVP. Immunohistochemical reactions for alpha-actin were performed to identify smooth muscle cells (clone 1A4, Dako) in order to detect microvascular remodeling (media disease) associated with MVP.

Results: At 1 month and 1 yr post HTx, stenotic MVP was present in 37% and 40% of biopsies, respectively.
During the first year post HTx, 50% of pts presented elevated levels (>10 U/L) of ET_AR-Abs and 53% high levels of AT_1R-Abs. Pts with high AT_1R-Abs presented more often with acute cellular rejection than pts without (77% vs. 33%, p=0.06). Increased density of muscularized microvessels were found at 1 month post HTx in pts with high ET_AR-Abs (88% vs. 44%, p=0.06) and at 1 yr post HTx in pts with high AT_1R-Abs (80% vs. 27%, p=0.05). CRP pre-HTx was higher in pts with elevated ET_AR-Abs (3.9±0.9 vs. 0.9±0.3 mg/dL, p=0.01) or AT_1R-Abs (3.8±0.7 vs. 0.8±0.3 mg/dL, p=0.01), implicating putative permissive role of inflammation.
Conclusions: HTx recipients frequently develop non-HLA antibodies targeting ET_AR and AT_1R after transplantation. Elevated levels of ET_AR-Abs and AT_1R-Abs are associated with stronger alloimmune response and earlier onset or faster progression of microvascular remodeling post HTx.

O-99 CORRELATING MICROARRAY GENE EXPRESSION DATA WITH HISTOPATHOLOGICAL LESIONS IN HUMAN HEART ALLOGRAFT BIOPSIES

Michael H. Mengel[1], Jessica Chang[1], Daniel Kim[2], Banu Sis[1], Thomas Mueller[1], Jeffrey Burton[2], Wayne Tymchak[2], Philip F. Halloran[1].
[1]Alberta Transplant Apllied Genomics Centre, University of Alberta, Edmonton, AB, Canada; [2]Cardiology, University of Alberta, Edmonton, Canada

The histopathological criteria for assessing endomyocardial biopsies are empiric and arbitrary lacking external biological validation. Aim was to correlate gene expression with histopathological lesions based on ISHLT criteria in human heart allograft biopsies.
We used Affymetrix microarrays in 101 cardiac allograft biopsies from 45 patients to assess genome-wide changes in expression. Histological lesions were assessed following the ISHLT consensus criteria. Gene-based and get set-based expression data were correlated to histological lesions.
Gene sets reflecting the major biological processes during allograft rejection like T cell, B cell, macrophage infiltration, and injury responses of the myocardium showed strong correlations with each other indicating a stereotyped response during rejection: increased expression of inflammatory gene sets is correlated with signs of tissue injury. The extent of Quilty lesions (type A vs. B) positively correlated with the molecular inflammatory burden (T-,NK-, B cell, macrophage, interferon-γ transcripts; r=0.38-0.58, $p<0.001$) and inversely with the decreased expression of myocardium associated transcripts (metabolism and solute carrier transcripts (r= -0.28-0.44, $p<0.03$). Essentially no correlation was found between ISHLT grades of rejection and rejection associated gene sets. Of the top 100 transcripts correlating with ISHLT grade of acute rejection 80% were not annotated as rejection-related transcripts. In contrast, of the top 100 transcripts correlating with the former degrees of Quilty lesions (A/B) 24% were annotated as T cell, 9% as macrophage, 6% as NK cell associated, 5% as interferon-γ inducible transcripts, and 29% as myocytal transcripts.



A: Annotation of top 100 probe sets correlating positively and negatively with the ISHLT-grade of **acute cellular rejection**
B: Annotation of top 100 probe sets correlating positively and negatively with the extent of **Quilty lesions**
C: Annotation of top 100 probe sets correlating positively and negatively with the extent of **interstitial edema**

The assessment of genome-wide microarray data as an objective measurement of rejection-related changes in heart allograft tissue reveals Quilty type B lesions and interstitial edema but not ISHLT grades of rejection to be associated with molecular profiles of rejection and tissue injury.

O-100 ADVANCED GLYCATION END-PRODUCTS IN PATHOLOGY OF THE TRANSPLANTED HEART

Michael Zakliczynski, Jerzy Nozynski, Dominika Konecka-Mrowka, Marian Zembala. *Dept. of Cardiac Surgery & Transplantation, Silesian Center for Heart Disease, Zabrze, Poland*

Background: Abnormal glycemia in orthotopic heart transplant (OHT) recipients may accelerate a process of non-enzymatic conjugation of glucose in cardiac tissues, resulting in advanced glycation end-products (AGEs) formation. However role of AGEs in development of typical complications after OHT – acute rejection (AR) and coronary vasculopathy (CAV) – is unknown.
Aim of the study: To localize AGEs in late endomyocardial biopsies (EMBs) of OHT recipients in order to correlate their presence with AR and CAV in subjects with and without diabetes (DM).
Material and methods: Elective EMBs performed 3y post OHT in 65pts. with DM (60M/5F, 49±8y/o) and 24pts. without DM (21M/3F, 42±10y/o) were involved in the study. Localization of AGEs in myocardial paraffin sections was assessed immunochemically using mouse monoclonal anti-AGE antibodies (clone 6d12). The presence of AGEs in cardiomyocytes, stromal cells, connective tissue cells and capillaries was described with semi-quantitative scale.
Results: Occurrence of AGEs was similar in both groups – cardiocytes 74 vs. 63%, stroma 34 vs. 33%, connective tissue 14 vs. 9%, and capillaries 32 vs. 33% – in DM vs. non-DM pts., while intensity of staining was onsignificantly higher in DM subjects. Overall number of AR episodes and mean EMB score were significantly correlated with AGE presence in cardiomyocytes (r=0.31/0.25, p=0.011/0.049, Spearman test), but only in DM group. There was no any significant relation between AGEs occurrence and CAV diagnosis in DM pts., while time free from any angiographically confirmed CAV, and CAV related event was significantly longer in non-DM pts. with AGEs in cardiomyocytes and/or capillaries (p=0.017/0.014/0.03/0.014 respectively, log-rank test).
Conclusion: AGEs occurrence in DM subjects is related to AR, but do not predict CAV, while in non-DM patients it is note correlated with AR, but prolongs freedom from CAV.

O-101 PROTECTIVE EFFECTS OF N-ACETYLCYSTEINE AFTER EXPERIMENTAL HEART TRANSPLANTATION

Zhanqing Li[1], Arash Nickkholgh[1], Xue Yi[1], Helge Bruns[1], Marie-Luise Gross[2], Markus W. Büchler[1], Peter Schemmer[1]. *[1]Dept. of General, Visceral and Transplantation Surgery, Ruprecht-Karls-University, Heidelberg, Germany; [2]Dept. of Pathology, Ruprecht-Karls-University, Heidelberg, Germany*

Background: Heart transplantation is the most successful therapeutic option for individuals with end-stage cardiac disease. Although the results of transplantation have improved dramatically over the past few years, obstacles exist. Ischemia/reperfusion injury (IRI) plays a major role for the development of graft dysfunction following transplantation. N-acetylcysteine (NAC) protects heart from IRI after acute myocardial infarction. Thus, this study was designed to evaluate its effect on hearts after transplantation.
Methods: Lewis rats (n=20) were randomly divided into 2 groups of n=10 animals. Experimental groups were given NAC (300mg/kgBW; i.v.) 30 min before organ harvest. Controls were given the same volume of Ringer. Hearts were stored in 4°C HTK solution for 18 hrs before heterotopic transplantation. Blood was drawn at 6, 12, and 24 hrs post-reperfusion to analyze serum enzymes to index graft injury. Tissue samples were taken at 24 hrs after transplantation for histology and immunohistochemistry. Graft survival was determined by daily palpation of the heartbeat. Analysis of variance (ANOVA) or χ^2 (or Fisher's exact) test were used as appropriate. Results are presented as mean ± SEM.
Results: NAC significantly improved graft survival. Further, NAC markedly reduced serum levels of TnT, CK, CK-MB, LDH and transaminases at 6 and 12 hrs after reperfusion. Histology confirmed that NAC protects against heart injury after transplantation. Immunohistochemistry documented the significantly higher expression scores for eNOS (3.6±0.2 vs. 2±0.2) and significantly lower scores for MMP2 (2.1±0.2 vs. 3.8±0.1) and MMP9 (1.6±0.2 vs. 3±0.1) after NAC. TUNEL assay was significantly lower for NAC (20±7 vs. 155±37).
Conclusion: Administration of a single-dose NAC in donors before organ harvest decreases IRI after heart transplantation, most likely through antioxidative, anti-apoptotic mechanism.

O-102 ISCHEMIC TOLERANCE OF OLDER DONOR HEARTS IN A RAT TRANSPLANTATION MODEL

Gábor Szabó, Shivakannan Loganathan, Achim Koch, Matthias Karck. *Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany*

Background: Donor shortage led to an extension of donor pool including older donors. We investigated the ischemic tolerance of young and older donor hearts in a rat heart transplantation model.
Methods: Intraabdominal heterotopic transplantation was performed in Lewis

rats after one hour or eight hours of ischemic preservation. Donor hearts from young (3 months, n=12) and old donors (18 month, n=12) were assessed after one hour of reperfusion. Left ventricular pressure (LVP), its first derivative (dP/dt), end-diastolic pressure (LVEDP), coronary blood flow (CBF), endothelium-dependent vasodilatation to acetylcholine (ACH) and endothelium-independent vasodilatation to sodium nitroprusside (SNP).
Results: After one-hour presrvation, LVP (84±7 vs. 83±4 mmHg) and dP/dt (1679±313 vs. 1740±116 mmHg/s) CBF (3.01±0.28 vs. 2.86±0.35, ml/min/g) did not differ between the groups. However, ACH resulted in a significantly lower response of CBF in the old donor group (33±4 vs. 51±15%, $p<0.05$). After 8-hour preservation, two of the old donor hearts showed no mechanical actipity upon reperfusion. LVP (55±6 vs. 72±5 mmHg, $p<0.05$) dP/dt (899±221 vs. 1530±217 mmHg/s, $p<0.05$), CBF (1.05±0.16 vs. 2.39±0.28, ml/min/g, $p<0.05$) and response to ACH (21±11% vs. 44±4%, $p<0.05$) was significantly reduced in the old donor heart group in comparison to young controls. Response to SNP differ neither after one nor eight hours of reperfusion.
Conclusions: Thus, the ischemic tolerance of older donor hearts is reduced especially after longer preservation times, where as endothelium is more vulnerable then the myocardium. This should be taken into account in the allocation of older donor hearts.

Session 13. Infections in liver transplantation

O-103 WEANING OF HBV PROPHYLAXIS AFTER LIVER TRANSPLANTATION IS FEASIBLE IN THOSE PATIENTS TRANSPLANTED FOR HEPATITIS B CIRRHOSIS WITH UNDETECTABLE cccDNA IN GRAFT TISSUE

Lenci Ilaria, Mario Angelico, Luca Toti, Tommaso Maria Manzia, Manuele Berlanda, C.F. Perno, Daniele Di Paolo, Arianna Brega, Laura Tariciotti, Giuseppe Tisone. *U.O.C.Trapianti d'Organo, Tor Vergata University, Rome, Italy*

Background: The risk of HBV reactivation after liver transplantation is due to persistence of the viral genome as covalently closed circular (ccc) DNA. We investigated the feasibility of complete withdrawal of HBV prophylaxis in a cohort of transplanted patients considered at low risk of HBV recurrence.
Methods: We studied 30 patients (age 54±9 yrs) transplanted for HBV-related cirrhosis already found to have undetectable intrahepatic total and ccc-DNA. All were HBsAg positive and HBeAg negative and with undetectable HBV-DNA at transplant. The mean follow-up after transplant was 112 months (range=39-179). All patients underwent HBIg withdrawal and were initially continued on lamivudine, with quarterly monitoring of serum HBsAg and HBV-DNA. After 24 weeks from HBIg withdrawal, a further liver biopsy for intrahepatic total and cccDNA was obtained and serum HBV DNA was also investigated. Patients found to be negative for all these assays withdrew also lamivudine and were then monitored for additional 16-40 weeks, with monthly assays of serum HBsAg and HBV-DNA.
Results: One patient became HBsAg-positive during the first 24 weeks following HBIg withdrawal. HBIg were promptly reinstituted and the patient returned to be HBsAg negative, without displaying any biochemical, or virologic event. All other 29 patients had undetectable total and cccDNA in the liver biopsy obtained 24 weeks after HBIg withdrawal. All therefore underwent also lamivudine withdrawal. After a median follow-up of 24 weeks (range=16-40) all 29 patients continued to be negative for serological markers of HBV reinfection and serum HBV-DNA and no ALT flares occurred.
Conclusion: In this cohort of patients with undetectable HBV viremia at transplant and no evidence of intrahepatic cccDNA $\geq$3 years after transplant, cautious weaning HBV prophylaxis is feasible and safe.

O-104 OUTCOME OF LIVER TRANSPLANTATION IN HIV VERSUS NON-HIV PATIENTS

Umberto Baccarani, Gian Luigi Adani, Marcello Tavio, Dario Lorenzin, Angela Londero, Francesco Bragantini, Chiara Comuzzi, Anna Rossetto, Andrea Risaliti, Claudio Avellini, Federico Pea, Vittorio Bresadola, Pierluigi Viale. *Transplantation, Univeristy Hospital, Udine, Italy*

The aim of this study is to analyze LT in HIV+ patients compared with a matched HIV- case-control group. From 2004 to 2008, 27 HIV+ patients submitted to LT versus 23 HIV- patients matched for age (45, range 39-58 vs 50, range 37-57), gender, HCV (HCV+ 70% vs 61%) and HCC (40% vs 39%). Immunosuppression was based on tacrolimus in 75% and 74% and cyclosporine in 25% and 26% of cases in HIV+ and HIV- patients respectively (p=0.935). Results (HIV+ vs HIV-): the median waiting list time was 3 months for both groups (p=0.83). MELD was 17 (range 7-32) vs 17 (range 8-29) (p=0.80). Donor age was 46 (range 19-73) vs 55 (range 17-75) (p=0.45). Cold ischemia time was 457±96 vs 463±95 min (p=0.83), duration of transplantation was 389±84 vs 392±81 min (p=0.91) and units of blood transfused were 5, range 0-25 vs 3, range 0-28, p=0.44. The ICU and the total hospital were 5±3 versus 5±2 (p=0.45) and 18±9 versus 16±7 days (p=0.64). The median follow-up was 21 months (range 2-47) and 29 months (range 3-39 p=0.93). Biopsy-proven acute rejection was diagnosed in 2 (10%) and 5 (21%) cases (p=0.298). The estimated 1, 2 and 4 years patients and grafts survival were respectively 90%, 82.5% and 82.5% for HIV+ versus 100%, 94% and 79% for HIV- (p=0.64), and 95%, 87% and 87% for HIV+ versus 95%, 89% and 82% for HIV- (p=0.89). Regarding HCV recurrence the median grading and staging score, at the latest liver biopsy, were respectively 3 (range 2-6) versus 3 (range 1-5), (p=0.201) and 1 (range 0-4) versus 1 (range 0-6), (p=0.515) for HIV+ vs HIV-. We suggest that LT in HIV+ patients, with a pre-transplant CD4 count >200 has similar medium term results than a matched HIV- population.

O-105 PROGNOSIS OF SEVERE RECURRENCE OF HCV INFECTION AFTER LIVER TRANSPLANTATION IN A LARGE COHORT OF HIV INFECTED PATIENTS

Teresa Maria Antonini[1], Anne-Marie Roque-Afonso[4], Mylene Sebagh[1,2,3], Bruno Roche[1,2,3], Didier Samuel[1,2,3], Jean-Charles Duclos-Vallee[1,2,3]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Unite 785, INSERM, Villejuif, France; [3]UMR-S 785, Univ Paris-Sud, Villejuif, France; [4]Laboratoire de Virologie, AP-HP Hopital Paul Brousse, Villejuif, France*

Liver transplantation (LT) in HIV infected patients is characterized by recurrence of HCV infection on the liver graft. The aim of this study was to determine the prognosis of severe fibrosis and fibrosing cholestatic hepatitis (FCH) after liver transplantation in HIV infected patients.
Patients and methods: Between December 1999 and January 2008, 68 HIV+ infected patients with a median age of 44.7 years (29-62), predominantly male (82%) underwent Liver Transplantation because of HCV related cirrhosis (HCV+ (n=43), HBV/HCV or HBV/HDV/HCV (n= 5), alcohol (n=5), hepatocellular carcinoma (n=15)
Results: 15 patients developed severe fibrosis: F3 (n=3) and F4 (n=12) with a mean delay of 18.9 (2-48) months after LT. Nine patients developed FCH with a mean delay of 6 (2-14) months after LT. Among 12 patients with anti-HCV therapy, 1 patient had a sustained response, 2 patients were relapsers and the 9 remaining patients developed severe intolerance (n=2) or were non responders (n=7). Twelve patients died (80%) with a mean delay of 25 (4-88) months after LT: 9 patients because of HCV recurrence. Among patients who died because of severe HCV recurrence, 7 patients developed FCH and 2 patients developed mitochondrial toxicity. At time of diagnosis of FCH, mean HCV viral load was 6.9 log10 copies/mL (5.6-9).
Conclusions: In our cohort of 68 liver transplanted HIV-HCV coinfected patients, 19.1% developed cirrhosis and 13% a fibrosing cholestatic hepatitis with a mean delay of 18.9 (2-48) and 6 months (2-14) respectively. A high mortality rate (80%) was observed in this subroup of patients.

O-106 PEG-IFN-alfa 2b WITH RIBAVIRIN FOR THE PREVENTION OF HISTOLOGICAL FIBROSIS PROGRESSION IN PATIENTS WITH HCV HEPATITIS RECURRENCE. A MULTICENTER RANDOMIZED CONTROLLED STUDY. INTERIM REPORT

Luca S. Belli[1], Riccardo Volpes[2], Ivo Graziadei[3], Peter Starkel[4], Andy K. Burroughs[5], Patrizia Burra[6], Stefano Fagiuoli[7], Alberto B. Alberti[1], Maria Guido[6], Luisa Pasulo[7], Wolfgang Vogel[3], Bruno Gridelli[2], Umberto Cillo[6], Luciano De Carlis[1], Giovambattista Pinzello[1]. *[1]Hepatology and Transplantation Unit, Ospedale Niguarda, Milan, Italy; [2]Transplantation Unit, ISMETT, Palermo, Italy; [3]Surgery and Gastroenterology, University Hospital, Innsbruck, Austria; [4]Hepatology and Transplantation Unit, Clinique Univ. Saint Luc, Brussels, Belgium; [5]Liver Transplantation Unit, Royal Free Hospital, London, United Kingdom; [6]Gastroenterology and Surgical Unit, Università, Padova, Italy; [7]Gastroenterology and Liver Unit, Ospedali Riuniti, Bergamo, Italy*

Purpose: To investigate the efficacy of the combination PEG-IFN alfa-2b plus Ribavirin in the prevention of fibrosis progression in patients with mild-moderate recurrent hepatitis C as histologically assessed at 1 year after liver transplantation (LT).
Methods: Antiviral therapy was compared with no treatment in an open, randomized controlled multicenter trial. Patients were randomized 1 year after LT only if Grading >3, Staging < 4 (Ishak) and bil<3mg/dL. Treated patients received escalating doses of PEG-IFN alfa 2b (up to1.5 mcg/kg/w) and ribavirin (up to 800 mg daily). Assessment of treatment efficacy was based on histological examinations of all paired liver biopsies obtained at randomization (BX12), 1 year (BX24) and 2 years (BX36) afterwards and was performed by an external pathologist. Of the 72 patients consecutively enrolled from 7 Centers, the first 39 from 4 Centers completed the follow up and were considered in this interim analysis: 18 received antiviral treatment (GROUP I) and 21 were followed up as controls (GROUP 2). A safety stopping rule was established for control patients progressing beyond stage 3 during the follow up.
Results: 148 consevcutive HCV transplants were considered. 22 died within

Table 1. Comparison of paired biopsies (at randomization and 2 years afterwards): Fibrosis Ishak score changes in treated (Group 1) and untreated patients (Group 2)

	Group 1 (18 pts)	Group 2 (21 pts)
Worse	6	5
Stable	11 (SVR = 5)	15
Improved	0	0
Other	1	1

Worse = at least 2 points worse; stable = 0 or 1 point change; improved = at least 2 points better; other = rejection. Note: 7 pts in Group 1 and 4 pts in Group 2 were S3 at randomization.

the first 12 months. 126 patients were evaluated for randomization but only 39 participated in the study according to the inclusion/exclusion criteria. 5 patients in GROUP 1 achieved SVR (27%). Results of fibrosis progression in treated and untreated patients are reported in table 1. All 5 pts achieving SVR in GROUP 1 remained stable at BX36.

Conclusions: The impact of combined antiviral therapy in preventing fibrosis progression in patients with mild-moderate histolgically proven recurrent hepatitis C after 2 years of follow up seems limited.

O-107 ANTIVIRAL TREATMENT (AT) FOR HCV RECURRENCE FOLLOWING LIVER TRANSPLANTATION: THE IMPACT ON FIBROSIS PROGRESSION

Eleonora De Martin[1], Marco Senzolo[1], Francesca R. Ponziani[2], Raffaella Viganò[3], Luca Belli[3], Giovambattista Pinzello[3], Michele Colledan[4], Francesca Donato[5], Daniele Di Paolo[6], Mario Angelico[6], Maria Rendina[7], Maurizio Pompili[2], Antonio Gasbarrini[2], Francesco P. Russo[1], Umberto Cillo[1], Stefano Fagiuoli[4]. *[1]Dept. of Surgical and Gastroenterological Sciences, University-Hospital, Padua, Italy; [2]Liver Transplant Center, Policlinico A. Gemelli, Rome, Italy; [3]Hepatology and Abdominal Organ Transplantation Unit, Ospedale Niguarda Cà Granda, Milano, Italy; [4]Gastroenterology and Liver Transplant Surgery, Ospedali Riuniti, Bergamo, Italy; [5]Migliavacca Center for Liver Disease, Ospedale Maggiore Policlinico, Milan, Italy; [6]Gastroenterology Unit, Policlinico Tor Vergata, Rome, Italy; [7]Gastroenterology Unit, Policlinico Universitario, Bari, Italy*

Background: The benefit of AT in patients with histological HCV recurrence following LT is still controversial. Aim To assess the effect of AT on histological HCV recurrence after LT.

Methods: A multicenter retrospective study was performed in HCV+ recipients who underwent AT (IFN or Peg-IFN/Ribavirin; 1999-2007) after LT. Protocol liver biopsies (LB) performed before, at the end, and 12 months after AT discontinuation (Ishak's score).

Results: 191 patients (136 M, 55 F, mean ± SD age 54±7.6 years) were included. AT was started at 44±63 months from LT, the pre-AT mean staging (S) was 2.46. HCV-RNA was negative in 84 patients after 6 months of AT, in 112 patients at the end of AT (ETR), in 73 patients after 12 months of AT withdrawn (SVR). In ETR+ vs ETR- patients, at the end of AT, S was 2.44 vs 3.03 (p=0.044); at 12 months after the end of AT, S was 2.24 vs 3.15 (p=0.021) respectively. In SVR+ vs SVR– patients, at the end of AT, S was 2.32 vs 2.86 (p=0.049); at 12 months after the end of AT, and S was 2.06 vs 2.94 (p=0.022). The S progression comparing the pre-AT vs the end of treatment AT vs 12-month after AT LB was 2, 2.13, 1.87 in ETR+ (p=0.47) and 1.61, 1.94, 2.44 in ETR– and drop out patients (p=0.01), moreover S was 1.94, 2.28, 1.78 in SVR+ (p=0.18) and 1.72, 1.93, 2.35 in SVR- and drop-out patients (p=0.027).

Conclusion: In patients with ETR following AT, at least no progression of fibrosis due to HCV recurrence following LT was seen. Moreover, in SVR–, a significant fibrosis progression was observed between the end of treatment and the 1-year after AT liver biopsy.

O-108 IN VIVO PROTECTION AGAINST HCV BY BROADLY NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES

Norman Kneteman, M. Law, T. Maruyama, J. Lewis, E. Giang. *Department of Surgery, University of Alberta, Edmonton, AB, Canada; Department of Immunology and Molecular Biology, Scripps Research Institute, La Jolla, CA, USA; Department of Immunology and Melocular Biology, Scripps Research Institute, La Jolle, CA, USA; Department of Surgery, University of Alberta, Edmonton, AB, Canada; Department of Immunology and Melocular Biology, Scripps Research Institute, La Jolla, CA, USA; Department of Surgery, University of Alberta, Edmonton, AB, Canada*

Introduction: HCV recurrence post transplant reduces graft and patient survival. Extreme variability due to quasispecies evolution challenges efforts at both vaccine design and passive immunotherapy. Previous clinical trials attempting to prevent HCV re-infection after transplant with human anti-HCV immunoglobulin preparations failed.

Methods and results: Utilizing a Fab phage-display library, 115 clones with specific binding to HCV E2 were isolated and 3 groups recognizing specific antigenic regions were generated. After conversion to full length IgG1s, binding affinities ranged from 0.4-6 nM, but only mAbs specific for antigenic region 3 (AR3) reacted with both genotype 1a and 2a E1-E2 complex and cross – neutralized JFH-1 virus and many HCV pseudotypes. We tested the ability of these broadly neutralizing AR-3 specific mAbs to protect against heterologous HCV quasispecies. 3 groups of 6 SCID/uPA human liver chimeric mice with high level human hepatocyte repopulation received 200mg/kg of 2 AR3 mAbs (AR3A, AR3B) or isotype control Ab IP 24 hours prior to IV inoculation with genotype 1a infected human serum (2 X 10^5 copies).

All control mice demonstrated high titre HCV. 2/5 AR3A and 3/4 AR3B treated mice remained HCV negative for 6 weeks. In a second study, 1/8 AR3A treated mice vs. 5/9 control mice ($P<0.05$) developed high titer infection.

Two groups of 5 mice each received irrelevant antibody or AR3B prior to inoculation with genotype 3a laden human serum. 3/5 and 5/5 control mice demonstrated high titer infection on days 14 and 70, with 0/5 and 2/5 AR3B treated mice so infected.

Conclusions: These results demonstrate the ability to protect against a heterologous HCV quasispecies swarm across genotypes 1a and 3a with mAbs against AR3 of HCV E2 and raise the potential for such mAbs to protect from HCV reinfection after liver transplant.

O-109 LONG TERM SURVIVAL AND FIBROSIS PROGRESSION ANALYSIS IN RETRANSPLANTED PATIENTS FOR HCV RECURRENCE

Rodolphe Sobesky[1,2,3], Valerie Delvart[1], Paul Carrier[1], Teresa Maria Antonini[1], Bruno Roche[1,2,3], Faouzi Saliba[1,2,3], Philippe Ichai[1,2,3], Daniel Azoulay[1], Rene Adam[1,2,3], Anne-Marie Roque-Afonso[5,2,3], Mylene Sebagh[4,2,3], Catherine Guettier[4,2,3], Denis Castaing[1,2,3], Didier Samuel[1,2,3], Jean-Charles Duclos-Vallee[1,2,3]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Unite 785, INSERM, Villejuif, France; [3]UMR-S 785, Univ Paris-Sud, Villejuif, France; [4]Laboratoire d'Anatomie Pathologique, AP-HP Hopital Paul Brousse, Villejuif, France; [5]Laboratoire de Virologie, AP-HP Hopital Paul Brousse, Villejuif, France*

Patients with positive HCV-RNA prior to liver transplantation (LT) have post-LT recurrence and the natural history of HCV liver graft infection could be severe. The question of the retransplantation (reLT) is discussed.

Aims: To study 1) survival of HCV infected patients retransplanted for severe recurrence of HCV infection and 2) fibrosis kinetic on the second liver graft.

Methods: ReLT was proposed for 30 patients (45±8 years) in a monocentric population of 504 HCV infected patients transplanted for end stage liver cirrhosis or hepatocellular carcinoma. ReLT was proposed for surgical complications (n=11), for severe complications of HCV related liver graft infection (n=13) and because of chronic rejection or alloimmune hepatitis (n=6). Survival of retransplanted patients was compared to an HCV LT group (n=125) matched on donor age. Fibrosis progression on the first and the second graft was studied.

Results: Among the 13 patients retransplanted for severe HCV reinfection, the interval between the 2 LT was 95 (±68) months. The mean MELD score before reLT was 22.5 (±6.2). Four patients had combined kidney and liver transplantation. Five patients deceased after reLT, three within 10 months because of infectious complications and 2 later because of severe HCV recurrence. Survival rate after reLT was 67% at 1 year and 57% at 5 years, respectively. Since the first LT, global survival in the retransplanted group was 65% and 72% in the matched group (p=ns). In retransplanted patients, the mean fibrosis progression before and after reLT was 1.27 (±1.0) and 1.2 (±1.4) METAVIR unit per year, respectively (p=ns).

Conclusions: Survival after retransplantation of patients for severe HCV recurrence on the liver graft was 67% at 1 year and 57% at 5 years, respectively. Retransplantation does not affect fibrosis progression on the second graft.

O-110 GB-VIRUS-C VIREMIA AFTER LIVER TRANSPLANTATION IS ASSOCIATED WITH BETTER 10-YEAR-SURVIVAL

Kinan Rifai[1], Heiner Wedemeyer[1], Juergen Klempnauer[2], Michael P. Manns[1], Hans L. Tillmann[1,3]. *[1]Gastroenterology, Hepatology & Endocrinology, Hannover Medical School, Hannover, Germany; [2]Visceral & Transplant Surgery, Hannover Medical School, Hannover, Germany; [3]Hepatology, Duke University, Durham, USA*

Purpose: We earlier demonstrated a beneficial influence of the GB virus C (GBV-C) on the long-term course of HIV infection. Here we describe the long-term outcome of patients with GBV-C after liver transplantation (OLT).

Methods: We studied overall survival rates of 102 patients after OLT. GBV-C envelope antibodies (anti-E2) and RNA were tested directly before transplantation and after transplantation. Before OLT, 29/100 patients had anti-E2-antibodies and 9 patients were viremic. No patient presented both GBV-C antibodies and RNA. Survival rates were assessed 10 years after OLT by using Kaplan-Meier and Cox's regression analysis to determine long-term effect of GBV-C status.

Results: Of the 64 patients without neither anti-E2 nor viremia before OLT, 11/64 (17%) patients presented anti-E2 and 28/64 (44%) showed viremia after

OLT. In 2/64 patients (3%), both anti-E2 and GBVC-RNA were detected. Furthermore, 2/29 patients (7%) presenting anti-E2 before OLT became viremic after OLT. Overall 1-, 3- and 10-year patient survival rates were 88%, 80% and 69%. Mean patient and graft survival was 8,9±4,7 and 8,4±4,9 years, respectively. A worse outcome was found for patients requiring retransplantation ($p<0.0001$) and those with liver tumors ($p<0.0001$). If patients surviving at least 3 years were analysed by Kaplan-Meier survival function, those who were GBV-C-RNA positive post-OLT had a significantly longer survival than RNA-negative patients ($p<0.05$). In addition, relevance of GBV-C viremia regarding survival was confirmed in Cox's regression including patient age and sex, need of retransplantation and presence of tumor disease showing that GBV-C viremia ($p<0.05$) was independently associated with patient survival (besides retransplantation and tumor disease; both $p<0.001$).
Conclusion: Patients with active GBV-C infection after liver transplantation had an improved long-term outcome, actually in line with similar data from renal transplant recipients.

O-111 REEVALUATION OF RISK FACTORS OF FUNGAL INFECTION AFTER LIVER TRANSPLANTATION (LT) WITHIN THE MELD ERA

Faouzi Saliba[1,4,5], Valerie Delvart[1], Philippe Ichai[1,4,5], Najiby Kassis[2], Francoise Botterel[3], Marie Kouassi[1], Denis Castaing[1,4,5], Didier Samuel[1,4,5]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Departement Microbiologie, AP-HP Hopital Paul Brousse, Villejuif, France; [3]Departement Parasitologie Mycologie, AP-HP Hopital de Bicetre, Le Kremlin Bicetre, France; [4]UMR-S 785, Univ Paris-Sud, Villejuif, France; [5]Unite 785, INSERM, Villejuif, France*

Invasive fungal infection (IFI) is a common cause of morbidity and mortality after LT.
Patients and methods: From 1999 to 2005, 667 consecutive LT performed in 593 patients were studied. Mean age was 48.6±13.4 years, predominantly male (68.6%). Mean MELD score was 20.5±10.9. 198 LT recipients (29.7%) received fungal prophylaxis predominantly with ABLC (73.7%) or fluconazole (25.3%) with a mean duration of 22±12 days.
Results: Patient survival was respectively 88% and 73% at 1 and 5 years. Five-year survival rate was significantly lower in patients with IFI compared to those without IFI (69% vs. 48%, logrank p=0.009). Fungal prophylaxis reduced significantly the incidence of invasive fungal infection and colonization during the first year ($p<0.0001$). In the univariate analysis, risk factors predictive of developing an IFI were prior to LT: UNOS1 patients (p=0.003), mechanical ventilation (p=0.02), antibiotherapy ($p<0.0001$), hepatic encephalopathy (p=0.007), total and conjugated bilirubin (p=0.003), creatinine (p=0.007), prothrombin time (p=0.018), hemoglobin (p=0.0005) and MELD score >17 (p=0.006), during LT: roux-en-Y bilio-digestive anastomosis (p=0.038), length of intervention (p=0.02), blood transfusions (p=0.001) and after LT: monoclonal and polyclonal antibodies (p=0.01), CMV infection (p=0.003), bacterial infection (p=0.0006) and bacteremia ($p<0.0001$). The mean MELD score was respectively higher in patients with IFI 23.7±11.4 vs. those without IFI 19.4±10.4. In the multivariate analysis, high MELD score ($p<0.0001$), the absence of fungal prophylaxis ($p<0.0001$), bilio-digestive anastomosis (p=0.01) and bacterial infection (p=0.01) during the first month were independent risk factors of developing an IFI.
Conclusion: IFI severely impacts morbidity and mortality after LT. Targeted prophylaxis of high-risk patients significantly reduces the incidence of IFI after LT. High MELD score is an independent risk factor of IFI.

O-112 EFFICACY AND LONG-TERM OUTCOME OF AMPHOTERICIN B LIPID COMPLEX (ABLC) PROPHYLAXIS IN HIGH RISK LIVER TRANSPLANT RECIPIENTS IN THE CURRENT PRACTICE

Faouzi Saliba[1,4,5], Valerie Delvart[1], Philippe Ichai[1,4,5], Najiby Kassis[2], Francoise Botterel[3], Marie Kouassi[1], Daniel Azoulay[1], Rene Adam[1,4,5], Denis Castaing[1,4,5], Stephane Bretagne[4], Didier Samuel[1,4,5]. *[1]Centre Hepato-Biliaire, AP-HP Hopital Paul Brousse, Villejuif, France; [2]Departement Microbiologie, AP-HP Hopital Paul Brousse, Villejuif, France; [3]Departement Parasitologie Mycologie, AP-HP Hopital de Bicetre, Le Kremlin Bicetre, France; [4]Departement Parasitologie, AP-HP Hopital Henri Mondor, Creteil, France; [5]UMR-S 785, Univ Paris-Sud, Villejuif, France*

Fungal prophylaxis with Amphotericin B lipid formulations has been recommended to high-risk liver transplant recipients mainly those with severe hepatic failure, renal failure and retransplantation.
Patients and methods: From 1999 to 2005, 667 consecutive liver transplantations performed in 593 patients were studied.10.8% of the patients underwent a second transplant. Mean age was 48.6±13.4 years, predominantly male (68.6%). 18.2% of the patients were UNOS1 (in the ICU) prior to liver transplantation. Mean MELD score was 20.5±10.9. 146 high risk liver transplant recipients (21.9%) received fungal prophylaxis with low dose ABLC (1mg/kg/day the first week, then 2.5 mg/kg twice a week) for a mean duration of prophylaxis of 23±12 days.
Results: Candida infection was significantly (p=0.011) reduced from 41.2% in the non-treated group to 29.5% in the ABLC group. Invasive candidiasis necessitating a systemic antifungal treatment was significantly ($p<0.0001$) reduced from 28.6% in the control arm to 11.6% in the ABLC group during the first three months and from 30.5% to 15.1% during the first year post LT (p=0.0002). Candidemia developed respectively in 5 patients (3.4%) and 12 patients (2.6%) in the ABLC and non-prophylactic group (p=NS). ABLC reduced significantly invasive abdominal and biliary Candida infection during the first three months (9.2% to 4.1%; p=0.049). Proven Aspergillosis was lower in the ABLC group (1.4% vs 2.8%) without reaching significance. Mean creatinine did improve in the ABLC group from 146±133 μmol/L at Day 1 after liver transplantation to 112±35 μmol/L at M3 (p=0.005).
Conclusion: Targeted prophylaxis of high-risk patient with low dose ABLC for 3 weeks is safe and reduces significantly the incidence of invasive fungal infection after liver transplantation.

O-113 RETROSPECTIVE COHORT INVESTIGATION OF RISK FACTORS FOR SURGICAL SITE INFECTION AFTER LIVER TRANSPLANTATION

Walter C. Hellinger[1], Michael G. Heckman[2], Hani P. Grewal[3], Winston R. Hewitt[4], Christopher B. Hughes[4], C. Burcin Taner[3], Darrin L. Willingham[3], Justin H. Nguyen[3]. *[1]Infectious Disease, Mayo Clinic Florida, Jacksonville, FL, USA; [2]Biostatistics, Mayo Clinic Florida, Jacksonville, FL, USA; [3]Transplantation, Mayo Clinic Florida, Jacksonville, FL, USA; [4]Surgery, Emory University School of Medicine, Atlanta, GA, USA*

Purpose: Surgical site infections (SSIs) after liver transplantation (LT) identified by standardized surveillance methodology (SSM) have been associated with increased risk for death and graft loss. Risk factors (RFs) for these infections have not been identified in large cohorts of liver transplant recipients.
Methods: A retrospective cohort analysis of 1000 consecutive patients undergoing first liver transplantation between 2003 and 2008 was performed. SSIs were prospectively identified by SSM. Patient, donor and operative characteristics (potential RFs) previously associated with SSI were collected from transplant center databases. Associations of potential RFs with the development of SSI after LT were investigated using Cox proportional hazards models; relative risks (RR's) and 95% confidence intervals (CI's) were estimated.
Results: Median age was 56, 67% of patients were male, and median MELD was 17. LT's were performed by 7 different surgeons. 156 patients (16%) developed SSI: 40 (26%) superficial, 11 (7%) deep and 105 (67%) organ/space. In multivariable Cox proportional hazards analysis, associations were identified between SSI and liver disease diagnosis (P=0.045), operative time (P=0.008), and surgeon ($P<0.001$). Liver disease diagnosis with the highest risk of SSI relative to the most frequent, referent diagnosis (chronic hepatitis C) was Laennec's cirrhosis (RR: 1.56, 95% C: 1.00 – 2.43). Longer operative times were associated with a higher risk of SSI (RR: 1.16 [1 hour increase], 95% CI: 1.04 – 1.29). Compared to the most active, referent surgeon, the RR's of surgeon for SSI ranged greatly from 0.38 to 1.44.
Conclusion: Laennec's cirrhosis and increased operative time were associated with significant risk for SSI. The risk of SSI varied greatly according to surgeon. Identification of surgical practices that directly and indirectly (via increased operative time) increase the risk for SSI is needed.

Session 14. Operational tolerance: approaches & mechanisms

O-114 HUMAN MACROPHAGES DRIVEN TO A NOVEL STATE OF ACTIVATION SUPPRESS T CELL RESPONSES *IN VITRO*

Paloma Riquelme[1], James A. Hutchinson[1], Fred Fändrich[2], Edward K. Geissler[1]. *[1]Department of Experimental Surgery, University Hospital Regensburg, Regensburg, Germany; [2]Clinic for Applied Cell Therapy, University Hospital Kiel, Kiel, Germany*

Earlier studies have shown that human macrophage preparations can promote a state of alloantigen-specific unresponsiveness in recipients of renal transplants and may facilitate the safe, early minimisation of immunosuppression in these patients (Hutchinson JA, *Tr. Int.*, 2008). To better define the putative effector population within these mixtures, and so to optimise the cell product for clinical applications, purified $CD14^+$ monocytes were grown under defined culture conditions until a steady, relatively homogeneous population was obtained: these cells have been called *regulatory macrophages* (M-*regs*). Here, it is shown that human M-*regs* are uniquely identified by their mode of derivation, morphology, cell-surface marker phenotype, cytokine secretion profile and high expression of particular retinol dehydrogenases. The acquisition of this pheno-

type by peripheral blood monocytes is a gradual process, which depends upon plastic adherence and exposure to components of human serum. M-*regs* express only low levels of CD80 and HLA-DR, and do not stimulate allogeneic T cell proliferation *in vitro*; on the contrary, M-*regs* potently suppress T cell proliferation, apparently through the elimination of activated T cells. In various respects, the M-*reg* responds to activating stimuli, such as LPS, quite differently to comparator macrophage types and this may, in part, be explained by the absence of CD14, TLR2 and TLR4 expression. It is concluded that the human M-*reg* represents a novel, stable state of macrophage differentiation, and that M-*regs* may be particularly suitable for immune-conditioning therapies in transplantation.

O-115 THE ROLE OT LTβR SIGNALING IN THE FORMATION OF ECTOPIC LYMPHOID TISSUE WITHIN CARDIAC ALLOGRAFTS

Reza Motallebzadeh[1], Sylvia Rehakova[1], Thet Su Win[1], Chris Callaghan[1], Siva Sivaganesh[1], Eleanor Bolton[1], Nancy Ruddle[2], Andrew Bradley[1], Gavin Pettigrew[1]. *[1]Department of Surgery, Cambridge University, Cambridge, United Kingdom; [2]Department of Immunobiology, Yale University, New Haven, USA*

Foci of ectopic lymphoid tissue (ELT) are found at sites of chronic inflammation and in solid organ transplants. Lymphoid organ development is dependant upon signaling through the lymphotoxin-β receptor (LTβR). This study aims to characterise ELT in a model of chronic allograft vasculopathy, and to determine whether blocking LTβR signaling can inhibit ELT development.

Bm12 heart allografts, excised from B6 recipients at day 20, 40 and 100, were stained with H&E and lymphoid aggregates characterised by immunohistochemistry. Presence of ELT was confirmed by: discrete aggregates of B220+ B cells, CD4+ T cells, and PNAd/MadCAM-1-expressing High Endothelial Venules (HEVs). Lymphatic vessel (LV) density was assessed by immunofluorescence staining with LYVE-1 mAb. Blockade of LTβR signaling was achieved with weekly intraperitoneal injection of 100μg LTβR-Ig fusion protein. Control animals received a non-specific IgG-protein complex. We have previously reported that MHC II-mismatched bm12 heart allografts in B6 recipients provoke anti-nuclear autoantibody; autoantibody responses were compared by quantifying binding to nuclear antigen expressing HEp-2 cells.

Aggregates of B cells + CD4 T cells, with associated HEVs, were present in 12/13 grafts and fulfilled criteria for ELT. The number of foci of ELT within grafts increased with time. Some LVs stained for HEV markers suggesting derivation from a common progenitor vessel. LV density was less in syngeneic hearts, and neither lymphoid aggregates nor HEVs were present. Compared to control-treated animals, LTβR-Ig treatment abrogated the formation of lymphoid aggregates + HEVs (0.4 vs 2.1 per field, p=0.04) and inhibited LV proliferation (LV density = 1427 vs 2952 μm^2, p=0.02). Nevertheless in both groups, allografts provoked comparable autoantibody responses.

The formation of ELT within cardiac allografts is reduced by inhbiting LTβR signaling, suggesting a novel pathway for the prevention of allograft vacsculopathy.

O-116 CRITICAL ROLE OF NEWLY IDENTIFIED $CD4^+CD25^{++}CD45RA^+$ NAIVE REGULATORY T CELLS IN OPERATIONAL TOLERANCE AFTER PEDIATRIC LIVING-DONOR LIVER TRANSPLANTATION

Hanaa Abdelhakeem Nafady-Hego[1], Ying Li[1], Shimon Sakaguchi[2], Kathryn Wood[3], Shinji Uemoto[4], Takaaki Koshiba[1]. *[1]Innovation Center for Immunoregulation Technologies and Drugs, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Experimental Pathology, Institute for Frontier Medical Science, Kyoto University, Kyoto, Japan; [3]Transplantation Research Immunology Group, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford, United Kingdom; [4]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Background: In humans, the phenotypes of conventional regulatory T cells (conventional-Tregs) were reported to be $FOXP3^+CD4^+CD25^{+++}$ CD45RA[oline]. Recently, the existence of CD45RA (naive phenotype) expressing $FOXP3^+CD4^+CD25^{++}$ regulatory T cells (naive-Tregs) has been identified as a distinct cell fraction from conventional-Tregs.However, the role of naive-Tregs in transplant (Tx) tolerance remains elusive.

Methods: The peripheral blood mononuclear cells (PBMCs) were derived from 23 patients who could successfully stop immunosuppression (IS) (Gr-Tol), 13 patients who failed to stop IS (Gr-Intol) after pediatric living-donor liver-Tx and 19 age-matched healthy volunteers (Gr-Vol). To examine the frequency of naive-Tregs, PBMCs were phenotyped by FACS analysis. Naive-Tregs were isolated by a cell sorter and reconstituted with syngeneic $CD4^+$ cells at a 1 to 5 ratio. MLR of reconstitued cells to donor APC or haploidentical alloantigen was compared with that of $CD4^+$ cells alone.

Results: The frequency of naive-Tregs ($CD4^+CD25^{++}CD45RA^+$ cells) within the lymphocytes was significantly decreased in Gr-Intol, compared with those in Gr-Tol and -Vol (Gr-Tol, -Intol and -Vol; 5.5%, 2.3% and 4.3%, Gr-Intol *vs* Gr-Tol, and -Vol, $p<0.001$ and $p<0.05$). Naive Tregs vigorously suppressed MLR of $CD4^+$ cells to donor-antigen or haploidentical alloantigen in Gr-Tol or Gr-Vol. In contrast, naive Tregs in Gr-Intol failed to suppress MLR of $CD4^+$ cells to donor antigen (suppression rate; Gr-Tol, -Intol, and -Vol; 65%, 5%,and 38%, Gr-Intol *vs* Gr-Tol, Vol, $p<0.01$).

Conclusions: Both reduction in the frequency and the lack of suppressive property of naive Tregs were observed in patients who failed to stop IS, compared with those in patients who could successfully stop IS. This is the first report providing detailed evidence that similar to conventional Tregs, newly identified naive-Tregs play a critical role in the maintenance of operational tolerance after pediatric living-donor liver Tx.

O-117 ADOPTIVE TRANSFER OF*EX VIVO* DONOR ALLOANTIGEN-STIMULATED $CD4^+CD25^{++}$ REGULATORY T CELLS AMELIORATES REJECTION OF LUNG TRANSPLANTATION BETWEEN FULLY MISMATCHED MINIATURE SWINES

Naoki Satoda[1], Ying Li[2], Tsuyoshi Shoji[1], Xiangdong Zhao[1], Hiroyuki Kanazawa[3], Hidenori Ohe[3], Toru Bando[1], Junichi Tottori[4], Shimon Sakaguchi[5], Eiji Kobayashi[6], Shinji Uemoto[3], Hiromi Wada[1], Hiroshi Date[1], Takaaki Koshiba[2]. *[1]Department of Thoracic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]2Innovation Center for Immunoregulation Technologies and Drugs, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [4]CLAWN Institute, Japan Farm, Isa, Kagoshima, Japan; [5]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; [6]Organ Replacement Research Center, Jichi Medical School, Shimono, Tochigi, Japan*

Background: An adoptive cell transfer of regulatory T cells (Tregs) has been successful in rodent Tx models,but little is known about its potential in large animal Tx models.

Method: Fully mismatched miniature swines (two haplotypes; C1 and C2), weighing 20-30kg, were used as recipients and donors.Orthotopic lung Tx was performed. The peripheral blood mononuclear cells were obtained from recipients by apheresis and $CD4^+CD25^{++}$ cells were isolated by magnet beads system on pre-Tx day 2. They were stimulated by donors APC with IL-2 and rapamycin until post-operative day (POD) 10 when cultured $CD4^+CD25^{++}$ cells (10^6 cells/kg) were transferred into recipients in the presence of leukopenia induced by cyclophosphamide. High-dose tacrolimus was used from Tx to POD 6, which was followed by low-dose tacrolimus from POD 7 to 21 (Group-Tregs+TAC,n=5). Graft survivals were compared with those in other groups where low dose tacrolimus alone (Group-TAC,n=4), Tregs transfer alone (Group-Tregs,n=3), and neither low dose tacrolimus nor Tregs transfer (Group-non TAC,non Tregs, n=2) was given.

Results: In Group-non TAC,non Tregs, graft was rejected on POD15.5. Adjunction of low dose tacrolimus alone or Tregs transfer alone did not prolonged survivals (Group-TAC and-Tregs; POD16.3 and 15). However, in the presence of Tregs transfer and low dose tacrolimus, survival was the greatest (Group-Tregs+TAC;POD 41.6, $p<0.01$ *vs* Group-TAC, -Tregs and -non TAC,non Tregs). In this group, one recipient exhibied clinical sign of GVHD on POD38 which was self-limiting within a week, and accepeted graft (>POD60).

Conclusions: Adoptive transfer of *ex vivo* donor alloantigen-stimulated $CD4^+CD25^{++}$ Tregs combined with low dose tacrolimus ameliorates rejection of immunogenic lung Tx in clinically relevant miniature swines. In addition, a question arises as to whether GVHD represents a major side effect of Tregs transfer.

O-118 SELECTIVE CD28 BLOCKADE SYNERGIZES WITH REGULATORY T CELL ACTIVITY IN A CTLA-4 DEPENDENT MANNER

Nahzli Dilek, Nicolas Poirier, Philippe Hulin, Bernard Vanhove, Gilles Blancho. *UMR643, INSERM, Nantes, France*

Aim: Targeting CD28-B7 costimulation without perturbation of CTLA-4/B7 inhibitory pathway might favour tolerance induction. This strategy has been applied to primate allograft models and showed 1/ a prevention of acute rejection, even after immunosuppression withdrawal 2/ an induction of donor-specific hyporesponsiveness 3/ an increase of intragraft and peripheral Tregs. However, the demonstration that blocking CD28/B7 interactions promotes immunoregulatory function of Treg was still awaited.

Results: Looking at human T cells behaviour in contact with cognate APC by Time-Lapse, we observed that selectiv CD28-blockade prevented the formation of durable immunological synapses, which resulted in a T cell motility increase (83 ± 5 *vs* $324\pm57\mu m$ for control and CD28 antagonist, respectively; $p<0.001$), a decrease of T/APC contact time (16 ± 0.9 *vs* 7 ± 1.6 min, respectively; $p<0.001$) and a decrease of T cell calcium peaks (0.37 ± 0.03

vs 0.15±0.02 peaks/min of contact, respectively; $p<0.001$). These effects on contact time and on motility were dependent on the availability of CTLA-4 ligands since antagonist of CTLA-4 reversed the effect of CD28-blockade. This is in accordance with findings that CTLA-4 signalling overrides the TCR-induced stop signal. In contrast, the reduction of calcium peaks induced by CD28 antagonists could not be reversed by the simultaneous blockade of CTLA-4. In other experiments, we observed that the suppressive activity of Treg cells *in vitro* was increased by CD28 antagonists and blocked by CTLA-4 antagonists ($p<0.01$). We also observed that CD28-blockade on Tregs during their priming with matured allogeneic dendritic cells conferred more suppression to the Treg cells whereas blocking CTLA-4 had no effect.
Conclusion: The mechanism of action of selective CD28-blockade on T cells involves both CTLA-4-dependent (formation of stable T/APC contacts, T cell motility and preserved Tregs function) and independent (calcium flux and T cell proliferation) components.

O-119 BONE MARROW DERIVED MESENCHYMAL STEM CELLS ATTENUATE RENAL ALLOGRAFT INJURY AFTER PROLONGED COLD ISCHEMIA

Yoshiaki Hara[1,3], Meaghan Stolk[1,3], Maria Dams[1], Elke Effenberger[1], Juliane Ladhoff[1], Katja Kotsch[1], Anja Reutzel-Selke[2], Hans-Dieter Volk[1,3], Martina Seifert[1,3]. *[1]Institute of Medical Immunology, Universitätsmedizin Charite Campus Mitte, Berlin, Germany; [2]Department of General, Visceral and Transplantation Surgery, Universitätsmedizin Charite Campus Virchow, Berlin, Germany; [3]Institute of Medical Immunology, Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany*

Background: Ischemia reperfusion injury is still a major contributor to the development of primary failure and delayed graft function in kidney transplantation. Mesenchymal stem cells (MSCs) are multipotent stem cells that have been shown to express both immunomodulatory and tissue repair potential. Therefore, the aim of this study was to verify the immunomodulatory features*in vitro* and to evaluate the therapeutic efficacy in kidney transplantation after prolonged cold ischemia.
Materials & methods: Bone marrow derived rat MSCs were co-cultured with mixed lymphocyte cultures (MLC) of CFSE labeled Lewis lymph node cells with irradiated DA lymph node cells. At day 4, proliferation of CFSE (+) recipient cell subpopulations was determined by FACS. Identical experiments were performed in transwell-setting. In addition, re-stimulation assays after 4 day MLC and 2 days rest with DA cells have been done. For *in vivo* studies, renal grafts of DA were 24-hours cold-preserved and transplanted to Lewis rats. Syngeneic MSCs were administered 7 days prior, immediately after and 1 day after operation. Grafts were harvested on day 3, and examined by qRT-PCR and histology.
Results: Syngeneic and allogeneic MSCs induced a dose-dependent inhibition of responder T cell proliferation and transwell assays demonstrated cell-cell contact is required. Responder cells of rat MSC co-cultures showed delayed proliferation after allo-restimulation suggesting inhibition of memory T cell induction without complete anergy. MSCs administration in kidney transplantation model significantly reduced intragraft mRNA-expression of IFNγ, IL1β, ICAM1, CCL19 and CCL21 (10.4, 3.1, 1.6, 3.0 and 2.3 fold, respectively) compared to the control. Moreover cellular infiltration markers like CD3 and CD25 were distinctly reduced.
Conclusions: Our results indicate that MSCs therapy ameliorates ischemic damage of kidney and also inhibits allo-responses. Immunomodulatory effects might be exerted through cell-cell contact in the rat model.

O-120 THE REGULATORY FUNCTION OF TOLEROGENIC CD8+CD45RC^{low} T CELLS IS INFLUENCED BY CELL CONTACT WITH NAIVE CD4+CD25- T CELLS

Xian Liang Li, Séverine Ménoret, Dominique Chabannes, Marcelo Hill, Claire Usal, Régis Josien, Ignacio Anegon. *INSERM U643, CHU Nantes, Institut de Transplantation et de Recherche en Transplantation (ITERT), Nantes, France*

Introduction: Tolerogenic CD8+CD45RC^{low} T cells mediate long-term allograft survival after blockade of CD40/CD40L interaction in a rat heart transplantation model but the regulatory mechanisms remain largely unknown.
Results: The suppressive function of tolerant CD8+CD45RC^{low} T cells was observed in the presence of plasmacytoid DCs and CD4- myeloid DCs. CFSE-labelling of naive CD4+CD25- T cells showed that the donor alloantigen specific suppressive function of tolerogenic CD8+CD45RC^{low} T cells was only mediated by pDCs, which significantly increased the expression level of Foxp3 in tolerogenic CD8+CD45RC^{low} T cells in a coculture system.
Both IDO- and IFNγ-dependent regulatory mechanisms coexisted in vivo since blockade of either IDO or IFNγ did not totally abrogate the tolerance induction. Different regulatory mechanisms were observed in vitro if naïve CD4+CD25- T cells contacted CD8+CD45RC^{low} T cells or not. Without contact with naïve CD4+CD25- T cells, the production of indoleamine 2,3 dioxygenase (IDO) by pDCs was responsible for the suppression by tolerogenic CD8+CD45RC^{low} T cells and suppression was IFN-γ independent. With contact of naïve CD4+CD25- T cells, suppression by tolerogenic CD8+CD45RC^{low} T cells was IDO independent and IFN-γ dependent. The concentration of IFN-γ in the culture medium not related to the suppressive function. Soluble IFN-γ alone did not exert the suppressive function. The regulatory mechanism by IFN-γ was independent of TGFβ.
Conclusion: Contact of tolerogenic CD8+CD45RC^{low} T cells with naïve CD4+CD25- T cells change the suppressive mechanisms from and IDO-dependent to an IFNγ-dependent mechanism. IDO-dependent mechanisms may be responsible to maintain tolerance and of inhibiting remote naïve CD4+CD25- T cell activation. IFN-γ-dependent mechanism may account for local suppression which directly inhibits the proliferation of naïve CD4+CD25- T cells.

O-121 MYCOPHENOLIC ACID-INDUCED REGULATORY CD4+ T CELLS CONFER TOLEROGENIC PROPERTIES TO HUMAN DENDRITIC CELLS

Roxane Lemoine[1], Florence Velge-Roussel[1], Hubert Nivet[1,2], Yvon Lebranchu[1,2], Christophe Baron[1,2]. *[1]UPRES EA 4245 "Cellules Dendritiques, Immunomodulation et Greffes", UFR de Médecine, Université François Rabelais, Tours, France; [2]Service de Néphrologie et d'Immunologie Clinique, CHRU Bretonneau de Tours, Tours, France*

Purpose: Regulatory T cells are able to suppress effector T lymphocyte responses. However, their effects on dendritic cells (DC) are not completely understood. We have recently demonstrated that mycophenolic acid-treated DC induced human CD4$^+$ regulatory T cells. In this study, we further analyzed the effects of these regulatory T cells on the phenotype and function of human DC.
Methods: The maturation of human monocyte-derived DC was induced by TNFa with or without MPA. Regulatory CD4$^+$ T cells (iTreg) and effector CD4$^+$ T cells (Teff) were obtained by repetitive allostimulation with MPA-DC or TNF-DC respectively and then co-cultured for 2 days with immature or LPS-matured DC. Their T cell-priming ability was analyzed in allogeneic MLR after negative selection of DC. Cytokine secretions were measured by ELISA and DC surface markers expressions were assessed by flow cytometry.
Results: We demonstrated that iTreg did not modify expression of CD80, CD83, CD86 and CD25 on immature DC contrary to Teff which induced a strong maturation. CCR5 expression on immature DC was increased following incubation with iTreg. Incubation with Teff led to a significant increase of IL-6, IL-12 and IFN-g secretions contrary to iTreg which only induced the production of IL-10. These results taken together showed that iTreg and Teff had opposite effects on immature DC.
iTreg increased CCR5 expression and significantly decreased expression of CD80, CD83, CD86 and CD25 on LPS-DC. iTreg inhibit IL-6, IL-12 and IFN-g secretions of LPS-DC while Teff had increased those secretions. Importantly, DC modified by iTreg did not support the proliferation of allogeneic CD4$^+$ T cells whereas DC modified by Teff did. These data suggested that Treg "reprogram" mature DC into semi-mature DC.
Conclusion: CD4$^+$ T cells activated by MPA-DC conferred tolerogenic properties to human DC.

O-122 LIVER SINUSOIDAL ENDOTHELIAL CELLS TOLERIZE B CELLS SPECIFIC FOR BLOOD GROUP CARBOHYDRATE ANTIGENS AFTER ABO-INCOMPATIBLE LIVER TRANSPLANTATION

Yuka Igarashi, Toshimitsu Irei, Masataka Bansyodani, Yuka Tanaka, Hideki Ohdan. *Second Surgery, Hirsohima University, Hiroshima, Japan*

We have recently demonstrated that a tolerant state among B cells responding to blood group-A antigens develops following blood group A-to-O pediatric liver transplantation. Blood antigen-reactive B cells might be tolerized through their interaction with the liver sinusoidal endothelial cells (LSECs), which exclusively express blood antigens. To address this possibility, we have used α1,3-galactosyltransferase-deficient (*GalT*$^{-/-}$) mice, since the Gal epitope is very similar in structure for blood antigens. Immune fluorescence staining of the wild-type *GalT*$^{+/+}$ mouse livers reveals that Gal epitopes predominantly express on the LSECs, resembling blood antigens in human livers. The LSECs isolated from *GalT*$^{+/+}$ mice were adoptively transferred via the portal vein into the congeneic *GalT*$^{-/-}$ mice (4×10^6 cells); they were intraperitoneally injected 2 days before the transplantation with the pyrrolizidine alkaloid monocrotaline, which impaired host LSECs, conferring proliferative advantage to the transplanted LSECs. Three to four weeks after the adoptive transfer of LSECs, the recipient mice underwent myeloablative radiation and reconstitution with bone marrow cells (BMCs) with/without splenocytes from *GalT*$^{-/-}$ mice. After the immunization with α-Gal-expressing rabbit RBCs, high levels of anti-Gal Abs were detected in the sera of the *GalT*$^{-/-}$ mice that had not received LSECs but were repopulated with BMCs from *GalT*$^{-/-}$ mice. In contrast, anti-Gal Abs were persistently undetectable in the sera of the *GalT*$^{-/-}$ mice that had received

LSECs from *GalT*$^{+/+}$ mice and were consequently repopulated with BMCs from *GalT*$^{-/-}$ mice. However, in the *GalT*$^{-/-}$ mice repopulated with both BMCs and splenocytes from *GalT*$^{-/-}$ mice, anti-Gal Abs were highly detectable, despite LSEC engraftment from *GalT*$^{+/+}$ mice. These findings suggest that after ABO-incompatible liver transplantation, the LSECs tolerize newly developing B cells but do not tolerize preexisting mature B cells specific for blood group carbohydrate antigens.

O-123 Th17 AND REGULATORY T CELLS RELATIONSHIP DURING ALLOGRAFT REJECTION

Louis-Marie Charbonnier, Benoit Vokaer, Fleur S. Benghiat, Michel Y. Braun, Michel Goldman, Alain Le Moine. *Institute for Medical Immunology, Université Libre de Bruxelles, Brussels, Belgium*

Aim: We previously observed in vitro that whole CD4+CD25+ prevented Th1 and Th2 alloreactivity but not Th17 cells. This suggests a Th17-mediated alternative pathway of allograft rejection. Herein we investigated the implication of IL17 in skin allograft rejection in case of different CD25+ on CD25- CD4 T cell ratios and in minor antigen model of graft.
Methods: Single MHCII incompatible skin grafts were performed into mice reconstituted with various ratios of CD4+CD25- and CD4+CD25+ T cells. In vitro T cell alloreactivity, intragraft mRNA expression and histology were analysed. In addition, we performed male skin graft into female recipients and compared IL17A$^{-/-}$ and WT donors and recipients.
Results: Reconstitution with 1/1 ratio of CD4+CD25+ and CD4+CD25- T cells failed to delay MHC-II disparate allograft rejection, compared to reconstitution with CD4+CD25- only. In mice reconstituted with 1/1 ratio, we found a significant increase of Th17 in draining lymph nodes (0.7 *vs* 5.2%; p=0.0025) together with intragraft IL17A mRNA expression, (6.5 fold increased; $p<0.016$) compared to mice reconstituted with CD25- alone. Despite the increased amounts of IL-17, neither the absence of T cell-derived IL17A nor IL-17A neutralization did delay allograft rejection. Similarly, T cell-replete IL17-deficient mice rejected MHC-II or MHC-I-disparate skin allografts in a normal tempo. However, in case of minor antigen disparaty, the IL17A-neutralization significantly delayed rejection kinetics (Day30: 80% vs 20% of graft survival; $p<0.05$) and dramatically decreased graft-infiltrating neutrophils.
Conclusion: We show that Tregs do not prevent, but rather strengthen Th17 alloreactivity in vivo. IL-17 is neither the dominant nor the only effector pathway in case of major mismatch, but becomes an important player in case of shrunk alloreactive repertoire. Further experiments are in progress to understand these mechanisms.

O-124 INHIBITION OF TLR-STIMULATED NK CELLS CYTOTOXICITY: A NOVEL IMMUNOREGULATORY FUNCTION OF MESENCHYMAL STEM CELLS

Massimo Giuliani[1], Noufissa Oudrhiri[2], Amelia Vernochet[1], Simone Negrini[1], Bernard Charpentier[1], Annelise Bennaceur[2], Antoine Durrbach[1]. [1]*U542, INSERM, Villejuif, France;* [2]*U935, INSERM, Villejuif, France*

Bone Marrow Mesenchymal Stem Cells (BM-MSC) have been demonstrated to regulate T and NK cells in transplantation. On the other hand, IL-2/15-activated NK cells can kill BM-MSC. TLR represent an alternative mechanism of NK cells activation, with respect to the classical IL-2/15 activation. In humans, ten members of TLR family have been described that recognizes distinct PAMPs and mediates the NK cells activation. NK cells express TLR-3, -7 and -8 that lead to their NK cells activation and cytotoxicity. TLR-3 is activated by poly (I:C), induces signal via a MyD88-indipendent pathway and up-regulates IFN-γ production. In contrast, TLR-7/8 cascade is mediated via a MyD88-dependent pathway and induces IFN-γ production by activated NK cells.
The goal of this study was to evaluate the interactions between NK and MSC derived from different sources. We isolated MSC from Bone Marrow and from Embryonic Stem Cells and investigated their interactions with allogenic NK cells. NK cells were stimulated with TLR3 or TLR7/8 agonists and cultured in the presence or not of MSC to test their cytokine production, phenotype and cytolytic functions. We show for the first time that BM and ES-MSC inhibit type I interferon production and affect Natural Cytotoxic Receptors (NCR) expression in IL 12/TLRs activated NK cells. MSC also decrease cytolytic activity [CD107a (LAMP-1) degranulation] and lysis of K562 cells. In contrast, MSC failed to inhibit pre-activated NK cells cytolytic functions. These results suggest a novel type of inhibition of NK cells by adult and embryonic MSC, that could lead to a dramatically consequence in the immune response and renforce their use in transplantation.

Session 15. Deceased donation: procurement, allocation, safety & best practise

O-125 CRITICAL CARE STAFFS' ATTITUDES TO ORGAN DONATION IMPACT ON NATIONAL DONATION FIGURES – DATA FROM THE DONOR ACTION DATABASE

Leo Roels[1], Caroline Spaight[1], Jacqueline Smits[2], Bernard Cohen[1]. [1]*Donor Action Foundation, Linden, Belgium;* [2]*Eurotransplant International Foundation, Leiden, Netherlands*

Study aims: To investigate whether Critical Care (CC) staffs' attitudes to organ donation, the concept of brain death (BD) and self-reported skills in donation related tasks may impact on national donation rates.
Methods: Donor Action (DA) Hospital Attitude Survey (HAS) data was collected from 19,537 CC staff in 245 hospitals in 11 countries (Australia, Belgium, Croatia, Finland, France, Israel, Italy, Japan, Norway, Poland, Switzerland) between November 2006 and October 2008. Data examined included average support to donation, respondents' willingness to donate their own, their children's and relatives' organs, the acceptance of the BD concept and reported confidence levels with donation-related tasks. Countries' donation performance was expressed as a Procurement Efficiency Index (PEI) (organs procured and transplanted/deaths from eligible causes/million population/year)*.
Results: A strong positive association was found between national PEI rates and CC staffs' average support to donation (R=.700, P=.0141), acceptance of the BD concept (R=.742, P=.0069), notifying a transplant coordinator (R=.722, P=.01), explaining BD to family (R=.763, P=.0045), introducing organ donation to family (R=.867, P=.0002) and obtaining consent to donation (R=.796, P=.0021).
Conclusions: DA's HAS methodology is a powerful and standardized tool to assess CC staffs' attitudes and donation related skills in different environments. HAS outcomes are strong predictors of national donation rates, as demonstrated in this study. Measures to improve countries' donation performance should focus on guidance and education of CC staff so as to ensure that these practitioners have sufficient knowledge and confidence with donation related issues.
References: Countries' Donation Performance in Perspective: Time for more Accurate Comparative Methodologies (Editorial). *L. Roels e.a.,. Am J Transplantation 2007; 7:1439-1441*

O-126 KIDNEY, KIDNEY-PANCREAS AND LIVER-KIDNEY TRANSPLANTATION IN HIV INFECTED INDIVIDUALS: THE ITALIAN EXPERIENCE

P.A. Grossi[1], C. De Cillia[2], S. Venettoni[2], L. Rizzato[2], D. Donati[3], D. Dalla Gasperina[1], M. Tozzi[4], S. Sandrini[5], U. Boggi[6], P. Castelli[4], S.M. Giulini[10], P. Gerunda[7], F. Bresadola[8], L. Lazzaroni[1], A. Vaglio[9], R. Dionigi[4], A. Nanni Costa[2]. [1]*Infectious Diseases, University of Insubria, Varese, Italy;* [2]*Centro Nazionale Trapianti, Istituto Superiore di Sanità, Roma, Italy;* [3]*Transplantation Nephrology, Ospedale di Circolo, Varese, Italy;* [4]*Surgery, University of Insubria, Varese, Italy;* [5]*Nephrology, Ospedali Civili, Brescia, Italy;* [6]*Surgery, University of Pisa, Pisa, Italy;* [7]*Surgery, University of Modena, Modena, Italy;* [8]*Surgery, University of Udine, Udine, Italy;* [9]*Transplantation Nephrology, University of Parma, Parma, Italy;* [10]*Vascular Surgery, University of Brescia, Brescia, Italy*

Introduction: Until a few years ago, HIV infection was considered an exclusion criteria for organ transplantation. However, more recently, because of the significant increase in life expectancy of HIV-infected persons with highly active antiretroviral therapy (HAART), kidney, kidney-pancreas, heart, lung and liver transplantation have been introduced in this patients population in several centers around the world.
Aim of the study: To evaluate the possible extension of the indications of kidney transplantation to HIV-infected individuals, the *Italian National Centre for Transplantation* has designed a protocol to be applied on a national basis.
Material and methods: Inclusion criteria required a CD4 count 200/mm^3 and undetectable HIV viral load for at least 3 months for patients on HAART. The program was voluntarily adopted by 6 transplant centres.
Results: From January 2006 through November 2008 a total of 24 HIV infected patients (16 male and 8 female, mean age 42.9 years, range 27-56) underwent cadaveric kidney transplantation (including three kidney-pancreas and two liver-kidney) after a median waiting time of 219 days (range 1-891). Median CD4 cells count at the time of transplantation was 385 (range 210-830) and the HIV-RNA was undetectable in all recipients. HAART was started in all recipients after transplantation and HIV-RNA remain undetectable in all patients. Six patients (25%) experienced an episode of biopsy proven acute rejection (steroid resistant in one). Drug-drug interactions between antiretrovirals and immunosuppressive agents required frequent dosage modifications. Graft and patient survival was 100% at a median follow-up of 395 days after transplantation (range 80-1040).

Conclusion: Despite the limited number of patients and the shortness of the follow-up, our study confirms excellent short term results of kidney transplantation in HIV-infected individuals

O-127 VIROLOGICALLY COMPROMISED DECEASED ORGAN DONORS IN THE UNITED KINGDOM

Sonia J. Wakelin[1], James J. Powell[1], Mark Jones[2], Rachel Johnson[2], John L.R. Forsythe[1]. *[1]Transplant Unit, Edinburgh Royal Infirmary, Edinburgh, United Kingdom; [2]Department of Statistics and Clinical Audit, NHS Blood and Transplant, Bristol, United Kingdom*

Purpose: There is growing disparity between organ supply and demand in the United Kingdom leading to increased usage of extended criteria donors including those where there is an increased risk of transmitting blood borne viruses. Using registry data from UK Transplant, this study evaluates the use of virologically compromised organs over a 10 year period.
Results: Between 1998 and 2007, 21057 organs were utilised from 7676 organ donors of which 151 (1.97%) were virologically compromised. Fewer virologically compromised donors were used in the first (n=43) compared to the second (n=108) half of the decade. The majority of donors were HBcAb positive (n=110, 72.8%), with a smaller proportion being HBsAg positive (n=2, 1.32%). 38 donors (25.16%) were HCV positive. Of 649 virologically compromised organs offered, 357 (55%) were utilised. Transplant survival rates for livers and kidneys from virologically compromised and non-virologically compromised donors are shown in the table. The majority of virologically compromised livers (n=75, 70%) were transplanted into virologically compromised recipients. Subgroup analysis revealed 1 and 5 year transplant survival rates of 82% (95% CI=73-90) and 52% (95% CI=35-69) for HBV positive liver allografts. For HCV positive liver allografts, 1 and 5 year transplant survival rates were 87% (95% CI=74-100) and 70% (95% CI=37-100). Survival rates were similar irrespective of whether livers were transplanted into virologically compromised or non-compromised patients.

	Number	1 yr survival	95% CI	5 yr survival	95% CI	P value
Livers						
Virologically compromised	107	84%	77-91	66%	54-79	p=0.5
Non-virologically compromised	6455	80%	79-81	68%	67-69	
Kidneys						
Virologically compromised	182	85%	80-90	66%	57-75	p=0.04
Non-virologically compromised	11659	89%	88-89	76%	75-76	

Discussion and conclusions: These data suggest that liver and kidney allografts from virologically compromised donors provide acceptable results and that there may be scope for increased use of such organs.

O-128 HBV AND HBC POSITIVE ORGAN GRAFTS IN FRANCE

François Lamy, Fabienne Pessione, Aurélie Deshayes, Hervé Creusveaux, Marina Roche, Marie Thuong, Bernard Loty. *Medical and Scientific Direction, Agence de la Biomédecine, Saint-Denis La Plaine, France*

Objective: French law has authorized for a period of 4 years (2006 – 2009) the use of kidneys, lungs, heart and liver for transplantation purposes from donors positive for Hepatitis B Viral (HBV) markers or Hepatitis C Viral (HCV) markers. The Agence de la biomedecine monitors these grafts to measure whether they present no more risk than a graft from a negative donor.
Methods: All recipients transplanted in France in 2006 and 2007 with an organ recovered from a donor positive for HCV or HBV markers are monitored for main hepatitis viral markers at 3 to 24 months after transplantation. All negative to positive viral marker transitions are studied and investigated during this period. Graft survival comparisons between this cohort and the main national cohort are also produced.
Results: 216 HBV positive donors and 454 grafts were observed during the period, mainly kidneys and livers. The majority (83%) of donors were positive for both HBc and HBs antibodies (AcHBc and AcHBs). The majority of recipients (50%) were positive for isolated AcHBs. Follow-up: 25 recipients (5%) showed a positivation of AcHBc and 3 recipients (<1%) showed a negative to positive HBs antigen transition (naïve liver recipients). No difference was found in graft survival for liver or kidney recipients versus the national cohort. 6 HCV and 12 grafts were observed during the period. One recipient died prematurely after the graft (death not related to the organ), all the other recipients did not show adverse reactions.
Discussion: A report will be produced and used by the French health ministry to assess whether these grafts can be maintained beyond the considered derogation. Patients treated with prophylactic antiviral drugs and grafted with HBV positive organs only show AcHBc positivation – the 3 cases of HBs antigen positivation were from patients without prophylactic treatment.

O-129 A UK REGISTRY STUDY OF THE INCIDENCE OF TRANSMISSION OF INTRACRANIAL MALIGNANCY

Rebecca Roberts[1], Karen Wright[2], David Greenberg[2], Claire Hamilton[3], Dave Collett[3], Christopher Watson[1]. *[1]University of Cambridge Department of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Eastern Cancer Registry and Information Centre, National Cancer Information Service, Cambridge, United Kingdom; [3]Directorate of Audit and Statistics, NHS Blood and Transplant (UK Transplant), Bristol, United Kingdom*

Background: Anecdotal case reports and registry data show the potential for transmission of donor CNS malignancy, and the Council of Europe guidelines advise against the use of such donors, in particular high grade gliomas such as Glioblastoma Multiforme. We examined the UK data to establish the incidence of transmission of primary intracranial malignancy from the donor to the recipient.
Methods: Data from the National Transplant Database were merged with data held by the cancer registries of England, Wales and Northern Ireland to identify all donors with a history of intracranial malignancy. The recipients of organs from these donors were then identified and screened to identify post transplant malignancy.
Results: From 1/1/95 to 31/12/01 there were 5936 donors whose data could be analysed by the cancer registries. From these, 194 donors were identified as having a history of primary intracranial malignancy. These tumours included 24 Grade 4, 3 Grade 3 and 2 Grade 1 Gliomas, in addition to 9 medulloblastomas and a further 82 of indeterminate histology. Organs from these 194 donors were transplanted into 411 recipients, 24 of whom were subsequently notified as developing a malignancy within 2 years of transplant. Of these 24 malignancies 9 were tumours identified in the explanted liver immediately post transplant (8 hepatomas & a cholangiocarcinoma); 6 were lymphoproliferative disorders, 3 skin cancers together with carcinomas of bronchus, breast, pancreas and bladder. There was no incidence of transmission of any primary intracranial malignancy.
Conclusion: These data suggest that donor intracranial malignancy has a low incidence of transmission, and organs from such donors may be used, but the recipients should be warned of the potential risk of transmission.

O-130 GENDER-BASED DISPARITIES IN ACCESS TO LIVER TRANSPLANTATION FROM THE WAITING LIST

Amit K. Mathur[1], Douglas E. Schaubel[2,3], Qi Gong[3], Mary K. Guidinger[2,4], Robert M. Merion[1,2,4]. *[1]Division of Transplantation, Department of Surgery, University of Michigan, Ann Arbor, MI, USA; [2]Scientific Registry of Transplant Recipients, Ann Arbor, MI, USA; [3]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA; [4]Arbor Research Collaborative for Health, Ann Arbor, MI, USA*

Objectives: Gender-based disparities exist in many high-complexity health care services. We sought to determine whether gender disparities exist in access to deceased donor liver transplantation (DDLT).
Methods: The Scientific Registry of Transplant Recipients database was used to analyze 53,129 adult candidates waitlisted before (8/1997-8/2001) or after (2/2002-2/2007) implementation of liver allocation based on mortality risk (Model for End-stage Liver Disease [MELD]). The primary outcome was DDLT. Cox models were fitted to determine covariate-adjusted differences in DDLT rates by gender.
Results: Females represented 41% of waitlisted patients in the pre-MELD era and 35% in the MELD era. In an analysis spanning both eras, females had significantly lower adjusted DDLT rates (hazard ratio [HR] 0.72; 95% confidence interval [CI] 0.56-0.93; p=0.01). Females had significantly lower adjusted DDLT rates in both the pre-MELD era (HR 0.66; 95% CI 0.51-0.87; p=0.003) and in the MELD era (HR 0.85; 95%CI 0.81-0.88; p=0.003). In the MELD era, significant access disparities for women were seen only at MELD scores above 14 (Fig. 1). Interaction tests between race and gender were not significant in either era.

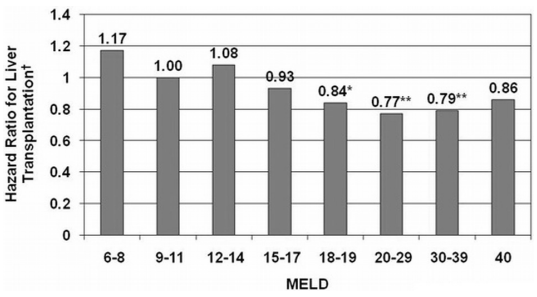


Figure 1. Lower rates of liver transplantation in women with high MELD scores. *p<0.10, **p<0.05. †HR calculated relative to men. HR > 1.00 denote higher rates for women, and HR < 1.00 denote lower rates for women.

Conclusions: Impaired DDLT access among female candidates has been ameliorated in the MELD era, but a significant disparity remains. The concentration of this disparity among candidates at higher risk of waiting list mortality in the current MELD era is troubling, and causative factors merit further study.

O-131 EXPERIENCE OF A HIGH VOLUME LIVER TRANSPLANTATION CENTER WITH THE INTRODUCTION OF THE MELD ALLOCATION SYSTEM IN EUROPE (EUROTRANSPLANT COLLABORATIVE REGION)

Frank Ulrich, Panagiotis Fikatas, Michael Hippler-Benscheidt, Andreas Pascher, Gero Puhl, Olaf Guckelberger, Ulf Neumann, Peter Neuhaus, Johann Pratschke. *Dept. of General, Visceral and Transplantation Surgery, Charité, Campus Virchow Clinical Centre, Berlin, Germany*

Purpose and methods: The aim of this study was to investigate specific alterations and implications after introduction of the MELD allocation in the Eurotransplant region. We analyzed a period of 18 months before and after the start in December 2006 concerning demographic data, waiting list characteristics, posttransplant complications and outcome before and after introduction of the MELD allocation.

Results: The mortality on the waiting list was lower by trend in the MELD allocation period (9.2 vs. 13.2%; p=0.17). Recipients allocated by MELD (n=199) showed several significant differences compared to the UNOS-allocated group (n=223): higher recipient and donor age, decreased waiting time, longer cold ischemia and higher percentage of alcoholic cirrhosis. The labMELD-Score at listing was higher in the MELD-allocation period (with exceptions: 17.3 vs. 19.7, p=0.05; without exceptions: 19.1 vs. 22.1, p=0.03). MELD-allocated patients had an increased need for transfusions and required more posttransplant dialyses (32.7% vs. 42.2%; p=0.04). Beside a higher rate of biliary leakage and ITBL, the incidence of graft vessel thrombosis was lower. The 1-year patient and graft survival (UNOS vs. MELD) was 89.6 vs. 83.8% (p=0.19) and 77.1 vs. 77.1%.

The incidence of PNF was comparable, while the retransplantation rate was higher in the UNOS group (p=0.004). Time on the waiting list was significantly reduced for patients with HCC and alcoholic cirrhosis. ROC-analysis showed that MELD is a moderately adequate predictor for 3-months-, but not for 1-year survival.

Conclusion: The introduction of a MELD-based allocation in the Eurotransplant region resulted in a decreased mortality and a significantly reduced time on the waiting list. Despite higher donor and recipient age, higher transfusion demand and need for dialysis, patient survival was lower by trend only, while graft survival remained comparable.

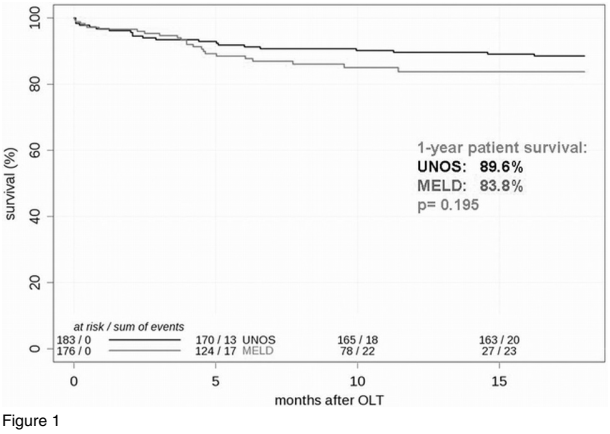


Figure 1

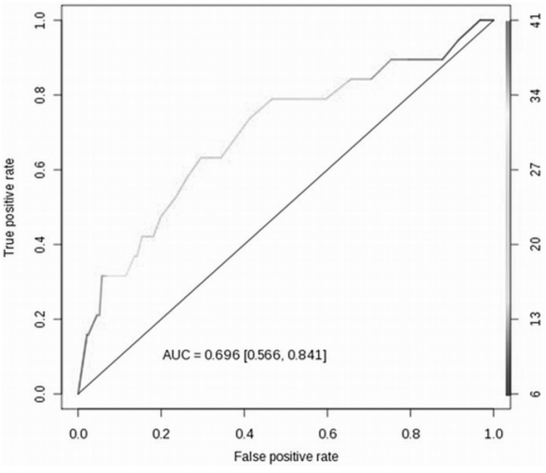


Figure 2. ROC analysis: MELD score and patient survival (3 months).

O-132 KIDNEY DONATION AND TRANSPLANTATION IN EUROTRANSPLANT: MINIMIZING DISCARD RATES BY USING A RESCUE ALLOCATION POLICY

Maurits T. Vinkers[1], Jacqueline M. Smits[1], Ineke C. Tieken[1], Jan de Boer[1], Hans de Fijter[3], Dirk Ysebaert[2], Axel Rahmel[1]. *[1]Research, Eurotransplant, Leiden, Netherlands; [2]Surgery, University Hospital, Antwerp, Belgium; [3]Nephrology, LUMC, Leiden, Netherlands*

It is a challenge for every organ exchange organization to maximize the donor utilization rate. We investigated the benefit of a rescue allocation policy and to study the impact of donor factors on the risk of kidney discard.

All renal donors offered for allocation to Eurotransplant between 2006-2007 were included [N=4057]. Allocation is *patient-oriented* based on a point-score system including recipient and donor factors. In case, an organ offer is rejected 5 times for medical reasons, allocation can be switched to rescue allocation, i.e. the organ is then offered in a *center-oriented* way. A logistic regression model was built to test whether donor factors could predict both the probability of the need for rescue allocation and the probability of kidney discard.

Rescue allocation policy was applied to 665 donors (16.4%); within this group transplant rate was 54.3%, resulting in a donor discard rate of 304 donors (7.5% of the total study group). The multivariate model showed that donors aged <10 years and donors with a high creatinine (>1.5 mg/dL) were significantly more likely to be allocated via the rescue allocation system (OR=6.6, $p<0.001$ and OR=3.2, $p<0.001$, respectively). Moreover, a positive virology led to an increased probability of rescue allocation (HBsAg+ OR=14.8, $p<0.0001$, HCV+ OR=28.2, $p<0.0001$). The odds of kidney discard were significantly associated with donor age ($p<0.001$), non heart beating donor ($p<0.001$), serum creatinine ($p<0.001$), diabetes (p=0.025), HbsAg+ ($p<0.001$) and HCV+ ($p<0.001$).

Rescue allocation is an effective way to achieve a lower donor discard rate. But even with a rescue allocation scheme, several donor factors were significantly associated with a higher kidney discard rate. The combination of liberal donor criteria and rescue allocation policy can reduce the loss of kidneys thereby decreasing the waiting list for kidney transplantation.

O-133 PREEMPTIVE CADAVERIC RENAL TRANSPLANTATION: FAIRNESS AND UTILITY IN THE CASE OF HIGH DONATION RATE

Elisabetta Bertoni, Giuseppina Rosso, Aida Larti, Alberto Rosati, Maurizio Salvadori. *Renal Unit, Careggi University Hospital, Florence, Italy*

Preemptive from deceased donors is rarely performed because of organ shortage. Aim of our study is to describe the characteristics of a preemptive cadaveric program started in our region in the last years because of an huge increase in organ donation rate.

Tuscany, an Italian region, experienced in the last years an increase of donation rate up to 37.5 pmp. Patients on waiting list for transplantation slightly decreased (from 445 to 423 patients in the last 2 years). Starting from 2006 a cadaveric preemptive transplant program has been activated for patients belonging to stage 5 of CKD.

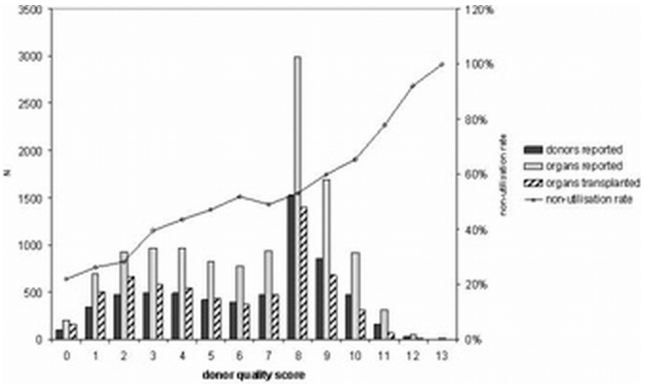
From october 2006 to october 2008, 163 patients entered on waiting list for renal transplantation. 120 of them (73.6%) were on dialysis while 43 patients (26.4%) were not yet on dialysis (preemptive).Of 43 preemptive patients, 8.6% started dialysis treatment while waiting on list within 3.1±1.9 months. Overall 58/163 (35.6%) patients were transplanted during the period after a waiting time of 10.3±6.4 months. 43 patients were on dialysis, 15 were preemptive (25.8%). At the Cox multivariate analysis the probability of transplantation was similar for preemptive and dialysed patients (RR 1.02; p=NS). One and two years graft (94% vs 92%) and patient survival (94% vs 92%) were similar but DGF was lower in preemptive group (13 vs 42%;p=0.007). The one year serum creatinine was 1.56±0.43 in the preemptive group and 1.68±0.92 in the dialysis group (p=NS).No differences were on donor age and rejection rate.

The preemptive listing rate for cadaveric renal transplant is over 35%, one of the highest level reported in literature. In the last two years preemptive transplantation was 25.8%, whereas reported rate is less than 7-8%. This is the result of the optimal cooperation between nephrologists and transplant centers in our region.

O-134 INCREASING THE DONOR POOL BY THE USE OF A LUNG DONOR QUALITY SCORE

Jacqueline M. Smits[1], Wim Van der Bij[2], Dirk Van Raemdonck[3], Maurits Vinkers[1], Axel Rahmel[1]. *[1]Statistics and Research, Eurotransplant, Leiden, Netherlands; [2]Internal Medicine, UMCG, Groningen, Netherlands; [3]Thoracic Surgery, University Hospital Gasthuisberg, Leuven, Belgium*

Background: Lung donors are still a scarce resource, in 2008 in Eurotransplant only 22% of all donors was a lung donor. Optimal donor recognition is a prerequisite for obtaining a high lung donation rate.
Aim: In order to increase the objectivity in evaluating a potential lung donor, a lung donor quality score was calculated for all reported lung donors and the outcome of their donation process was evaluated.
Material and methods: A lung donor quality score developed by Oto *et al.* But adapted to the Eurotransplant population was applied to all lung donors reported to Eurotransplant in the period January 1,1999 and December 31, 2007 [N=6234]. Points in this donor scoring system were assigned as follows: donor age (y): [<45,0; 45-54,1; 55-59,2; ≥60,3]; history of smoking [no, 0; yes, 1]; Chest X-ray [clear, 0; edema or shadow, 1; atelectasis, 2; consolidation, 3]; bronchoscopy [clear, 0; non-purulent secretions, 1; purulent secretions, 2; inflammation, 3; tumor, 3]; PaO_2/FiO_2 (mmHg) [>450, 0; 351-450,2; 301–350,4; ≤300,6]. The non-utilisation rate is obtained by taking 1 minus the number of transplanted lungs devided by the number of reported lungs.
Results: The number of reported lung donors, reported lungs and transplanted lungs stratified by value of the lung donor quality score are shown in the figure. There is an inverse relation between the value of the lung donor score and the likelihood that a reported lung donor is ultimately used for transplantation.



Conclusions: The lung donor quality score can help in screening for potential lung donors. Therefore, we recommend calculating a lung donor quality score for each potential lung donors, and to consider all donors with score that indicates high quality, i.e. a low value, for lung transplantation. In the future, this lung donor quality score might be used in the lung allocation scheme.

O-135 HOW EFFECTIVE IS A THEORY-BASED INTERVENTION TO INCREASE ADOLESCENTS' INTENTION TO REGISTER AS A DONOR?

Fabienne Dobbels[1], Frank Van Gelder, Jacques Pirenne[2]. *[1]Center for Health Services and Nursing Research, Katholieke Universiteit Leuven, Leuven, Belgium; [2]Abdominal Transplantation, University Hospitals of Leuven, Leuven, Belgium*

Introduction: Interventions to promote organ donation have seldom been tested. Our RCT tested the efficacy of a theory-based intervention on adolescents' intention to register as a donor.
Methodology: Using the integrated model of behavioral prediction, we surveyed which factors were influencing intention towards registration in all fifth grade adolescents (age ±17 years) of 14/16 high schools in Leuven (Belgium) (N= 1328) (multilevel logistic regression analysis). All schools were randomized (cluster design) afterwards into a control (N= 6/7) and an intervention group (N= 6/7). The 1, 5 hour intervention (1 year after baseline), using the results of the baseline assessment, consisted of a young patient's witness, a letter of a mother of a young donor, targeted information ruling out wrong beliefs, an explanation of the law and how one can register in favor or against donation. Intention was re-evaluated 1 year after baseline.
Results: Female gender, being engaged in social activities, not believing that donation is mutilating the body, a better knowledge of the law, a more favorable attitude towards donating the organs of family members and donating their own organs, paying less attention to the family's opinion, a higher self-efficacy, knowing somebody who has been a donor, and having had a conversation with the family, were all significant determinants of a positive intention, explaining 81% of the variance (N= 1328). At baseline, 23% had a positive intention (based on 824 students who completed both questionnaires). Intention to register as a donor increased to 40% in the intervention group, while in the control group intention remained stable (24.8%) (RR= 1.98; 95% CI: [1.5; 2.7]).
Discussion: These results show that a tailored, theory-based intervention program has a strong effect on intention to register as a donor in adolescents, but should be replicated.

Session 16. Risk factors, mechanisms & treatment of kidney humoral rejection

O-136 EXPERIENCE WITH 50 CONSECUTIVE ABO INCOMPATIBLE KIDNEY TRANSPLANTATIONS AT THE KAROLINSKA UNIVERSITY HOSPITAL

Gunnar Tydén[1], Gunilla Kumlien[2], Helena Genberg[1], Mats Efvergren[3], John Sandberg[1], Johan Nordström[1], Amir Sedigh[1], Lars Wennberg[1], Henrik Gjertsen[1]. *[1]Transplantation Surgery, Karolinska University Hospital, Stockholm, Sweden; [2]Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden; [3]Renal Medicine, Karolinska University Hospital, Stockholm, Sweden*

Purpose: To evaluate a protocol for ABO-incompatible kidney transplantations, using antigenspecific immunoadsorption, rituximab instead of splenectomy and a conventional immunosuppressive protocol.
Methods: The protocol called for a preconditioning starting with one dose of rituximab, 375 mg/m^2, given one month before transplantation, followed by tacrolimus, mycophenolate mofetil and conventional prednisolon tapering starting 10 days pretransplant. Antigen-specific immunoadsorption was performed on pretransplant days –6,-5,-2 and –1. Following the last session 0.5 g/kg of intraveneous immunoglobulin was given. Postoperatively three more apheresis sessions were given every third day.
Results: Fifty consecutive patients have been transplanted with this protocol. The A/B antibodies were readily removed by the antigen specific immunoadsorption before transplantation and remained at a low level post transplantation. We have not seen any humoral rejections or late rebound of anti-A or anti-B antibodies. Two kidneys have been lost for technical reasons (one venous and one arterial thrombosis) and another two have been lost due to patient non compliance, all the remaining 46 having normal function at a follow-up of up to 91 months.
Conclusion: We conclude that following one dose of rituximab and antigen-specific immunoadsorption, blood-group incompatible renal transplantations can be performed with standard immunosuppression and with excellent short and long term results.

O-137 NATIONAL REGISTRY OF ANTIBODY INCOMPATIBLE RENAL TRANSPLANTATION, 2001-2008: PRELIMINARY ANALYSIS

Rachel Johnson[1], Rob Higgins[2], Susan Fuggle[1], David Taube[3], Jack Galliford[3], Nizam Mahmode[4], Andrew Bradley[1]. *[1]Organ Donation and Transplant Directorate, NHS Blood and Transplant, Bristol, United Kingdom; [2]Dept Transplantation, University Hospital, Coventry, United Kingdom; [3]West London Renal and Transplant Centre, Hammersmith Hospital, London, United Kingdom; [4]Transplant Unit, Guy's and St Thomas' Hospital, London, United Kingdom*

Antibody incompatible renal transplantation (AIT) is widely practised, but there remain uncertainties about outcomes. The UK Registry is the first comprehensive national registry for HLA antibody and ABO AIT.
Comprehensive data for all UK transplants are already collected. An additional AIT dataset was introduced in 2008, collecting data on transplants since 2001 with ABO incompatibility (ABOi), or donor specific HLA antibodies (DSA) detectable in the immediate pre-transplant period (HLAi). Those with historic positive, current negative DSA were not included.
190 transplants were performed in 14 centres. 11 centres reported 109 HLAi transplants (range per centre 1-58); 9 centres reported 75 ABOi transplants (range 1-38), and 3 centres performed 6 transplants with simultaneous HLAi and ABOi. The annual transplant rate rose to 60 in 2007, and 61 in the first 8 months of 2008.
ABO antibodies were removed with plasmapheresis in 76% of cases, antigen-specific absorption in 18%, and 6% had no antibody removal. In HLAi transplantation, 29% of cases had a positive pre-treatment cytotoxic crossmatch, 49% positive flow cytometric crossmatch, and 22% had DSA detected only by microbead or other solid phase assay. Intravenous immunoglobulins and monoclonal antibodies against CD20 and CD52 were all used, but not by all units.
Three year graft survival (death and graft loss) was 93% (95% CI 83-97%) for ABOi, and 83% (95% CI 70-90%) for HLAi. In antibody compatible transplants

performed in the UK in the same time period, 3 year graft survival was 91% (95% CI 90-92%) for living donor transplants, and 84% (95% CI 83-85%) for deceased donor transplants.
In summary, over half the units in the UK have performed AIT. Three year graft survival rates for ABOi and HLAi were comparable with those in antibody compatible living donor and deceased donor transplantation respectively.

O-138 THE EVALUATION OF TWO DIFFERENT PRECONDITIONING REGIMENS FOR ABO-INCOMPATIBLE LIVING KIDNEY DONOR TRANSPLANTATION. A COMPARISON OF SPLENECTOMY VS. RITUXIMAB-TREATED NON-SPLENECTOMY PRECONDITIONING REGIMENS

Toshihito Hirai[1], Hideki Ishida[1], Yuuki Miyauchi[1], Tomokazu Shimizu[1], Hiroki Shirakawa[1], Kazuya Omoto[2], Kazunari Tanabe[1]. *[1]Urology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan; [2]Transplantation, Ohkubo Hospital, Shinjuku-ku, Tokyo, Japan*

Background: The aim of this study was to evaluate the long-term results of ABO-ILKT with splenectomy, and also compare the putcome of ABO-ILKT with splenectomy versus Rituximab-treated non-splenectomy.
Methods: We performed ABO-incompatible living donor kidney transplants at our institution and affiliated hospital between January 2001 and December 2006 (n=70).
Between January 2001 and December 2004, all patients underwent pretransplant double filtration plasmapheresis (DFPP) and splenectomy at the time of transplant (n=46) (ABO-ILKT SPX group). Between January 2005 and December 2006, splenectomy was not perfromed and a protocol that involved pretransplant low dose Rituxumab was employed (n=24) (ABO-ILKT RIT group). ABO-compatible living kidney transplants (n=55) performed between January 2001 and December 2004 were employed as a control group (ABO-C group). We retrospectively compared the patient survival rate, graft survival rate and the incidence rate of acute rejection in each group.
Results: Patient survival was 100% in all groups. Three-year graft survival was 98.2, 93.5 and 95.8% in the ABO-C, ABO-I-SPX and ABO-I-RIT groups. Five-year graft survival rate was 93 and 91.3% in the ABO-C and ABO-I-SPX groups. Renal allograft function was comparable among the three groups. However, compared to the ABO-I-RIT group, the incidence of acute antibody mediated rejection (acute-AMR)or chronic antibody mediated rejection (chronic-AMR) was significantly higher in the ABO-C and ABO-SPX groups.
Conclusion: Although long-term outcome of the ABO-I-SPX group was excellent and showed no significant difference compared to the ABO-C group, splenectomy is not essential for the successful ABO-ILKT. The rituximab-treated patients showed was excellent short-term graft survival and renal function, and the incidence of AMR in the ABO-I-RIT group was significanly reduced compared to the ABO-I-SPX group.

O-139 ABO INCOMPATIBLE TRANSPLANTATION WITHOUT RITUXIMAB OR SPLENECTOMY – EXCELLENT CLINICAL AND HISTOLOGICAL OUTCOMES BEYOND 12 MONTHS

Shlomo (Solomon) J. Cohney, Rowan G. Walker, Gavin Becker, Rosemary Masterson, Amanda Robertson, Shaun Flint. *Nephrology, Royal Melbourne Hospital, Melbourne, VC, Australia*

Introduction: Until recently ABO incompatible renal transplantation (ABOi) was performed with splenectomy, and/or Rituximab. Our ABOi patients now receive a Tacrolimus based regimen, identical to that used in our "ABO compatible" live donor transplant recipients, except for antibody removal.
Results: 50 patients have received ABOi transplants, with 100% patient and graft survival. Four have received Rituximab, six others were transplanted with one or more coexisting donor specific anti-HLA Ab. Thus 40 ABOi recipients have been transplanted with neither Rituximab or splenectomy. Within this group, thirty have had transplants beyond 1 year, fifteen beyond 2 years, and 8 patients have had functioning transplants for around 3 years.
There have been 2 episodes of AbMR, both within the first 10 days post transplant with neither causing ill effect on long-term function or subsequent histological appearance. There have been 7 episodes of cellular rejection, 2 subclinical; one occurring beyond 12 months following a change in immunosuppression to facilitate pregnancy. The latter resulted in significant loss of graft function. All others have maintained stable function, except one who developed BK nephropathy. There have been 5 other cases of BK viraemia which resolved without sequelae. Protocol biopsies have been performed, and in the 14 patients with biopsies beyond 1 year there is no transplant glomerulopathy. There has been one case of CMV disease in a patient who failed to receive prophylaxis and no other opportunistic infections. Three patients are on treatment for post-transplant Diabetes Mellitus.
Conclusion: ABO Incompatible Kidney Transplantation (including moderate to high titre) can be performed with conventional immunosuppression with good patient and graft outcomes beyond the first year.

O-140 PRESENSITIZED KIDNEY GRAFT RECIPIENTS WITH HLA CLASS I AND II ANTIBODIES ARE AT INCREASED RISK OF GRAFT FAILURE. A COLLABORATIVE TRANSPLANT STUDY REPORT

Caner Süsal, Bernd Döhler, Gerhard Opelz. *Department of Transplant Immunology, University of Heidelberg, Heidelberg, Germany*

Purpose and methods: We have previously reported that kidney transplant recipients with ELISA-reactive HLA-class I and -II antibodies are at an increased risk of graft failure. Our center uses this parameter routinely in an algorithm for the transplantation of highly immunized patients. To verify whether positivity for both classes of HLA-antibodies prior to transplantation is a valid indicator for identification of high-risk patients, the impact of preformed HLA-antibodies on graft survival was analyzed in a completely new series of 5.315 kidney transplantations performed between 2000-2008.
Results: In line with our previous findings, 121 first transplant recipients positive for both HLA-class I and -II antibodies had a poor 2-year graft survival rate of 76.5±4.0%, compared with an 87.5±0.5% rate in 4.175 recipients who were negative for both antibody classes ($p<0.001$). Good survival rates were observed in HLA-class I-positive/II-negative and HLA-class I-negative/II-positive recipients. Importantly, graft survival was good in HLA-class I- and -II-positive patients when they received a kidney with a 0-1 HLA-A+B+DR-mismatch and poor when they received a kidney with 2-4 or 5-6 mismatches. In the multivariate analysis, HLA-class I-positive/II-positive recipients showed a significantly increased hazard-ratio of 1.73 ($p<0.001$). HR were 1.77 for first transplants, 1.96 for retransplants, and 1.57 and 2.64, respectively, when 2-4 and 5-6 mismatched transplants were analyzed. Among patients with a functioning graft at month 3, as many as 48% of HLA-class I-positive/II-positive patients experienced an acute rejection episode, strikingly higher than the 16% rate in antibody negative patients ($p<0.001$).
Conclusion: These data confirm our previous observation that kidney transplant recipients who simultaneously possess HLA-class I and II antibodies in their pretransplant serum are at an increased risk of graft failure and require special attention.

O-141 NATURAL HISTORY OF SUBCLINICAL ANTIBODY-MEDIATED REJECTION (SAMR) IN KIDNEY TRANSPLANT RECIPIENTS WITH DONOR SPECIFIC ANTIBODIES (DSA)

A. Loupy[1,2,3], C. Suberbielle[4], G. Hill[3], C. Lefaucheur[5], D. Anglicheau[1,2], J. Zuber[1,2], Mo Timsit[6], F. Martinez[1,2], E. Thervet[1,2], P. Bruneval[1,3], C. Legendre[1,2], D. Nochy[1,3]. *[1]Université, Paris Descartes, Paris, France; [2]Transplantation Rénale, Hôpital Necker, Paris, France; [3]Anatomopathologie, HEGP, Paris, France; [4]Histocompatibilité, Saint Louis, Paris, France; [5]Saint Louis, Transplantation Rénale, Paris, France; [6]Urologie, Hôpital Necker, Paris, France*

Over the last years, results have improved in KTR with pre-existing DSAs. However, in these recipients, the natural history of SC AMR still deserves to be evaluated.
Methods: From January 2002 to March 2007, 54 patients with preformed DSAs received an ABO compatible deceased donor kidney transplant. Immunosuppression combined a biological induction, a CNI, an IMPDH inhibitor and low-dose steroids. Due to the presence of DSAs, all patients received 4 additional courses of 2g/kg/day IVIgs during the first 3 months. In this prospective observational study, all patients underwent a screening biopsy, a measured GFR and DSA testing by Luminex SA at 3 months and one year.
Results: The peak class I or II MFImax DSA value was 8557±705. After a mean follow-up of 30.2±16 months, graft and patient survival were 90.8% and 96.3%, respectively. Clinical AMR was observed in 10 patients (18.5%). At 3 months 31.1% of patients met the criteria of SAMR. Patients with 3-month SAMR had at 1 year: a higher C4d (1.6±0.99 vs 0.29±0.49, p=0.01), ptc score (1.90±0.98 vs 0.64±0.85, $p<0.01$), and arteriosclerosis score (1.37±0.91 vs 0.50±0.58, p=0.03), higher rate of IFTA (100% vs 33.3% $p<0.01$) and a higher rate of transplant glomerulopathy (40% vs 0%, p=0.02) compared to patients without 3-month humoral lesions.
Patients with SAMR at 3 months exhibited at 1 year a higher class II MFImax DSA (2904±1001 and 0±0, p=0.05), a lower mGFR (39.2±13.9 and 61.9±19.2 mL/min/1.73m^2, $p<0.01$), and finally a higher SCr at last follow-up (163±55 vs 117±42 μmol/L, p=0.04) compared to patients without SAMR.
Conclusion: SAMR is a frequent entity in KTR with preexisting DSAs, and promotes subsequent GFR impairment and development of chronic AMR at one year. Screening biopsies may be useful to recognize patients more likely to develop SAMR.

O-142 PREFORMED DONOR-SPECIFIC ANTIBODIES BY SINGLE ANTIGEN BEADS WITH A HIGH TITER ARE THE BEST PREDICTOR FOR HUMORAL REJECTION AFTER KIDNEY TRANSPLANTATION

Sebastian Riethmüller[1], Thomas Fehr[2], Barbara Rüsi[3], Carine Hadaya[4], Jean Villard[5], Sylvie Ferrari[5]. *[1]Division of Visceral and Transplantation Surgery, University Hospital, Zurich, Switzerland; [2]Division of Nephrology, University Hospital, Zurich, Switzerland; [3]Interdisciplinary HLA Laboratory, University Hospital, Zurich, Switzerland; [4]Division of Nephrology, Geneva University Hospital and Medical School, Geneva, Switzerland; [5]Transplantation Immunology Unit and Service of Laboratory Medicine, Geneva University Hospital and Medical School, Geneva, Switzerland*

Purpose: Detection of donor-specific anti-HLA antibodies (DSA) with single antigen beads (SAB) has markedly improved the assessment of sensitized renal allograft recipients. However, the relevance of preformed DSA by SAB to predict posttransplant outcome remains controversial. Luminex cross-match (LXM) testing is a new technique to measure sensitization against a given donor and may help to identify relevant DSA prior to transplantation. The aim of this study was to assess sensitivity, positive and negative predictive values (PPV, NPV) of pretransplant LXM, FACS cross-match (FXM) and DSA titer by SAB for renal allograft rejection within the first year post-transplantation.
Methods: All recipients of a living donor kidney in Zurich and Geneva between 2005 and 2007 were screened for the presence of HLA antibodies by Luminex or ELISA. In positive patients, FXM and LXM were performed retrospectively, the specificity and titer (mean fluorescence intensity, MFI) of anti-HLA antibodies were determined by SAB. XM tests in DSA+ and DSA- patients were then correlated with allograft outcome one year post-transplant.
Results: 36 HLA-sensitized patients were identified, and 19 of them had DSA (44%). Among DSA+ patients, 32% subsequently developed humoral and 42% cellular rejection within the first year, whereas in DSA- patients the respective numbers were 12% and 41%. With higher titer of DSA, the PPV for humoral rejection increased, but sensitivity decreased dramatically. LXM and FXM by themselves were inferior to DSA testing alone in terms of PPV, NPV and sensitivity. Use of LXM or FXM in the subgroup of DSA+ patients provided no additional diagnostic value (Table). For acute cellular rejection none of the tests was predictive.

Predictibility of humoral rejection by DSA, LXM and FXM

	PPV [%]	NPV [%]	Sensitivity [%]
DSA >500 MFI	32	88	75
DSA >2000 MFI	38	87	63
DSA >5000 MFI	50	86	50
DSA >10000 MFI	100	82	25
LXM	15	74	29
FXM	29	80	57
LXM (DSA >2000 MFI)	40	71	50
FXM (DSA >2000 MFI)	38	60	60

MFI = mean fluorescence intensity.

Conclusion: Preformed DSA with a high titer detected by SAB is the best predictor of humoral allograft rejection after kidney transplantation.

O-143 ACUTE HLA ANTIBODY-MEDIATED REJECTION: CELLULAR INFILTRATION OF RENAL ALLOGRAFTS BY NEUTROPHILS AND CD3+VE CELLS

Rob Higgins[1], Klaus Chen[1], Daniel Zehnder[2], Rizwan Hamer[1], Nithya Krishnan[1], Habib Kashi[1], Chris Imray[1], Lam Chin Tan[1], For T. Lam[1], Dave Lowe[3], David Briggs[3]. *[1]Transplant Unit, University Hospital, Coventry, United Kingdom; [2]CSRI, Warwick Medical School, Coventry, United Kingdom; [3]Histocompatibility Laboratory, NHS BT, Birmingham, United Kingdom*

Background: The histological appearances of antibody-mediated rejection are described in the Banff 07 classification of renal allograft pathology. The aim of this study was to compare the classification with the findings in our programme of HLA antibody incompatible transplantation.
Methods: Renal biopsies from patients with donor specific antibodies (DSA) to HLA were scored by Banff 07, were stained for CD45, and a subset for CD20, CD68 and CD3.
Results: 36 patients had 72 renal biopsies. In 29 biopsies were performed 30 minutes after graft reperfusion, mean number of CD45+ cells per glomerulus was higher than in control antibody compatible grafts ($p<0.04$), was associated with the DSA level ($p<0.01$), and 8/9 patients with greater than 5 CD45+ cells per glomerulus had rejection or oliguria, compared to 11/20 with less than 5 CD45+ per glomerulus ($p<0.01$).
In the first 10 days post-transplant, although peritubular capillary (PTC) leucocyte margination grade 3 and C4d deposition were specific for rejection, their sensitivities were low. PTC C4d staining was not seen in the first 5 days after transplant, even in the presence of rejection, but was present in the majority of later biopsies with rejection. Glomerular leucocytes were 1% CD20+, 66.6% CD68+, and 32.4% CD3+; the interstitial leucocytes were 1.8% CD20+, 50.2% CD68+, and 48% CD3+.Glomerular polymorphonuclear neutrophils (PMN) made up a mean of 52% of CD45+ cells on day 0 biopsies, and 48% on later biopsies.
Conclusions: Glomerular margination of lympho-histiocytic (CD45 +) cells occurred early after transplantation and was associated with DSA level and early graft dysfunction. A significant proportion of the leucocytes in glomeruli and renal interstitium were CD3+. C4d was not seen in PTC for the first few days after transplantation, even in the presence of cellular margination, and this requires further investigation.

O-144 COMPARATIVE STUDY OF LIVING RELATED-DONOR/SPOUSAL-DONOR KIDNEY TRANSPLANTATION AND TRANSPLANTATION WITH/WITHOUT DESENSITIZATION BY RITUXIMAB. SINGLE-CENTER ANALYSIS OF THE INCIDENCE OF ACUTE REJECTION

Toshihito Hirai[1], Hideki Ishida[1], Junpei Iizuka[1], Daisuke Tokita[1], Houichi Shimizu[1], Hiroki Shirakawa[1], Kazuya Omoto[2], Kazunari Tanabe[1]. *[1]Urology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan; [2]Transplantation, Ookubo Hospital, Shinjuku-ku, Tokyo, Japan*

Background: Kidneys from spousal donors are being used for transplantation. However, spousal kidneys, especially from husband to wife, are at a high risk of acute rejection because of sensitization. On the other hand, an immunosuppressive protocol including the anti-CD20 antibody rituximab has just been introduced as an alternative induction protocol to splenectomy, etc., for highly sensitized recipients. Herein, we evaluated the risks of spousal transplantation and the effect of rituximab.
Subjects: We divided the 417 transplantations conducted between 2000 and 2008 at our institution into two groups by the donor type: related donor group (N=314) and spousal donor group (N=100). After 2005, rituximab was included in the protocol for ABO-incompatible cases and cases that were considered to be highly sensitized because of the presence of donor-specific antigens. The incidence rate of acute rejection (AR rate) was compared retrospectively between the two donor groups and before/after introduction of the rituximab.
Results: AR rate in the related donor group was less than that in the spousal donor group (28.1% vs. 39.0%, respectively: $p<0.05$). The overall AR rate improved after 2005, after the introduction of the rituximab (related donor group: 38.8% to 10.7%, $p<0.01$, spousal donor group: 56.3% to 23.1%, $p<0.01$), however, the incidence in the related donor group was still lower than that in the spousal donor group ($p<0.05$). In the spousal-donor group, AR rate in the husband-to-wife cases was almost equal to that in the wife-to-husband cases (36.1% vs. 40.6%, $p>0.05$).
Conclusion: Spousal-donor kidney transplantation is still associated with a higher risk, in terms of the incidence of AR, than related-donor transplantation, even after the introduction of a rituximab-containing protocol. Husband-to-wife transplantation may not be as risky if appropriate desensitization is performed.

O-145 THE EFFECT OF SIMVASTATIN ON DESENSITIZATION OF PANEL-POSITIVE KIDNEY TRANSPLANT CANDIDATES

Jamshid Roozbeh, Azar Sattarinezhad, Mohammad Mehdi Sagheb, Ahad Eshraghian, Ghanbarali Raeesjalali, Saeed Behzadi, Heshmatollahi Salahi, Ali Bahador, Saman Nikeghbalian, Seyed Ali Malek-hosseini. *Organ Transplant Research Center, Shiraz University of Medical Science, Shiraz, Fars, Islamic Republic of Iran*

Background: Patients with panel reactive antibodies (PRA) have many difficulties to find a crossmatch-negative kidney for transplantation and are at the risk of transplantation rejection more than other transplanted patients. We evaluated the effect of simvastatin on PRA and post transplant outcome of these sensitized patients.
Materials and methods: In a prospective manner, 82 patients with end stage renal disease (ESRD) with a PRA $\geq$ 25% were evaluated. In a one year follow up the patients were treated with simvastatin. At the end of the second and 12th month PRA was rechecked. Those patients who underwent transplantation continued to take simvastatin six month after transplantation. Serum creatinine levels were checked at monthly intervals post operation.
Results: Fifty (61%) of sensitized patients were male. The patients were predominantly in the middle-aged group, mostly on a hemodialysis program. 39% of patients had a history of a previous renal transplant and 13.4% had a history of blood products transfusion. The major known cause of ESRD was glomerulonephritis.
All of them took simvastatin for 2 months. The mean PRA decrement was 14.22 ± 19.63 during this period. 34.1% showed a complete response (i.e. PRA $<25\%$). Six patients gave up the follow up (F/U) program and one patient died (not due to simvastatin side effects). Other 75 patients continued to take simvastatin for 12 month. At the end of the 12th month the mean PRA decrement was (26.57 ± 26.10) which was significant in comparison with the initial values ($P<0.05$). Overall 53 patients showed a complete response to simvastatin.

Twenty five patients underwent renal transplant. 3 patients discontinued the F/U program. Of the other 22 patients, only two patients became dependent on hemodialysis again.
Conclusion: Simvastatin can safely be used to lower PRA and improve post transplant outcome.

O-146 A MESF OF GREATER THAN 1.2 FOR DONOR B-CELL REACTIVITY AS DETECTED BY FLOW CYTOMETRY IS ASSOCIATED WITH A SIGNIFICANTLY HIGHER BPAR RATE IN THE FIRST 6 MONTHS POST RENAL TRANSPLANTATION

Yasha Johari, David Bryson, Michael L. Nicholson. *Division of Renal Transplantation, Leicester General Hospital, Leicester, Leicestershire, United Kingdom*

Aims: To assess the effects of a "Postive" B Cell crossmatch (flow cytometry) on acute rejection rates in the first 6 months post renal transplantation.
Methods: Pre-implanataion donor B-Cell flow cytometry was performed on all renal transplant receipients between the dates 2005 and 2007. MESF values over 1.2. were regarded as a "Postive" (B-Cell –ve <1.2, n=76, B-Cell +ve >1.2, n=118). There were no significant differences in age, proportion of predialyisis patients, no of previous transplants or proportion of cadaveric or live donors. All patients received simulect induction, cellecept, tacrolimus and prednisolone de novo. Table 1 demonstrates the differences in the number of HLA mismatches between B-Cell positive and negative cohorts.

Table 1. HLA mismatches in B-Cell +ve and –ve cross-matches

	Number of Mismatches		p value
	B Cell –ve <1.2 N=76 (%)	B Cell +ve >1.2 N=118 (%)	
HLA A	68 (90%)	94 (79%)	0.074
HLA B	76 (100%)	104 (88%)	<0.002
HLA DR	55 (72%)	81 (71%)	0.580

Banff grading for acute rejection data was obtained by manually reviewing hospital pathology databases for biopsy results.
Results: There were no siginifcant differences between graft or patient survival (B-Cell –ve vs B-Cell +ve 1 vs 3 and B-Cell –ve vs B-Cell +ve 4 vs 3, n.s.).

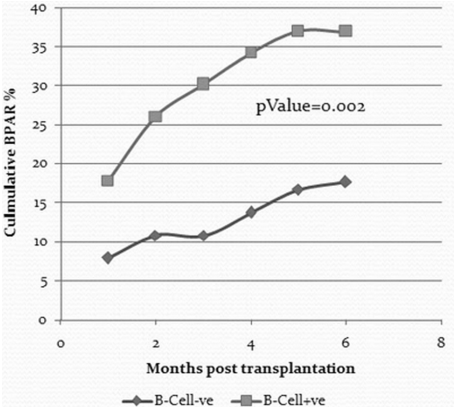


Figure 1. Rejection rate and B-Cell MESF ratio.

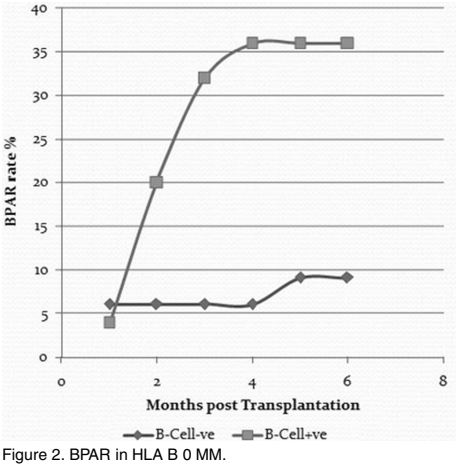


Figure 2. BPAR in HLA B 0 MM.

Figure 1 demonstrates the culmulative biopsy proven acute rejection (BPAR) rate.
There were no rejection episodes in B-cell-ve HLA 000 mismatched (n=19) patients whereas there were 5 rejection episodes in the B-Cell positive cohort (0% vs 28%, p value 0.046 Fisher's exact test).
This significant difference remained throughout all fully matched patient in the HLA A, B and DR loci (see Table 2).

Table 2. Rejection rates in HLA 0 MM patients

	Rejection Rate B-Cell +ve	Rejection Rate B-Cell –ve	p value
HLA 0 MM	0%	20%	0.0137
HLA B 0 MM	10%	26%	0.02
HLA DR 0 MM	3%	34%	0.00060.

The trend for differences between the B-Cell-ve and +ve cohort disapeared as the degree of mismatching increased (HLA A 2 MM, 60% vs 36%, p value = 0.3. HLA B 2 MM 28% vs 37%, p value = 0.7 and HLA DR 2 MM 10% vs 33%, p value = 0.3).
Conclusion: B-Cell +ve crossmatches are a significant risk factor for acute rejection, especially in well macthed recipients.

Session 17. Liver transplantation: immunobiology, genetics, inflammations & bioartificial devices

O-147 GRAFT FIBROSIS IN OPERATIONALLY TOLERANT PATIENTS AFTER LIVER TRANSPLANTATION; ANTIGEN-DEPENDENT OR INDEPENDENT?

Hidenori Ohe[1], Hiroto Egawa[1], Aya Miyagawa[2], Hironori Haga[2], Shinya Okamoto[1], Shinji Uemoto[1], Takaaki Koshiba[3]. *[1]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Laboratory of Anatomic Pathology, Kyoto University Hospital, Kyoto, Japan; [3]Innovation Center for Immunoregulation Technologies and Drugs, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Background: Protocol biopsy revealed that a subset of operationally tolerant patients after pediatric living-donor liver-Tx, albeit showing normal liver test, exhibited graft fibrosis in a greater extent than patients on maintenance immunosuppression (IS). However, it remains elusive whether fibrosis in such tolerant patients is antigen-dependent or independent, since time between protocol biopsy and Tx was longer in tolerant patients compared with that in patients on maintenance IS.
Methods: Biopsy was performed in operationally tolerant patients and patients in weaning process of IS. Fibrosis was evaluated by Ishak's staging. Minimal maintenance IS (MM-IS) (once a day low dose taclorimus) was started in tolerant patients or patients in weaning process in case that either single biopsy demonstrated the presence of bridging fibrosis or repeated biopsy demonstrated progression of fibrosis. Then, follow-up biopsy was performed to examine whether fibrosis would be improved after starting MM-IS.
Results: 8 tolerant patients and 14 patients in weaning process started MM-IS. MM-IS was started in 6 patients because of bridging fibrosis and in 16 patients because of progression of fibrosis. Follow-up biopsy which was performed 14 months (a mean) after starting MM-IS demonstated that improvement of fibrosis was seen in 8 patients (36%), while no change was seen in 13 patients (59%).Only in 1 patient (5%), fibrosis became worse.
Conclusions: In a subset (36%) of operationally tolerant patients or patients in weaning process following pediatric living-donor liver-Tx, graft fibrosis was improved after starting minimal maintenance IS. This supported that fibrosis in operationally tolerant patients was antigen-dependent and thereby, this is in accordance with a concept that non-toxic very low dose IS should be continued to maintain good graft function permanently, which was referred to as prope tolerance by Sir RY Calne.

O-148 IMPACT OF "SINUSOIDAL/PARENCHYMAL RELAXATION" ON MICROCIRCULATORY PERFUSION FAILURE IN HEPATIC ISCHEMIA/REPERFUSION

Koichiro Hata[1,2], Rene H. Tolba[2,3], Shunichi Saito[1], Tomohide Hori[1], Shintaro Yagi[1,3], Yuzo Yamamoto[4], Shinji Uemoto[1]. *[1]Department of Surgery, Division of Hepato-Pancreato-Biliary Surgery and Transplantation, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]House of Experimental Therapy, University of Bonn Medical Center, Bonn, Germany; [3]Institute for Laboratory Animal Science and Experimental Surgery, University of Aachen, Aachen, Germany; [4]Department of Gastroenterological Surgery, Akita University School of Medicine, Akita, Japan*

Purpose: Sinusoidal "no-reflow" is a well-known determinant pathology in

hepatic ischemia/reperfusion (I/R), which is inevitable in liver transplantation. We recently reported pyrrolidine dithiocarbamate (PDTC) could induce heme oxygenase-1 (HO-1) substantially and rather specifically into liver tissue *in vivo*, resulting in remarkable sinusoidal dilatation. We herewith report the significant impact of such strong vaso-dilatation/relaxation in hepatic parenchyma on I/R-mediated microcirculatory failure.

Methods: Male Wistar rats were pretreated with either PDTC (150mg/kg, intramuscularly) or vehicle (controls). After 36-hour interval, the rats were exposed to 70%-lobar hepatic I/R for 60 minutes. We examined hepatic microcirculation quantitatively using Intravital Microscopy and laser-Doppler flowmetry. Bile flow, transaminase release, tissue ATP contents, liver histology, and animal survival were also investigated.

Results: PDTC/HO-1 produced remarkable sinusoidal dilatation, up to 104.8% (zone-1), 185.7% (zone-2), 194.0% (zone-3) laterally, and 110.1% elongation of hepatic acini longitudinally (%-control in diameter/length).

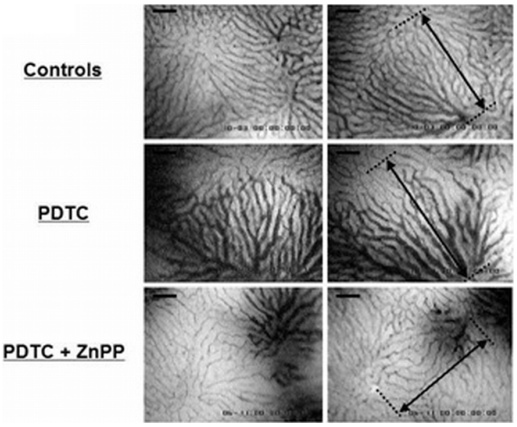


Figure 1

This sinusoidal/parenchymal "relaxation" was expected to decrease their vascular resistance down to 82.9% (zone-1) and 7.1% (zone-3) rheologically, thus to increase sinusoidal flow up to 133.1% theoretically. In fact, parenchymal flow increased up to 112.4% before ischemia and 130.3% after I/R, the latter is comparable with the theoretical prediction. In line, sinusoidal perfusion rate, transaminase release, tissue ATP concentration, liver histology and animal survival were all significantly improved.

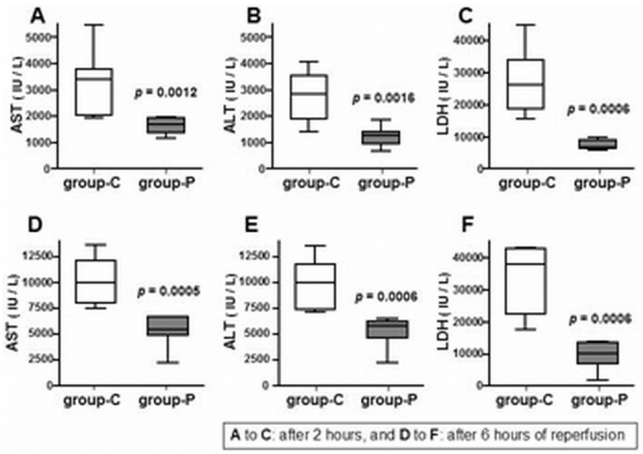


Figure 2

Of quite interest, PDTC/HO-1 promoted significantly more bilirubin production not only than in ischemic controls but than in non-ischemic, normal livers, reflecting highly-upregulated heme-oxygenase activity, as well as the stoichiometrical relationship between its products, carbon monoxide and bilirubin.

Conclusions: PDTC/HO-1 produced marked sinusoidal dilatation/relaxation, thus preventing I/R-mediated microcirculatory "shut-down". These results truly reflect the huge impact of sinusoidal vascular resistance on hepatic tissue perfusion, and suggest the remarkable potential of "sinusoidal/parenchymal relaxation" as a novel therapeutic approach against hepatic I/R.

O-149 VIABILITY ASSESSMENT OF HUMAN LIVERS BY RELEASE OF AST DURING HYPOTHERMIC MACHINE PERFUSION

Qiang Liu[1], Katrien Vekemans[1], Veerle Heedfeld[1], Tine Wylin[1], John Brassil[2], Peter Demuylder[2], Bert Theunis[2], Jonathan Vercruysse[2], Jos van Pelt[3], Jacques Pirenne[1], Diethard Monbaliu[1]. *[1]Abdominal Transplant Surgery, Catholic University Leuven (KUL), Leuven, Belgium; [2]Organ Recovery Systems Europe, Organ Recovery Systems, Zaventem, Belgium; [3]Hepatology, Catholic University Leuven (KUL), Leuven, Belgium*

Introduction: Extended-criteria donor livers are increasingly used to decrease organ shortage but objective criteria reliably reflecting graft viability *prior to* Tx are lacking. In analogy with the kidney, we hypothesized that analysis of hepatocellular enzymes released from human livers during Hypothermic Machine Perfusion (HMP) could be used as surrogates of viability *prior to* Tx.

Aims: To determine whether Aspartate amino transferase (AST) release during HMP can reflect quality and viability of human livers.

Methods: HMP was conducted on 11 human livers initially allocated for Tx but discarded due to unexpected findings: >50% steatosis (n=5); other reasons (n=5); failed rescue-allocation (1 not-transplantable patient). In retrospect, these livers were regarded as absolutely not-(n=7) or potentially well-transplantable (n=4). Following 13h15'±5h50' of cold storage, livers were HMP preserved for 24h with non-oxygenated 4-6°C KPS-1™. Cumulative AST release was determined in perfusate obtained during HMP (30',1h, 6h, and 24h). These parameters were studied in 2 different groups: First in > *vs* <50% steatosis (n=5 in each group), second in absolutely not- *vs* potentially well-transplantable livers.

Results: During HMP of >50% steatotic livers, AST release was higher ($p<0.05$) compared to <50% steatotic livers (4672±2049 *vs* 251±170 U/L, 4834±2171 *vs* 293±191 U/L, 7101±3207 *vs* 409±239 U/L, 8802±5070 *vs* 727±376 U/L, at 30', and 1h, 6h, 24h). AST was higher ($p<0.05$) when comparing absolutely not- *vs* potentially transplantable livers (4672±2049 vs 251±170 U/L, 4843±2171 *vs* 293±191 U/L, 7101±3207 *vs* 409±239 U/L, 6618±5572 *vs* 511±224 U/L, at 30', and 1h, 6h, 24h).

Conclusions: This study indicates that AST in HMP perfusate is a simple/reliable marker to assess the quality of liver grafts *prior to* Tx and may potentially discriminate transplantable *vs* non-transplantable livers, thereby allowing wider and safer use of extended-criteria donor livers.

O-150 MITOCHONDRIAL FUNCTION DURING ISCHAEMIA-REPERFUSION IN DCD LIVERS

Debabrata Roy[1], Karl Morten[2], Constantin Coussious[3], Reza Morovat[1], Jens Brockman[1], Peter J. Friend[1]. *[1]Nuffield Department of Surgery, John Radcliffe Hospital, Oxford, United Kingdom; [2]Nuffield Deparmetof Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, United Kingdom; [3]Department of Bioengineering, John Radcliffe Hospital, Oxford, United Kingdom*

Introduction: DCD (Donation after cardiac death) livers are extremely susceptible to ischaemia-reperfusion injury and the role of the mitochondria is believed to be central to his. We have investigated mitochondrial functional changes during ischaemia-reperfusion and the relationship of these to hepatocellular injury in post-ischaemic livers.

Methods: Porcine livers (Group W, n= 5) were subjected to 60 minutes of warm ischaemia and then connected to a normothermic extracorporeal perfusion circuit for 24 hours for assessment of function. Group C (Control, n=5) did not receive the warm ischaemic injury but were otherwise treated the same way. Both groups were subjected to transient cooling (60 minutes) during the bench work prior to reperfusion. Mitochondria were isolated from sequential liver biopsies and analysed for ATP content, mitochondrial function (respiratory control ratio (RCR), mitochondrial level of complex II, aconitase and superoxide dismutase (SOD). The perfusate was analysed for serum transaminase, bile production and base deficit.

Results: Cellular ATP levels reduced significantly during 60 minutes of warm ischaemia ($p<0.01$), but with minimal change in mitochondrial function. However, subsequent cold preservation produced a significant decline in mitochondrial function (RCR 3.97±0.43 vs. 2.45±0.21 $p<0.001$) with a parallel decrease in complex II, aconitase and SOD2. Mitochondrial function did not recover during reperfusion after cooling and this was associated with raised transaminase release ($p<0.05$) in the perfusate. By comparison, Control livers (without warm ischaemia) maintained normal mitochondrial function during cold preservation and subsequent reperfusion with minimal hepatocellular damage.

Conclusions: The progressive damage that is seen in livers that experience sequential warm followed by cold ischaemia (the DCD donor) is mirrored by tests of mitochondrial function. This may have important implications in developing mitochondrial based therapeutic strategies for resuscitation of NHBD livers.

O-151 ROLE OF HIPOXIA INDUCIBLE FACTOR (HIF) IN FATTY LIVER PRESERVATION: AN EX VIVO APPROACH USING IGL-1 SOLUTION

Amine Zaouali[1], Ismail Ben Mosbah[1], Hassen Ben Abdennebi[2], Isabel Freitas[3], Maria Pia Vairetti[4], Olivier Boillot[5], Carmen Peralta[6], Joan Roselló-Catafau[1]. *[1]Liver Unit, IIBB-.CSIC, Barcelona, Spain; [2]Human Physiology, Fac. of Pharmacy, Monastir University, Monastir, Tunisia; [3]Animal Biology Dept., Pavia University, Pavia, Italy; [4]Internal Medicine & Therapeutics Dept., Pavia University, Pavia, Italy; [5]Transplantation Unit, Edouard Herriot, Lyon, France; [6]Liver Unit, IDIBAPS, Barcelona, Spain*

Hipoxia inducible factor (HIF) generated during hypoxia is responsible for the tissue adaptation to ischemia. However, recent studies point out that HIF involvement in enhancing of liver tolerance against reoxygenation injury. IGL-1 solution has been successfully employed to prevent fatty liver cold ischemia-reperfusion injury (IRI), but the protection mechanisms are still understood.
Steatotic livers from Zücker rats (n =6) were preserved for 24 hours at 4°C in IGL-1 and UW solutions and then "ex vivo" reperfused for 2 hours at 37°C. HIF and nitric oxide (nitrites/nitrates and NOS activity), hemeoxygenase HO-1 were measured and correlated with liver (AST/ALT) and function (bile production)injury; as well as, other factors lied to the known exacerbated susceptibility of steatotic livers against IRI, as the oxidative stress (MDA) and mitochondrial damage (GLDH), respectively. Fatty livers preserved in IGL-1 showed significant HIF levels (3,85±0.9 ug HIF/ protein) when compared to those obtained with goal UW solution (1,48±0.48 ug HIF/ug protein). Surprisingly, the addition of an antiischemic drug as trimetazidine (10-6M) to the original IGL-1 solution (IGL-1+TMZ) augmented HIF levels (7,02±0,82) when compared to IGL-1 alone (3,85±0.9 ug HIF/ug protein). These HIF increases were concomitant with nitrites/nitrates levels observed for the IGL-1+trimetazidine solution (403,3±21,7 pmol/mg) when compared to those determined for IGL-1 (259,8±33,8 pmol/mg), respectively. These results showed that the NO is contributing to the HIF stabilization in normoxic reperfusion. The augmented presence of HIF in IGL solutions was associated with the induction of liver hemeoxygenase (HO-1) in IGL-1 preserved livers, with and without trimetazidine.
In conclusion, the suitable fatty liver preservation using IGL-1 or IGL-1+TMZ seems to be associated with an increased production of HIF, HO-1 and NO, respectively.

O-152 IMPACT OF CCR5Δ32 MUTATION ON ISCHEMIC TYPE BILIARY LESIONS AFTER LIVER TRANSPLANTATION

Ines Ruth[1], Maria Hoppe-Lotichius[1], Christian Mönch[2], Gerd Otto[1]. *[1]Transplantation and Hepatobiliopancreatic Surgery, Universitätsmedizin Mainz, Mainz, Germany; [2]Visceral and General Surgery, Ohann Wolfgang von Goethe University, Frankfurt, Germany*

Ischemic-type biliary lesions (ITBL) occur after orthotopic livertransplantation (LT) with a reported incidence of up to 26% and lead to considerable morbidity, graft loss, and even mortality. A multi-factorial etiology is assumed. CC-chemokines and their receptors have been identified to play a key role in cell survival and proliferation. The CC-chemokine receptor 5delta32 (CCR5Δ32) mutant has been shown to be a major risk factor for the development of ITBL after LT.
338 LT recipients (374 LT including 36 retransplantations) transplanted between September 1997 and December 2007 (116 female and 222 male) were included in the study. ITBL were identified by increased parameters of cholestasis followed by imaging of the bile ducts.
CCR5 wild-type and CCR5Δ32 were identified in 290 and 48 patients, respectively. ITBL occurred in 35 of the 322 transplantations performed in patients with wild-type and in 12 of 52 grafts in patients with CCR5Δ32 mutation (p=0.022). 70% of all ITBL occurred within 6 months, 90% of all ITBL occurred within 12 months.
Patient survival was reduced in patients with CCR5Δ32 to 61% after 5 years. In patients with CCR5 wild type the survival was 75% after 5 years (p=0.011). Graft survival was equally reduced.
Rate of rejection was not affected (p=0.282). CCR5Δ32 showed no influence on reinfection, rate of retransplantation, and fibrosis (within a year) after liver transplantation in patients with hepatitis C (p=0.721). In patients with wild type arterial back-table pressure perfusion was capable of preventing ITBL (p=0.009).
In a large collective CCR5Δ32 was shown to still be a significant risk factor for the development of ITBL following LT. The mutation not only leads to a reduction of patient and graft survival but also to an increased retransplantation rate.

			ITBL		Total	
Δ32			no	yes		
No	arterial back-table pressure perfusion	yes	75	2	77	*P*=0.009
		no	213	32	245	
	Total		288	34	322	
Yes	arterial back-table pressure perfusion	yes	6	3	9	*P*=0.422
		no	34	9	43	
	Total		40	12	52	

O-153 TUMOR NECROSIS FACTOR ALPHA-INHIBITORS AS IMMUNOMODULATORY ANTI-REJECTION AGENTS IN INTESTINAL TRANSPLANTATION

Undine A. Gerlach, Sven Kohler, Peter Neuhaus, Johann Pratschke, Andreas Pascher. *Department of General, Visceral and Transplantation Surgery, Charite Campus Virchow Klinikum, Universitaetsmedizin Berlin, Berlin, Germany*

Tumor necrosis factor alpha (TNFa) has been shown to play a key role as a marker cytokine during organ rejection. Experimental and clinical data indicate that TNFa inhibition contributes to the treatment and prevention of acute rejection in different types of solid organ transplantation.
Infliximab (a chimeric antiTNFa antibody) has been successfully used in intestinal allograft recipients as induction therapy and as anti-rejection agent.
We exmanined 7 patients (5 male, 2 female) out of 22 short bowel or multivisceral transplant recipients who received infliximab. All 7 patients underwent single intestinal transplantation, due to ultra-short bowel syndrome at an average age of 32±5.2 years. Indication for infliximab therapy was steroid (n=3), OKT 3 (n=3) refractory rejection or ulcerative inflammation of the ileal graft (n=1).
Application of infliximab started at a mean of 20.2±15.5 months after transplantation. Repeat dosing was timed according to TNFa and LPS binding protein serum levels. In one patient, adalimumab (a human monoclonal anti-TNF-a antibody) was applied once upon detection of antichimeric antibodies, which were determined using an anti-TNFa antibody ELISA.
The number of infliximab administration averaged 7.4±5.5, at a dosage of 3-5 mg/kg body weight. 6 patients responded instantly to infliximab, showing improvement of graft histology and resolution of ulceration on endoscopy, whereas in 1 patient OKT 3-refractory rejection resulted in graft loss and death despite infliximab infusions.
One of the 6 patients received adalimumab once, as a consequence to the loss of efficacy of infliximab owing to the development of antichimeric antibodies.
In conclusion, TNFa-inhibition is an effective therapeutic option for steroid or OKT 3-refractory rejection and chronic inflammatory graft alterations in intestinal allograft recipients.

O-154 DIFFERENTIAL GENE EXPRESSION PROFILE IN BIOMODULATED LIVERS AFTER NON-HEART BEATING TRANSPLANTATION

Katrien Vekemans[1], Diethard Monbaliu[1], Veerle Heedfeld[1], Hannah van Malenstein[2], Erica Balligand[1], Qiang Liu[1], Tine Wylin[1], Paul van Hummelen[3], Jacques Pirenne[1], Jos van Pelt[2]. *[1]Dept. of Abdominal Transplant Surgery, [2]Dept. of Hepatology, [3]VIB MicroArrays Facility, Catholic University of Leuven (KULeuven), Leuven, Belgium*

Purpose: A multifactorial biomodulation (M) protocol was developed to decrease ischemia/reperfusion injury after non-heart beating liver transplantation in pigs. This protocol resulted in prevention of primary-non-function (PNF) and increased survival (83%) compared to untreated controls ł(22%). To understand the protective mechanisms of this M-Protocol, we studied the differential expression of molecular pathways in C *versus* M livers.
Methods: Porcine livers exposed to 45'WI were cold stored, transplanted and either modulated or not. In the M-group, *donor livers* were flushed with *warm Ringers, streptokinase* and *epoprostenol* prior to cold storage; *in recipients*, *glycine*, α_1*-acid-glycoprotein*, a *MAPKinase-inhibitor*, α*-tocopherol*, *glutathione*, and *apotransferrin* were administrated. Liver biopsies were collected at baseline (BL) and 1h post reperfusion (PR) for analysis of mRNA-expression by Affymetrix microarray (M: $n=5_{BL}+3_{PR}$, C: $n=5_{BL}+2_{PR}$). Porcine genes were translated into their human equivalents. Gene expression changes between BL and PR were analyzed by GeneMaths. Gene expression data were mapped on a pathway database using Mappfinder. Array results were confirmed by real-time PCR on selected genes.
Results: In the M-group 4005 genes were significantly altered between BL and PR: 1513 genes were up-regulated and 2492 down-regulated. In the C-group 5180 genes were significantly changed: 3128 were down-regulated and 2052 up-regulated. After pathway mapping the following pathways clusters were identified: cell death, cell proliferation and inflammation (PR vs. BL ($p<0.05$)). In addition the amount of inflammation related pathways remained stable in C and M. The pathways related to cell death and cell proliferation decreased in the M-group *vs.* C-group (5 *vs.* 12 pathways, respectively). Overall the M-group revealed 18 pathways (PR compared to BL) whereas the C-group revealed 32 pathways.
Conclusions: A multi-factorial biomodulation protocol (that prevents PNF) resulted in less pathway up-regulation. This decrease was situated in cell proliferation and apoptosis pathways.

O-155 DIFFERENTIAL GENE EXPRESSION PROFILE IN WARM ISCHEMIA PORCINE LIVERS

Katrien Vekemans[1], Erica Balligand[1], Qiang Liu[1], Hannah van Malenstein[1], Diethard Monbaliu[1], Veerle Heedfeld[1], Tine Wylin[1], Paul Van Hummelen[2], Jacques Pirenne[1], Jos van Pelt[3]. *[1]Dept. of Abdominal Transplant Surgery, [2]VIB MicroArrays Facility, [3]Dept. of Hepatology, Catholic University of Leuven (KULeuven), Leuven, Belgium*

Purpose: The molecular and pathophysiological mechanisms underlying warm ischemia (WI) injury in the liver are not fully understood. To elucidate the pathways involved, we investigated gene expression by microarray in a porcine model of WI.
Methods: In anesthetized and ventilated pigs (33±4kg) the liver was randomly subjected to increased WI periods (0', 15', 30', 45' WI: n=3/group). Liver samples were collected for analysis of mRNA expression by Affymetrix microarray. Porcine genes were translated into human equivalents (Tsai et al.). Changes in gene expression between 0'WI and each WI group were analyzed by GeneMaths 3.5. To identify the affected molecular pathways, gene expression data were mapped on a pathway database using Mappfinder 2.1.
Results: 3530 genes were significantly altered between 0'WI and 15'WI. of them 1762 genes were up-regulated and 1768 were down-regulated ($p<0.05$). Between 0'WI and 30'WI 1924 genes were significantly up-regulated and 2217 were down-regulated. For 0' and 45'WI 1552 genes were up-regulated and 1262 genes were down-regulated. After pathway mapping, we identified pathways clustering for cell death, cell proliferation and inflammation being up-regulated in WI, which were not altered in 0'WI ($p<0.05$). Of note, pathways related to extracellular region/space and regulation of cellular process were only observed between 0'WI and 15'WI. In addition the amount of inflammation-related pathways decreased with increased WI. The inflammatory pathways include CXCL2, a chemokine involved in the neutrophil chemotaxis and MAP3K5, a protein that -when over expressed- induces apoptosis. The pathways related to lipid metabolism were down-regulated and increased in number with WI time.
Conclusions: Liver WI is accompanied by changes in gene expression affecting several molecular pathways. The most prominent are: lipid metabolism, cell death, cell proliferation and inflammation. This study provides new perspectives to alter mechanisms related to WI in liver transplantation.

O-156 PORCINE MODEL OF EXTRA-CORPOREAL MEMBRANE OXYGENATION (ECMO) IN THE UNCONTROLLED NON-HEART BEATING DONOR (NHBD); THE EFFECT ON LIVER VIABILITY

Aditya Kanwar[1], Christopher Ray[1], Stephen Ray[1], Mohamed Saleem Noormohamed[2], Susan Stamp[2], Soroush Sohrabi[1], Alex Navarro[1], Hugh Wyrley-Birch[1], Brian Shenton[2], Anne Cunningham[3], Noel Carter[3], Steve White[1], Jon Smith[4], David Talbot[1]. *[1]Liver/Renal/Pancreas Transplantation, Freeman Hospital, Newcastle upon Tyne Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom; [2]Department of Surgery, University of Newcastle upon Tyne, Newcastle Upon Tyne, United Kingdom; [3]Pharmacy, Health and Wellbeing, University of Sunderland, Sunderland, United Kingdom; [4]Department of Anaesthesia, Freeman Hospital, Newcastle upon Tyne Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom*

Purpose: ECMO has been instituted with success in NHBD's for both renal and liver transplantation. We sought to compare the effect of ECMO on liver viability with our current standard; intra-vascular and -peritoneal cooling.
Methods: 11 cross-Yorkshire landrace pigs were studied as 2 groups; ECMO (n=5) and Cold Preservation (CP) group (n=6). Under general anaesthesia, all animals underwent laparotomy to place microdialysis catheters and cannulate the great vessels, followed by abdominal closure and euthanasia. After 30mins of warm ischaemia, abdominal aorta was isolated both proximally and distally, and thrombolysis administered. In the CP group, a peritoneal cooling circuit was established along with continuous aortic infusion of cold HTK solution for 2 hours. In the ECMO group, the circuit was commenced to perfuse the abdominal organs using pig's own oxygenated normothermic blood for 2hours. Liver was then retrieved and re-perfused on an ex-vivo oxygenation circuit using a mixture of autologous blood and RS-I solution. Throughout the period, multiple readings and samples were taken to assess liver viability and function. Oxygen consumption, weight gain and bile production were analysed using ANOVA (with Bonferroni) and Mann-Whitney U, as appropriate.
Results: During liver reperfusion, bile production was significantly higher in the ECMO group (2 vs 0.3 mls, $z=-2.25$;$p<0.05$). Oxygen consumption appeared to be higher in the CP group, but was not significantly different ($p>0.05$). After 2hours of reperfusion, average weight gain was greater in the ECMO group but not statistically significant (16.1 vs 7.4 g/100g tissue, $z=-1.64$;p=NS). Other investigations in progress are liver function tests, analysis of microdialysis samples, electron microscopy and micro-array analysis.
Conclusion: Preliminary data appears to support ECMO as a promising preservation technique for NHBD livers. Definitive conclusions to be drawn when pending results are analysed.

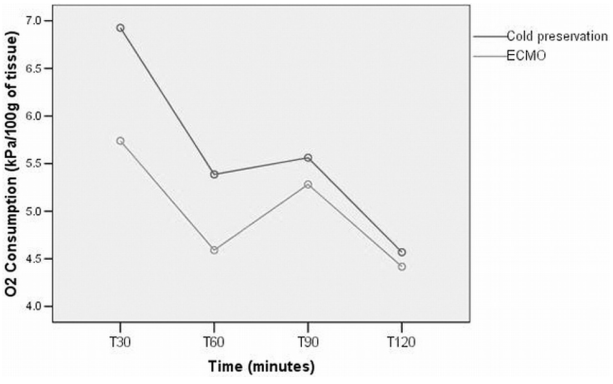


Figure 1. Liver-oxygen consumption on reperfusion.

O-157 CONSTRUCTION OF AN *IN VIVO* UTILIZING BIO-ARTIFICIAL LIVER BASED ON HEPATIC CELLS AND DECELLULARIZED LIVER MATRIX

Ji Bao, Yujun Shi, Huaiqiang Sun, Hong Bu, Youping Li. *West China Hospital, Sichuan University, Key Lab of Transplant Engineering and Immunology, Ministry of Health, Chengdu, Sichuan, China*

Background: Before obtaining of an appropriate donation, bio-artificial liver systems are reliable strategies to help the patient suffering end-stage liver diseases survive. Current bio-artificial liver systems are all used *in vitro*, here we introduce a bio-artificial liver based on tissue engineering to be used *in vivo*.
Methods: The median lobe, portal vein and suprahepatic vena cava of the rat liver were removed*in bloc*. The median lobe was perfusd from portal vein to vena cava with alkaline and non-ionic detergents to make an acellular, perfusable vascular architecture and intact scafford. The mechanical properties of the matrix were strengthened by biomimetic mineralization. Layer-by-layer heparin deposition was performed to make the matrix thromboresistant. The mixed cells isolated from fetal rats were co-cultured on the scafford *in vitro* for 48 hours to prepare a tissue engineering liver (TEL). Adult rats were used as TEL recipients after 90% of the liver was removed and the TEL was implanted in the portal system. The TEL was harvested for histology and function examination 72 hours after operation.
Results: The rats with only 90% hepatectomy died within 48 hours after operation, however, in TEL transplantation group, 60% of the recipients survived at 72 hours. In histology, the TEL showed notable hepatocytes proliferation and colonization characterized by clumps of hepatocytes surrounding capillaries, and some of the capillaries penetrated the clumps and changed into sinusoids. The TEL tissues also showed several characteristics of liver-specific functionality including synthesis of albumin, glycogen, thrombin and urea.
Conclusion: Our TEL, albeit without actual liver architecture, can substitute some functions of a naïve liver and help the recipient survive end-stage liver failure. This novel*in vivo* utilizing bio-artificial liver might be a promising liver substitution before either recovery of the native liver or transplantation.

Session 18. Improving donor kidney quality & palliation of I/R injury

O-158 MACHINE PERFUSION VERSUS COLD STORAGE PRESERVATION IN NON-HEART-BEATING KIDNEY DONATION AND TRANSPLANTATION: FIRST RESULTS OF A MULTICENTRE TRIAL IN EUROTRANSPLANT

Jacques Pirenne[1], Jacqueline M. Smits[2], Cyril Moers[3], Mark-Hugo J. Maathuis[3], Jürgen Treckmann[4], Bogdan P. Napieralski[4], Margitta van Kasterop-Kutz[2], Jaap J. Homan van der Heide[3], Ernst van Heurn[5], Günter R. Kirste[6], Axel Rahmel[2], Henri G.D. Leuvenink[3], Andreas Paul[4], Diethard Monbaliu[1], Ina Jochmans[1], Jean-Paul Squifflet[7], Rutger J. Ploeg[3]. *[1]Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium; [2]Eurotransplant International Foundation, Leiden, Netherlands; [3]University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [4]Department of General, Visceral and Transplantation Surgery, University Hospital Essen, Essen, Germany; [5]Department of Surgery, University Hospital Maastricht, Maastricht, Netherlands; [6]Deutsche Stiftung Organstransplantation, Frankfurt, Germany; [7]Abdominal Surgery and Transplantation, Centre Hospitalier Universitaire Sart Tilman, Liège, Belgium*

Delayed Graft Function (DGF) > Kidney Transplantation (KTx) causes mor-

bidity & cost, & negatively affects graft function/survival. Kidney grafts from Non-Heart-Beating-Donors (NHBD) exposed to warm+cold ischemia are particularly vulnerable to DGF. Compared to Cold Storage (CS), hypothermic Machine Perfusion (MP) may provide better preservation for NHBD kidneys, but evidence is limited in quality & numbers.
Aim: To compare efficacy of MP *vs* CS for preserving NHBD kidneys.
Methods: In an international/prospective/randomized/controlled trial we enrolled kidney pairs of 82 consecutive NHBD. All NHBDs were Maastricht-category-3 (awaiting cardiac arrest/planned therapy withdrawal). One kidney was randomly assigned to MP&the contralateral kidney to CS. Kidneys were allocated using standard allocation. At time of offer, preservation method (MP*vs*CS) and perfusion parameters were not revealed. 3-month data of all 164 recipients were analyzed.
Results: Donor age (y) was 43 (17-67). Baseline demographics were comparable between MP *vs* CS: Recipient age (y) 49 (24-73)*vs* 52 (24-77), p=0.81; preTx dialysis duration (days) 1542 (366-6402)*vs* 1448 (132-3904), p=0.48; first/reTx 34/48 *vs* 34/48, p=0.56; %PRA (0-5/6-84/85+) 71/11/0 *vs* 71/10/1, p=0.73; %0 HLA A, B, DR mismatches was 2.4 *vs* 3.7, p=0.5. Cold Ischemia Time (CIT) (h) was 15 (4.3-28.9) for MP *vs* 15.9 (8.6-46.6) for CS, p=0.7. DGF-incidence was 53.7% in MP *vs* 69.5% in CS recipients, p=0.027. DGF duration (days) was 9 (1-48) in MP *vs* 13 (2-43) in CS, p=0.04. DGF <7days occurred in 12/32 (27%) in MP *vs* 6/51 (10.5%) in CS, p=0.028. Creatinine clearance (ml/min) at d7, d14, 1mth, 3mth in MP *vs* CS was 13 *vs* 9, p=0.009; 23 *vs* 13, p=0.001; 46 *vs* 38, p=0.078; 57 *vs* 49, p=0.19, resp. PNF rate was identical after MP & CS (2.4%). Acute rejection rate was 7.3% in MP *vs* 12.2% in CS kidneys, p=0.22. Graft loss (<3mth) was identical after MP & CS (3.6%). Patient survival was 98.7% (MP) *vs* 100% (CS). Logistic regression analysis showed that MP (p=0.035; Odds ratio 0.476) & CIT (p=0.009; Odds ratio 1.118) independently influenced DGF.
Conclusion: This study demonstrates -for the first time- that MP of NHBD kidneys reduces *incidence*, *duration* & *severity* of DGF and ameliorates graft function after KTx. 1-year results will be presented.

O-159 GST AND HFABP VALUES DURING MACHINE PERFUSION OF DECEASED DONOR KIDNEYS ARE INDEPENDENT PREDICTORS OF DELAYED GRAFT FUNCTION, BUT NOT OF PRIMARY NON-FUNCTION AND GRAFT SURVIVAL

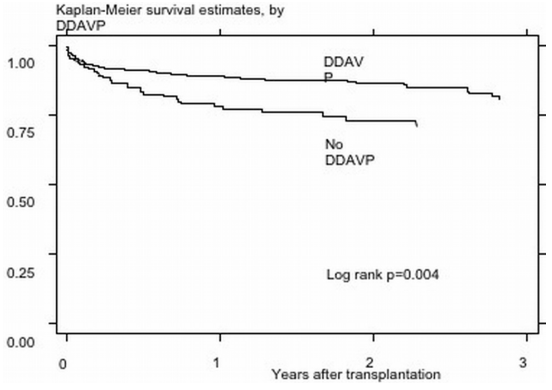
Cyril Moers[1], Oana C. Varnav[2], Jürgen Treckmann[3], Diethard Monbaliu[4], Gerhard Rakhorst[2], Andreas Paul[3], Jacques Pirenne[4], Henri G.D. Leuvenink[1], Rutger J. Ploeg[1]. *[1]Surgery, University Medical Center Groningen, Groningen, Netherlands; [2]Biomaterials, University of Groningen, Groningen, Netherlands; [3]General, Visceral, and Transplant Surgery, University Hospital Essen, Essen, Germany; [4]Abdominal Transplant Surgery–Transplant Coordination, University Hospital Leuven, Leuven, Belgium*

Introduction: Retrospective evidence suggests that glutathione S-transferase (GST) and heart-type fatty acid binding protein (HFABP) measured during kidney machine perfusion (MP) have predictive value for posttransplant outcome. However, these data are usually biased due to organ discard based on biomarker measurements, and previous analyses were not adjusted for likely confounding factors.
Methods: From 302 deceased donor kidneys included in an international prospective RCT (Machine Preservation Trial), GST and HFABP were measured in the perfusate at the end of MP. Donors were either heart-beating, or controlled non-heart-beating. We tested whether GST and HFABP levels were associated with delayed graft function (DGF), primary non-function (PNF), and graft survival (GS). A logistic regression model investigated whether the biomarkers remained independent predictors when adjusted for donor type, donor age, and cold ischemic time.
Results: For kidneys with DGF, median GST and HFABP concentrations were significantly higher (379 vs. 304 U/L, p<0.0005, and 7325 vs. 5176 pg/ml, p<0.005). In the logistic regression model, a GST or HFABP value above the median was independently associated with an increased risk of DGF (OR 2.0, p=0.03, and OR 2.8, p=0.001). There was no increased incidence of PNF in kidneys with high vs. low GST or HFABP values (1.4% vs. 3.4%, p=0.3, and 2.0% vs. 2.6%, p=0.7), neither was one year GS different (96% vs. 93%, p=0.2, and 95% vs. 94%, p=0.8).
Conclusion: This study for the first time shows that GST and HFABP in MP perfusate are independent predictors of DGF, but that these biomarkers do not predict PNF or GS. Although GST and/or HFABP values can be a valuable tool to help fine tune posttransplant management, measurement of these biomarkers should never lead to kidney discard.

O-160 DONOR EMPLOYMENT OF DESMOPRESSIN (DDAVP) IS ASSOCIATED WITH SUPERIOR LONG-TERM ALLOGRAFT SURVIVAL, BUT HAS NO EFFECT ON DELAYED GRAFT FUNCTION AFTER KIDNEY TRANSPLANTATION

Uwe Gottmann, Urs Benck, Hui Song, Simone Hoeger, Benito Yard, Peter Schnuelle. *Vth Medical Clinic (Nephrology), University Hospital Mannheim, University of Heidelberg, Mannheim, Germany*

Background: Optimizing the medical management of the brain-dead donor has the potential to improve the transplantation outcomes. Here we assess the concomitant administration of DDAVP in a multicenter cohort of 505 renal-transplant recipients. The present study is nested in the data-base of the RCT on donor pre-treatment with low-dose dopamine (ClinicalTrials.gov number, NCT00115115).
Methods: 264 eligible brain-dead donors included in the dopamine-trial were categorized on whether they had received DDAVP during intensive care. All donors were stable under low-dose norepinephrine and presented with a serum-creatinine <2.0mg/dL before organ procurement, and <1.3mg/dL on admission, respectively. We investigated the effects of DDAVP on DGF, and allograft survival after 3 years.
Results: The groups were very similar in demographic and clinical donor-recipient characteristics, but DDAVP treated donors significantly differed in the 24hr urine production (5119ml vs. 4079ml, p<0.001). There was no effect on DGF (30.6% vs. 29.6%, P=0.83), but DDAVP was associated with superior allograft survival after 3 years (81.0% vs. 71.1% p=0.004). Blood pressure and urine production were not discriminating variables in the multivariate Cox regression. To disclose a possible underlying mechanism, we incubated cultured renal endothelial cells with DDAVP at different concentrations, with varying temporal exposure, suggesting a time-dependent depletion of vWF from endothelial cells.



Conclusions: Donor DDAVP is associated with improved long-term allograft survival after kidney transplantation. We hypothesize that depletion of endothelial vWF pre-transplant attenuates subsequent PDGF-dependent downstream effects after transplantation, which play a crucial role in the generation of microvascular thrombosis, and in the formation of interstitial fibrosis.

O-161 IMMEDIATE DOPPLER ULTRASONOGRAPHY AFTER KIDNEY TRANSPLANT CAN HELP TO RESCUE THE GRAFT

Alireza Hamidian Jahromi, Nicos Kessaris, Rene Chang, Eric Chemla, Helen Gregson, Mohamed Morsy, Jiri Fronek. *Renal Transplant Department, St. George's Hospital, London, United Kingdom*

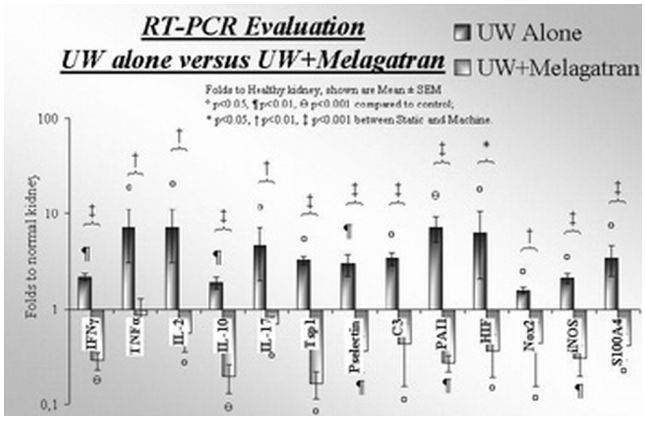
Irreversible damage to the renal transplant may be prevented if surgical correctable complications are detected early after the operation. Such complications include renal transplant vein or artery thrombosis. These are very important causes of graft loss in the early postoperative phase. Although the importance of performing a Doppler Ultrasound (DUS) in the post operation period has traditionally been accepted, the ideal timing of this study has not been identified.
We present a comparison of kidney transplants outcomes, from a single centre, during two different periods of our practice. During the first period we performed day-one post operation DUS and during the second period we carried out immediate post operation DUS.
Methods: A cohort of 151 renal transplant recipients who had immediate post operative DUS was studied. The transplant outcome in this group was compared with another cohort of 153 renal transplant recipients who had DUS on the first post operative day. In the situations where satisfactory perfusion of the transplanted kidney was not confirmed by the DUS, the decision was made for immediate re-operation.
Results: Before we established our immediate post-operative DUS protocol, we lost five kidneys all with serious vascular complications. Since we have

adopted this protocol, we have had four cases of immediate re-exploration after transplantation all of which have been rescued. The success rate of the re-exploration surgery was significantly higher in the group of patients with immediate postoperative DUS scan. (100% success rate versus 0.0% success rate, P = 0.008)
Conclusion: Immediate DUS scan after kidney transplantation can help in early detection and diagnosis of surgical correctable complications and is indispensable to allograft salvage.

O-162 THE WEAK SPOT OF ISCHEMIA REPERFUSION INJURY: THROMBIN INHIBITION RESCUES CHRONIC KIDNEY INFLAMMATION AND FIBROSIS

Frederic Favreau[1,3], Raphael Thuillier[1,3], Olivier Page[1,3], Lilach O. Lerman[2], Thierry Hauet[1,3]. *[1]Inserm U927; CHU de Poitiers; Univ Poitiers, Faculte de Medecine et de Pharmacie, F-86000, Poitiers, France; [2]Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA; [3]FLIRT (Federation pour L'Etude de l'Ischemie Reperfusion en Transplantation), France*

Purpose: Chronic kidney fibrosis remains the main issue in kidney transplantation and its severity is correlated to Ischemia reperfusion injury (IRI). We propose that coagulation, a pathway at the center of IRI, is a critical target for therapy.
Methods: We used an autologous Large White pig 'donor after cardiac arrest' transplantation model, in which kidneys underwent 60min warm ischemia then 24hours cold preservation in UW, with or without Melagatran (0.3mg/L), a thrombin inhibitor. Grafts were evaluated 3 month posttransplant.
Results: Compared to UW alone, Melagatran improved survival at 3 month (89% vs. 20%, $p<0.01$), decreased serum creatinine (130±6.5 vs. 435±47.5μmol/L, $p<0.01$), proteinuria (0.30±0.05 vs. 4.16±0.78 g/24h, $p<0.01$) and interstitial fibrosis (6.8±0.2% vs. 37.3±2.8% Sirius Red staining, $p<0.01$).
Proteomic analysis revealed that Melagatran reduced expression of TGFβ, CTGF (0.07±0.02 vs. 0.67±0.19 densitometric units, DU, $p<0.05$) and phospho-Smad3 activation (0.35±0.02 vs. 1.65±0.24 DU, $p<0.01$). Results also showed a downregulation of PAI-1 protein (0.09±0.02 vs. 0.29±0.06 DU, $p<0.01$). Genomic analysis demonstrated that Melagatran decreased chronic inflammation through downregulation of Th1 markers IFNγ, TNFα and IL-2; Th2 marker IL-10; Th17 marker IL-17 and complement molecule C3. Pro-inflammatory markers Thrombospondin (Tsp-1), P selectin were also reduced. Furthermore, Melagatran reduced the expression of fibrinogenesis marker PAI-1, hypoxic injury marker HIFα, epithelial mesenchymal transition marker S100A4 and oxidative stress markers Nox2 and iNOS.
Histological analysis established that treatment reduced CD3+ immunohistochemical score at 3 months (6.3±2.2 cells by high power field versus 16.6±5.8, $p<0.001$).



Conclusion: We conclude that inhibition of thrombin during kidney graft conservation significantly diminished IRI and reduced chronic graft fibrosis, with consequences on survival. Coagulation is thus a determining element of IRI, and the development of therapeutics to reduce its impact is critical to improve graft survival.

O-163 MINIMALLY INVASIVE ROBOTIC ASSISTED RENAL TRANSPLANTATION – THE INITIAL 5 CASES

Stuart Geffner, Harry Sun, Shamkant Mulgaonkar, Francis Weng, Erin Vermeulen. *Division of Transplantation, Saint Barnabas Medical Center, Livingston, NJ, USA*

Purpose: – We report the first five cases of a completely minimally invasive technique for renal transplantation utilizing robotic assistance. To our knowledge this is the first reported series of successful renal transplantation utilizing completely minimally invasive techniques.
Methods: – Robotic assistance was provided using the da Vinci S robotic surgical system (Intuitive Surgical, Inc.; Sunnyvale, California). Training in minimaly invasive surgery and the da Vinci S robotic surgical system was gained via dry lab, animal lab, and human cadaveric lab sessions and performance of basic robotic procedures in patients. In preparation for performing an entire renal transplant procedure with robotic techniques, individual portions of the procedure (dissection of the retroperitoneal space, dissection and control of the iliac vessels, vascular anastamosis, transplant ureterocystostomy) were performed with robotic assistance on animal and human subjects.
Results: – 5 cases were performed between 12/08 and 3/09. 4 female and one male patient ranged in age from 26 – 61years old. All renal allografts were procured from living donors with standard laparoscopic techniques. Four laparoscopic/robotic ports were placed in the left lower quadrant, right lower quadrant, and peri-umbilical region. After dissection of the retroperitoneal space, the allograft was placed in the right lower quadrant via a 6 cm incision in the right lower quadrant. All suturing of the single renal artery, single renal vein, and ureterocystostomy was performed "closed" with robotic assistance. Total anastamotic time for the artery and vein was approximately 60 minutes. All patients had immediate graft function. 4 patients were discharged on POD #3; one patient on POD #4. No complications have been identified.
Conclusion: – Robotic assisted, minimally invasive renal transplantation is feasible and can be performed with excellent immediate outcomes. This procedure may provide several benefits to patients.

O-164 STRATEGIES TO IMPROVE THE QUALITY OF ORGANS FROM DCD

Rui Maio[1], Nuno Figueiredo[1], Isa Santos[1], Christoph Thiemermann[2], Helder Mota-Filipe[3], Paulo Costa[1]. *[1]Departamento de Cirurgia e Transplantação, Hospital de Santa Maria, Lisboa, Portugal; [2]The William Harvey Research Institute, Barts and The London School of Medicine, London, United Kingdom; [3]Departamento de Farmacologia, Faculdade de Farmacia, Lisboa, Portugal*

Background: The organs from DCD can alleviate the critical shortage of grafts. However, DGF and PNF must be minimized. The aim of our study was to determine the effects in the graft and in the receptor of the pre-treatment of the donor with EPO and treatment of the recipient with 5-AIQ or with Tempol, in a porcine model of DCD kidney transplantation.
Material/Methods: 24 Landrace pigs were killed by lethal injection; kidneys were subjected to 30 min of WIT and then transplanted after 24 h of cold storage. In the pre-treated group, donors received a single dose of EPO (1000 IU/kg) 30 min before cardiac arrest. In the treated group, recipients received a continuous dose of 5-AIQ (5mg/kg/h) or Tempol (30mg/kg/h) 10 minutes before reperfusion and during 60 minutes. Blood, urine and renal tissue samples were collected for biochemical, histological and immunohistochemistry (PARS, iNOS and COX-2) evaluation. Data analysis was performed with Graph Pad and $p<0.05$ was considered statistically significant.
Results: Transplantation of kidneys from DCD resulted in: a significant rise of the levels of creatinine, NAG, GST, AST, LDH, ALT, fractional excretion of Na+, interleucin 1 and 6, MDA levels and MPO activity ($p<0.05$); a significant reduction in urine flow and creatinine clearance; and disturbances in the histological and imunohistological pattern. Administration of EPO before ischemia and 5-AIQ or Tempol before reperfusion reduced significantly the biochemical ($p<0.01$), histological and imunohistochemical evidence of glomerular dysfunction and tubular injury. They also reduced the systemic injury, the inflammatory response and the oxidative stress.
Conclusions: This study provides evidence that pre-treatment of the donor with EPO and treatment of the recipient with 5-AIQ or Tempol causes a substantial reduction of the dysfunction and injury associated with the transplantation of kidneys recovered after cardiac death.

O-165 ATORVASTATIN PROTECTS AGAINST REPERFUSION INJURY IN THE RAT KIDNEY THROUGH DIRECT INHIBITION OF ACTIVE CASPASE-3

John L. Haylor[1], Kevin P.G. Harris[2], Michael L. Nicholson[2], Helen L. Waller[2], Bin Yang[2]. *[1]Academic Nephrology Unit, University of Sheffield, Sheffield, United Kingdom; [2]Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom*

Background: Beneficial effects on ischaemia reperfusion injury (IRI) in the kidney have been reported for statins administered prior to the ischaemic insult. Since drugs given only perfuse the transplanted organ following its reperfusion in patients, such benefits may be over-estimated, and its mechanism is not well known.
Methods: Male rats underwent a right nephrectomy. The left renal hilus was clamped for 45 min and followed by reperfusion for 4h. Atorvastatin (AT) 10 mg/kg was administered intravenously after clamping but prior to reperfusion.

Histological injury, inflammation, apoptosis and oxidative damage were assessed. Caspase-3 activity, protein expression and nitric oxide involvemnet were measured as well.
Results: AT decreased tubulointerstitial damage (2.89±0.06 vs. 2.14±0.18), ED1+ cellular infiltration (5.4±1.2 vs. 1.4±0.4), tubular apoptosis (2.3±0.2 vs. 1.2±0.2), interstitial apoptosis (1.9±0.4 vs. 0.7±0.3) and tubular necrosis (4.3±1.2 vs. 1.2±0.5, cells/high power field). IRI was associated with an increase in caspase-3 activity due to 12 & 17 kD active subunits. Caspase-3 activity was reduced following IRI in animals treated with AT. However, there was no evidence of any reduction in active subunits. Protein S-nitrosylation, including a 12 kD band, was increased following IRI but not further affected by AT. Caspase-3 activity in the tissue homogenate of IR kidneys containing 20 μg protein and 5 ng recombinant human caspase-3 was dose dependently inhibited by AT, reaching a statistically significant difference at 1 and 4 mM respectively. Thus AT has a direct selective inhibitory effect on the caspase-3 enzyme itself. Serum cholesterol and triglyceride remain unchanged.
Conclusions: Acute administration of AT after ischaemia can improve reperfusion tolerance through a direct inhibition of active caspase-3. This effect is independent of any reduction in serum cholesterol and its long-term impact on renal function requires further investigation.

O-166 BRAIN DEATH PREDISPOSES THE KIDNEY TO A PRO-INFLAMMATORY RESPONSE UPON REPERFUSION

Dorottya K. de Vries[1], Jan H.N. Lindeman[1], Jan Ringers[1], Ton J. Rabelink[2], Alexander F.M. Schaapherder[1]. *[1]Department of Surgery, [2]Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands*

Purpose: It has become clear that brain death, as present in heart beating (HB) donors, is of important influence on post-transplantation graft dysfunction and graft survival. Brain death not only leads to changes in circulatory parameters in the donor, but may also provoke inflammatory changes in the kidney. To assess the influence of the inflammatory state of the graft after transplantation, local cytokine release from the reperfused kidney was compared between living donor (LD), HB and non-HB (NHB) donor kidney transplantation.
Methods: To assess the influence of graft type on the reaction on ischemia-reperfusion (I/R) injury, we systematically studied the cytokines released locally from the graft in early renal reperfusion. We collected paired arterial and renal venous blood samples at consecutive time-points during the first 30 minutes of reperfusion. Eight LD, HB or NHB donor kidney transplantation recipients were included in each group. A custom-made set of 9 different cytokines was measured.
Results: LD and NHB grafts showed a pleiotropic cytokine response with release of IL-6, MCP-1 and IL-1ra. In contrast, kidneys from brain dead donors released many different pro-inflammatory cytokines in vast amounts, such as G-CSF, IL-8, IL-9, IL-16 and MCP-1. It appears that the balance in LD grafts was in favor of protective IL-6 release with low MCP-1 release, while in brain dead donors the balance shifted to massive pro-inflammatory action and very low release of IL-6.
Conclusion: Our results indicated that brain dead influences the graft inflammatory state and thereby its reaction to I/R injury. Because we show that the mechanism leading to damage may be totally different in transplantation of kidneys form brain dead donors, our results emphasize that future therapy should focus on donor pretreatment using a specific approach for grafts from brain dead donors.

O-167 THE PHYSIOLOGICAL EFFECTS OF HYDROGEN SULPHIDE ON ISCHAEMIA REPERFUSION INJURY IN DONATION AFTER CARDIAC DEATH KIDNEYS

Sarah A. Hosgood, Phillip J. Yates, Michael L. Nicholson. *Department 3ls, Transplant Group, University of Leicester, Leicester, Leicestershire, United Kingdom*

Introduction: Therapies to alleviate ischemia reperfusion (I/R) injury and improve graft function have an important role in kidney transplantation. This study used a porcine model of donation after cardiac death (DCD) kidneys to investigate the effects of hydrogen sulphide on I/R injury.
Methods: Porcine kidneys were subjected to a significant level of warm ischaemia and cold ischaemic injury. They were then reperfused with oxygenated autologous blood on an isolated organ perfusion circuit. Kidneys were treated with the membrane gaseous mediator, Hydrogen Sulphide (H_2S). Doses of 1mM, 0.5mM and 0.1mM H_2S (n=4) were infused into the arterial arm of the isolated circuit 10 minutes before and after reperfusion. Renal haemodynamics and function were assessed over 3 hours of reperfusion and the results compared to a control group.
Results: 1mM and 0.5mM hydrogen sulphide significantly improved the renal blood flow and lowered intra-renal resistance ($P \leq 0.05$). Serum creatinine (Cr) fall and Creatinine clearance (CrCL) were significantly improved with the treatment of H_2S. (Area under the curve (AUC) Cr μmol/L·h; control 2257±52.1, 1mM 1549±286.6, 0.5mM 1647±310.1, 0.1mM 1812±328.7; P=0.013) (AUC CrCl ml/min/100g·h; control 1.5±1.5 1mM 5.2±2.35, 0.5mM 7.57±6.33, 0.1mM 5.57±2.87; P=0.04). Doses of 1mM and 0.1mM H_2S reduced renal tubular damage with significantly lower levels of total nitric oxide in the urine compared to the control (1mM; 25.45±4.02, 0.1mM; 13.58±10.02, Control; 45.42±10.71 μmol/L: P=0.002).
Conclusion: This study provides new evidence of the physiological role of hydrogen sulphide in ischaemically damaged porcine kidneys. Hydrogen sulphide improved renal blood flow and ameliorated the renal dysfunction associated with ischaemic damage. It therefore has potential as a new therapy against I/R injury in DCD kidney transplantation.

O-168 IMMUNOLOGIC CAMOUFLAGE AND EXTRACELLULAR COMPOSITION PROTECTS AGAIN ISCHEMIA REPERFUSION INJURY AND ALLOGRAFT FIBROSIS

Emilie Manguy[1,2], Raphael Thuillier[1,2], Giraud Sebastien[1,2], Frederic Favreau[1,2], Jean-Michel Goujon[1,2], Thibeau Desurmont[1,2], Michel Eugene[1,2], Thierry Hauet[1,2]. *[1]Inserm U927; CHU de Poitiers; Univ Poitiers, Faculte de Medecine et de Pharmacie, F-86000, Poitiers, France; [2]FLIRT (Federation pour L'Etude de l'Ischemie Reperfusion en Transplantation), France*

Purpose: The multiplication of preservation solution (PS) and the lack of well designed studies make the choice difficult. The aim of this study was to compare 3 PS in a preclinical model of renal allotransplantation.
Methods: 3 different PS were used: UW as gold standard, IGL-1® (Inst. G. Lopez, France; a UW-like solution with an extracellular ionic composition and 1g/L PEG35kDa as colloid) and SCOT® (a Krebs like solution with an extracellular ionic composition and 30g/L PEG20kDa as colloid). These were tested in a Large White pig model of low mismatch kidney allograft transplantation, for which kidneys were preserved at 4°C for 24h.
Results: After 3 months, graft survival was 20% in UW, 40% in IGL-1® and 80% in SCOT® ($p<0.05$ to UW). Plasma creatinine was 282.8±65.7μmol/L in UW, 261±5837μmol/La in IGL-1®, and 144.6±10.4μmol/L in SCOT® ($p<0.05$ to other PS). Proteinuria was 4.0±1.1g/24h in UW; 4.5±1.9g/24h in IGL-1® and 0.3±0.1 in SCOT® ($p<0.01$ to UW).
Histological analysis revealed intense fibrosis in UW-grafts (25,3±6,6% Sirius Red staining) and IGL-1®-grafts (31,7±8,2%), contrary to SCOT®-grafts (5,47±1,94%, $p<0.001$). UW- and IGL-1®-grafts were positive for Vimentin and Smooth muscle actin staining in the tubules, markers of Epithelial to Mesenchymal Transition. SCOT®-grafts were free of such staining.
RT-PCR analysis showed induction of CMH-assembly markers Tap1, Tap2 and β2-microglobulin in all grafts; SCOT-grafts showing decreased β2 microglobulin induction ($p<0.05$). Inflammation markers TLR2, MCP-1 and Thrombospondin were overexpressed. SCOT®-grafts showed less oxidative stress makers (lower HO-1 expression coupled with HO-2 expression maintenance at control levels). Finally, TGFβ1 expression was absent in SCOT®-grafts, whereas it was increased in UW- and IGL-1®-grafts, following fibrosis levels.

Table 1. RT-PCR analysis of 3 month pig kidney allografts (folds to control, healthy kidney, shown are mean ± SEM)

	Genes	UW	IGL-1	SCOT
CMH-I assembly	TAP-1	3.2±0.3*	6.5±3.4*	4.0±1.1*
	TAP-2	2.7±0.2*	4.6±2.4*	2.7±0.4*
	B2-microglobulin	3.2±0.6*	3.6±1.1*	1.9±0.4*¶‡
Innate immunity	TLR2	1.9±0.3*	3.5±1.6*	1.3±0.1¶
	TLR4	1.7±0.1*	1.7±0.6	1.6±0.2*
	MCP-1	1.5±0.3	1.1±0.2	0.6±0.1*¶
	Thrombospondin	0.4±0.1*	0.3±0.1*	0.1±0.1*¶‡
Oxidative stress	HO-1	0.9±0.1	0.8±0.1*	0.7±0.1*¶
	HO-2	0.6±0.1*	0.6±0.2*	0.8±0.1*¶
Fibrosis	TGFβ1	1.7±0.2*	2.4±0.9*	0.9±0.1*¶‡

Statistical analysis: *$p<0.05$ to control; ¶$p<0.05$ to UW; ‡$p<0.05$ to IGL-1.

Conclusion: In conclusion, presence of PEG 20kDa in an organ preservation solution of extracellular composition is of critical benefit to allografts.