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01 Extracorporeal membrane oxygenation in lung transplantation

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Background: Extracorporeal membrane oxygenation (ECMO) is currently accepted in lung transplantation either to bridge patients to transplantation or to treat postoperatively arising severe primary graft failure. Based on promising initial experiences we have since 2001 implemented ECMO as the standard of intraoperative extracorporeal support in lung transplantation (LuTX) patients with haemodynamic or respiratory instability with the potential to prolong ECMO support into the perioperative period. The aim of this paper is to summarize our total experience with the use of ECMO in LuTX.

Methods: We retrospectively reviewed all 306 patients undergoing primary lung transplantation from 1/2001 until 1/2006 with regard to the different forms of ECMO use. Results of all patients requiring ECMO were compared to those without ECMO during the observation period.

Results: ECMO was used in 147 patients in total. Two patients were bridged to transplantation. A total of 130 patients received intraoperative ECMO support. In 51 of these patients ECMO was prolonged into the perioperative period. Five of these patients required ECMO support again in the postoperative period due to graft dysfunction. CPB was used in 27 patients mainly with concomitant cardiac defects. Eleven of these patients needed therapeutic ECMO in the further course. A total of 149 patients without relevant risk factors were transplanted without any intraoperative extracorporeal support. Six of these patients required ECMO support in the postoperative period for treatment of PGD. Overall 3-months, 1-year and 3-years survival rates were 88.6, 82.1 and 74.63%. The mentioned survival rates were 85.4, 74.2 and 67.6% in the intraoperative + prolonged ECMO group; 93.5, 91.9 and 86.5% in the no support group and 74.0, 65.9 and 57.7% in the CPB group.

Conclusions: ECMO is a valuable tool in lung transplantation providing the potential to bridge patients to transplantation, to replace CPB with at least equal results and to overcome severe postoperative complications. Favourable survival rates can be achieved despite the fact that ECMO is used in the more complex patient population undergoing lung transplantation as well as to overcome already established severe complications.

02 Lipocalin-2 – a novel inflammatory regulator during ischemia and reperfusion and acute allograft rejection

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Background: The main focus of this work was to analyze the possible implication of Lipocalin-2 (Lcn-2) upregulation for the course of ischemia/reperfusion (IR) during heart transplantation as well as its role in acute allograft rejection. Lcn-2 expression was also analyzed in terms of acute rejection following liver transplantation in a clinical setting.

Methods: Male inbred C3H and C57BL/6 mice as well as the Lcn-2−/− mouse were used in our transplantation experiments. Western blot, RT-PCR and immunohistochemistry and TUNEL assay were performed to determine Lcn-2 expression and apoptosis in the graft. Cardiac rejection was classified following the ISHLT score (0–3). Additionally, Lcn-2 protein expression was analyzed in the serum of recipients at day 0–15 following liver transplantation using ELISA technology.

Results: Infiltrating polymorphonuclear cells (PMN) were the major contributors to Lcn-2 expression during IR peaking 24 h after reperfusion. The number of infiltrating PMN was significantly reduced in Lcn-2−/− recipients (by 79% at 12 h [$p<0.01$]). No difference was observed in the apoptotic rate between wildtype and Lcn-2−/− donors in our murine heart transplantation model. Concomitant upregulation of Lcn-2 in the serum of liver transplant recipients could be observed during acute rejection episodes in a timely dependent course (3 to 7-fold).

Conclusions: Our data suggest a chemoattractant function of increased Lcn-2 expression in the transplanted heart due to infiltrating PMN. Lcn-2 is a novel inflammatory regulator upregulated during IR and is proposed an early biomarker for acute graft rejection. Our observations shed light on a possible function of Lcn-2 in the recruitment of PMN to the site of IR and identify possible targets for therapeutic intervention.

03 Influence of ABO-compatible transplantation on long-term outcome in cardiac transplant recipients

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Background: Cardiac transplantation is an established life saving procedure for end-stage heart disease. Previous reports on the influence of the ABO system on outcome after cardiac transplantation indicated that blood group identical transplants had a better outcome than blood group compatible transplants. As the demand for donor organs largely exceeds the supply, it is our policy to use blood group compatible local donors for transplantation if no suitable ABO identical recipient is available. The aim of this study was to determine outcome after blood group compatible transplants.

Methods: Between 1984 and 2003 a total of 915 cardiac transplants have been performed at our center. Median follow-up was 127 months. Patients were analyzed according blood group matching (ABO identical vs. ABO compatible). Moreover subgroup analyses within the different groups were made to identify potential differences. Groups were analyzed for survival, graft rejection and graftvasculopathy (CAD). Kaplan-Meier analysis was used and log-rank test was performed to detect differences. A p-value of <0.05 was defined as statistical significant.

Results: A total of 81 transplants (8.9%) were blood group compatible. The majority ($n=32$) were 0A transplants (40%) followed by OB ($n=19$; 23%), AAB ($n=17$; 21%), BAB ($n=7$; 9%) and OAB ($n=6$; 7%). Overall survival comparison showed no significant difference in long-term survival (10-year) between the two groups (identical: 52.2% vs. compatible: 42.1%; p: n.s.). Yet there was a clear trend towards lower survival within the compatible group (10a survival: OB: 73.3%, AAB: 58.8%, BAB: 32.1%, OA: 27.6%; $p=0.0568$). In contrast, Freedom from acute rejection was significantly different between identical and compatible groups (71.4 vs. 49.9%; $p<0.0001$). There was no difference in incidence of CAD as well as severe CAD (10a: [CAD: 62.5 vs. 60.3%; n.s.], [CADsev: 76.8 vs. 77.9%; n.s.]).

Conclusions: The results of our analysis show that ABO blood group compatible transplants have similar outcomes in behalf of survival and CAD as ABO identical transplant. Yet rejection rates are significantly higher in ABO compatible transplants. This finding needs further investigation.

04 Impact of ACE-inhibitor and angiotensin receptor blocker therapy on development of proteinuria after switch to sirolimus in cardiac transplant recipients

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Background: Weaning of Calcineurin-inhibitor based immunosuppression with concomitant switch to sirolimus (SRL) due to renal impairment has been shown to be effective and safe after cardiac transplantation. However there have been reports of increased incidence of proteinuria after switch to Srl, associated with a further decrease of renal function. ACE-inhibitors (ACEi) as well as angiotensin receptor blockers (ARB) are generally used to treat proteinuria in the non-transplant setting. The aim of this analysis was to examine if ACEi and/or ARB therapy has an influence on development of proteinuria after switch to SRL.

Methods: Eighty-one long-term cardiac transplant patients were switched from Cyclosporine based immunosuppression to SRL based IS 8.8 ± 4.5 years after transplantation. Concomitant IS consisted of Mycophenolate-Mofetil ± steroids. In 61 patients pre- and serial post-switch measurements of proteinuria were performed. Data on anti-hypertensive medication was

recorded and switch-patients were divided into two groups (ACEi/ARB group vs. conventional antihypertensive therapy [CAT]). Differences in development of proteinuria and renal function were compared between both groups.

Results: Fifty-five patients (89%) received anti-hypertensive medication. Of these, 58% were treated with ACEi ($n = 32$) and 4 (7%) patients received a combination of ACEi and ARB's. Overall proteinuria increased significantly from median 0.13 g/d (range 0–5.7) pre-switch to 0.23 (0–9.88) 24 months post switch ($p = 0.0024$). There was no difference in proteinuria between the two groups before switch to Srl (ACEi/ARB: 0.13 [range 0–5.7] vs. CAT: 0 [0–4.98; p = ns]). ns]). After 24 months of follow-up there was a significant difference between the two groups (ACEi/ARB: 0.18 [range 0–7.25] vs. CAT: 0.42 [0–9.88; $p < 0.05$]). Renal function was similar before the switch in both groups (ACEi/ARB: 48.8 [range 20.6–132.1] vs. CAT: 40.6 [17.3–120.2; $p = $ ns]). After 24 months renal function was better in the ACEi/ARB group (58.8 [range 6.6–184.6]) compared to the CAT group (44.7 [range 10–84]; $p < 0.05$).

Conclusions: Development of proteinuria after switch to Srl can be significantly diminished with the use of ACEi/ARB therapy. Antihypertensive therapy of patients should be changed to an ACEi/ARB based one before Srl is initiated.

05 Tolerance through transplantation of genetically modified hematopoietic stem cells

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Background: Mixed (cellular) chimerism induced by allogeneic BMT leads to robust donor-specific transplantation tolerance. However risks associated with transplantation of allogeneic BM limit clinical application. Molecular chimerism is induced by transplantation of autologous (i.e., syngeneic in the murine system) hematopoietic stem cells genetically modified to express the antigen(s) of interest. Molecular chimerism thus overcomes the risk of GVHD, and requires less host conditioning for BM engraftment. This concept has been reported to induce tolerance in models of organ transplantation and autoimmune disease, but awaits further development to make clinical transition possible. We now started to investigate this promising approach by introducing an allergen into murine BM.

Methods: Balb/c donors were treated with 5-FU to enrich hematopoietic stem cells. Bone marrow cells (BMC) were isolated, cultivated and retrovirally transduced in vitro to express Phl p 5 (a major grass pollen allergen) in a membrane-anchored fashion (transduction efficiency: 35–55%). Myeloablated Balb/c mice received $2–4 \times 10^6$ transduced BMC iv, and were thereafter repeatedly injected sc with the recombinant allergen Phl p 5 and rBet v 1 (an unrelated control birch pollen allergen) (0.5 µg plus aluminumhydroxide, wks 6, 9, 12 and 22 after BMT). Multi-lineage chimerism was followed by FACS. Tolerance towards the introduced allergen Phl p 5 was determined by measuring of allergen-specific antibody levels in sera by ELISA. T-cell tolerance was determined by proliferation of splenocytes by stimulation with r Phl p 5. Tolerance at the effector cell level was tested in vitro by IgE-mediated mediator release from basophils, and in vivo by intradermal injection of allergens.

Results: All mice ($n = 10$) transplanted with Phl p 5-transduced BMC developed high levels of multi-lineage molecular chimerism which remained stable for the length of follow up (up to 9 months) (e.g. 11 mean% Phl p 5+ B cells and 22% T cells, 25 wks post-BMT). Serum levels of Phl p 5-specific IgE and IgG1 remained undetectable in all chimeras throughout follow-up while high levels of Bet v 1-specific IgE and IgG1 were measured. Mediator release assays revealed the absence of Phl p 5-specific degranulation in chimeras, while in contrast Bet v 1-specific degranulation was preserved. In T-cell proliferation assays chimeras showed specific non-responsiveness to Phl p 5 ($n = 3$; $p < 0.01$ vs non-transduced mice).

Conclusions: These proof-of-principle experiments demonstrate for the first time that molecular chimerism induces robust and long-lasting tolerance in allergy. They also re-emphasize that molecular chimerism is a powerful concept that allows establishment of tolerance at the B-cell, T-cell and effector cell levels, making it attractive for numerous indications, including tolerization of graft-versus-host and host-versus-graft alloreactivity in transplantation.

06 Messung der Intima-Media-Dicke bei Kindern nach Nierentransplantation (NTX) – ein Pilotversuch

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Grundlagen: Kardiovaskuläre Erkrankungen stellen einen bestimmenden Faktor für Langzeitmorbidität und Mortalität bei Kindern nach NTX dar. Die Intima-Media-Dicke Messung (IMT-Messung) der Carotis mittels Ultraschall stellt einen frühen Marker für das Ausmaß und Vorhandensein von atherosklerotischen Veränderungen dar. Ziel unserer Untersuchung war einerseits, die Reproduzierbarkeit der IMT bei wiederholten Messungen und zwischen zwei Untersuchern darzustellen, andererseits sollen die IMT-Werte von Kinder, die einer präemptiven NTX unterzogen wurden, mit jenen von nicht-präemptiv transplantierter Kinder verglichen werden.

Methodik: Im Rahmen eines 4-wöchigen Studienaufenthalts an der Charité Berlin wurde die Untersuchungstechnik der IMT erlernt. Zur Evaluation der Reproduzierbarkeit wurden bei 12 Patienten von 2 Untersuchern jeweils 2 Messungen pro Seite durchgeführt. Aus den erhaltenen 4 Messwerten wurde jeweils ein Mittelwert gebildet. Der interindividuelle Fehler und intraindividuelle Schwankung wurden mittels Bland and Altman-Funktion und deskriptiver Statistik ermittelt. An der Kinderdialyse Wien werden Kinder nach NTX in präemptiv

bzw. nicht-präemptiv unterteilt und nach Alter und Geschlecht passende Paare gebildet. An diesen Kindern wird die IMT gemessen und der Einfluss der Dialysebehandlung auf die Gefäßveränderung untersucht.

Ergebnisse: In der Bland and Altman Graphik zeigte sich ein interindividueller Fehler von 0,026 mm sowie eine intraindividuelle Schwankung von 0,035 mm (±0,02) beim erfahrenen und 0,04 mm (±0,023) beim unerfahrenen Untersucher. Bei Kindern nach NTX wurden bisher durchwegs Normwerte gemessen, in den präliminären Daten konnten wir keinen Unterschied zwischen präemptiver bzw. nicht-präepmtiver Gruppe finden.

Schlussfolgerungen: In unserer Untersuchung erwies sich der interindividuelle Fehler mit 0,026 mm geringer als die intraindividuelle Schwankung von 0,035 bzw. 0,04 mm (6,5% Schwankung), die Reproduzierbarkeit war somit gegeben. Bei Kindern nach NTX sind weitere Untersuchungen notwendig.

07 Phosphorylation of extracellular signal regulated protein kinases (ERK) and delayed graft function in human kidney allografts

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Background: Extracellular signal regulated protein kinases (ERK) belong to the family of mitogen activated protein kinases that are crucial mediators of survival and cell death. Experimentally, ERK signalling is altered during ischemia reperfusion injury. Aim of this study was to correlate ERK activation with early graft function in human kidney transplantation.

Methods: Cadaveric kidney allografts ($n = 28$, mean donor age 49.96 ± 13.73 a; mean cold ischemia time 863 ± 314 min) were biopsied during cold ischemia (CI) and 15 min after reperfusion (R). Samples were analyzed for phosphorylation by western blot using antibodies directed against total and phosphorylated forms of ERK. Phosphorylation (pERK) was quantified as area under the curve and expressed as percentage of total ERK protein. Early graft function was stratified by adequate initial diuresis (group A, $n = 17$) vs postoperative need for dialysis (group B, $n = 11$).

Results: PERK increased from 17% (CI) to 54% (*R*), for a mean 4.64 ± 2.90 – fold increase ($p < 0.0001$). Strong (>5-fold) increase was associated with male donors ($p = 0.048$) and donor natrium <150 mmol/l ($p = 0.009$), but not donor age ($p = 0.720$), perfusion fluid ($p = 0.385$), cold ischemia time ($p = 0.511$) or anastomosis time ($p = 0.072$). While pERK increase ($p = 0.097$) and pERK_R levels ($p = 1.000$) did not differ between groups, PERK_CI levels were significantly higher in group B ($p = 0.027$).

Conclusions: PERK significantly increases during ischemia/reperfusion in human kidney transplantation. While the total increase does not corresponded with early graft function, high phosphorylation levels during cold ischemia are associated with delayed graft function.

08 Allogeneic stem cell transplantation in patients with myelodysplastic syndromes: a single center experience

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of stem cell disorders characterized by bone marrow dysplasia, peripheral cytopenia, and an enhanced risk to transform to acute myeloid leukemia (AML). In most patients, treatment options are limited to supportive care and palliative cytoreduction. However, in a group of patients, intensive therapy can be offered. The only established curative treatment approach for these patients is haematopoietic stem cell transplantation (SCT).

Methods: In the present study, we retrospectively analyzed a cohort of 29 adult patients (18 males, 11 females) with MDS ($n = 26$) or MDS transforming into secondary AML ($n = 3$), who underwent SCT at our institution between 1988 and 2007. Sixteen patients had a HLA-identical related transplant donor, and 13 had a HLA-matched unrelated donor. The median age at time of SCT was 39 years (range: 19–62 years). According to the WHO classification, 4 patients had RA, 1 RARS, 3 RCMD, 1 RCMD-RS, 4 RAEB-1, 11 RAEB-2, 1 CMML, 1 MDS-unclassified, and 3 had AML following RAEB at SCT. According to the IPSS, which was available in 27 patients, 8 had intermediate-1 risk, 10 intermediate-2 risk, and 9 high risk MDS. Conditioning consisted of chemotherapy plus total body irradiation (24/29 patients) or chemotherapy alone (5/29 patients). Graft versus host disease (GvHD) prophylaxis consisted of a combination of low-dose methotrexate and cyclosporine A (26/29 patients) or cyclosporine A plus mycophenolat mofetil (3/29 patients).

Results: Patients were followed up with a median observation time of 23 months (range: 1–240). Currently, 15 patients (52%) are alive at 5–240 months after SCT. Only one patient with intermediate-2 risk relapsed and is now undergoing SCT from an unrelated donor. All other 14 patients are still in CR, 12 of them suffer from chronic GvHD. Of the CR patients, 7 had intermediate-1 risk, 3 intermediate-2 risk and 4 had high risk MDS at SCT. Of the 14 patients who died, post-transplantation relapse occurred in 4 patients, namely in 2 with high risk, 1 with intermediate-2 risk, and 1 with intermediate-1 risk MDS. Four patients died of treatment-related causes (multi-organ failure, sepsis, haemorrhage), i.e., 2 with intermediate-2 risk MDS, 1 with high risk MDS, and one in whom the IPSS score was not available.

Conclusions: In summary, a substantial number of patients with MDS achieve long term disease-free survival after SCT, confirming previous data. In our small cohort of patients, we also found that survival and outcome after SCT do not correlate with IPSS risk categories or the WHO classification. Therefore, we believe that new prognostic factors and score systems are required to optimally predict survival in MDS patients who are considered candidates for SCT.

09 Late referral to pediatric nephrologist and access to preemptive kidney transplantation

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Background: Kidney transplantation is the most desirable modality of renal replacement therapy (RRT) in developing and growing child, because it yields higher life expectancy and better quality of life. Recent studies showed that late nephrologists referral reduces the likelihood of kidney transplantation in adults. Aim of our study was to evaluate the association between late referral to pediatric nephrologists and the chance of preemptive kidney transplantation for children.

Methods: In this retrospective study, we assessed demographic, diagnostic and therapeutic variables at first referral and at initiation of RRT in all children at our tertiary care center for pediatric nephrology (''Kinderdialyse Wien'') between 1978 and 2005. We categorized the population whether the first RRT modality was preemptive kidney transplantation or not and termed a first pediatric nephrologists visit <3 months prior to RRT as ''late referral'' and a visit >3 months as ''early referral''. Descriptive statistics, correlation and logistic regression techniques were used for analysis.

Results: We evaluated 112 children (50 girls and 62 boys, aged 8.0 [median 8.7] years at first referral and 10.9 [median 11.8] years at initiation of RRT). The mean follow up at the Kinderdialyse Wien was 2.9 [median 1.5] years. In comparison to 85 children who had their first visit >3 months prior to RRT, 27 children with ''late referral'' showed significant differences for hemoglobin ($p = 0.0001$), bicarbonate ($p = 0.007$) and phosphorous ($p < 0.0001$) at first referral. Only 3 of these 27 children got the chance of preemptive transplantation, whereas 32 of 85 children (''early referral'') were preemptively transplanted ($p = 0.015$). Using a threshold of 12 months this difference for the likelihood of preemptive kidney transplantation was still significantly influenced by timing of referral ($p = 0.03$).

Conclusions: Earlier pediatric nephrologists care was associated with greater access to preemptive kidney transplantation.

10 Bariatric surgery for treatment of new onset type II diabetes mellitus associated with morbid obesity in kidney-pancreas transplant recipients

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Background: Combined kidney pancreas transplantation is the treatment of choice for end-stage diabetic nephropathy. Post transplant weight gain increases the risk for post transplant complications and death due to cardiovascular events. Gastric banding is an established treatment of moderate morbid obesity.

Methods: We report on two patients, who experienced significant weight gain with a body mass index $>40\,kg/m^2$ and developed type II diabetes mellitus following successful kidney-pancreas transplantation.

Results: Patient #1 underwent laparoscopic gastric banding and initially had good weight loss. However, lack of compliance with dietary guidelines led to transient failure of weight-loss therapy. With further adjustment of the gastric band good weight-loss and a reduction in insulin requirement was achieved. Patient #2 suffered from diabetic gastroparesis and esophageal motility disorder and therefore underwent laparoscopic implantation of a gastric pacemaker. Both morbid obesity and delayed gastric emptying were successfully treated. This patient also was healed from his type II DM.

Conclusions: Bariatric surgery seems a useful strategy in the treatment of new onset type II DM after successful pancreas transplantation in morbidly obese patients. The optimal bariatric procedure for this patient population remains to be determined.

11 Bariatric surgery and liver transplantation: single center experience with six cases

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Background: Obesity has evolved as major epidemic worldwide. Obesity as part of the metabolic syndrome can be associated with Non-alcoholic steatohepatitis (NASH), NASH can lead to end-stage liver disease requiring liver transplantation (LT). Bariatric surgery has been shown to effectively reduce body weight and improve some metabolic components associated with obesity.

Methods: Between 1998 and 2001, 467 liver transplants were performed in 402 individuals at our center. Within this series six patients were identified who had bariatric surgery pre ($n = 5$) or post ($n = 1$) LT. Procedures included gastric bypass ($n = 2$), jejunoileal bypass ($n = 2$), gastric banding ($n = 1$) and sleeve gastrectomy ($n = 1$).

Results: Median follow up for the six patients was 5.3 (range 4.0–6.5) years, five patients are currently alive, one died five years post LT from lung cancer. One patient required two retransplants due to PNF. Underlying diseases were: NASH in three patients, alcoholic liver disease in two patients and epitheliod hemangioedothelioma in the last patient. The median BMI at last follow up in these patients was 38.2 (range 28.4–53.3) kg/m^2. Three patients remained with the same weight (BMI), one was able to loose 18 kg and two patients gained more than 25 kg within five years post LT. Currently all six patients have a BMI $>35\,kg/m^2$. Accordingly, three suffer from hyperlipedemia, one has IDDM, two NIDDM and two are borderline diabetics. Three require antihypertensive medications, five suffer from depression and one from significant insomnia. All six patients require psychiatric evaluation and medications. Three are prescribed acid blocking medications. Five have renal insufficiency with one patient requiring hemodialysis.

Conclusions: Previous bariatric surgery is not a contraindication for LT; patients who previously had jejunoileal bypass and develop NASH are increasingly referred for LT. Morbid obesity cannot be considered a contraindication against LT. Bariatric surgery should be included in the management of patients who experience significant weight gain post LT, however, the optimal procedure still needs to be determined.

12 HCV recurrence post liver transplantation: a matter of the graft quality?

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Background: Recurrent hepatitis C is the most common cause of late graft loss after liver transplantation (LT). Advanced donor age has been accepted the major risk factor for development of bridging fibrosis due to HCV recurrence post LT. Much less is known about the significance of other donor associated risk factors.

Aim: To describe the long term outcome of LT for patients with HCV according to the utilization of grafts from extended criteria donor.

Methods: Longitudinal cohort study including 402 patients undergoing 467 LTs including 163 patients (193 transplants) with the diagnosis of HCV at Mayo Clinic Jacksonville between 1/98 and 12/01. All transplants were performed using the piggyback technique. Immunosuppression consisted of tacrolimus, mycophenolate-mofetil and tapered steroids.

Results: Two hundred and sixty grafts were from extended criteria donors (ECD). The median non adjusted MELD score of patients receiving ECD grafts was 14 vs. 16.6 for the remaining 207 transplants ($p = 0.003$) and in 9.2% as compared to 19.8% used for reLT ($p = 0.001$). One/five year graft survival did not differ between transplants for HCV ($n = 194$) and for non HCV associated ($n = 273$) liver disease with 78.9/60.3% and 71.1/61.2%. A single extended criterion had no influence on graft survival, however, when multiple extension criteria were found survival was significantly poorer. For transplants for HCV associated liver disease, donor age >70 had the most detrimental impact (5 year graft survival 30%), followed by serum Na >160 mmol/l (5 year graft survival 44%), donor body mass index >35 kg/m^2 and significantly elevated LFTs (5 year graft survival 48%), whereas pediatric grafts (5 year graft survival 90%) had a better outcome than grafts from non-ECD (5 year graft survival 70%), $p = 0.009$. Such a difference was not observed in patients transplanted for non-HCV related liver disease.

Conclusions: The recently proposed donor index is only valid for early graft losses. This study clearly shows that the underlying disease, in particular HCV infection must be taken in consideration in order to optimize allocation. In addition, extended follow up must be considered before making conclusions as the effects of HCV recurrence on graft survival may only be seen after several years.

13 Are radiological criteria reliable to predict survival for patients undergoing liver transplantation for hepatocellular carcinoma?

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Background: Liver transplantation (LT) is the best treatment of hepatocellular carcinoma (HCC) in the setting of liver cirrhosis. Milan criteria are used by most centers. Radiology may not correspond to the findings on pathology.

Methods: This longitudinal cohort study of 402 patients undergoing 467 LTs at our center between 1/98 and 12/01 includes 75 individuals with HCC.

Results: Twelve patients with incidental tumors, one patient with fibrolamillar HCC and another with cholangiocarcinoma as primary diagnosis were excluded from the analysis. Pre-LT chemoembolization was performed in 59 patients; one patient had radiofrequency ablation and one surgical resection. Twenty-nine patients met MC on radiology and pathology, 16 did not meet MC on both; 12 met MC on radiology but had HCC outside MC on pathology and four patients, who did not meet MC on radiology met MC on pathology. Nine patients had vascular invasion, which could not be predicted by radiology. Five year patient survival was 71% vs. 55% for MC met versus not met according to radiology, $p = 0.107$, n.s. and 82% vs. 46% for MC met versus not met according to pathology, $p = 0.002$. Sixteen patients had HCC recurrence; 13 died of HCC. On multivariat analysis, radiology was not predictive for survival as opposed to pathology. Concerning HCC recurrence, advanced stage, high alpha fetoprotein levels and donor age >65 years were predictive for early death.

Conclusions: Expanding tumor selection criteria beyond MC remains controversial. In contrast to radiology, the pathological staging accurately predicts the long-term survival of LT in patients with HCC.

14 Successful live donor transplantation of the sensitized renal recipient using B-cell directed immunotherapy

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Background: Patients with high levels of preformed antibodies to donor antigens have a high graft loss rate due to immunological complications.

Methods: Twelve patients (5 men and 7 women) with a median age of 46.7 (range 26.4–69.8) years with preformed donor alloantibodies underwent preconditioning prior to live donor renal transplantation (RTx). B-cell modulation included whole plasma exchanges (WPE), rituximab (anti-CD20), mycophenolate-mofetil (MMF) and intravenous immunoglobulins

(IVIG). Standard immunosuppression included thymoglobulin induction, tacrolimus, MMF and a steroid taper.

Results: There were four primary (all female) and eight retransplants. All patients had preformed antibodies against B-cells; two patients against T-cells (CDC and flow). On flow bead analysis, two patients had class I and ten class II antibodies. At time of RTx all patients had low level donor antibodies. Post RTx WPE/IVIG was repeated in case of rising antibody levels. After a median follow up of 168 (range 52–500) days, all patients are alive with functioning grafts (serum creatinine 0.7–1.8 mg/dl). During follow up, antibodies completely disappeared (five patients, antibody titres significantly declined (three patients) but in four patients the antibody production remained strong (in two cases associated with non compliance). Five of the twelve patients (42%) developed acute rejection episodes, which were treated with a steroid bolus ($n = 2$), ATG ($n = 2$) and WPE ($n = 1$). Infection rate was 67%; severe infectious complications included pulmonary nocardiosis ($n = 1$) and pulmonary aspergillosis ($n = 1$).

Conclusions: This protocol resulted in excellent short term graft and patient survival with acceptable morbidity.

15 Gute Ergebnisse können mit Nieren von über 80-jährigen Spendern erreicht werden

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Grundlagen: Zur Erweiterung des Spender-Pools bei bekanntem Organmangel wurden im Rahmen des ET – old for old – Programms auch Nieren von >80-jährigen Spendern angeboten. Wir analysierten retrospektiv die Ergebnisse von insgesamt 6 Patienten mit Transplantaten aus dieser Altersgruppe.

Methodik: Von 5/1999 bis 03/2007 erhielten von insgesamt 70 Nieren-Empfängern innerhalb des ET – old for old – Programms 6 Patienten (5 Frauen, 1 Mann) im mittleren Alter von 66,0 (65–68) Jahren Transplantate von regionalen Spendern im mittleren Alter von 81,3 (80–83) Jahren, wovon 4 an intrazerebraler Blutung, und 2 an Schädel-Hirntrauma verstorben waren. Anamnestisch waren weder Hypertonie, Adipositas, Diabetes noch Nikotinkonsum bekannt. Alle Spender waren CMV-IgG negativ und hatten ein mittleres Serumkreatinin von 0,76 (0,7–0,9) mg/dl. Abgesehen von einem Spender mit einer 60-minütigen hypotensiven Phase waren alle anderen Spender während des im Mittel 2,7 (1–4) Tagen dauernden Intensivaufenthaltes kreislaufstabil. Insgesamt 4 Biopsien zum Zeitpunkt der Transplantation wiesen histologisch jeweils weitgehend normale Gefässe auf, davon 2 Nieren je 20 % bzw. 8% sklerosierte Glomerula und 2 Organe eine Tubulusatrophie. Die Mismatches betrugen im AB-Lokus im Mittel 2,3 (0–3), im DR-Lokus 1,0 (0–2), die KIZ 11:35 (07:19–16:02) Stunden, die Anastomosenzeit 34,7 (26–54) Minuten. Die initiale Immunosuppression war bei 5 Patienten CNI-frei mit einem IL-II-Antagonisten + MMF + Cortison, CyA/FK wurden im Mittel ab Tag 8,2 (7–10) eingesetzt. Eine Patientin erhielt im Rahmen eines Studienprotokolls IL-II-Blocker + CYA + MMF + Cortison von Beginn an. Die mittlere Beobachtungszeit betrug 43,0 (12–70) Monate.

Ergebnisse: 2/6 Nieren wiesen einen ischämischen Tubulusschaden auf. Insgesamt 2 bioptisch verifizierte akute Abstoßungen wurden mit Steroidbolus bzw. Konversion auf FK reversiert. Ein Transplantat ging in Monat 9 an einer Polyomavirus-Infektion verloren. Eine Patientin verstarb im 27. Monat an einem zerebralen B-Lymphom mit funktionierendem Transplantat. Der mittlere Kreatininwert nach 2 Jahren betrug bei den lebenden Patienten 1,67 (0,7–2,4) mg/dl. Die infektiösen Komplikationen waren beherrschbar. Operationspflichtige Komplikationen (Hüftkopfnekrose, Cholezystitis, Blasenhalssklerose) resultierten aus vorbestehender Komorbidität.

Schlussfolgerungen: Gute Ergebnisse können mit Nieren von über 80-jährigen Spendern erzielt werden, wenn diese keine kardiovaskulären Risikofaktoren und eine gute Nierenfunktion aufweisen, die Ischämiezeiten kurz gehalten und postoperativ nephrotoxische Substanzen vermieden werden.

16 Natural killer cell dose and donor killer-cell immunoglobulin-like receptor (KIR) genotype in HLA-identical haematopoietic stem cell transplantation

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Background: Natural killer (NK) cells are considered a relevant component of the haematopoietic stem cell graft by promoting engraftment and possibly contributing to a graft-versus-malignancy effect. The role of killer-cell immunoglobulin-like receptor (KIR) ligand compatibility is still unclear, as is the impact of the NK cell dose of the graft.

Methods: To investigate these issues in HLA-identical sibling peripheral blood stem cell transplantation (PBSCT), 43 consecutive transplants for haematological malignancies were retrospectively analyzed. Twenty-four patients underwent myeloablative conditioning and 19 patients received busulfan/fludarabine-based reduced intensity conditioning (RIC).

Results: In patients with acute myelogenous leukaemia (AML) or myelodysplastic syndrome (MDS) ($n = 18$), regardless of conditioning type, no relapse occurred following transplants meeting both, a high (above median) natural killer (NK) cell count and missing KIR ligand, compared to all other AML/MDS patients (0% vs. 44%; $p = 0.049$). Quantitative graft composition was found to have significant impact exclusively in RIC transplants. Here, a trend towards reduced relapse incidence was found in patients receiving high numbers of NK cells ($p = 0.09$). Overall survival (OS) was superior in recipients of high T-cell numbers ($p = 0.01$). Non-relapse mortality (NRM) was significantly reduced in patients receiving high T-cell numbers ($p = 0.046$). Multivariate analysis, including KIR-ligand matching, showed the probability of relapse to be significantly decreased only in patients receiving high NK cell numbers ($p = 0.039$).

Conclusions: These data suggest that both, the number of transplanted NK cells, and their KIR genotype in relation to the given HLA-type, play a role in the graft-versus-malignancy effect in HLA-identical PBSCT. Quantitative graft composition influenced outcome only in RIC transplants, while missing KIR ligand(s) improved the relapse incidence particularly in AML and MDS patients.

17 Role of cytomegalovirus donor and recipient status and HLA match on graft survival following 1053 consecutive single renal transplants

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Background: HLA alleles function as transplant antigens and presenters of viral antigens and therefore play an important role in renal transplantation.

Methods: Graft survival following 1053 consecutive renal transplants performed between 1994 and 2004 was analyzed according to the CMV donor and recipient status and HLA match in a longitudinal cohort study.

Results: First transplants had significantly better survival than second and third transplants (one/five year 93.3/85.5% vs. 90/80.4% vs. 81.3/65.1%), $p = 0.0003$. The 108 living donated grafts had a five year survival of 89 vs. 83% for cadaveric grafts ($p = 0.06$). A significant improvement in the survival was observed during the study period with patients transplanted from 1994 to 1996 ($n = 288$) showing a one/three year survival of 90/84%, from 1997 to 1999 ($n = 276$) of 91/89%, from 2000 to 2001 ($n = 218$) of 92/87% and from 2002 to 2004 ($n = 271$) 96/94%, $p = 0.0029$, despite a significant rise in the donor age from mean 38.9 years to 45.6 years, $p < 0.0001$ and recipient age from mean 44.4 years to 47.8, $p = 0.001$ during the study period. Age of CMV positive donors was 44.4 years as compared to 40.4 years for their CMV negative counterparts, $p < 0.0001$. The improvement of the survival was restricted to CMV positive grafts. The most common HLA allele was A2 (donors 45%, recipients 46%), all other alleles had a lower frequency. There was no major difference in the distribution of the various alleles between donors and recipients except for B12, 15, 17 and B40. Neither HLA class one nor class two matching had an impact on survival. None of the individual HLA alleles of donors and recipients or match was associated with a particular difference in the outcome.

Conclusions: The CMV match had a significant impact on survival during the early period, however, this effect was almost completely abolished by improved CMV detection assays and prophylaxis during the later period. Using modern immunosuppression the effect of HLA matching on graft survival is only minimal.

18 Prognostic value of indocyanine green plasma disappearance rate (PDR) and MELD in patients with liver cirrhosis

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Background: The estimation of short-term prognosis in liver cirrhosis is important for the timing of liver transplantation. The Model for End-Stage Liver disease (MELD) score derived from bilirubin, creatinine, and INR, has been found useful to estimate 90-day survival. The hepatic clearance of indocyanine green (ICG) has been proposed several decades ago as a quantitative test to estimate liver function. Recently, the interest in this test was renewed by the introduction of a noninvasive method using finger pulse densitometry. The aim of this prospective study was to compare the prognostic value of ICG clearance versus MELD for prediction of 90-day survival.

Methods: The study population comprised consecutive patients ($n = 69$) with liver cirrhosis including outpatients admitted to the liver clinic, hospitalized patients being evaluated for liver transplantation, and patients with acute-on-chronic liver failure admitted to the ICU. The plasma disappearance rate (PDR) of ICG was measured following injection of 0.25 mg/kg ICG using the LiMon system (Pulsion, Munich, Germany). Simultaneously, the MELD score was determined using the Mayo Clinic website calculator. The primary endpoint was 90-day survival.

Results: Forty-four patients (64%) of the study cohort survived for more than 90 days whereas 25 patients (36%) died within 90 days. Both PDR and MELD differed significantly for patients surviving more or less than 90 days (PDR 7.5 ± 4.6 vs. 4.6 ± 1.6 [%/min]; MELD score: 14 ± 9 vs. 29 ± 11). Superior diagnostic accuracy for prediction of 90-day survival was found for MELD (AUROC 0.85) versus PDR (AUROC 0.79). Only a weak correlation was found between PDR and MELD ($r = 0.35$) suggesting that PDR contains additional prognostic information independent from MELD. Logistic regression analysis yielded a prognostic model comprising bilirubin, white blood count (WBC), and PDR, calculated as $1.2 * \ln(\text{bilirubin [mg/dl]}) + 0.136 * \text{PDR[\%/min]} - 0.284 * \text{WBC} - 4.58$. This model showed superior diagnostic accuracy for prediction of 90-day survival (AUROC = 0.88) as compared to MELD (AUROC = 0.85).

Conclusions: The prognostic value of PDR for prediction of short-term survival in patients with liver cirrhosis was found to be inferior to that of MELD. A new model including bilirubin, WBC and PDR seems to have more diagnostic accuracy for estimation of 90-day survival as compared to MELD and therefore could enrich the optimal timing of a liver transplantation.

19 Sirolimus in patients after liver transplantation due to Hepaitis C

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Background: Cirrhosis due to infection with HCV is one leading indication for liver transplantation. Triggered by the immunosuppression the virus recurs in nearly all patients mostly resulting in graft cirrhosis requiring a retransplantation. The benefit of use and its effect on the viral load of SIR for patients with HCV are still undetermined.

Methods: We retrospectively reviewed the medical reports of patients who were transplanted in our centre due to HCV

cirrhosis from 01/02, 12/06. All patients received the same induction therapy. Primary therapy with TAC and MMF was switched to a SIR based 1 month after transplantation. Extracted data included liver synthesis parameters, SIR target level, HCV PCR and survival outcomes.

Results: Fourteen patients were transplanted from 01/02, 12/06. All patients survived until now. The starting dose was 1–2 mg/day with a target level of 5–9 ng/ml. Ten patients stayed on combined MMF/SIR immunosuppression, 2 had to be switched to TAC because of MMF side-effects, 2 were combined with CYA. The transaminase levels were stable in 12 patients. Only 1 patient developed a complete HCV recidive and 1 biopsy proven rejection was treated with steroids. The HCV PCR levels were measured every 6 months and were constant for 13 patients. The viral recurs was treated by immunosuppressive reduction resulting in a stable virus load. The biopsy showed a mild fibrosis unlikely for the moderate viral activity.

Conclusions: SIR has shown to be a potent immunosuppression for LT but is normally only administered to avoid side-effects of CNIs. Rare is known about its effect on HCV. This retrospective analysis suggests a potential of SIR to be evaluated as immunosuppressant for HCV positive LT candidates to avoid early recurrence.

20 Enterocolitis after allogeneic hematopoietic stem cell transplantation due to human herpes virus 6 infection

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) offers cure for many patients with hematologic and oncologic diseases. Myeloablative conditioning is associated with substantial toxicity involving predominantly the gastrointestinal tract. During aplasia patients are at high risk for opportunistic infections. Human herpesvirus 6 (HHV6) was first isolated in 1986 and is capable of establishing a lifelong latent infection after primary disease in children seen as roseola infantum and febrile illness.

Methods and results: We present the case of a 40-year-old male suffering from secondary acute myelogenous leukaemia (AML) and receiving a hematopoietic stem cell transplant from an unrelated male donor with a HLA-B-allele mismatch in second complete remission (CR). Conditioning was myeloablative consisting of cyclophosphamide and total body irradiation (TBI). Cyclosporine A and methotrexate were administered for graft-versus-host disease (GVHD) prophylaxis. On January 9, 2007 8×10^6 CD34+ peripheral blood stem cells were transplanted.

On day +5 after HSCT the patient experienced a septicemic episode with staphylococcus epidermidis in his blood culture that responded rapidly to antimicrobial therapy. On day +10 after HSCT his diarrhea worsened with up to 3700 ml stool volume per day, nausea, and abdominal pain. Stool cultures performed repeatedly revealed a low concentration of candida albicans and candida glabrata responding to caspofungin and enterococcus faecium responding to teicoplanin. A CT scan of the abdomen was normal besides cholecystolithiasis without inflammation. On day +17 after HSCT endoscopy with tissue biopsy showed no ulcerations and no signs of GVHD or cytomegalovirus infection in the gastric, duodenal, and colonic areas. Absolute neutrophil counts (ANC) increased above 1000/µl on day +23 after HSCT.

Despite normalization of CRP and a decrease of the stool volume to 1400 ml per day the patient continuously complained about nausea and abdominal pain. Virologic exams remained negative until day +50 after HSCT when HHV6 DNA was detected by polymerase chain reaction (PCR) in the peripheral blood (PB). A retrospective analysis of the GI mucosa by immunohistochemical staining for HHV6 was performed.

The patient's viral infection resolved within the following weeks and he never experienced acute or chronic GVHD.

Conclusions: To our knowledge, this is the first reported case of enterocolitis due to HHV6 in an immunocompromised patient. Thus, HHV6 infection should be kept in mind in patients with appropriate clinical symptoms early after allogeneic HSCT.

21 A six year old lung transplanted boy with an EBV-associated bronchitis

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We report of a 6-year-old boy with Arnold Chiari Malformation Type I and primary pulmonary hypertension who underwent bilateral sequential double lung transplantation at the age of four years due to respiratory insufficiency.

Eighteen months after lung transplantation EBV-reactivation occurred. By reducing the mycophenolic acid dose the number of EBV-copies decreased initially, but raised subsequently. In parallel a drop in lung function (−30%, baseline FVC: 113%, FEV1: 107%, MEF50: 67%, MEF 25: 72%) occurred and a multi-slice CT scan revealed an increase of lymph nodes with regard to number and size (axillary, mediastinal and paratracheal, 2 lymph nodes >1 cm diameter). However no clear evidence for a PTLD was substantiated. Nevertheless FK 506 blood levels were lowered (6–8 ng/ml) with no effect on lung function. EBV copy counts remained stable at high levels and a clinical picture of an acute bronchitis (cough, oxygen-dependency, fever), non-responsive to antibiotic treatment, arose. Repeated bronchoscopy did not provide any evidence for transplant rejection, bacterial, fungal, viral infections other than EBV or cardiac decompensation. However numerous EBV-positive lymphocytes were present in the mucosa with no evidence for PTLD or interstitial lung disease.

Because of the insufficient therapeutic intervention an approach by elimination of B-cells via application of rituximab was performed. Treatment was applied four times at a concentration of 375 mg/m^2 in weekly intervals. EBV-levels in respiratory fluid dropped and clinical symptoms revealed within days and lung function improved above baseline level.

EBV-triggered bronchitis after lung transplantation should be considered as a result of EBV-reactivation.

22 Health related quality of life, psychosocial adjustment and intellectual outcome in children after renal transplantation

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Background: Knowledge regarding health-related quality of life (QOL), psychosocial adjustment (PA) and intellectual outcome in children after renal transplantation (TPL) is limited.

Methods: Thirty-seven paediatric patients were investigated at a median age of 14.5 years (range 6.5–17). Median duration after TPL was 4.5 years (range 0.5–12.8). Child- and parent-rated QOL were evaluated by The Netherlands Organization for Applied Scientific Research Academical Medical Centre (TNO-AZL) Child Quality of life Questionnaire. PA was assessed by the Child Behaviour Checklist (CBCL) providing parental reports of a child's behaviour. In 28 children, intellectual performance was assessed using the Wechsler Intelligence Scale for Children (WISC-III). Socioeconomic status (SES) was calculated by means of a 12-point scale based on paternal occupation and maternal education. Two children with severe intellectual impairment due to cerebral palsy were excluded from the analysis for median intellectual outcome.

Results: In patients self-ratings, only the QOL subscales physical complaints and global positive emotional functioning were impaired compared to healthy controls, whereas parents rated 5 of a total of 7 subscales as abnormal (motor functions, autonomy, cognitive functions, social functions, and positive emotions). PA was impaired for internalizing behaviour problems. Steroid treatment, young age at TPL, cadaveric related donation, conflictuous family climate and maternal distress had significant negative impact on QOL and PA ($p < 0.05$).

Median IQ of the patients was 98 (range 71–133). An average IQ ($=$ IQ $=$ 80–120) was found in 21 (75%) children. The two children excluded from analysis where the only with an IQ below 70. Full-scale IQ and SES correlated strongly. There was no correlation between QOL, PA and IQ.

Conclusions: Health related quality of life and psychosocial adjustment were impaired in children after renal transplantation. Parents judged their children's quality of life more critically as the children themselves. Treatment modalities and family environment had significant impacts on quality of life and psychosocial adjustment. Intellectual outcome of paediatric patients after TPL was within the normal range.

23 Intraoperative assessment of ICG elimination as a predictor of outcome after liver transplantation

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Background: Early allograft dysfunction after liver transplantation (OLT) cause marked morbidity and mortality. A reliable test to assess graft viability in the perioperative period is needed. The indocyanine green (ICG) elimination tests have been suggested as a predictor of graft viability and outcome, but their significance in the early post-reperfusion period have not yet been evaluated. The goal of this study was to determine the predictive capability of intraoperative determination of plasma disappearance rate (PDR) of ICG on one-year-survival in liver transplant recipients.

Methods: We retrospectively analyzed data from 99 liver transplant recipients (26 female and 73 male; mean age $52 + 9$ years) with a mean MELD score of $17 + 7$. The PDR ICG was measured within 30 min after graft reperfusion by transpulmonary double indicator (thermo-dye) dilution technique. A 4F aortic catheter with an integrated fiber-optic device and a thermistor was inserted via a femoral sheath for invasive aortic PDR ICG assessment. The fiber-optic device was connected to a computer system (COLD-Z021, PULSION Medical Systems, Munich, Germany). For the PDR ICG assessment 0.5 mg/kg of ICG in cooled saline (10–15 ml) was injected through a central venous catheter. Data are presented as mean + standard andard deviation, unless indicated otherwise.

Results: The mean PDR ICG within 30 min after reperfusion was $23.4 + 9\%/\text{min}$. Patients with more than 1-year-survival (73 patients) had significantly higher PDR ICG values than those who died (26 patients) within one year after OLT, $24.9 + 8$ vs. $18.8 + 9\%/\text{min}$ ($p < 0.001$), respectively. Receiver operating characteristic (ROC) statistics using the PDR ICG value within 30 minutes after reperfusion in each transplant recipient revealed an area under the curve (AOC) of 0.709 (95% confidence interval 0.609–0.796) with a cutoff point of $<20.3\%/\text{min}$. The sensitivity and specifity for this test were 72 and 67.6%, respectively. The positive predictive value, negative predictive value, positive likelyhood ratio and negative likelyhood ratio were 42.9, 87.7, 2.22 and 0.41%, respectively.

Conclusions: The PDR ICG measured within 30 min after graft reperfusion as a marker of liver function und perfusion is a moderate predictor of overall one-year-survival after OLT. Due to the high negative predictive value is this test suitable as screenning for patients at high risk of liver graft dysfunction and consecutive related complications.

24 Hohe Mortalität der TIPS-Intervention nach Lebertransplantation

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Grundlagen: Die TIPS Anlage ist eine etablierte Therapie für Patienten mit therapierefraktärem Aszites und nicht beherrschbarer Varizenblutungen. Portal-hypertensive Komplikationen nach Lebertransplantation (LT) sind selten und in der Regel Folge rascher hepatischer Fibrose und/oder Niereninsuffizienz. Daten zum TIPS nach LT liegen in der Literatur nicht vor. In dieser retrospektiven Studie wurde die Effektivität und Sicherheit des TIPS nach LT evaluiert.

Methodik: Bei 10 Patienten (6 Frauen; durchschnittliches Alter: 57 Jahre) wurde ein TIPS nach LT implantiert, ein Patient erhielt nach reLT neuerlich einen TIPS. Die Grunderkrankungen waren HCV-Zirrhose ($n = 4$), Fettleberzirrhose ($n = 2$) und PBC ($n = 2$), sowie eine Hämochromatose und autoimmune Hepatitis bei je einem Patienten. Die Indikation zur TIPS Anlage waren refraktärer Aszites ($n = 7$), Hydrothorax ($n = 2$) und eine Kolonvarizenblutung ($n = 1$). Bei vier Patienten bestand

eine neuerliche HCV-Zirrhose, bei drei eine duktopenische Abstoßung, bei je einem Patienten intrahepatische Gallengangstenosen bzw. eine Pfortaderstenose. Die mediane Zeitspanne von LT bis zur TIPS Anlage betrug 15 (4–158) Monate.

Ergebnisse: Bei 3/7 (42%) der Patienten mit therapierefraktärem Aszites konnte ein Therapieerfolg erzielt werden. Sonographisch konnte kein bzw. nur noch minimaler Aszites nachgewiesen werden. Beide Patienten mit Hydrothorax waren trotz TIPS therapierefraktär. Die Kolonvarizenblutung konnte erfolgreich behandelt werden. Der mediane portosystemische Druckgradient konnte von initial 11,5 (0–22) auf 8,3 (5–13) mm Hg gesenkt werden. Bei einem Patienten musste eine Reintervention (PTA) durchgeführt werden. Zwei Patienten wurden bei duktopenischer Abstoßung bzw. eines HCV Rezidivs im Stadium der Zirrhose retransplantiert. Der Patient mit chronischer Abstoßung zeigte 14 Monate nach reLT eine stabile Graftfunktion; der Patient mit HCV Rezidiv erhielt wegen eines raschen HCV Rezidivs im Zirrhosestadium erneut einen TIPS und verstarb 2 Monate nach reTIPS am Multiorganversagen. Bei 70% der Patienten kam es im post TIPS Verlaufe zum Auftreten einer hepatischen Enzephalopathie. In einem Fall war ein Shuntverschluss notwendig. Nur ein Patient der Studiengruppe überlebte. Das mediane Überleben der Patienten nach TIPS betrug 3,3 (0,4 bis 20) Monate. Todesursachen waren Sepsis mit Multiorganveragen bei sieben, sowie eine massive pulmonale bzw. gastrointestinale Blutungen bei je einem Patienten.

Schlussfolgerungen: Obwohl bei 40% der Patienten ein initialer Therapieerfolg erzielt werden konnte, war das Überleben dieses speziellen Patientenkollektivs enttäuschend. Die Patienten verstarben zumeist an Sepsis bzw. Multiorganversagen.

25 Nocardiosis following solid organ transplantation

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Background: Nocardiosis is a rare infection that predominantly affects immunocompromised hosts. The aim of this study was to retrospectively review clinical course and outcome of nocardiosis in solid organ recipients from a single centre.

Methods: Nine cases of nocardiosis were identified in a series of more than 2000 consecutive solid organ transplants performed between January 1995 and July 2007. All cases of nocardiosis diagnosed at UVA from 1997 to 2007 were retrieved from the microbiology database.

Results: In total 65 cases of nocardiosis were identified and there were nine solid organ recipients including six patients who had undergone renal transplantation, one combined pancreas kidney, one liver and one lung transplantation, of which four were retransplants. The encountered species were *N. asteriodes* ($n = 6$), *N. nova* ($n = 2$) and in one case where no differentiation was performed. All patients had pulmonary nocardiosis including pneumonia ($n = 8$), lung abscess ($n = 1$). Seven of the nine patients had rejection prior to Nocardia infection and of those five received OKT3 or ATG. Four patients were smokers. Treatment consisted of trimethoprim-sulfamethoxazol in all patients except for the only patient in this series who died in whom diagnosis was established post mortem. This patient had a liver retransplantation for HCV recurrence and in addition to nocardiosis also suffered from aspergillosis, Clostridium difficile enterocolitis and staphylococcal sepsis.

Conclusions: Nocardiosis seems to be a rare complication following solid organ transplantation, and in most cases has a favourable outcome if timely diagnosed. Intensified immunosuppression and retransplantation were the main risk factors for nocardiosis in our series. Smoking may also predispose to this infection.

26 Peripheral blood stem cell mobilization with pegfilgrastim in two children with Ewing sarcoma

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Background: Several studies have shown that pegfilgrastim, the long acting agent of filgrastim (G-CSF), is as effective as filgrastim in children undergoing cytotoxic chemotherapy by reducing the duration of neutropenia without increased number of adverse events as bone pain and headache. Recent studies in adults have shown that pegfilgrastim used to mobilize CD34+ stem cells is as efficient as filgrastim. Studies showed a reduction of time to reach the peripheral blood CD34+ cell peak, resulting in earlier leukapheresis. Although the conventional use of pegfilgrastim in children is established, no data have been published yet describing the ability of pegfilgrastim to mobilize CD34+ stem cells.

Methods: Two patients aged 8 and 12 years suffering from Ewing sarcoma were treated according the Euro Ewing 99 protocol. Pegfilgrastim was applied on day 4 after the third course of VIDE in a dosage of 200 µg/kg bodyweight.

Results: Patient 1 had peripheral stem cell collection on days 9, 10 and 11, Patient 2 on days 11, 12 and 13. A total of 23.91 (8.83/9.34/5.74) CD34+ cells and of 30.06 (17.60/9.16/3.30) CD34+ cells/kg bodyweight was collected, respectively. Both patients did not show any adverse events. One patient developed prolonged marrow aplasia after the sixth course of VIDE. Despite donation of pegfilgrastim (day 4) and filgrastim (days 11–15 subcutanously, days 16–18 continuous intravenous infusion) she did not recover her WBC. After stem cell rescue with 8.83 CD34+ cells/kg bodyweight on day 19 she had complete hematologic reconstitution 10 days later.

Conclusions: Our preliminary data show that CD34+ mobilisation with a single dose of pegfilgrastim can also be performed in children, avoiding daily unpleasant stitches. Further investigations have to be done.

27 Introduction of laparoscopic living donor nephrectomy increased the number of living donor kidney transplantations

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Background: Laparoscopic living donor nephrectomy has led to the increase of live donor transplantation in many renal

transplant centers in industrialized countries. The number of live kidney donation at the KH Ellisabethinen averaged 2 per year with conventional nephrectomy.

Methods: In March 2006, we started our laparoscopic live kidney donor nephrectomy programme. Until June 2007 seven living laparoscopic donor kidney transplantations were performed and two will be done in mid July. Intraoperative and postoperative data including ischemic time, perioperative complication rate, length of hospital admission and graft function as well as donor and recipient survival were analyzed.

Results: All harvested grafts were left kidneys. The donor age was 52.9 years in mean (range 30–69). Four donors were male, 3 female. Operative time for laparoscopic nephrectomy was 174 min in mean (range 147–210). Time from vascular clamping to perfusion of the graft was 164 sec in mean (range 120–180). Two kidneys showed two renal arteries and an angiomyolipoma was resected from one graft on table. Except one patient with temporarily slightly increased serum creatinine no perioperative complications were noticed. Median length of hospital stay was 7.7 days (range 6–13). All recipients underwent their first kidney transplantation. The mean age was 42.7 years in mean (range 21–58). All grafts showed immediate function up to now in the follow up.

Conclusions: Laparoscopic donor nephrectomy is the preferred route of organ harvesting for live donations and should be state of the art in renal transplant centers offering live donor kidney transplantations.

28 An uncommon cause of paraplegia late after allogeneic stem cell transplantation

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In June 2005 a 37 year old patient was hospitalized due to fever and leucocytosis. Bone marrow examination revealed an acute myeloid leukemia with abnormal eosinophils (cytogenetics: 46 XY, inv 16). Following induction chemotherapy with daunoblastine, high dose cytarabine and etoposide a complete remission was achieved. The patient received three cycles of consolidation chemotherapy. Fourteen month ago the patient was treated with etoposide containing chemotherapy due to testicular cancer. Assuming a t-AML with worse prognosis an HLA identical sibling stem cell transplantation was performed in January 2006.

The patient received standard conditioning therapy with busulfan and cyclophosphamide and engrafted on day eleven. GVHD-prophylaxis consisted of ciclosporin and methotrexate. An acute GVHD grade III appearing on day 74 was treated successfully with methylprednisolone 2 mg/kg body weight. Developing chronic GVHD the patient received continuously oral prednisolone in a dosage from 37.5 to 50 mg/day for the following months. The patient showed moderate clinical signs of cushing syndrome. Additional administration of Sirolimus and Rituximab were less effective against severe sicca symptomatic. Lung involvement of GVHD was confirmed by bronchoscopy on day 261.

An aggravation of dyspnoea led to hospitalisation on day 347. CT scan revealed central necroticizing pulmonary infiltrates. Nocardiosis could be confirmed by bronchoscopic and sputum culture. There was no clinical improvement after six days of anti infectious therapy when the patient developed severe backpain and progressive weakness in both legs with paraparesis at level Th5 within few hours. Beside an osteoporotic vertebral compression fracture CT and MRI scan showed a thecal sac compression by tumorous enlargement of epidural fat from Th3 to Th8. An immediate laminectomy and decompression was performed without any neurological improvement. Histological evaluation revealed normal fatty tissue correlating with the diagnosis of spinal epidural lipomatosis (SEL). The patient died on day 358 after transplant due to additional septicemia and respiration failure.

SEL is defined by thickness of more than 6 mm mainly of the thoracal or lumbal epidural fat tissue. Even if exogenous steroid administration of various duration or endogenous hypercorticism is found in most cases of this rare disease the pathomechanism remains unclear.

29 Changes in antimicrobial application and surveillance of infection in liver transplantation, a 20 year experience from a single centre

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Background: Infection is the most common complication in patients undergoing liver transplantation (LT). There has been an enormous improvement in the outcome of LT due to improved surgical technique, perioperative management and more powerful immunosuppression. Infection monitoring has dramatically changed during the past two decades and many new antimicrobial agents have become available.

Methods: Between January 1987 and December 2004 a total of 703 liver trans-plants were performed at the Innsbruck Medical University, 668 of them were studied in detail. For the purpose of the study, patients were divided into three groups according to the date of transplant; group 1 (202 LTs, 1987–1996), group 2 (239 LTs, 1997–2000) and group 3 (228 LTs, 2001–2004). The outcome of the three cohorts with emphasis infectious complications was analyzed.

Results: The rejection rate and infection rates dramatically declined during the past two decades. Patients are much shorter hospitalized and the one year survival now approaches 90%. The number of obtained surveillance cultures has been drastically reduced; however continuous CMV monitoring was introduced. Gram positive cocci have been increasingly isolated during the study period. A shift from antibacterial to antiviral agents was observed. Less cephalosporins and more quinolons and ureidopenicillins are used, aminoglycosides have virtually disappeared. Details are shown in the following Table.

Period	1987–1996	1997–2000	2001–2004	Total
No. of LT evaluated (performed)	202 (223)	238 (249)	228(231)	668 (703)
No. of patients	189	225	211	625
Male/female	132/70	164/74	164/64	460/208
Median age (range)	51 (0.5–94)	54 (0.4–73)	56 (0.5–76)	54 (0.4–94)
One year graft survival	82.2%	88.2%	90.4%	87.1%
No. of patients died until 2005	43.6%	30.3%	16.2%	29.5%
Rejections	107 (52.9%)	69 (28.9%)	27 (11.8%)	203 (30.4%)
Perioperative infection episodes	280	288	174	742
Episodes/transplant	1.4	1.2	0.8	1.1
Antimicrobial Chemotherapy				
Antibacterials (days/patient)	24.9	13.2	16.1	17.7
Antifungals (days/patient)	4.7	3.3	4.5	4.1
Antivirals (days/patient)	10.8	11.6	13.3	11.9
Total number of microbiological specimens	14712	7164	3715	25591
n/transplant	73	30	16	38
% sterile	59.4	69.8	73.3	64.3
Total number of isolated pathogens	7234	3977	1320	12531
Spectrum of pathogens				
Gram positive cocci	45.9	59.1	59.5	51.5
Gram positive rods	0.3	1.2	0.1	0.6
Gram negative rods	14.9	25.0	28.0	19.5
Non fermentative bacilli	9.6	6.3	4.4	8.0
Anaerobes	0.1	0.1	0.3	0.1
''normal flora''	20.2	0.0	3.0	12.0
Fungi	9.1	8.4	4.7	8.4

Conclusions: The significant survival benefit of LT patients can to be greatly referred to improved diagnostic, therapeutic and prophylactic measures to combat infectious complications.

30 Liposomal Amphotericin B in liver transplant recipients: a single center study

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Background: Infections, especially those caused by fungi, remain common complication and causes of death in liver transplant (LT) recipients. Amphotericin B is still the antifungal agent with the broadest spectrum of activity, although new agents have been recently developed.

Methods: Between January 1987 and December 2004 a total of 703 liver transplants were performed at the Innsbruck Medical University, 668 of them were studied in detail. Thirty-eight patients were treated with liposomal Amphotericin B (Ambisome®) for antifungal prophylaxis or therapy of proven fungal infection. For the purpose of the study, patients were divided in three groups according to the date of transplant; group 1 (202 LTs, 1987–1996), group 2 (239 LTs, 1997–2000) and group 3 (228 LTs, 2001–2004).

Results: During the study period a stepwise change from directed therapy of mycological proven fungal infection to antifungal prophylaxis was observed. Directed therapy was the approach in all nine cases in the first group. All fatal invasive filamentous fungal infections were observed during the first and second time period when only 16% (4 of 25 patients) received prophylactic liposomal Amphotericin B. Prophylaxis was increasingly given in the second (4 of 16 cases) and third group (10 of 13 cases); during the last time period, no patient died from invasive fungal infection.

Conclusions: Therapy of proven filamentous fungal infection with Amphotericin B failed in six patients – five of them high risk patients – died despite therapy. No invasive filamentous fungal infection and only three superficial Candida infections occurred in the prophylactic group. LT recipients who are at high risk for Aspergillus infection should receive prophylaxis with liposomal Amphotericin B.

31 ATG-Induktionstherapie nach Herztranplantation – Auswirkungen auf die Begleitimmunsuppression und daraus resultierende Frühergebnisse

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Background: Induction-therapy after heart transplantation is still a controversial theme – it's used by 50% of all centres worldwide. But there do not exist exact data about the optimal

dosage and the duration of use. The aim of this study is to compare different doses and durations of the use.

Methods: Between 1998 and 2002, 239 patients were heart-transplanted. 195 of them were included in our study, because all peri- and post-operative data (especially ATG-dosages) was available. The median age was 54 years, 18% ($n = 37$) were female. The median follow-up-time was 51 months. We separated the patients into following groups: days of induction (gross and net: 3, 4–6 and more days), ATG total dose (<600 vs. 600–1000 vs. >1000 mg), daily average dose (<145 vs. 145 to 175 vs. >175 mg), dose referring to the body weight (<1.92 vs. 1.92 to 2.36 vs. >2.36). Survival, rejections, infections (total number and severe cases), CMV-disease, graft-vasculopathy (total and severe) and tumours were analysed. Kaplan-Meier-Analyses were used and we compared the groups by using log-rank und Wilcoxon. Results with $p < 0.05$ have a significant impact.

Results: Overall survival was 85% after 5 years and was nearly similar in all groups. We found significant differences at the net-group (group 1 [means 3 or less days]: 100% vs. group 2: 85% vs. group 3: 70%; $p = 0.034$). Incidence of rejections was 15% after 5 years. There was a significant difference as well at the net-group (93 vs. 90% and 77%; $p = 0.025$) as at the total dose group (93 vs. 94 vs. 75%; $p = 0.002$). We saw 24 and 33% severe infections after 1 and 5 years follow-up. Significant differences were found within the net-group (90 vs. 63 und 57%; $p = 0.027$), the gross-group (88 vs. 44%; $p = 0.04$) and the daily average dose group (70 vs. 70 vs. 40%; $p = 0.016$). CMV-diseases had an incidence of 10% after 1 year. The net and the gross-group showed significant differences: net (100 vs. 90 vs. 80%; $p = 0.008$), gross (100 vs. 92 vs. 80%; $p = 0.007$). Both, ACADs and severe ACADs displayed a significant difference comparing the doses referring to the body weight (52 vs. 87 vs. 82%; $p = 0.029$; 55 vs. 92 vs. 87%; $p = 0.0033$).

Conclusions: Recapitulatory we could show that different dosages and durations of the use of ATG have an impact on the outcome of heart-transplanted patients. More analyses will be necessary in order to find out the reasons for all those differences.

32 IgE-mediated allergies in lung-transplanted adults

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Background: IgE-mediated allergy has repeatedly been reported in organ-transplanted children despite strong immunosuppressive therapy. The prevalence of clinically symptomatic allergy after solid organ transplantation in a pediatric population seems to be around 10%. Interestingly, transplantation-associated allergy has not been reported in adults, suggesting a particular propensity in childhood.

Methods: In the present cross-sectional study we assessed the prevalence of type 1 allergy to common nutritive and inhalant allergens in adult lung transplant recipients (age: 25–50 years at enrolment and 19–49 years at transplantation). In addition, we analyzed the association of sensitization and allergy prevalence to patient age, time since transplantation, and immunosuppressive therapy. Instruments included standardized interviews (modified ISAAC criteria), skin prick tests, and specific and total serum IgE measurement.

Results: Ten of 42 patients (23.8%) displayed elevated specific IgE levels and/or positive skin prick test results against one or more allergens. Five individuals (11.9%) additionally reported corresponding clinical symptoms including allergic rhinoconjunctivitis and food allergy. No statistically significant association with gender, age, kind of immunosuppressive therapy, or time since transplantation was found.

Conclusions: The phenomenon of transplantation-associated allergy is not age-restricted and thus should be assessed more thoroughly in all age groups.

33 Serum creatinine at time of switch to sirolimus is predictable for successful rescue therapy in liver transplant patients with deteriorating kidney function

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Background: Immunosuppression with calcineurin inhibitors (CNI) following liver transplantation is associated with nephrotoxicity. The aim of this study was to analyze whether late conversion to a Sirolimus (SRL) based and CNI free immunosuppressive protocol would have a beneficial effect on deteriorating renal function in patients after orthotopic liver transplantation.

Methods: Seventy-eight patients after orthotopic liver transplantation (OLT) between 2001 and 2005 were switched to SRL from their former CNI based immunosuppression. Indication for switch was Serum creatinine (SCr) elevation above 1.2 mg/dl. SCr levels were measured at 3, 6, 12, 24 and 36 months after liver transplantation.

Results: Median time between date of liver transplantation and switch was 37.01 (12.91,85.62: q1,q3)months. Median follow up was 12 (3.92,24.45: q1,q3)months. Primary analysis showed mean SCr at time of switch of 2.20 ($r = 1.20$–4.79) mg/dl, 1.93 mg/dl at 3 months ($p = 0.01$), 1.79 mg/dl at 6 months ($p = 0.009$), 1.79 mg/dl at 12 months ($p = 0.02$), 2.28 mg/dl at 24 months ($p = 0.4$) and 3.00 mg/dl at 36 months ($p = 0.24$) respectively.

In a subgroup analyses we compared two groups:

Group A ($n = 35$): Patients with a mean SCr at time of switch of 1.77 ($r = 1.5$–2.0) mg/dl revealed significant improvement in renal function with a mean SCr of 1.45 mg/dl at 3 months ($p < 0.000$), 1.42 mg/dl at 6 months ($p < 0.000$), 1.42 mg/dl at 12 months ($p < 0.000$), 1.37 mg/dl at 24 months ($p = 0.007$), and 1.36 mg/dl at ($p = 0.09$), respectively.

Group B ($n = 26$): Patients with a mean SCr at time of switch of 3.00 ($r = 2.01$–4.79) mg/dl. Further mean SCr was 2.67 mg/dl at 3 months ($p = 0.167$), 2.46 mg/dl at 6 months ($p = 0.09$), 2.57 mg/dl at 12 months ($p = 0.516$), 3.47 mg/dl at 24 months ($p = 0.10$), and 4.09 mg/dl at 36 months ($p = 0.139$), respectively.

Conclusions: Primary analysis showed short term beneficial effect of conversion to SRL that could not be seen in long term follow up. Subgroup anaylisis showed that patients with a SCr beween 1.5 and 2.0 mg/dl had significant benefit from conversion to SRL. SCr at time of switch is predictive for the benefit of a late SRL conversion as rescue therapy in OLT patients with deteriorating kidney function.

34 Outcome of hepaticojejunostomy for biliary tract obstruction following liver transplantation

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Background: Nowadays, endoscopic retrograde cholangiography (ERC) is seen as golden standard for the majority of biliary tract interventions following liver transplantation. Especially in hilar biliary strictures, endoscopic treatment might not lead to a definitive cure in all patients. The aim of this study was to determine the efficacy and the long-term outcome of hepatico-jejunostomy (HJS) for post-transplant biliary tract obstruction.

Methods: Thirty-nine patients were retrospectively studied who underwent conversion to bilioenteric diversion for biliary strictures and concrements in a series of 807 liver transplantations between 1993 and 2006. Resolving of cholestasis and the incidence of recurring biliary obstructions were analyzed.

Results: Surgery was performed due to anastomotic strictures in 8, non-anastomotic strictures in 22 and biliary tract concrements in 9 patients. Cholestasis instantly resolved in 30 of the patients (77%). After a long-term follow-up of median 38 months, 30 of the patients (77%) required no further intervention for biliary obstruction following HJS. Nine patients (23%) presented with recurrent strictures ($n = 7$) or biliary concrements ($n = 2$).

Conclusions: HJS did prevent reintervention for recurrent biliary complications in the longer follow-up in 77% of the patients. We therefore recommend early HJS for recurrent post-transplant biliary tract obstruction not treatable by a limited number of ERC. Definitive assessment of the efficacy of early HJS requires further prospective randomized clinical trials comparing HJS and ERC.

35 Degenerative cardiac pigment lipofuscin vontains cytokeratin-18 and caspase-cleaved cytokeratin-18

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Background: Previous studies of our group have demonstrated elevated serum levels of caspase-cleaved cytokeratin-18 (ccCK-18) in patients suffering from acute coronary syndrome (ACS) (Eur J Clin Invest 2007 May; 37(5): 372–380). The intermediate filament cytokeratin-18 (CK-18) is a cytoskeletal component mainly expressed in epithelial cells. CK-18 was also identified in coronary vascular smooth muscle cells and endothelial cells within cardiac microvasculature but not in cardiac myocytes. Apoptosis describes the programmed active dying mechanism of a cell accompanied by various morphological alterations including membrane blebbing and cell shrinkage. Caspase-mediated cleavage of CK-18 occurs during apoptosis and leads to formation of a specific neo-epitope of the caspase-cleaved cytokeratin-18, recognized by the antibody M30. We sought to investigate if cytokeratins, previously primarily described in epithelial cells, and caspase-cleaved cytokeratin-18 are present in tissue samples obtained from patients with various forms of cardiomyopathy (CMP).

Methods: Paraffin-embedded myocardial samples of patients with hypertrophic ($n = 15$), dilatative ($n = 15$) and ischemic CMP ($n = 15$) were included in this study. Tissues were taken from the left ventricle of cardiac explants and fixed immediately. Control samples ($n = 3$) were acquired from solid organ donors without pathology. For immunoblotting, myocardial and liver specimens obtained from autopsies of patients with ischemic CMP were analyzed ($n = 3$). Immunohistochemistry was performed using monoclonal antibodies detecting CK-18, CK-8 and ccCK-18. For immunoblotting analysis, a horseradish-peroxidase conjugated anti-mouse Fc secondary antibody was used. Tissue of cirrhotic liver served as positive control because it contains abundant CK-18 and ccCK-18.

Results: Immunohistochemical analysis revealed weak presence of CK-18 and strong presence of ccCK-18 in ischemic, dilatative and hypertrophic CMP. The signaling co-localized with findings of lipofuscin in cardiac tissue. CK-8 was not detected in any form of CMP. Immunoblotting analysis of myocardial and liver tissue lysates confirmed the histological data and revealed positive bands for CK-18 and ccCK-18.

Conclusions: This report represents the first description of the co-localization of CK-18, ccCK-18, and lipofuscin in ischemic and other forms of CMP. We are currently investigating if detection of serum ccCK-18 concentrations can differentiate between the various forms of CMP.

36 Anti-thymocyte globulin impairs T-cell/antigen-presenting-cell interaction: disruption of immunological synapse and conjugate formation

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Background: Anti-thymocyte globulin (ATG), the immunoglobulin G (IgG) fraction of sera from rabbits or horses immunized with human thymocytes or T-cell lines, are currently employed for the treatment and prevention of acute steroid resistent graft rejection organ rejection. The Key mechanisms of action are T-cell depletion and functional alterations of the remaining cells. However, the mechanism(s) underlying its immunomodulatory capacities are still ill-defined. T-cell activation requires complex molecular rearrangements at the interface with professional antigen-presenting cells (APC), the so-called immunological synapse (IS), which is a requirement for stable T-cell/APC interactions and full stimulation. Here we investi-

gated, whether ATG affects formation of the IS and T-cell/APC conjugate formation.

Methods: Two preparations of ATG were used in this study. ATG Fresenius (batch SU 01 A-1, Fresenius Biotech, Munich, Germany) and Thymoglobulin (CH-B.: TH118-H05, IMTIX, SangStat, Lyon, France), which have been produced by immunization of rabbits with a Jurkat T-cell line and human thymocytes and are referred to as ATG-1 and ATG-2, respectively. Peripheral T-cells or Jurkat T-cells were incubated with different ATG concentrations. FACS analysis was performed to assess T-cell surface molecule expression essential for IS development. IS and conjugate formation were directly addressed in two experimental models to analyze molecule relocalization into the IS and the efficiency of conjugate formation.

Results: Treatment of peripheral T-cells and Jurkat T-cells with different concentrations of ATG-1 and ATG-2 led to a time- and concentration-dependent downregulation of the adhesion molecules CD11a, CD50 and CD49d. Moreover, we found that the expression of the co-stimulatory molecule CD28 was significantly reduced by ATG treatment. ATG-treated (3 hours) T-cells exhibited significantly reduced relocalization of CD3 and CD11a into the IS even at low ATG concentrations. While low ATG concentrations did not effect conjugate formation, ATG-treated T-cells were impaired in their capability to form stable conjugates with DC at higher ATG concentrations in accordance with inhibited IS formation.

Conclusions: These data demonstrate that ATG profoundly blocks the earliest stages of the T-cell/APC interaction indicating that apart from its lymphocyte depleting capacity a discrete IS disturbing potential may become operative. Furthermore, this peculiar property may help to understand the functional inactivation of peripheral T-cells that have escaped cellular depletion after ATG treatment. Collectively, blocking IS formation at distinct stages may mediate effects on T-cell activation of currently used immunosuppressant, apart from their capacity to block gene transcription, cytokine signalling, and DNA replication.

37 ABO-incompatible living donor kidney transplantation using immunoadsorption – first case performed in Austria

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There is increasing evidence that living donor kidney transplantation across the ABO barrier can be successfully performed using blood group specific immunoadsorption (IA) combined with immunomodulation using the CD20 antibody Rituximab and intravenous immunoglobulin (IVIG). Here, we report on the first successful case of ABO-incompatible kidney transplantation at the Medical University Vienna, applying a modification of the protocol earlier published by Tyden et al. (Transplantation. 2007, 83; 1153). A 66-year-old male recipient (blood group 0 Rhesus positive) received a kidney from his 64 year-old wife (blood group A1, Rhesus negative) following a course of pre-emptive IA. Initial IgG anti-A titers were 1:512. Conventional CDC-PRA and CDC-crossmatch revealed no detectable HLA sensitization. The patient received a single infusion of Rituximab (375 mg/m^2) 3 weeks before transplantation. Basal immunosuppression with tacrolimus (target level: 12–15 ng/ml), MMF (2×1 g), and steroids (25 mg per day) was initiated 2 weeks before transplantation. The first IA (GlycoSorb ABO; Glycorex Transplantation AB, Lund, Sweden) was performed on day -8, followed by two subsequent sessions (8 l plasma volume treated per session). Titration at day -5 revealed a substantial reduction of IgG anti-A titers to 1:16. Due to a considerable rebound between daily sessions (retrospective analysis), we decided to continue treatment on a daily base including IA treatment also immediately before transplantation. Using a modification of the original protocol, a dose of 0.5 g/kg body weight IVIG was administered during the 4th IA session in order to deplete the potential content of anti-A antibodies. Combined immunosuppressive treatment led to a stable reduction of titers to 1:8 the day before transplantation. The post-transplant course was uncomplicated and serum creatinine levels dropped promptly (serum creatinine at day 7: 1.2 mg/dl; creatinine clearance 60 ml/min). Interestingly, following transplantation, anti-A titers constantly remained below 1:4, so that, in contrast to previously reported cases, we decided not to perform post-transplant IA. A protocol biopsy performed 2 weeks after transplantation revealed no features of humoral rejection (C4d negative). Even though long-term results are not yet available, the early follow-up of our case suggests high efficiency of the GlycoSorb protocol, and reinforce that ABO-incompatible transplantation represents a safe strategy to expand the donor pool.

38 Combination of Everolimus with low-dose calcineurin-inhibitors in lung transplant recipients with chronic renal insufficiency

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Background: Chronic renal insufficiency induced by calcineurin-inhibitors (CNIs) is a common complication in lung transplant patients. Everolimus, a proliferation signal inhibitor with less renal toxicity, was recently introduced in lung transplantation. To date little is known about the benefit of Everolimus combined with low dose CNI in this patient population.

Methods: Eleven lung transplant recipients (6 male vs.5 female, underlying diseases: 9 with COPD, 1 with apha1-antitrypsin deficiency and 1 with primary pulmonary hypertension) with a mean age of 56 ± 8.9 years were enrolled in this study. All Patients received standard dose of CNIs and were switched to a combined immunosuppressive regimen with Everolimus and reduced doses of CNIs. Target trough levels of Everolimus were 5–7 ng/ml. Tacrolimus was reduced to target trough levels of 4–8 ng/ml and Cyclosporine to 80–120 ng/ml. Kidney function parameters (creatinine, BUN), number of thrombocytes, cholesterol and triglyceride values and lung function parameters

(VC, FEV1, MEF 50, TLC) on the day of switch and 1, 3, 6 and 12 months after switch were documented.

Results: Two patients died due to chronic kidney failure during the observation period, 1 patient was already on dialysis before transplantation. All patients showed improvement or stabilization of kidney function after switch to a combined Everolimus and reduced CNIs regimen. Kidney parameters compared to baseline function (time of switch):

Creatinine (baseline: 3.1 ± 0.7 mg/dl) decreased to 2.5 ± 0.7 mg/dl ($p = 0.041$) after 1, to 2.9 ± 1.1 mg/dl ($p =$ ns) after 3, to 2.6 ± 0.6 mg/dl ($p =$ ns) after 6 and to 2.2 ± 0.5 mg/dl ($p =$ ns) after 12 months (baseline: 2.8 ± 0.5, including only 6 patients) following switch. BUN (baseline: 54.3 ± 16.7 mg/dl) decreased to 42.9 ± 14.1 mg/dl ($p =$ ns) after 1, to 45.8 ± 18.2 mg/dl ($p =$ ns) after 3, to 41.6 ± 11.1 mg/dl ($p = 0.05$) after 6 and to 35 ± 10.3 mg/dl ($p =$ ns) after 12 months (baseline: 49.6 ± 15.3, including only 6 patients) following switch. The results were only partly significant perhaps due to the small number of patients. The number of thrombocytes, cholesterol and triglyceride values did not change significantly after 1, 3, 6 and 12 months. All patients had stabile lung function parameters during the observed periods.

Conclusions: This investigation suggests that the combination of Everolimus with reduced doses of CNIs could be a valuable alternative immunosuppressive regimen to reduce nephrotoxic side effects in patients with chronic kidney failure.

39 Long term outcome after pulmonary retransplantation

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Background: Pulmonary retransplantation remains the only therapeutic option in some cases of severe primary graft dysfunction (PGD), advanced bronchiolitis obliterans sydrom (BOS) as well as in some cases of severe airway problems (AWP), mainly cicatriceal stenosis. However its value has been questioned due to overall scarcity of donor organs and reports on unsatisfying outcome. We analysed our institutional experience with pulmonary retransplantation to evaluate its value for different indications.

Methods: We retrospectively analysed all 46 patients undergoing pulmonary retransplantation out of 567 consecutive primary lung or heart-lung transplantations performed in our department from 8/1995 to 8/2006. We stratified patients according to indication for retransplantation and analysed the outcome.

Results: Forty-six patients with a mean age of $41 + 16$ years (18 male, 28 female) underwent pulmonary retransplantation (14 BLTX, 32 SLTX) for primary graft dysfunction ($n = 23$), bronchiolitis obliterans syndrome ($n = 19$) and airway problems ($n = 4$). Mean time to retransplantation was $26 + 27$ days in the PGD group, $1069 + 757$ days in the BOS group and $220 + 321$ days in the AWP group. 30 days, 1 year and 5 years survival after retransplantation were 52.2, 34.8 and 29.0% in the PGD group and 89.2, 72.5 and 61.3% in the BOS group. All 4 patients in the AWP group are still alive (p BOS vs. PGD = 0.02; p BOS vs. AW$p = 0.27$; p PGD vs. AW$p = 0.06$).

Conclusions: Pulmonary retransplantation for bronchiolitis obliterans offers long term survival rates in the range of primary lung transplantation for selected patients. Long term survival rates for retransplantation due to primary graft dysfunction are significantly lower, warranting restrictive use in this indication. In our experience with a limited number of patients, retransplantation for airway problems has excellent results. Pulmonary retransplantation for chronic problems is a worthwhile effort, provided that patients are carefully selected. Retransplantation for PGD should be avoided.

40 CMV hyperimmunoglobulin: mechanisms in allo-immune response in vitro

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Background: Cytomegalovirus hyperimmunoglobulin (CMVIg) containing drugs are routinely administered in cardiac transplantation for prophylaxis against CMV disease. Yet little is known about their influence on transplant relevant immune functions. The aim of this study was to evaluate the effect of CMVIg on cellular immunity in in vitro experiments and to define their role in tolerance inducing mechanisms.

Methods and results: CMVIg reduces proliferation in mixed lymphocyte reactions and anti-CD3 blastogenesis assays and is related to decreased production of immune modulating cytokines IL-2, INFγ and IL-10. This anti-proliferative effect is associated with a cell-cycle arrest in the G0/G1 phase and induction of apoptosis in CD8+ and natural killer cells. Co-incubation with CMVIg causes downregulation of cell bound immunoglobulin and Fcγ RIII surface expression on natural killer cells and leads to attenuation of antibody dependent cellular cytotoxicity effector functions.

Conclusions: We conclude that CMVIg induces immunological features on leukocytes in vitro that are known to be related to tolerance induction. Our observations extend the current concept of CMVIg as passive CMV prophylaxis to a therapeutic drug compound capable to reduce allogeneic immune response.

41 The cutting (w)edge – comparative evaluation of renal baseline biopsies obtained by two different methods

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Chronic donor-related damage has been demonstrated to prognosticate the graft survival of transplant kidneys. Baseline wedge biopsies taken at the time of transplantation are a reliable tool to assess the overall degree of chronic lesions, such as proportion of glomerular scars, interstitial fibrosis/tubular atrophy or atherosclerosis. In the present study, we report about a new, standardized method for obtaining baseline kidney biopsy

specimens at the time of transplantation. Instead of taking wedge biopsies, a skin punch biopsy tool was utilized for this purpose, to obtain a biopsy sample also representative of the deeper cortical zones. Besides providing a more adequate sampling of small arterial vessels, this method allows a prompt sutural closure of the biopsy defect, preventing postoperative hemorrhagic complications.

Here we compared 80 biopsy specimens taken by either method (35 wedge biopsy specimens vs. 45 punch biopsies) with respect to the number of glomerula and arterial vessels they contained, as well as their predictive value regarding chronic donor-related damage of transplanted kidneys.

Although wedge biopsy samples contained a significantly higher number of glomerula (57.2 compared to 37.4 in punch biopsies), there was no difference between the number of small arteries (3.2 compared to 2.8, respectively). Moreover, ''punch'' biopsy samples, when compared to traditional wedge biopsies, displayed a significantly lower standard deviation regarding the number of glomerula contained, indicating lesser inter-individual variation of biopsy sample size. In effectively detecting chronic donor-related damage, both methods were proven reliable as shown by analysis of subsequent core biopsy samples of the same patients.

Therefore, we conclude that the use of skin punch biopsy tools for obtaining baseline biopsies from transplanted kidneys is a safe and effective method for assessment of donor-related damage of the organ.

42 Vascular complications following 217 consecutive pancreas transplantations

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Background: Following pancreas transplantation (PTx) a wide spectrum of vascular complication must be expected including vascular thrombosis of the graft artery or vein, thrombosis of the preexisting AV-fistula, and ongoing deterioration of peripheral arterial disease (PAD).

Methods: Two hundred and seventeen enteric drained whole PTx performed from 1997 to 2004 using ATG induction, Tacrolimus, MMF and steroids prophylactic immunosuppression were analyzed. All grafts were revascularized in an end-to-side fashion with the inferior vena cava and via a donor iliac Y-graft with the right common iliac artery. Patients received i.v. heparin intraoperatively, followed by low lose heparin subcutaneous for one week. Platelet aggregation inhibitors were continued if premedicated.

Results: Actuarial patient, pancreas and kidney graft survival at one year were 96.4, 88.5 and 94.8%, rejection rate was 30%. A total of 35 patients with vascular complications were identified (16.1%). Vascular thrombosis accounted for eight pancreas graft losses and anastomotic rupture associated with intraabdominal infection caused two graft losses. One renal graft was lost due to embolic infarction caused by endocarditis. There were 15 cases of thrombosis of the hemodialysis access; 53% of AV-fistula occlusions occurred within 24 hours post transplant, and 73% within the first week. In 14 cases the av-fistula was recanalized by thrombectomy. Four patients with severe PAD underwent arterial reconstruction. Two patients underwent major and three minor amputations median five years post PTx.

Conclusions: Vascular graft thrombosis remains a common cause of pancreatic graft loss. For early onset av-fistula occlusions, thrombectomy is successful but a controversial undertaking. Progression of PAD may be prevented in some cases after PTx.

43 Superiority of ATG induction therapy in lung transplant recipients with cystic fibrosis

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Background: The use of induction therapy after lung transplantation is discussed controversly. We hypothesised that induction with rabbit-antithymocyte-globulin used in patients with cystic fibrosis would reduce acute rejection episodes in the early postoperative course an decrease the use of steroids and the rate of severe infections. We retrospectively analysed the files of all CF patients transplanted between 1996 and 2005.

Methods: In a 10 year period between 1996 and 2005 seventy patients with CF underwent transplantation. Demographics: m/f = 34/36. 66 (94%) pat received a double lung transplantation, 2 (3%) a heart-lung transplantation and 2 (3%) a single lung TX. Mean age at time of TX was 25.3 ± 8.4 years (range 9–50 years). Five patients (7%) were ventilator dependent, 3 of them on ECMO support at time of transplantation. In 39 patients (55%) (group A) induction therapy with rabbit-antithymocyte-globulin (ATG) was administered, 6 pats (8%) received Daclizumab (group B) and in 25 (37%) cases no induction agent was (group B) used. The mean time of follow up was 37.5 ± 30.5 months.

Results: Patients who received ATG (group A) as induction had 1, 3 and 5 year survival of 97, 97 and 80%, patients who received Daclizumab or no induction agent (group B) had a 1, 3 and 5 year survival of 61, 61 and 55% (log rank group A vs. group B $p = 0.0014$). The use of induction therapy with ATG compared to no induction therapy or Daclizumab resulted in fewer episodes of acute rejection > grade A1 within the first 100 days post-transplant ($p = 0.013$). In group A one patient died due to infectious complications on day 147 after TX, in group B 12 patients died during the first 6 months postoperatively (reason for death: septicaemia $n = 8$, POF $n = 2$, others $n = 2$). Freedom from BO(S) was 98, 92 and 84% after 1, 3 and 5 years, respectively. There was no statistical difference in the incidence of BO(S) between groups A and B.

Conclusions: The use of ATG as an induction agent demonstrated excellent results in regard to freedom from acute rejection and long-term survival (1 year survival 97 vs. 61%, $p = 0.0014$) compared to no induction or induction therapy with Daclizumab. A significant lower rate of deaths due to infectious complications in the ATG group accounts for this improvement of survival.

44 Cytomegalovirus prevention in high risk lung transplant recipients

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Background: CMV infections are common after lung transplantation and have an influence on acute rejection rates and chronic organ dysfunction. We performed a prospective trial of valganciclovir prophylaxis in high risk lung transplant recipients (CMV D) using a 3 months ($n = 16$) and 12 months ($n = 12$) therapy in matched groups.

Methods: Only patients who survived more than 90 days were included. The study population received either 3 ($n = 16$) months (group A) or 12 ($n = 12$) months (group B) oral valganciclovir 900 mg per day in combination with CMV hyper immune globulin (Biotest – Cytotect) for 4 times (day 1, 7, 14 and 21 post TX). CMV load test (PCR) was done at every scheduled visit. Primary endpoint was the incidence of CMV viremia/disease 6 months after cessation of CMV prophylaxis. Secondary endpoints were incidence of acute rejections, kidney function, leucopenia and survival 1 year after TX.

Results: The incidence of CMV viremia was 11/16 in group A (68%) and 2/16 in group B (16%) ($p = 0.001$) 6 months after valganciclovir cessation. The incidence of symptomatic CMV disease was 7/16 (44%) in group A and 1/12 in group B (8%) ($n = 0.03$). In both groups viremia, while on prophylaxis, was uncommon (1 in group A and 1 in group B). Histological proven acute rejections episodes $\geq$A2 ISHLT were found in 4 patents of group A and in no patent of group B during the first year. All 4 patients in group A had more than one rejection episode (two had 2 AR, one had 3 and one 4 episodes of AR). Two patients in group A received ATG therapy for recurrent rejections. One survival rate was 93% in group A and 92% in group B ($p =$ NS). One year after TX kidney function was equal in both groups (mean Kreatinin value group A vs. B 1.4 mg/mL vs. 1.42 mg/mL, $p =$ NS). Episodes of leucopenia were found in 6 patients in each group (group A vs. group B = 37% vs. 50%.

Conclusions: A 12 months CMV prophylaxis with oral valganciclovir is effective in significantly reducing CMV viremia and CMV disease in high risk lung transplant recipients. In addition we observed a reduction of acute and recurrent rejection episodes potentially due to less CMV viremia and subsequent immunomodulatory effects. Despite prolonged treatment with valganciclovir there was no increase of side effects like kidney dysfunction or leucopenia. No case of ganciclovir resistance was observed in both study arms.

45 Assoziation von MMF vs. Azathioprin mit Transplantatverlust bei Biopsie-gesicherter chronischer Transplantatabstoßung

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Grundlagen: Die chronische Transplantatabstoßung stellt die häufigste Ursache für einen späten Transplantatverlust nach Nierentransplantation dar. Mycophenolat Mofetil (MMF), ein Proliferationshemmer, hat seit seiner Einführung in den frühen 90ern ältere Metaboliten, wie z. B. Azathioprin, aufgrund einer niedrigeren akuten Transplantatabstoßungsrate verdrängt. Ob MMF das Fortschreiten einer Transplantatverschlechterung bzw. einen Transplantatverlust bei bestehender chronischer Transplantatabstoßung verhindert ist allerdings unklar.

Methodik: Wir führten eine retrospektive, offene Kohortenstudie an 297 Patienten aus dem Österreichischen Dialyse und Transplant Register mit Biopsie-gesicherter chronischer Transplantatabstoßung durch. 94 der 297 Patienten mit chronischer Transplantatabstoßung erlitten seit 1990 einen Transplantatverlust. Analysiert wurden klinische Parameter und deren Assoziation mit einem späten Transplantatverlust bei Biopsie-gesicherter chronischer Transplantatabstoßung, wobei verschiedene Cox Proportion Hazard Modelle, wie etwa Propensity Score und Marginal Structure Modelle, berechnet wurden um für mögliche Confounder wie etwa die Indikation der immunsuppressiven Therapie zu berücksichtigen. Zusätzlich wurden sämtliche Co-Variablen inklusive Medikation und Laborparameter als zeitabhängige Variablen eingeschlossen.

Ergebnisse:

Parameter	p value	HR	95% CI	
AZA vs. MMF	0,837	0,95	0,58	1,56
Jahr der Transplantation	0,001	1,15	1,06	1,26
Verwendung von ACEI/ARB (Ja vs. Nein)	0,724	0,90	0,50	1,62
Anzahl der Antihypertensiva	0,056	1,16	0,99	1,35
Spenderalter pro Dekade	0,388	1,06	0,93	1,22
Typ II Diabetes (Ja vs. Nein)	0,191	1,51	0,81	2,80
BCAR	0,242	1,31	0,83	2,08
Proteinurie zwischen 500 und 3500 vs. <500 mg/d	0,015	1,86	1,13	3,06
Proteinurie >3500 vs. >500 mg/d	0,016	2,10	1,15	3,84

Schlussfolgerungen: In keiner unserer Analysen war MMF im Vergleich zu AZA mit einem niedrigeren Transplantatverlust assoziiert.

46 Neurological complications after lung transplantation

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Background: Following lung transplantation (LuTx), several neurological complications can occur in the post-transplant period. In this study we analyzed the frequency and the factors related to the development of neurological complications after LuTx.

Methods: We retrospectively reviewed 87 consecutive patients undergoing primary lung transplantation from 01/2006 till 12/2006. Underlying diseases were cystic fibrosis in 23% ($n = 20$), idiopathic fibrosis in 23% ($n = 20$), COPD in 35% ($n = 31$) and other indications in 19%. Mean age was 44.7 ± 16 years. Analysis included the incidence of seizures, polyneuropathy (PNP) and ischemic or hemorrhagic strokes and

statistical evaluation was performed by Mann–Whitney-White and Kaplan–Meier-Survival-Curve method. The impact of possibly related factors to the development of neurological complications was explored.

Results: Sixteen events occurred in 13 patients (14.9%): 8 PNP (50%), 4 seizures (25%) and 4 strokes (25%). Seven women and 6 men with a mean age of 41.5 ± 15 years presented neurological complications. The mean time to development of a neurological complication was 36.9 ± 77.4 days (range: 6–93). Young patients developed seizures (seizure vs. non seizure: 25.2 ± 8.2 years vs. 45.7 ± 15.7 years, $p = 0.022$). Seizures were significantly related to the underlying disease (CF vs. non CF: 15 vs. 1.5%, $p = 0.001$). PNP was associated with time on ICU (PNP vs. non PNP: 29.7 ± 28.8 days vs. 11.0 ± 12.0 days, $p = 0.018$). 50% ($n = 4$) of patients, who were intubated prior to LuTx suffered from PNP ($p = 0.031$). Patients transplanted on assist device showed in 13.3% ($n = 4$) ischemic or hemorrhagic strokes, 3 in the early (day 6, 9, 11) and 1 in the late postoperative period (day 290). Strokes did not occur in patients, who were transplanted without assist device ($p = 0.008$). No difference was found in survival of patients with neurological complications.

Conclusions: The incidence of neurological complications after LuTx was significant. Young patients with CF have a high incidence of seizures, whereas transplantation on assist device is associated with strokes in the postoperative period. PNP was significantly related to time on ICU and to intubation before LuTx. Survival analysis did not show any differences between patients with or without neurological complications.

47 Kinderherztransplantation – Erfahrungen aus Innsbruck

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Grundlagen: Die Herztransplantation ist eine akzeptierte chirurgische Methode in der Behandlung der terminalen Herzinsuffizienz im Erwachsenenalter. Aber auch im Kindesalter können eine Reihe angeborener wie auch erworbener Erkrankungen zur Entwicklung einer terminalen Herzinsuffizienz führen. Das Ziel dieser Untersuchung bestand darin, die Ergebnisse der Kinderherztransplantationen an unserer Klinik zu evaluieren.

Methodik: Wir untersuchten alle Kinderherztransplantationen in Innsbruck von 1996 bis Juni 2007. Demographische Faktoren, kardiale Grundkrankheiten, Wartezeit, Bridgingsysteme wie auch der postoperative Outcome werden beschrieben.

Ergebnisse: Im Untersuchungszeitraum wurden 15 Herztransplantationen bei 14 Kindern durchgeführt. Eight Kinder waren männlichen, 6 Kinder weiblichen Geschlechts. Das mittlere Alter betrug 10,3 Jahre bei einem Altersspektrum von 0,8 bis 17 Jahren. Die zur Transplantation führende Grunderkrankung bestand bei 9 Kindern in einer dilatativen Kardiomyopathie, bei 2 Kindern in einer restriktiven Kardiomyopathie. Eine Transplantation wurde bei Ebsteinanomalie und eine bei Zustand nach Virusmyokarditis durchgeführt. Ein Mädchen, welches 1996 erstmalig herztransplantiert wurde, wurde 2007 aufgrund einer Transplantvaskulopathie retransplantiert. Weiterhin wurde ein Junge, welcher 2002 in einem anderen Zentrum transplantiert wurde, 2005 an unserer Abteilung retransplantiert. Die mittlere Wartezeit zur Transplantation betrug 177 Tage mit einem Spektrum von 4 bis 652 Tagen. Ein Mädchen ist am Tag nach der Transplantation an der Folge eines sudden cardiac death bei perakuter Abstoßung verstorben. Im Langzeitverlauf ist kein Kind verstorben. Acht Kinder wurden mit einem mechanischen Unterstützungssystem zur Transplantation gebridgt. Hierbei handelte es sich in einem Fall um eine ECMO, zwei LVADs und fünf BVADs.

Schlussfolgerungen: Die Herztransplantation ist eine gute therapeutische Option für Kinder mit terminaler Herzinsuffizienz, welche mit akzeptablen perioperativem Risiko zu guten Langzeitergebnissen führen kann. Die Wartezeiten zur Transplantation sind bei Kindern länger als bei Erwachsenen, wodurch sich der hohe Anteil an mechanischen Unterstützungssystemen in der kinderherzchirurgischen Population erklären lässt.

48 Serum matrix metalloproteinase levels to predict complications after peripheral blood stem cell transplantation

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Background: Matrix metalloproteinases (MMP) are a family of zinc-containing enzymes involved in many processes such as tissue remodeling, tumor invasion and immune regulation due to activation of cytokines. Therefore, MMP and their tissue inhibitors (TIMP) may play a role in stem cell transplantation. Indeed, MMP-2 and TIMP-1 are essential for the invasive capacity of human mesenchymal stem cells. MMP-1 and -19 and TIMP-1 and -3 are expressed in intestinal graft-versus-host disease (GvHD) lesions, and the synthetic MMP inhibitor KB-R7785 prevents acute GvHD in mice undergoing bone marrow transplantation. Data on MMP and TIMP serum levels after allogeneic and especially after peripheral blood stem cell transplantation (PBSCT), however, are lacking.

Methods: Aim of this study was the evaluation of a possible correlation between in vivo MMP and TIMP levels and the occurrence of complications after PBSCT. Therefore, MMP-2, MMP-9 and TIMP-1 levels were determined by commercially available enzyme-linked immunosorbant assays in sera of 16 patients at various time points before and after HLA-identical PBSCT with reduced-intensity conditioning.

Results: The median MMP-2 production in the sera of the 16 patients was $231.8 \pm 20.4\,\mathrm{ng/ml}$ and did not significantly differ from the MMP-2 levels in the sera of two normal controls ($185.2 \pm 18.9\,\mathrm{ng/ml}$). No relevant changes were observed between day $-4/-5$ ($200.0 \pm 56.8\,\mathrm{ng/ml}$) and day $+90$ ($236.9 \pm 121.5\,\mathrm{ng/ml}$) MMP-2 levels. The median TIMP-1 production in the sera of the 16 patients was slightly, but not significantly higher ($278.0 \pm 16.1\,\mathrm{ng/ml}$) than the TIMP-1 levels in the sera of two normal controls ($198.9 \pm 2.0\,\mathrm{ng/ml}$) and did not differ from pretransplant ($209.7 \pm 122.1\,\mathrm{ng/ml}$) and posttransplant ($265.5 \pm 109.6\,\mathrm{ng/ml}$) levels. In contrast, MMP-9 serum levels were not as uniform as MMP-2 and TIMP-1. Median MMP-9 levels of the 16 patients were dramatically lower ($245.2 \pm 391.4\,\mathrm{ng/ml}$) than of the normal controls

(1476.9 ± 60.7 ng/ml) and changed during the transplant procedure. The median MMP-9 levels at day $-4/-5$ (121.0 ± 152.5 ng/ml) dropped to 1.4 ± 15.1 ng/ml on day $+7/+8$ and rose from day $+10/+12$ (14.4 ± 162.7 ng/ml) to 284.0 ± 246.2 ng/ml on day $+90$. The highest MMP-9 levels were detected between day $+18$ and day $+50$ where most of the complications developed. From six patients extremely high MMP-9 levels were measured. These levels were released just before or concomitant with acute GvHD and/or cytomegalovirus infection.

Conclusions: In conclusion, measurement of MMP-9 levels in sera of HLA-identical PSCT patients maybe a simple and useful tool to identify patients developing acute GvHD and/or cytomegalovirus infection already before onset of these complications.

49 An irradiation-free murine protocol for the induction of mixed chimerism and tolerance

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Background: Bone marrow transplantation (BMT) together with costimulation blockade has been reported to induce mixed chimerism and tolerance without irradiation if (clinically unworkable) mega doses of donor bone marrow (BM) are transplanted. Both rapamycin and NK cell depletion have previously been shown to each enhance BM engraftment on their own. We thus investigated whether the combination of NK cell depletion plus rapamycin allows BMT without irradiation with a clinically feasible BM dose.

Methods: All groups of C57BL/6 mice received approx. 20×10^6 fully mismatched Balb/c bone marrow cells (d0), costimulation blockade consisting of anti-CD154 (CD40L) mAb (MR1; 1 mg; d0) and CTLA4Ig (0.5 mg; d + 2). *Group #1:* 1 Gy TBI (total body irradiation, d-1) without additional treatment; *Group #2:* 1 Gy TBI plus anti-NK1.1 (PK136; 0.25 mg d-1, +2); *Group #3:* no TBI plus anti-NK1.1, *group #4* no TBI plus anti-NK1.1 and rapamycin (5 mg/kg/d on days -1, 0 and $+2$). Multi-lineage chimerism was followed by flow cytometry. Tolerance was assessed by grafting donor and 3rd party skin.

Results: Mixed chimerism developed in 17/19 mice of group #2 (1 Gy, anti-NK1.1) but failed to develop without anti-NK1.1 (0/14, group #1; $p < 0.001$). Donor skin was accepted in 16/19 mice for more than 130 days (group #2), whereas 3rd party skin was rejected in all mice. Without TBI, 4/8 recipients treated with anti-NK1.1 developed multi-lineage chimerism (group #3). When unirradiated recipients were treated with anti-NK1.1 plus rapamycin, 7/7 mice showed multilineage chimerism which persisted long-term (e.g. 2% B cell, 11% myeloid chimerism at 18 wks post-BMT, group #4). All chimeras of this group showed prolonged donor skin survival (median 109 days) while rapidly rejecting 3rd party skin.

Conclusions: Depletion of NK cells in combination with rapamycin allows induction of chimerism and tolerance through BMT and costimulation blockade without host irradiation using clinically feasible doses of BM, thus providing a relevant irradiation-free protocol.

50 A new protocol for the induction of mixed chimerism using clinically available biologicals

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Background: Bone marrow transplantation (BMT) with costimulation blockade (anti-CD40L with or without CTLA4Ig) provides the mildest strategy to date for the induction of mixed chimerism and tolerance. The clinical translation of such protocols has been prevented in large part because clinical development of several anti-human CD40L monoclonal antibodies (mAb) has failed due to severe thromboembolic side effects. We therefore aimed to develop a costimulation blockade-based mixed chimerism regimen free of anti-CD40L treatment. LFA-1 (heterodimer consisting of CD11a and CD18) has multi-faceted roles in the immune response including T cell adhesion and costimulation. Blocking LFA-1 with anti-LFA-1 mAbs prolongs experimental allograft survival, and an anti-human LFA-1 mAb has recently been approved for psoriasis (efalizumab, Raptiva). We thus wanted to evaluate whether anti-LFA-1 allows mixed chimerism and tolerance without anti-CD40L treatment.

Methods: All groups of C57BL/6 mice received a non-myeloablative dose of total body irradiation (3 Gy; d-1), approx. 20×10^6 fully mismatched Balb/c bone marrow cells (d0), CTLA4Ig (abatacept, Orencia; 0.5 mg; d + 2, 4) and rapamycin (5 mg/kg/d; d-1, 0, +2). *Group #1:* additionally received anti-CD40L (MR-1, 1 mg d0), anti-murine LFA-1 (M17/4; 0.5 mg; d-1, +2) and anti-NK1.1 (PK136; 0.25 mg; d-1, +2); *Group #2:* like group #1 but without anti-CD40L; *Group #3:* anti-NK1.1 (0.25 mg; d-1, +2); *group #4:* anti-LFA-1 (0.5 mg; d-1, +2). Multi-lineage chimerism and deletion of donor reactive T-cells was followed by flow cytometry.

Results: Mixed chimerism developed in 3/3 mice of group #1 (anti-CD40L, anti-LFA-1, anti-NK1.1), and in 6/14 without anti-CD40L (group #2). Anti-NK1.1 without anti-CD40L failed to induce chimerism (group #3; 0/6). Notably, all mice treated with anti-LFA-1 without anti-CD40L (5/5, group #4) showed high multi-lineage mixed chimerism and deletion of donor reactive T-cells. A detailed evaluation of tolerance is pending.

Conclusions: Anti-LFA-1 mAb, together with CTLA4Ig and rapamycin, allows for the first time induction of mixed chimerism with a clinically feasible regimen of costimulation blockade.

51 Expansion of immature/transitional B-lymphocytes in patients with active chronic GVHD

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Background: Chronic graft-versus-host disease (cGVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT) and an important cause of non-relapse mortality. The diagnosis of cGVHD depends on clinical signs and histopathological confirmation. As a consequence of the poorly understood pathophysiology, the biomarkers for cGVHD

are lacking. Increasing amount of evidence implies a role of B-lymphocytes in cGVHD pathogenesis. We focus on the changes in B-lymphocyte subpopulations in cGVHD patients. In our previous study percentages of both non-class-switched (CD19 + /IgD + /CD27+) and class-switched memory B-lymphocytes (CD19 + /IgD-/CD27+) were significantly lower in patients with active cGVHD when compared to patients never experiencing cGVHD ($p = 0.003$, 0.001). The ratio of immature B lymphocytes/memory B-lymphocytes (CD21-/CD27+) was significantly higher in patients with active cGVHD when compared to patients never experiencing cGVHD ($p = 0.0046$). cGVHD and its therapy interfere with immunological reconstitution and causes profound immunodeficiency. In the current study we investigated how cGVHD affects the normal pattern of immune reconstitution in B-cell subpopulations within the first years after HSCT.

Methods: We report on the analysis of 200 sampling events (range of sampling time: 3 months to 12 years after HSCT) from 98 patients (median age at HSCT 42 years, range 17–62 years). One hundred and thirty three samples were obtained during active cGVHD and 67 samples at the time points without cGVHD. Evidence of cGVHD in at least one organ as defined by the NIH Consensus Development Project was sufficient to assign the sampling event into the active cGVHD group. Peripheral blood leukocytes were analyzed by multiparameter flow cytometry after staining for CD19, staining for surface Ig and the B-lymphocyte memory marker CD27 as well as staining for CD21, which is absent on immature/transitional B-lymphocytes. The patients were scored for cGVHD activity according to the NIH Consensus Development Project criteria at every sampling event.

Results: While the ratio CD21−/CD27+ as well as the percentage of immature/transitional (CD21−) B-lymphocytes decreases in the first years after HSCT in patients without cGVHD, these values remain high over the years after HSCT in patients with active cGVHD. Significant interaction exists between the value of CD21−/CD27+ ratio and time after transplant ($p = 0.001$) as well as between the percentage of immature B-lymphocytes (CD21−) and time after transplant ($p = 0.001$). In logistic regression analysis a higher CD21−/CD27+ ratio and higher percentage of immature B-lymphocytes (CD21−) significantly correlates with active cGVHD. The odds ratio rises from 1.028 to 14.532 between 2nd and 7th year after HSCT.

Conclusions: Our study demonstrates that cGVHD activity interferes with the reconstitution of the B-lymphocyte compartment leading to a disturbance of B-cell homeostasis. The most pronounced sign is a long lasting expansion of the immature/transitional B-lymphocytes. Prospective studies with larger patient numbers for monitoring of B-cell reconstitution in the cGVHD setting after alloHSCT are warranted.

52 Mixed immune cell population in intimal arteritis of renal allografts

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Background: Vascular rejection is characterised by immune cells infiltrating the arterial intima of allografts. Traditionally these cells are assumed to be lymphocytes. This belief is also reflected by the common use of anti lymphocytic drugs for treating vascular rejection in kidney transplantation. A recent study however found in a small number of cases that the majority of intimal immune cells would be macrophages rather than T-cells. The aim of our study was to determine the proportion of T-cells and macrophages in a larger number of unselected cases of vascular renal allograft rejection.

Methods: Our study population consisted of 42 biopsies with vascular rejection (Banff Type II). Inclusion criteria were: among all biopsies with the diagnosis of vascular rejection performed between 1997 and 2003, we selected all cases with at least two freshly cut sections available, each containing at least one artery with intimal arteritis. Typing of immune cells was performed on paraffin sections by immunohistochemical double labelling with antibodies against CD68 (monocytes/macrophages) and CD3 (T-cells). C4d staining was also performed on paraffin sections. For evaluation we calculated the ratio of CD68 and CD3 positive cells in all arterial cross sections. Cases that did not contain at least a total of 15 intimal immune cells were excluded from evaluation.

Results: The CD68/CD3 ratio ranged from 0.4 to 8.25 (median 1.1). That means that macrophages outnumbered T-cells in 57% of cases whereas T-cells predominated in the remaining 43%. The median value was used as cut-off level for defining two groups for statistical evaluation. Predominance of monocytes was associated with re-transplantation but not with C4d deposits or PRA-levels. The type of infiltrating immune cells did not depend on age of donors or recipients, duration of cold ischemia or number of mismatches. Evaluating the clinical course after transplantation, we found similar serum creatinine values at time of biopsy and up to 36 months thereafter in both groups. Kaplan–Meier analysis revealed no statistically significant difference in transplant and patient survival between groups and showed only a trend towards less favourable outcomes if monocytes predominated.

Conclusions: In contrast to previous reports we found no general predominance of either T-cells or macrophages in intimal infiltrates. Predominantly monocytic infiltration of the intima was associated with re-transplantation but not with indicators of humoral response and had no clear-cut influence on renal function and graft survival.

53 Non TBI-based RIC-transplantation in 42 patients with advanced or refractory/relapsed malignant diseases

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Background: Reduced intensity conditioning for stem cell transplantation (SCT) is feasible for patients with relapsed, refractory or advanced malignant disorders, who are at risk of developing severe toxicity after myeloablative therapy.

Methods: We treated 42 pts. (19 male and 23 female) at a median age of 52 y (21–72) with high risk acute leucemia (14 AML; 4 ALL), chronic myeloid (6 MPD; 6 MDS), lymphatic (5 NHL; 2 MM; 1 CLL) or other malignant diseases (3 RCC; 1 medulloblastoma) with non TBI based dose- reduced condition-

ing regimens followed SCT from 30 related (29 HLA-matched, 1 mismatched) and 12 unrelated (9 HLA-matched, 3 mismatched) donors. Conditioning regimens consisted of fludarabine combined with busulfan or HD-ARA-C for myeloid diseases or combined with cyclophosphamide or melphalan for lymphoid disorders and solid tumors. ATG (rabbit) (20 mg/kg i.v. for 2 days) was added in unrelated donor transplantations. Graft versus host disease (GvHD) prophylaxis included 3 mg/kg CsA from day −1 i.v. and mycophenolat mofetil 2 g/die p.o. from day +1 to +30.

Results: Forty patients had advanced disease mainly refractory or relapsed. Only 2 elderly pts. were in CR 1. In all cases PBSC were administered with a median of 4.59×10^6 CD 34 positive cells/kg (1.26–9.45) and 26.7×10^7 CD 3 positive cells/kg (12.48–54.9). Median time to leukocyte engraftment (>1 G/l) was 11 days (6–18). Platelet count >50 G/l was reached at a median of 16 days (10–233). Severe acute GvHD (grade III–IV) developed in 29%, limited chronic GvHD in 14% and extensive cGvHD in 14% of all evaluable patients. Infectious complications (24 patients) e.g. FUO occurred in 5, septicaemia in 14 and pneumonia in 12 patients (5 fungal pneumonia). In 9 patients CMV reactivation was seen with CMV disease (enteritis, hepatitis) in two patients. 16 patients (38%) are alive, 13 in CR with a median of 11.6 mo (1–75). 26 patients (62%) died, 13 due to relapse or progression and 13 due to TRM. Causes of death were septicaemia with or without GvHD. Prognostic factors like comorbidity index, donor/receptor polymorphism or KIR genotypes were of limited value in this patient group.

Conclusions: Non TBI-based Ric-SCT with Fludarabin based combinations is suitable for patients of higher age or high comorbidity, when standard conditioning regimens are not practicable. However, TRM rate as well as GvHD incidence are still high in patients with far advanced malignant disease, suggesting to perform Ric-SCT at an earlier stage, whenever applicable.

54 Hemophagocytic lymphohistiocytosis following antineoplastic treatment and stem cell transplantation in children – a rare but life-threatening complication

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a disorder characterized by fever, pancytopenia, hepatosplenomegaly and hemophagocytosis in bone marrow, liver and/or lymph nodes. Diagnosis is made by measurement of specific disease markers including ferritin, soluble interleukin 2 receptor and by detection of hemophagocytosis in bone marrow. Treatment aims to suppress hyperinflammation by chemoimmunotherapy. The occurence of HLH during childhood cancer treatment is a life-threatening event posing major diagnostic and therapeutic challenges. There are only few data published in the literature.

Methods: Between 1995 and 2006, six children (4 females, 2 males) aged between 0.5 and 16 years developed HLH during childhood cancer therapy. Underlying diseases were acute lymphoblastic leukemia ($n = 2$), acute myelogenous leukemia ($n = 2$), medulloblastoma ($n = 1$) and Ewing sarcoma ($n = 1$). Four patients had HLH while on conventional chemotherapy. Two patients developed HLH 57 and 24 days after allogeneic stem cell transplantation. Diagnosis of HLH was established by evaluation of HLH-specific serum parameters and detection of hemophgagocytosis in bone marrow. Different infectious agents were identified as possible triggers for HLH.

Results: Clinical symptoms included fever refractory to antibiotic and antimycotic treatment, hepatosplenomegaly and prolonged pancytopenia in all patients. Three patients also had CNS-symptoms. Treatment of HLH included antiinfective therapy, in addition 5/6 patients were treated with dexamethasone for 5–70 days, 3/6 children received etoposide and 2/6 children received infliximab and daclizumab, respectively. Only 3 patients survived, whereas 3 children died 2, 5, and 47 days after diagnosis of HLH.

Conclusions: HLH is a not well-known but severe complication of childhood cancer therapy. Early diagnosis and immediate initiation of adequate treatment including steroids and etoposide are necessary. The future role of new biologicals such as infliximab or daclizmab remains to be defined.

55 Danon-Krankheit: Späte Diagnose bei HCMP

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Grundlagen: Bei der seltenen Danon-Krankheit besteht eine primäre Defizienz an Lysosomen-assoziiertem Membran Protein-2. Ursache hierfür sind Mutationen im Bereich des LAMP-2 Gens, welches sich auf Xq24 befindet. Die klinische Symptomatik umfasst hypertrophe Kardiomyopathie, Skelettmyopathie sowie mentale Retardierung in unterschiedlicher Ausprägung.

Fallbericht: Wir berichten über einen männlichen Patienten, der im Alter von 9 Jahren an unserer kardiologischen Abteilung vorgestellt wurde. Echokardiographisch zeigte sich eine massive konzentrische Linksventrikelhypertrophie, laborchemisch eine Erhöhung der Transaminasen (ALT, AST), der Creatininkinase (CK) sowie Lactatdehydrogenase (LDH). Die daraufhin durchgeführte Abklärung inklusive Muskelbiopsie bei hypertropher Kardiomyopathie (HCMP) verlief negativ. Bei Progression der Kardiomyopathie und Entwicklung von malignen Herzrhythmusstörungen erfolgte im Alter von 12 Jahren die Herztransplantation.

Zu diesem Zeitpunkt wurde aufgrund einer rezenten Publikation der Verdacht auf Danon Krankheit gestellt. Sowohl im Skelettmuskel als auch den Leukozyten zeigte sich eine Defizienz des LAMP-2 Proteins. Im Rahmen der genetischen Ana-

lyse des LAMP-2 Gens konnte eine bisher nicht beschriebene Mutation gefunden werden, sodass schlussendlich die Danon Krankheit als Ursache der HCMP feststand.

56 Tetrahydrobiopterin pretreatment abrogates parenchymal damage by peroxynitrite formation following murine pancreas transplantation

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Background: Tetrahydrobiopterin (BH4) is an essential cofactor for nitric oxide synthases and thus a critical determinant of NO production. Recently, we have shown that BH4 depletion contributes to ischemia reperfusion injury (IRI) after pancreas transplantation. In this study we investigated the therapeutic potential of both donor BH4 supplementation previous to organ retrieval or recipient BH4 supplementation previous to organ reperfusion.

Methods: Male syngeneic C57BL6 (H-2b) mice, 10–12 weeks old were used as size-matched donor and recipient pairs. Murine cervical heterotopic vascularized pancreas transplantation was performed with a modified no-touch technique. Pancreatic grafts were subjected to 16 h prolonged cold ischemia time (CIT) and different treatment regiments: untreated (I), BH4 50 mg/kg i.m. before organ retrieval (II) and BH4 50 mg/kg i.m. previous to organ reperfusion (III). Non transplanted animals served as controls (IV). After 2 h of reperfusion intravital fluorescence microscopy was used for analysis of graft microcirculation by means of functional capillary density (FCD) and capillary diameters (CD). Quantitative assessment of parenchymal damage was analysed by histology (H&E) and by performing nitrotyrosine-immunostaining for peroxynitrite formation. BH4 tissue levels were assessed by HPLC.

Results: After prolonged CIT pancreatic grafts treated with BH4 before organ retrieval (II) as well as before reperfusion (III) displayed markedly higher values of FCD and CD compared to non treated animals (I) ($p < 0.01$, < 0.05 respectively). Histological evaluation showed increased inflammation, interstitial edema, hemorrhage, acinar vacuolization and focal areas of necrosis after 16 h CIT in group I, which could be significantly diminished by both BH4 treatment regiments. Likewise parenchymal damage (peroxynitrite formation) was significantly attenuated ($p < 0.05$). Intrapancreatic BH4 tissue levels were substantially increased in the BH4 treated donor organs.

Conclusions: BH4 pretreatment both of the donor prior to organ procurement or of the recipient previous to reperfusion significantly reduces postischemic deterioration of the graft and might be a promising novel strategy in attenuating IRI in clinical pancreas transplantation.

57 Intrapyloric injection of Botulinum Toxin A ameliorates diabetic gastroparesis in simultaneous pancreas kidney transplant patients

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Background: Diabetic gastroparesis (DGP) represents a chronic gastrointestinal disorder defined by delayed gastric emptying in the absence of mechanical obstruction. However, following successful simultaneous or sequential pancreas kidney transplantation DGP remains a major concern in one third of these patients. In cases refractory to prokinetic and anti-emetic medication alternative options like intrapyloric injection of botulinum toxin A (BoTx) were recently reported. However, results regarding BoTx therapy in these patients still remain contradictory. Here we report on the application of this treatment option in three simultaneous pancreas kidney (SPK) transplant recipients.

Methods: All three patients (m) suffered from severe and persistent gastroparesis following successful transplantation with stable graft function. Cardinal symptoms of gastroparesis like nausea, vomiting, early satiety, bloating and abdominal distension as well as quality of life (QOL) before injection and during follow up were quantified by a subjective severity scale. To exclude other possible underlying causes gastric emptying was determined by X-ray and scintigraphic examination prior to BoTx treatment. BoTx therapy consisted of up to 100 U which were injected equally distributed over the four quadrants of the pylorus. A first control X-ray was performed 24 hours later.

Results: Substantial therapeutic effects were evident within two weeks following BoTx injection in all patients. While the mean symptom score before BoTx injection was 28.67 (range 23–36) after the treatment it amounted to 11.38 (range 4–22). The first patient reported a consistent decrease of clinical symptoms paralleled by an improvement in QOL after a total of 120 U BoTx applied within two gastroscopies. The second patient experienced significant ameliorations of symptoms and QOL after just a single BoTx injection of 100 U. The third patient required a second injection (each 100 U) due to recurrent symptoms within the first month. However, after a mean follow up of 107 days all treated patients are doing well and display considerable improvements of all cardinal symptoms and even more important conspicuous amelioration of their reported QOL.

Conclusions: Intrapyloric BoTx injection represents a safe and straight forward treatment option for SPK patients suffering from severe and persistent DGF and should therefore be considered in patients refractory to prokinetic and anti-emetic medication.

58 Incidene and outcome of de-novo malignancy after cardiac transplantation – 20 years experience of a single centre

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De novo malignancies after transplantation are a growing problem of solid organ transplant recipients, due to longer sur-

vival follow-up under chronic immunosuppression. The aim of this study was to analyze a population of 921 consecutive one-month survivors after cardiac transplantation at a single transplant center from 1984–2003 for the development of de-novo cancer. Cancer was divided into three groups (skin cancer, Lymphoma and solid organ cancer.

Overall freedom from cancer was 90% at 5 years, 73% at 10 years and 62% at 15 years. Median time to cancer development was 63 months (range: 12–163 months) for skin cancer, 23.8 months (range: 2.7–106 months) for lymphoma and 44.2 months (3 to 153 months) for solid organ cancer. There was the same incidence of cancer development for patients transplanted during different time areas (1984–91: 5a: 8%, 10a: 27%; 1992–98: 5a: 13%, 10a: 28%; 1998–2003: 5a: 8%). Overall survival after cancer was 76, 57 and 49% at 1, 3 and 5 years respectively. Patients with skin cancer had significant better survival (1a: 93%, 3a: 81% and 5a: 67%) than patients with lymphoma (1a: 53%, 3a: 35% and 5a: 31%) or solid organ cancer (1a: 49%, 3a: 37% and 5a: 29%; $p < 0.0001$). Solid organ cancers consisted of 31% lung-, 20% urogenital (UG)-, 19% gastrointestinal (GI) and 30% other forms of cancer. Worst outcome was seen in lung cancer patients (12 m survival: 58%, 36 m: 11%) followed by GI cancer (12 m: 36%, 36 m: 36%) and UG cancer (12 m: 67%, 36 m: 42%).

An increased incidence of de novo cancers in the chronically immunocompromised patient demands careful long-term screening protocols which will help to facilitate the diagnosis at an early stage of the disease and possible better long-term outcome.

59 Alpha-Gal specific humoral immune response after implantation of bioprostheses in cardiac surgery

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Background: We have previously shown that the alpha-Gal (Galα; 1,3-Galβ; 1-4GlcNAc-R) epitope is a relevant xenoantigen present on bioprostheses utilized in adult cardiac surgery (Eur J Clin Invest 2005 Jan; 35[1]: 17–23). We sought to investigate whether a specific humoral alpha-Gal immune response is initiated after bio-valve implantation.

Methods: We collected plasma samples from patients who underwent bio-valve implantation prior and three months after cardiac surgery ($n = 15$). Plasma obtained from recipients of mechanical valves ($n = 8$) and coronary artery bypass graft (CABG) patients ($n = 15$) served as controls in this study. ELISA was utilized to quantify alpha-Gal specific total IgM, IgG and subgroup (IgG1, IgG2, IgG3, IgG4) immune response after heart operation. Three bio-valve tissue samples were obtained from patients who had to undergo re-operation because of valve malfunction within 1 month ($n = 2$) and 12–15 month ($n = 3$). We utilized confocal laser scanning microscope (CLSM) to detect presence of the alpha-Gal epitope (IB4) and cell nuclei (DAPI).

Results: We found in plasma analysis that implantation of bio-valves resulted in a significant increase of alpha-Gal specific IgG immune response as compared to control groups (pre-versus postoperative values [three months], $p < 0.001$); no alteration of alpha-Gal specific IgM content was observed ($p =$ not significant, NS). When comparing alpha-Gal specific IgG1, IgG2, IgG3 and IgG4 protein content, only IgG3 evidenced a significant increment in the bio-valve study cohort versus controls ($p < 0.01$). In CLSM analysis we demonstrated that within 1 month after implantation of bioprostheses the tissue matrix contained IB4/DAPI positive cells within the collagen matrix. Contrary, in patients who underwent re-operation after a mean of 12 month, porcine tissue showed only collagen and complete lack of IB4/DAPI.

Conclusions: Our results evidenced that implantation of bioprostheses elicits a specific humoral immune response against alpha-Gal bearing cells as compared to controls within 3 months after cardiac surgery. The clinical relevance of alpha-Gal specific IgG immune response was corroborated by complete absence of IB4/DAPI positive cells in bio-valve tissue obtained after re-operation.

60 Prevention of ischemia-reperfusion (I/R)-associated injuries: role of intracellular signaling pathways

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Background: Ischemia (I) and reperfusion (R) trigger a series of events, which culminate in severe injuries to the affected organs in organ transplantation. Cell death, metabolic alterations and inflammation result in impairment of short- and long term function. The group of mitogen activated protein kinases (MAPKs) are central regulators of these events. MAPKs have been implicated through aberrant activation in many pathophysiological settings including I/R-associated organ damage. We hypothesize that MAPK signaling is altered during renal I/R injury.

Methods: To asses intracellular signaling and kidney function a rat ischemia reperfusion model was used. Briefly after right nephrectomy, left kidneys of Wistar rats were subjected to 45 minutes of ischemia followed by reperfusion. Before and after reperfusion MAPK activity was assessed with phosphorylation-specific antibodies. Creatinine and Urea was measured at defined timepoints.

Results: In summary, reoxygenation was characterized by a dramatic increase in the activity of ERK ($p < 0.05$ vs. non ischemic control), JNK ($p < 0.05$) and p38 ($p < 0.0001$) during early reperfusion. This was associated with a significant raise in creatinine and urea. Preliminary data suggest that the use of small molecular weight p38-MAPK inhibitors (SB 203580 and SB 239063) have a beneficial effect on the ischemia reperfusion injury, showing a decrease in creatinine and urea as assessed 24 hours after reperfusion.

Conclusions: Renal I/R injury is associated with a significant increase of MAPK activity, suggesting that they may provide a promising target for ameliorating reperfusion injury.

61 Feasibility and tolerability of granulocyte transfusions in children and adults – an update

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Granulocyte transfusions (GT) from granulocyte colony stimulating factor (G-CSF)- or prednisone-stimulated donors have been shown to increase the absolute neutrophil count (ANC) prior to expected hematopoietic regeneration in neutropenic patients after chemotherapy or hematopoietic stem cell transplantation, thus GT offer a therapeutic option for the control of severe infections. In this report we re-assess the efficacy of granulocyte support and the incidence, spectrum and severity of acute side effects of 70 episodes of GT based on data from 60 children (mean age 6.28 years, range 0.13–17.73) and 10 young adult patients (age 21 years, 18.09–28.06 years) between 1995 and 2005. We analyzed in detail the impact of parameters such as patients' body weight, presence of organ dysfunction, as well as dose intensity of GT (number of neutrophils/kg transfused within first five days) on ANC increment, infection control, and survival. We detected a correlation of the ANC increment to the neutrophil content of the first GT dose but not of the 5-day cumulative neutrophil dose per kg recipient body weight. The 5-day cumulative neutrophil dose and the ANC increment did not correlate to 28-day survival, suggesting that the effect of GT might not be measured in a neutrophil-dose-dependent manner. Sepsis-related organ dysfunction at time of GT did not affect ANC increment but correlated with overall mortality in children and adults. Adverse reactions were rare; severe events ($\geq$III°) occurred as refractory fever (6%) and pulmonary complications (2%). These data suggest that GCSF-elicited GT are a feasible, well tolerated means to shorten neutropenia in children and low-weight adult patients.

62 Prevention of tolerance after stimulation of NKT cells with alpha-Gal is mediated by natural killer cells

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Background: Bone marrow transplantation (BMT) together with costimulation blockade can reliably induce mixed chimerism and tolerance in certain experimental models. Recently we delineated the role of NKT cells and found that stimulation of recipient NKT cells with alpha-gal leads to loss of tolerance. We wanted to determine which cell population is mediating this rejection, especially whether NKT cells directly affect rejection or whether they do so indirectly through activation of other effector cells.

Methods: C57BL/6 mice received a total body irradiation of 3 Gy or 1 Gy (day -1), approx. 20×106 fully mismatched Balb/c bone marrow cells (day 0) and costimulation blockade consisting of anti-CD154 mAb (MR1, day 0) and CTLA4Ig (day $+2$) or T-cell depletion with anti-CD4 and -CD8 on days -5 and -1. Groups were additionally treated with alpha-galactosylceramide (specifically stimulating NKT cells) or a vehicle (5 µg on day -1, $+2$, $+7$, $+14$), and anti-asialoGM1 50 µl (depletes NK but not NKT cells) (days 0, $+4$, $+8$, $+12$). Multi-linage chimerism and skin graft survival were followed for more than 120 days.

Results: Stimulation of NKT cells with the synthetic glycolipid alpha-galactosylceramide at the time of BMT with costimulation blockade prevented chimerism and tolerance, also after in vivo T-cell depletion (anti-CD4 + CD8) (0/5 chimeric), suggesting that T-cells are not critical for rejection. When NKT cells were stimulated after in vivo depletion of NK cells plus T cells (anti-CD4 + CD8 plus anti-asialo GM1), chimerism and tolerance were not abrogated (5/5), indicating that activated NKT cells trigger bone marrow rejection via NK cells. The negative effect of alpha-gal administration is restricted to the early phase of tolerance induction as late treatment (10 weeks post BMT) had no effect on chimerism levels and skin graft survival.

Conclusions: NKT cells have no critical regulatory role in tolerance induction through non-myeloablative BMT. Stimulation of NKT cells prevents tolerance induction through activation of NK cells, raising concern that concomitant infections with certain bacteria known to activate NKT cells would be of concern in patients receiving such regimens.

63 Beeinflusst die Migration das Outcome nach kindlicher Nierentransplantation? Ergebnisse von dreißig Jahre

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Grundlagen: Vor 30 Jahren wurde zum ersten Mal gezeigt, dass auch die Ethnizität – neben anderen spender- und patientenabhängigen Faktoren – die Transplantatsüberlebensrate wesentlich beeinflusst. In dieser Studie untersuchten wir den Einfluss der internationalen Migration auf das pädiatrische Patienten- und Transplantatüberleben.

Methodik: Diese Analyse wurde retrospektiv an 197 Kindern der Kinderdialyse des AKH-Wien durchgeführt, die seit Gründung der Kinderdialyse-Wien 1978 bis 2006 insgesamt 234 Nierentransplantationen (NTX) unterzogen wurden. Von diesen 197 Kindern hatten 48 (24%) eine Migrationshintergrund.

Ergebnisse: Die Patientenüberlebensrate der Migrantenkinder lag 1, 5 und 10 Jahre nach der NTX bei 98, 88 bzw. 80%, die der einheimischen Kinder bei 91, 91 bzw. 82% ($p = 0{,}92$). Die 1, 5 und 10 Jahresüberlebensraten der Transplantate der Migrantenkinder betrug 85, 80 und 80%, während diese Raten bei einheimischen Kindern bei 85, 69 und 59% lagen ($p = 0{,}55$).

Schlussfolgerungen: Unsere Ergebnisse zeigen keinen Unterschied zwischen den beiden Gruppen bezogen auf Patienten- und Transplantatüberleben. Wir planen daher, weitere Outcome-Parameter und bekannte Risikofaktoren insbesondere psychosoziale Einflüsse in Bezug auf Migration zu untersuchen.

64 Decrease of cardiac transplant numbers: a donor problem?

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Background: The number of cardiac transplants has dropped over the last years. The objective of this single-center analysis was to analyze cardiac donor offers over the last 11 years and their potential impact on decreased transplant numbers.

Methods: Between 1996 and 2006 a total of 1594 cardiac donors were offered (age 40.4 ± 2.9 a, 56.5% males, 171 ± 1 cm, 71 ± 2 kg, 45.8% cerebrovascular accidents, 42.5% trauma, 11.7% other). Changes of organ offers, organ acceptance, causes of refusal and parameters for donor organ quality were compared between two time periods: early (1996–2000) and late (2001–06).

Results: The annual average number of donor offers decreased significantly (160.4 ± 10.2 vs. 132 ± 15.8, $p < 0.01$), mainly due to reduction of local donor offers (54.5 vs. 38% of all offers). Organ offer refusal rate increased from 65.3% to 74.5% ($p = 0.038$). Significantly more organs were refused on site during the early time period (24.8% vs. 16.4%; $p < 0.05$). Bad quality was the main reason for refusal of organ offer (69%), followed by mismatch (18%). There were no differences between the two time intervals in behalf of reason of refusal. Yet significantly more local (72%) were refused for quality reasons (Eurotransplant offers [ET]: 42%; $p < 0.03$). Significantly more ET offers (25%) were refused due to size/weight mismatch (local: 13%; $p < 0.01$). Main quality reasons for refusal were high inotropic support, old age, bad cardiac contractility and coronary lesions (9% each). Old age as reason of refusal was the only variable that significantly increased over time (6.6% vs. 12%; $p < 0.03$) although donors $>$ 50a remained stable over time (30% vs. 33% of total).

Conclusions: Cardiac donor offers decreased significantly over the last years, and more organ-offers were refused (mainly due to quality reasons) by our center. There is need for action to increase local donor numbers.

65 Reducing renal impairment in liver transplant: 6 month interim data from a multi-centre randomised controlled study

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Background: Renal failure may develop late after liver transplantation and is a significant cause of morbidity and premature mortality. Major risk factors include the dose and level of calcineurin inhibitor in the six months following transplantation.

Methods: We undertook a prospective study in which patients undergoing a primary liver transplant were randomised to one of three groups: A) tacrolimus at the standard dose (target trough whole blood level $>$ 10 ng/ml) for the first month, group B) tacrolimus target level $\leq$ 8 ng/ml and mycophenolate mofetil (MMF) 1 g bid iv until at least Day 5; 1 g bid orally thereafter and C) daclizumab on Day 1 and Day 7, MMF as in Group B and tacrolimus introduced on Day 5 with target trough level $\leq$ 8 ng/ml. Corticosteroids were given in all groups, according to local protocol. The primary endpoint was change in creatinine clearance at one year.

Results: We report the preliminary results after 6 months on study. Between February 2004 and February 2006, 525 patients were randomised in 30 centres; 183, 170 and 172 were allocated to groups A, B and C, respectively. On an analysis of patients for whom complete 6 month data is available, patient survival at 6 months is 92.8, 92.3 and 95.5% in Groups A, B and C. Graft loss which led to death or retransplantation occurred in 6, 5 and 4 patients. 57.2, 57.2, and 59.4% experienced serious adverse events. Renal function, assessed by calculated glomerular filtration rate (GFR) was 103.01, 106.72 and 97.71 ml/min immediately before transplantation in the three groups respectively and at 6 months was 81.04, 82.48 and 86.04. The change from baseline in GFR (ml/min) at 6 months was -26.7, -21.6 and -13.2 in groups A, B and C respectively. Although the difference in change from baseline between groups A and B was not statistically significant ($p = 0.227$), the difference between groups A and C was significant ($p = 0.004$).

Conclusions: These findings remain preliminary and do not necessarily predict the conclusions from the full analysis at 12 months, the defined endpoint of the study, but suggest that the probability of late-onset renal failure may be reduced by delayed introduction of tacrolimus if the recipient is treated with MMF and daclizumab.

66 Polyclonal FoxP3 transduced murine CD4+ cells are similarly potent as natural Tregs in suppressing alloreactivity

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Background: Antigen-specific TCR-transgenic T-cells retrovirally transduced to express FoxP3 exert potent suppressor function in certain models but little is known about the ability of polyclonal FoxP3-transduced T-cells to suppress alloreactivity. We therefore compared polyclonal murine FoxP3-transduced CD4+ cells to natural CD4+ CD25+ Tregs in allogeneic suppressor assays. In previous studies we demonstrated a critical role for Tregs in the induction of tolerance after bone marrow transplantation with costimulation blockade. Here we show as initial step the in vitro generation and characterization of FoxP3 transduced CD4 + cells with the aim of examining their potency in a murine transplantation model.

Methods: CD4+ cells were isolated from B6 spleen and lymph nodes by MACS and cultured in the presence of anti-CD3, anti-CD28 and IL-2. Cells were infected twice (d2 and 3) with high-titer VSV-G pseudotyped retrovirus (MOI 5–10). The retroviral vector used encodes for FoxP3 and eGFP under the control of an internal ribosomal entry side (IRES). Transduction efficiency was up to 30%, cells were sorted for eGFP expression, phenotypically analyzed by FACS and characterized functionally in vitro. Natural Tregs (CD4 + CD25 high) were isolated from B6 spleen and lymph nodes by FACS sorting. Purity of sorted populations was $>$98%. Suppressor function

was examined in fully allogeneic proliferation assays in which 4×105 freshly isolated B6 splenocyte responders were co-cultured with different numbers of FoxP3-transduced or natural Tregs in the presence of 4×105 irradiated Balb/c splenocyte stimulators. After 72 h cells were pulsed with 3H-thymidine, incorporation was measured 18 h thereafter. Stimulation indices (SI) were calculated in relation to medium controls.

Results: Flowcytometric analysis showed a Treg-like phenotype in polyclonal FoxP3 transduced CD4+ cells (upregulation of CD25, CTLA4, CD62L) and intracellular staining verified FoxP3 expression in GFP positive cells. FoxP3-transduced CD4+ cells suppressed alloreactivity in a dose-dependent manner. Co-culture of FoxP3-transduced B6 CD4+ cells in a 1:2 vs. 1:1 vs. 2:1 ratio with freshly isolated B6 splenocytes resulted in a significant suppression of proliferation in response to fully allogeneic Balb/c stimulators. SI 0.44 vs. 0.22 vs. 0.03 with FoxP3-transduced Tregs, SI 0.17 vs. 0.06 vs. 0.04 with natural Tregs, SI 2.43 without Tregs, $p < 0.001$ for all ratios vs. controls without Tregs (data are representative for 2 independent experiments).

Conclusions: These results suggest that polyclonal FoxP3-transduced T-cells suppress a polyclonal alloresponse in vitro with comparable potency as freshly sorted CD4 + CD25 + Tregs. Based on these findings polyclonal FoxP3 transduced cells are an attractive candidate for use in in vivo models of transplantation tolerance.

67 CMVIg and IVIg induce CD32-mediated platelet aggregation *in vitro*: implication of therapy induced thrombocytopenia and thrombosis *in vivo*

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Background: We have previously shown that antithymocyte globulin (rATG), a drug widely used in prophylaxis and treatment of acute allograft rejection, causes platelet aggregation *in vitro* via the low-affinity Fc IgG receptor (CD32). We suggested that this pathway of aggregation is causative for platelet depletion after rATG application (Am J Transplant 2003 June; 3[6]: 754–759). Since various immunoglobulin drug compounds are currently finding increased acceptance in treating a) allograft rejection (intravenous immunoglobulin, IVIg); b) CMV infection (passive immunization, Cytomegalovirus immunoglobulin, CMVIg) and c) haematological and neurological diseases (IVIg), we sought to investigate if commercially available IVIg (Pentaglobin®) and CMVIg (Cytotect®, Cytoglobin®) compounds can cause similar effects of platelet aggregation in *in vitro* assays. Furthermore, it was of interest to us to find a mechanism by which clinically observed complications such as acute venous/arterial thrombosis and platelet depletion can be explained.

Methods: The influence of CMVIg (Cyotect®, Cytoglobin®) and IVIg (Pentaglobin®) on platelet aggregation was studied in a four-channel aggregometer. Expression of platelet surface activation marker CD62P was determined by flow cytometry analysis. Electron microscopy (ELMI) was utilized to determine platelet morphology. Furthermore, supernatants obtained from platelets incubated with CMVIg and IVIg were evaluated for presence of CD40L secretion (ELISA). All experiments were performed utilizing packed platelet concentrations from the blood bank and physiological concentrations of CMVIg and IVIg.

Results: Treatment of packed platelets with CMVIg (Cytotect®, Cytoglobin®) and IVIg (Pentaglobin®) markedly induced aggregation, featured an up-regulation of surface activation marker CD62P, secretion of platelet-bound sCD40L (CD154) and increased signs of aggregation in electron microscopy analysis (All assays were performed with a sample size $n = 8$, for each drug compound, $p < 0.001$). The capacity of CMVIg and IVIg to induce platelet aggregation was completely abrogated by adding antibodies against the low-affinity Fc IgG receptor (CD32) in all performed *in vitro* assays.

Conclusions: Our results suggest that CMVIg and IVIg suspensions with activating Fc domains were able to bind to CD32 on platelets, respective Fab-fragments directed towards carbohydrates present on platelets (e.g. CD40L/CD154, MHC-class I), and were responsible for the activation and aggregation of platelets in *in vitro* assays. These *in vitro* results indicate a mechanism by which immunoglobulin infusion induces platelet depletion and acute venous/arterial thrombosis *in vivo*.

68 Human platelet lysate (HPL) preserves *in vitro* potency of umbilical cord blood-derived MSC

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Background: Umbilical cord blood (UCB) is an easily accessible alternative source for multipotent mesenchymal stromal cells (MSC) and is generally believed to provide MSC with a higher proliferative potential compared to adult bone marrow (BM). Limitations in cell number and strict dependence of expansion procedures from selected lots of fetal bovine serum (FBS) have hampered the progress of clinical applications with UCB-derived MSC.

Methods: Isolation efficacy and proliferative potential of human UCB-MSC were analyzed as compared to BM-MSC under optimized ex vivo culture conditions. We further investigated human platelet lysate (HPL) as an alternative to replace FBS for clinical scale UCB-MSC expansion. Clonogenicity was determined in CFU-F assays. UCB-MSC were analyzed for their multipotent differentiation capacity and their function was tested in haematopoiesis support, vascular-like network formation and immune modulation potency assays.

Results: MSC could be propagated from UCB with HPL- as well as FBS-supplemented medium in 46% of UCB samples. Once established, the proliferation kinetics of UCB-MSC

resulted in more than 50 population doublings after 15 weeks. A clinical quantity of 100 million MSC with retained differentiation potential could be obtained from UCB within approximately seven weeks. Accelerated *ex vivo* expansion of haematopoietic UCB-derived $CD34^+$ cells as well as immune inhibition and vascular-like network formation could be shown for UCB-MSC propagated under both culture conditions.

Conclusions: We demonstrate for the first time that human MSC can be obtained and propagated to a clinical quantity from UCB in a completely FBS-free system. Functional data indicate the applicability of clinical grade UCB-MSC propagated with HPL-supplemented medium for haematopoiesis support, immune regulation and vascular regeneration.

69 Haematopoetic stem cell transplantation in CLL

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Background: We retrospectively evaluated the role of autologous stem cell transplantation (ASCT) and allogeneic reduced intensity transplantation (allo-RIC) in patients with chronic lymphatic leukaemia (CLL).

Methods: Between 1999 and 2006 19 patients (14 male, 5 female) diagnosed with CLL underwent 21 haematopoetic stem cell transplantations (HSCT) at our institution. The mean age at time of transplantation was 62.5 years (range: 39–67) and the mean time from diagnosis of CLL to HSCT was 53.6 months (range: 5–14). Fourteen patients underwent autologous transplantation (ASCT) whereas allo-RIC was performed in six patients (four siblings, two MUD). One patient underwent ASCT and MUD-RIC which was due to PD after ASCT and two allo-sibling RICs were performed in another patient, which was due to primary graft failure after the first stem cell transplantation. Pre-transplant remission status before ASCT was CR in three patients, VGPR in two patients, PR in five patients, and PD in four patients. Before allo-RIC, one patient was in CR, one patient in VGPR, and four patients in PR. For ASCT conditioning regimen consisted of fractionated TBI (8–12 Gy) and cyclophosphamide (120 mg/kg). For allo-RIC conditioning therapy consisted of fludarabine ($150\,mg/m^2$) and TBI (4 Gy), or fludarabine ($90\,mg/m^2$) and cyclophosphamide ($900\,mg/m^2$). In allo-RICs graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine A and mycophenolate mofetil. For MUD transplantation low dose ATG Fresenius ($4 \times 5\,mg/kg$ days -4 through -1) was added to this regimen.

Results: Transplantation-related-Mortality (day 100) was absent in both, the ASCT and the allo-RIC group. With a mean follow up of 40 months (range: 10–94) for ASCT and 36 months (range: 16–57) for allo-RIC, respectively, the CLL progression free survival is 50% in the ASCT and 83.3% in the allo-RIC group. The overall survival in patients undergoing ASCT is 64.2% and reaches 100% in patients where allo-RIC has been performed. Remarkably, no single patient treated with allo-RIC experienced acute or chronic GVHD.

Conclusions: Our data suggest that HSCT, particularly allo-RIC, displays a promising therapeutic option in patients with CLL.

70 Minimum requirements for fully humanized clinical scale MSC propagation

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Background: *Ex vivo* expansion of human MSC is currently considered as a strict prerequisite for blood and marrow MSC therapy. This study was performed to define the minimum requirements for producing sufficient MSC numbers for therapeutic application in a completely animal serum-free standard system from small bone marrow aspiration volumes within clinically acceptable time.

Methods: In compliance to good manufacturing practice (GMP) we established a time and resource saving efficient 'one step procedure' for MSC propagation. Bone marrow was seeded without manipulation directly in human platelet lysate (HPL) and L-glutamine supplemented minimum essential medium without antibiotics. Clinical scale MSC-HPL were harvested already after primary culture. MSC-HPL quality, identity, purity and function were assessed according to a defined panel of release criteria. The impact of the primary marrow plating density on the frequency of fibroblast colony-forming units (CFU-F) was studied.

Results: Starting from 790 to 950 million nucleated cells in aspiration volumes of 14 to 17 mL (male donors; age: 30, 36, and 47 years), three clinical scale expansions resulted in 906, 963 and 256 million MSC-HPL, respectively. This yield was achieved within a single culture period of 12 to 16 days. MSC-HPL quantity represents one to three application doses of >250 million MSC-HPL each. MSC-HPL viability was 95–97.7% and flow cytometry revealed a CD73+/CD90+/CD105+ phenotype with less than 2% hematopoietic cell contamination. Bacterial, fungal and mycoplasma contamination was excluded and endotoxin levels remained below 0.03 EU/mL. The BM plating density inversely correlated with the CFU-F frequency. The differentiation potential of MSC-HPL into adipo-, chondro- and osteogenic lineages was verified.

Conclusions: The opportunity to achieve up to three application doses of MSC without animal serum in a standardized one step procedure within 2 weeks supports therapeutic approaches that depend on the fast and safe availability of sufficient MSC doses in the clinical setting.

71 Internationale Fremdspendersuche bei 1622 Patienten: Überblick über die letzten 10 Jahre Fremdspendersuche

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Wir berichten über die internationale Fremdspendersuche für Patienten mit malignen hämatologischen Erkrankungen, die einen

Fremdspender benötigen. Seit 10 Jahren werden in Österreich zur Auswahl eines gewebeverträglichen Spenders von Knochenmark oder Blutstammzellen 5 Loci des menschlichen Gewebeüberträglichkeitssystems, des HLA-Systems, herangezogen. Seit diese strengeren Auswahlkriterien in Österreich Anwendung finden, wurden 1622 Patienten zur internationalen Spendersuche angemeldet. Ergebnisse von 1182 Patienten, bei denen der Suchvorgang bereits abgeschlossen ist, liegen vor. Insgesamt konnte bei 888 (75,1%) Patienten ein gewebeverträglicher Spender gefunden werden, allerdings fand nur bei 682 (57,7%) Patienten eine Transplantation statt. Die Spender kamen aus 21 Ländern der Erde, die meisten Spender ($n = 351, 51{,}5\%$) kamen aus Deutschland, gefolgt von den USA und Großbritannien. Das Verhältnis zwischen der Zahl der angemeldeten und der Zahl der transplantierten Patienten schwankt zwischen 39,7% im Jahr 2001 und 54,2% im Jahr 2004. Die Dauer der internationalen Spendersuche konnte von anfänglich 83 Tagen im Jahr 1997 auf 54 Tage im Jahr 2006 gesenkt werden.

72 A new cryoprotectant solution for PBSC with 5% DMSO increases the viability of CD34+ cells and nucleated cells after freezing-thawing compared to conventional 10% DMSO

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DMSO (Dimethyl Sulfoxide) is the standard cryoprotectant for PBSC (peripheral blood stem cells). Efforts were made to reduce the concentration of DMSO because most recipients get minor to moderate toxicities after reinfusion, in rare cases even serious side effects have been observed. We compared a new cryoprotectant solution, Cryostor^{TM}CS10 with 5% DMSO end concentration, with our conventional 10% DMSO-autologous plasma solution in vitro.

Aliquots of 11 autologous and 2 allogeneic PBSC were splitted and cryopreserved in test tubes after mixing with the equal volume of cryoprotectant solution A (consisting of 20% DMSO and 80% autologous plasma) or B (consisting of Cryostor^{TM}10 with 10% DMSO) resulting in 10% and 5% DMSO end concentration respectively. The following assays were tested before manipulation (starting point), and after freezing-thawing process in group A and B: nucleated cells (NC) by a cell counter, CD34, CD45, CD34- and CD45-viability (7-amino-actinomycin D) by flow cytometry, trypan blue exclusion dye and clonogenic capacity in stem cell cultures. A two-tailed Student's t-test was used to analyze the results.

At the start the NC and CD34 concentration was $235 \pm 49 \times 10$ E9/l and $1.1 \pm 0.8\%$ resp. The samples were stored for 77 ± 27 days in vapour phase of liquid nitrogen. The recovery of viable (7-AAD negative) CD45+ and CD34+ cells for group A and B was 77 ± 17 vs. $82 \pm 19\%$ ($p = 0.04$) and 87 ± 44 vs. $108 \pm 46\%$ ($p = 0.04$) resp. Trypan blue exclusion dye showed cell viability of 72 ± 13 vs. $84 \pm 10\%$ ($p < 0.01$). No significant difference was observed in clonogenic capacity (13 ± 8 vs. $11 \pm 8\%$, $p = 0.21$) and NC recovery (97 ± 18 vs. $100 \pm 18\%$, $p = 0.34$).

Our results show slightly significant better recovery of viable CD45 and CD34 cells and higher trypan blue cell viability but no difference concerning the proliferative capacity in the 5% DMSO group (Cryostor^{TM}) vs. 10% DMSO after freezing-thawing. The formula of the Cryostor solution is not published by the producer, it is described as a cell-specific, optimized preservation medium. The highest DMSO-concentration available in Cryostor-products is 10%, this comes to 5% DMSO end concentration after mixing with PBSC. The new product is intended for in vitro use only, but DMSO is not released for clinical application either. To our knowledge a clinical trial of Cryostor products is lacking but in our opinion meaningful as reducing DMSO without loss of quality would be a benefit for patients.

73 De novo Tacrolimus basierte Immunsuppression nach Nierentransplantation (NTX) bei Kindern

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Grundlagen: Seit Ende 2005 wird an der Universitätsklinik für Kinder und Jugendheilkunde initial Tacrolimus nach NTX bei Kindern eingesetzt. Das Ziel der Untersuchung ist es, Effektivität und Sicherheit dieser Immunsuppression zu evaluieren.

Methodik und Ergebnisse: Es wurden Daten von 13 Kindern (8 m, 5 w) mit 13 Transplantaten (9 LRD, 4 DD), die von 12/2005 bis 05/2007 nierentransplantiert wurden, ausgewertet.11 Patienten wurden ersttransplantiert, 2 Patienten retransplantiert. Die Patienten waren im Median bei NTX 9,3 Jahre (3,2–21,4). 6 Kinder wurden präemptiv transplantiert, 4 Kinder hatten Hämodialyse und 3 Kinder Peritonealdialyse als primäre Nierenersatztherapie. Die Patienten erhielten ein sequentielles Schema mit Daclizumab-Induktion, Tacrolimus (initial 0,3 mg/kg/d), Prednisolon und MMF.

Alle Patienten hatten eine sofortige TX-Funktion, die GFR (Schwartz-Formel) betrug 106,8 ml/min/1,73 m^2 nach 1 Monat. Das Serumkreatinin lag ab Tag 14 über 15 Beobachtungsmonate hin stabil bei 0,87 mg/dl. 2 Patienten hatten eine biopsieverifizierte akute interstitielle Transplantatabstoßung BANFF-Borderline, ein Patient hatte eine akute vaskuläre Transplantatabstoßung Typ 2A nach BANFF 97. 2 Patienten hatten eine CMV-Infektion und bei 2 traten Hyperglykämien auf, die diätetisch behandelbar waren. 4 Patienten wurden wegen einer chronischen TX Nephropathie auf Sirolimus konvertiert.

Serummagnesiumwerte waren am Tag 14 nach NTX signifikant niedriger (1,00 vs 0,64 mg/dl, $p = 0{,}0003$). Ebenso signifikant war der Anstieg der Cholesterinwerte am Tag 14 und nach 1 Monat (148 vs. 182 und 197 mg/dl).

Schlussfolgerungen: Unsere präliminären Daten zeigen, dass eine initiale Immunsuppression mit Tacrolimus sicher und effektiv ist.

74 To drain or not to drain – abdominal drainage after liver transplantation

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Background: Traditionally, an abdominal drainage catheter is routinely inserted in patients after liver transplantation

(LT) to drain ascetic fluid and to detect postoperative hemorrhage and bile leakage. However, the benefits of this surgical practice have not been evaluated and awaited advantages could be overshadowed by drainage-associated morbidity. Further recent data provide evidence that abdominal drainage after hepatic resection is contraindicated in patients with chronic liver disease.

Methods: Between 2000 and 2004, 126 patients that had undergone orthotopic LT were analyzed retrospectively for this study. After pair-match respecting MELD (16.1 vs. 16.4, mean), age (52.5 vs. 52.9 years), indication for LT and gender, the patients were randomized in two groups with either closed suction abdominal drainage (drainage group, $n = 63$) or no drainage (non drainage group, $n = 63$) after liver transplantation. Analyses of risk of systemic or local infection, time at ICU, hospital stay, necessity of blood and plasma transfusion and short time survival were performed.

Results: The indication for LT was distributed as follows for patients with and without drain respectively: alcoholic cirrhosis 40.9% vs. 42%, virus-hepatitis-cirrhosis 22.7% vs. 22%, HCC in cirrhosis 9.1% vs. 24% and other 27.3% vs. 12%. Neither systemic infection parameters (blood culture, urine bacteriology or central catheter bacteriology) nor local infection parameters (drain bacteriology or smear test) differed statistically significant between the two groups. Concerning the time of inpatient-treatment a statistically significant difference between the patients with drainage and those without drain application (30.6 ± 52.9 vs. 18.3 ± 19.2 days/mean $\pm$ SD, respectively, $p = 0.003$, Wilcoxon rank) was calculated. There was no statistically significant difference concerning MELD ($p = 0.845$, sign test), age ($p = 0.313$, sign test), number of blood concentrates transfused ($p = 0.142$, sign test) or FFP-usage ($p = 0.625$, sign test). Patients with abdominal drainage had significantly more often been treated with thrombocyte concentrates (0:0–1 vs. 8.9:0–46 range, $p = 0.007$, sign test). There was no significant difference in short time overall survival between the two groups ($p = 0.934$, log rank).

Conclusions: In our patient cohort we could see a significant difference in the hospitality stay between patients with or without abdominal drainage after orthotopic LT. However, the benefits of the surgical practice of abdominal drainage after LT should be further evaluated by a prospectively randomized study.

75 CD26-inhibition in a congenic murine model of mixed chimerism

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Background: Enhanced stem cell engraftment would be important for the success of bone marrow transplantation under minimal conditioning. CXCL12/SDF-1α has a considerable impact on homing of hematopoietic stem cells to bone marrow. CD26/dipeptidylpeptidase IV (DPPIV) expressed on the surface of hematopoietic cells cleaves CXCL12/SDF-1α and thus negatively regulates homing and engraftment. Inhibition of CD26 with Diprotin A has been reported to promote engraftment of isolated stem cells in lethally irradiated recipients. We now examined if inhibition of CD26 enhances engraftment of unseparated congenic bone marrow after non-myeloablative total body irradiation.

Methods: B6 CD45.1 mice received a non-myeloablative dose of total body irradiation (1Gy, day -1) and 15×10^6 CD45-congenic bone marrow cells from CD45.2 donors. Donor bone marrow for one group was incubated in vitro with Diprotin A (consisting of 3 amino acids, Ile-Pro-Ile, for blocking CD26; for 15 minutes). Multi-lineage macrochimerism was followed by flow cytometry.

Results: Preliminary data reveal similar rates and levels of multi-lineage chimerism with or without CD26 inhibition. 10/10 mice of the group receiving bone marrow treated with Diprotin A and 9/9 mice receiving untreated bone marrow developed chimerism. On day 76 mean chimerism of the group treated with Diprotin A reached about 26% in the CD4 T-cell, 17% in the CD8 T-cell, 37% in the B-cell and 28% in the myeloid-cell lineage. The group receiving untreated bone marrow showed comparable levels of chimerism (30% in the CD4 T-cell, 21% in the CD8 T-cell, 44% in the B-cell and 36% in the myeloid cell lineage). Control assays measuring CD26 activity after in vitro Diprotin A treatment are ongoing.

Conclusions: Until now, we have found no evidence that treatment of unseparated bone marrow with Diprotin A leads to an enhaced stem cell engraftment in a murine model by using non-myeloablative conditioning.

76 Proteinurie und Nierenfunktion nach Konversion zu einem Everolimus-basiertem immunsuppressivem Regime nach oHTX

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Grundlagen: mTOR-Inhibitoren [Rapamycin, Everolimus] stellen eine neue Alternative in der immunsuppressiven Therapie nach oHTX da. Rapamycin, welches schon seit einiger Zeit zur Verfügung steht, führt neben einer reduzierten Nephrotoxizität zu einer erhöhten Proteinurie. Ziel dieser Studie war neben der Nierenfunktion die Proteinurie unter Everolimus zu evaluieren.

Methodik: 30 Patienten [26 männl., 4 weibl.] nach oHTX wurden in 2 Gruppen unterteilt. Gruppe A [$n = 15$; Mittleres Alter 56,8 a] erhielt Everolimus in Kombination mit CsA und Aprednislon, Gruppe B [$n = 15$; Mittleres Alter 62,4 a] CsA, MMF und Aprednislon. Patienten der Gruppe A erhielten 1,0 bis 1,5 mg Everolimus pro Tag verteilt auf zwei Einzeldosen mit einem angestrebten Talspiegel von 3–8 ng/ml. Proteinurie, Kreatininwerte, Bluthochdruck und Immunsuppressiva-Talspiegel wurden retrospektiv über einen Zeitraum von 2 Jahren halbjährlich ausgewertet.

Ergebnisse: Die Gruppen unterschieden sich hinsichtlich Kreatininwerten [p: 0,36], GFR [p: 0,48], arterielle Hypertonie und Proteinurie [p: 0,267] nicht. Ebenfalls kam es in keiner der Gruppen zu einer Zunahme der Proteinurie über den Beobachtungszeitraum. Die in Gruppe A nach 24 Monaten erzielte Reduktion des CsA Talspiegels auf 77,9 ng/ml in Gruppe A

vs. 114,4 ng/ml in Gruppe B [$p < 0{,}001$] war mit keiner erhöhten Abstoßungstendenz korreliert. Keine statistischen Unterschiede gab es bezüglich Hospitalisierungsraten und Infekten.

Schlussfolgerungen: Eine Proteinurie so wie unter Rapamycin konnte für Everolimus nicht observiert werden. Nierenfunktionsparameter zeigten sich bei Dosisreduktion von CsA stabil.

77 The prognostic validity of ventricular evoked response (VER) signals in heart transplantation

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Background: Computerized Heart Allograft Rejection Monitoring (CHARM) used for non invasive rejection monitoring in HTX recipients is based on the analysis of ventricular evoked response (VER) signals. The aim of this study was to evaluate the prognostic validity of the TslewC, a parameter calculated out of VER.

Methods: During oHTX two unipolar fractally coated screw-in leads were implanted epimyocardially, one to the left and one to the right ventricular and connected to a telemetric pacemaker. Recordings of IEGMs were performed routinely at hospital. Data processing was done, artifacts were eliminated and trend curves of the patients were displayed. TslewC was calculated from the tangent of VER and evaluated from 105 patients. The patients were divided in survivors and non-survivors. For statistic analysis two-tailed *t*-test was performed.

Results: Patients in the non-survivors group compared to the control group showed a significant lower TslewC in the final follow-up [$p < 0.001$]. The differences were even more impressive in patients with a recorded IEGM 30 days prior to death or with a cardiac death reason. Tests were performed to find an optimal prognostic threshold of the TslewC and finally found the value of 26 mV.

Conclusions: TslewC can function as prognostic factor after oHTX. Further studies should be initialized to provide the prognostic threshold so that patients would not be forced to show up routinely all 4 weeks for follow up visits. Patients would only have to be admitted to hospital if the TslewC is under this prognostic threshold.

78 Use of extracorporeal photoimmunotherapy in the treatment of refractory recurrent rejections in heart transplant patients

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Background: The availability of numerous and novel immunosuppressive drugs has not totally prevented the presence of recurrent rejection resistant to conventional immunosuppressive therapy. Outcome of patients with these complications is grave. The aim of the present pilot study was to explore the feasibility of a new extracorporal photoimmunotherapy (ECP, photopheresis) schedule to prevent recurrent rejection and improve outcome in this selected group of patients.

Methods: Three patients (3 males, age range: 21–38 years) after heart transplantation (range 8 months to 7 years) suffering from recurrent refractory rejections were entered into this study after signing an informed consent. Extracorporal Photoimmunotherapy (ECP, photopheresis) was performed with a Therakos (Exton, Pa., USA) Photopheresis unit (Uvar XTS). Whole blood was removed from the patient and centrifuged for separation of its components; the obtained white blood cell (buffy coat)/plasma fraction was then irradiated with ultraviolet A light after the addition of a predetermined amount of 8-methoxypsoralen (8-MOP). Subsequently the photoactivated buffy coat cell/plasma fraction was re-infused into the patient. The red blood cells were returned untreated. Prior to ECP, an endomyocardial biopsy, a coronary angiography and echocardiography were performed. Patients were treated in intervals of two weeks on two consecutive days (one cycle). After 4 cycles an endomyocardial biopsy was performed in every patient. Routine work up with echocardiography and blood sampling was done before and after every cycle and whenever necessary. No changes in maintenance immunosuppressive therapy (Tacrolimus/Sirolimus/Corticosteroids, Tacrolimus/Mycophenolate Mofetil/Corticosteroids, Tacrolimus/Everolimus/Corticosteroids) was undertaken during the treatment period.

Results: ECP was tolerated well by all patients. The mean follow up period was 7 months of ECP with 12.3 cycles of therapy. One patient did not show a benefit still having episodes of rejections, while the remaining two showed stable graft function with no further signs of rejections.

The first patient had 3 rejections, 1 treated with corticoid bolus therapy before and no sign of rejections after ECP. The second showed 9 rejections before ECP, one treated with corticoid bolus therapy. After beginning with ECP there were still 3 rejections, but all of lower grades than before. The third patient had 2 rejections before ECP. After beginning of ECP he showed rejections again ($n = 2/3$ treated with corticoid bolus therapy). All patients had their levels of immunosuppressive therapy within the targeted range before and after ECP.

Conclusions: This early experience in a small number of patients showed a clear benefit in the majority of patients. Further studies in a larger patient population are mandatory to research the impact of this technology.

79 First experience with the Transmedics Organ Care System (OCS) in Austria

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Background: Early graft dysfunction is still a major cause of death after cardiac transplantation. Long ischemic time (>4 hours) is closely associated with graft dysfunction. Therefore, ischemic time poses a major limitation for cardiac transplantation.

Methods: The OCS is a portable organ perfusion and monitoring system for donated hearts. It was constructed with the intention to preserve the donated heart under conditions very

similar to physiologic ones during transportation. The heart is continuously perfused with warm, oxygenated donor blood, to which a nutritional solution and drugs were added.

Results: Four patients could be successfully transplanted within the PROTECT trial at the Medical University of Vienna. Cold ischemic time could be significantly reduced and averaged at 78 minutes with a mean warm perfusion period of 223 minutes. 3 patients could be extubated within 24 hours. A survival rate of 100% could be achieved.

Conclusions: With the Transmedics Organ Care System a completely novel and safe tool is present to offering the possibility of harvesting and transporting a donor heart in a very gentle and physiological way. Furthermore the OCS provides monitoring and checking the donor organ ex vivo. Due to this re-evaluation of the heart a modulation and optimization of the organ could be possible and in case of malperformance the heart transplantation can early be postponed to avoid primary organ failure. The donor pool might be expanded due to reduction of ischemic time.

80 Implementing an immunosuppressant TDM HPLC-MS/MS platform in a clinical environment: method comparison as key step for the performance evaluation

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Background: Within the past year, the ZIMCL has validated a novel HPLC-MS/MS routine assay for immunosuppressant TDM. It has been put into operation in 7/2007 and has been replacing three individual immunoassays previously used for the quantification of cyclosporin A, tacrolimus, and everolimus. In addition, this assay allows monitoring of sirolimus drug levels, which has not been addressed before. To mature such a novel HPLC-MS/MS assay to full clinical routine applicability, detailed performance evaluation studies are needed. This holds especially true, if the European in vitro diagnostics directive 98/79/EG has to be met as it is the case in Austria. Central to these investigations are comparative measurements with current TDM assays in use. These inter-assay comparisons have to be performed over prolonged time spans to include samples from most (ideally all) transplantation types to be expected at the respective hospital site.

Methods: In the case of the ZIMCL HPLC-MS/MS assay performance evaluation study, a two-step strategy was chosen. After performing a basic analytical validation of the assay, routine patient specimen (about 4000 tacrolimus, 4000 cyclosporin A, and 500 everolimus samples) were measured in parallel on the HPLC-MS/MS assay and the respective immunoassay (tacrolimus: MEIA/IMX; cylosporin A: FPIA/AxSym; everolimus: FPIA/TDX) over a time span of eight months. Data analysis also encompassed calibration and control results gathered in this time period (several thousand calibration and quality control data points) to evaluate the long-time stability and overall variability of the assay. Individual longitudinal patient profiles were used to evaluated bias fluctuations over time (i.e. bias development after transplantation), between patients groups (i.e. outpatients vs. ICU patients), and between transplantation types.

Results: More than 98% of all calibrators were found within $\pm 15\%$ of the target value and about 98% of the commercial quality control materials were measured within the assigned 2S ranges (usually $\pm 20\%$ of the target value). The established relative method bias distributions (derived from Bland-Altmann plots) were found to mirror the literature (cyclosporin: $-17 \pm 17\%$, tacrolimus: $-8 \pm 22\%$, everolimus: $-26 \pm 23\%$). The assay showed a long time measurement imprecision of about 7%.

Conclusions: The prolonged period of parallel measurement did prove, that the now fully validated and operational novel HPLC-MS/MS assay is a valuable analytical tool for routine TDM. It is measuring over the desired analytical range with accuracy and precision data meeting international requirements for analytical method validation. HPLC-MS/MS derived immunosuppressant TMD results are not only more precise than immunoassay based data but are not influenced by analyte metabolite levels or endogenous factors like the haematocrit. Hence they show less fluctuations over time, which allows to monitor the important patient related drug level changes more closely.

81 Building of long auxiliary support of the liver and cell therapy in quality of "bridge" to the liver transplantation

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Background: The basic problem of a transplantation is constant deficiency of donor organs. Because of shortage of grafts of a donor liver up to 50% of potential recipients die, not having waited a suitable donor organ. Treatment of hepatic failure in a pretransplantational period till now is one of challenges and existing methods of treatment not always are effective. In this connection intensive researches on search of new efficient methods of treatment hepatic failure proceed. One of such methods is the transplantation xeno- and allogenic hepatocytes. Last years the special attention is involved with synthetic bio-supporting systems at a transplantation of autologisted hepatocytes that allows to win time before OLT.

Methods: Isolated hepatocytes obtained at rats from a resect field of a liver. After the control of vitality isolated liver cells seeding inside of biodegraded matrixes by their permeating by a suspension of cells in concentration 1×10^6 cell/ml. In experiences on cultivation of cells used biodegraded matrixes which have been developed by the center on research of biomaterials National Research Institute of Transplantology and Artificial Organs. Matrixes with cells seated in conditions in vitro and incubated them within 3–4 day. After end of term of an incubation in matrixes defined vitality and concentration of cells. Matrixes, with cells immobilized in them, were transplantated in a mesentery of an intestine earlier operated animal. For 3, 5, 7, 9, 12 and 21 days investigated dynamics of a germination of pots in a matrix and vitality of cells in a matrix, by

building virtual three-dimensional voxeled models from series of pictures of a macro-preparation.

Results: The procedure of separation of hepatocytes from a resect field of a liver is optimized. The procedure of determination of vitality and concentration of cells in matrixes is optimized. The protocol of operation on an implantation of biodegraded matrixes in a mesentery of an intestine to rats is developed. The procedure of research of dynamics of a germination of vessels in matrixes is developed. The germination of venous vessels in a matrix stocked with hepatocytes for 10 day after an implantation is statistically authentically revealed. Results of histological research have shown presence of viable hepatocytes on biodegraded matrixes.

Conclusions: Research has shown vitality of hepatocytes on a new three-dimensional biodegraded matrix and an opportunity of use of this method with the purpose of enriching a state of potential OLT recipients.

82 Identification of non-HLA antigens in renal transplantation by proteomics

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Background: The main cause of graft loss in renal transplantation is chronic allograft nephropathy which is present in almost 60% of patients at 10 years. The pathogenesis of chronic rejection remains unclear but recent data show an involvement of graft specific antibodies. While anti-HLA antibodies lead mainly to early acute graft rejection, late rejection seems to be associated with non-HLA immunity. However, the targets of these antibodies are unknown.

Aim of the study: Our study uses a systematic proteomic analysis to characterise non-HLA donor-specific antibodies in recipients before transplantation.

Methods: Serum was taken from 8 dialysis patients from the renal transplantation waiting list with high panel reactive antibody (PRA) values (>85%). In addition, lymphocytes were isolated from these patients as well as from 10 healthy volunteers. For characterisation of the antibody specificities lymphocytic proteins were separated by SDS-gel electrophoresis and incubated with patient's antibodies in a Western blot. To isolate the antigenic proteins an immunoprecipitation (IP) was performed using patients's antibodies. The precipitate was then separated by SDS-gel electrophoresis as well as by 2-dimensional gel electrophoresis.

Results and conclusions: The Western blots showed antibody binding to some lymphocytic proteins from healthy donors which was not visible in patient's own lymphocytes. We regarded these proteins as allo-specific antigens. Most of these antigens had a molecular weight different to that of HLA. Although all patients showed a specific pattern of allo-reactive antibodies there was considerable overlap. The IP allowed a successful isolation of most of the donor-specific antigens. These proteins could be used for screening of renal transplant patients for non-HLA antibodies. The possibility of identifying recipients at increased risk of late graft loss before transplantation could be used to devise specific immunosuppressive strategies for these patients.

83 Chimerism-studies after fludarabine-based reduced-intensity conditioning and allogeneic hematopoietic stem-cell transplantation in pediatric patients with hematologic and oncologic disorders

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Background: Reduced intensity conditioning regimens are increasingly used for conditioning prior to allogeneic stem cell transplantation in patients with serious comorbidities, heavy pretreatment or multiply relapsed disease. We report 13 pediatric and adolescent patients with hematologic and oncologic disorders who were treated with fludarabine-based conditioning regimens followed by allogeneic hematopoietic stem cell transplantation at our institution between 2003 and 2007.

Methods: The 13 patients (m:f = 8:5) with a median age of 10.8 years (range: 2.4–21.25 yrs) suffered from AML ($n = 7$), neuroblastoma IV ($n = 2$), 2nd relapse of ALL, autoimmune lymphoproliferative syndrome (ALPS), severe congenital neutropenia (SCN) and 2nd relapse of Hodgkin's disease, respectively ($n = 1$). Conditioning consisted of fludarabine and melphalan in all patients with the addition of thiotepa and OKT3 in 4, ATG in 4 and Campath in 1 patient(s). 10/13 patients had 10/10 HLA allelic matched donors: 5 matched siblings, 3 MUD and 2 HLA-identical mothers; whereas 3 patients received grafts from haploidentical parents. Stem cell sources were bone marrow ($n = 7$), peripheral stem cells (PSC) ($n = 5$) and a combination of both ($n = 1$), respectively. 4 patients received manipulated PSC-grafts, (2 runs of aphereses: the first CD-34 selected and the second CD3/19 depleted, respectively). 1 patient received an unmanipulated PSC-graft. The median number of CD34+ cells/kg BW was 5.87×10^6. (range 2.36–21.6).

Results: Leukocyte engraftment ($\geq$1000/µl) occurred median on day +10 (range +8 to +35). Donor chimerism was determined in weekly intervals by SNPs (12/13) and VNTR (1(13), respectively. Complete donor chimerism (CDC) defined as $\geq$98% by SNPs and 100% by VNTR was achieved in median on day +18 and maintained in all but 2 patients: in a patient with AML an autologous population up to 18% was detected from day +65 on; upon withdrawal of immunosuppression the patient slowly reachieved complete donor chimerism. The patient suffering from SCN developed incipient graft rejection from day +28 and was treated with donor lymphocyte infusions in incremental dosages reverting him to CDC, accompanied by an acute GVHD of skin, liver and gut. The 2 patients with neuroblastoma received donor-lymphocyte infusions for the treatment of persistent disease. All patients survive for a median of 753 days (range 19–1493 days). The patients with hematologic malignancies are in complete remission; however the 2 patients with neuroblastoma are in a state of stable or slowly progressive disease, respectively.

Conclusions: In these 13 pediatric and adolescent patients fludarabine-based reduced intensity conditioning regimens followed by allogeneic hematopoietic stem cell transplantation lead to rapid leukocyte engraftment followed by complete

donor chimerism in median on day +18 which was stable in 11/13 patients allowing for sustained disease control in the patients with hematologic malignancies. Patients after RIC achieved full donor chimerism as early as patients after myeloablative conditioning, even in the haploidentical donor setting.

84 Outcome of calcineurin inhibitor-free immunosuppression after renal transplantation in the eurotransplant senior program – a randomized prospective trial

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Background: The Eurotransplant Senior Program (ESP) started in 1999 with local allocation of kidneys from donors elder than 65 years to recipients elder than 65 years. The requirements for immunosuppression in this group of patients are high due to the large amount of comorbidities.

Methods: This prospective randomized trial compared the standard immunosuppressive protocol based on cyclosporine A (CyA) to a calcineurin inhibitor-free protocol based on sirolimus (SRL). Based on a single center database, 30 kidney allograft recipients transplanted at our center from 2003 to 2005 were included. They were randomized into two groups for initial immunosuppression: CyA group $n = 16$ patients (53%), SRL group $n = 14$ patients (47%). The mean age was 69 years (68 years in CyA/70 years in SRL group). Follow-up time was 12 months. The prevalence of obesity (body mass index > 30) was $n = 10$ (33%). Eleven patients (37%) were diabetics before transplantation. Our primary endpoint was the calculated GFR (GFR improvement) after 6 months. We also analyzed comorbidities, drug dosing, side effects and monitoring, cold ischemia time, HLA-mismatch, protocol discontinuance, biopsy-proven rejection, graft loss, other adverse events and patient death at 12 months postoperatively.

Results: Baseline characteristics did not differ between both groups. GFR (calculated) after 6 months showed no significant difference (40.0 in CyA group vs. 43.3 ml/min/1.73^2 in SRL group). Preterm withdrawal was found in 13% ($n = 2$) in CyA vs 71% ($n = 10$) in SRL group. The biopsy-proven rejection rate was $n = 4$ (25%) with CyA and $n = 3$ (21%) with SRL. Delayed graft function occurred in 37% (7 patients [44%] in CyA and 4 patients [29%] in SRL group). Graft loss was 25% ($n = 4$) in CyA group and 7% ($n = 1$) in SRL group. Median time to graft loss was 39 days (range: 10 to 100). Overall survival after 12 months is $n = 22$ (77%). 63% ($n = 10$) survived the follow-up period in the CyA and 86% ($n = 12$) in the SRL group.

Conclusions: This analysis indicates no significant difference between the two immunosuppressive protocols. Sirolimus based immunosuppression did not lead to an increased GFR. Both groups showed a high mortality and morbidity without any significant difference. Optimal immunosuppression for this selected and high risk patient group still has to be defined. Careful patient selection seems to be critical for the outcome.

85 Outcome after laparoscopic live-donor nephrectomy: a single center experience with 110 consecutive cases

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Background: Living donor kidney transplantation provides superior short- and long-term patient and graft survival when compared with cadaveric kidney transplantation. To keep the morbidity of live kidney donors as low as possible laparoscopic technique was introduced.

Methods: We examined the results of two surgeons who performed the first 110 cases of laparoscopic living donor nephrectomy (only left side) at our center between 06/2000 and 06/2007. Surgeon 1: 50 operations (45%), surgeon 2 (transplant surgeon): 60 operations (55%). Prospective databases were reviewed for both donors and recipients. Donor surgeries were performed transperitoneally using 3-port technique. Kidneys were extracted through a 6 cm Pfannenstiel incision.

Results: The mean donor and recipient age was 47 years (±13) and 38 years (±19). 71 (65%) of the donors were women and 39 (35%) men. Their mean body mass index was 26 kg/m^2 (±4). The average operative time was 213 minutes (110–390 min.), the average warm ischemia time was 169 seconds (60–720 sec.). Intraoperative complication rate was 10% – predominantly bleeding (36%). Conversion rate was 4%. Most (58%) of the operative complications occurred during the first 50 operations. All of the donors demonstrated rapid recovery. The average length of hospital stay was 6 days (3–13 days). The mean serum creatinine levels on admission, discharge from hospital, and at last follow up were 0.89 mg/dL, 1.3 mg/dL and 1.3 mg/dL, respectively. 105 transplanted kidneys demonstrated immediate onset of function, the mean creatinine on day 7 was 1.6 mg/dL. Delayed graft function was observed in 5 recipients (4.5%). Acute rejection rate was 29%. Posttransplant surgical complications occurred in 28 cases (27%), among them 29% were urological complications. The overall graft survival rate (mean follow up 21 months) was 93%.

Conclusions: According to our results, laparoscopic living donor nephrectomy is linked with acceptable immediate morbidity and excellent graft function when performed with sufficient experience.

86 Laparoscopic living donor nephrectomy for pediatric recipients: outcome analysis

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Background: Laparoscopic nephrectomy has become the technique of choice for live donor nephrectomy. Limited data are available regarding the role of this technique for pediatric recipients, who may pose special challenges.

Methods: From 06/2000 to 06/2007 110 laparoscopic living donor nephrectomies have been carried out at our center. We reviewed our experience of 18 pediatric recipients (age ≤18 years) who received a living donor kidney. Patient demo-

graphics, intra-operative events, serum creatinine decline and graft function were analyzed.

Results: The mean donor and recipient age was 39.7 (±5.6) and 10.8 (±5.4). All cases involved the left kidney. 94% ($n = 17$) were parental donors. 15 (83%) donors were women and 3 (17%) were men. Their mean body mass index was 25 kg/m^2 (±4). Mean operative time was 208 (±67) minutes and mean warm ischaemia time was 158 (±46) seconds. Donor surgery complication rate was 11%, all due to bleedings without further complications postoperative. The average length of hospital stay was 5 days (range 3–10 days). The mean serum creatinine on discharge was 1.1 mg/dL. In 2 out of 3 cases we reused c-section scars of female donors for mini-laparotomy. All grafts functioned immediately, with a mean creatinine at postoperative day 7 of 1.1 mg/dL. At last follow up (mean 26.4 months) the mean creatinine was 1.37 mg/dL. 88% ($n = 16$) of the children received their first kidney transplant. One patient was transplanted the third, another one the second time. Acute rejection incidence was 28%. 11% ($n = 2$) of the patients underwent posttransplant surgery. One recipient suffered from wound healing disturbances, the other one required reimplantation of the ureter. 2 recipients (11%) died due to infection and another one lost the graft after rejection. The overall graft survival rate after one year was 94%.

Conclusions: Laparoscopic living donor nephrectomy is a safe modality for pediatric recipients and provides organs with excellent function.

87 Enterocolitis due to simultaneous infection with Rotavirus and Clostridium difficile in adult and pediatric solid organ transplantation

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Background: Diarrhea is a well known complication of immunosuppression but also frequently caused by pathogens such as Clostridium difficile (CD) and Rotavirus (RV).

Methods: Three adult and five pediatric solid organ recipients (SOR) developed diarrhea with simultaneous identification of CD and RV. RV was identified using an immunochromatografic- or enzyme-linked-immunosorbent-assay, CD using a rapid immunoassay or enzyme immunoassay.

Results: One adult renal, one adult kidney-pancreas, one adult liver and five pediatric liver recipients were affected. Onset of RV/CD infection ranged from two weeks to four years post transplant. All patients presented with enterocolitis causing significant fluid and electrolyte loss. In adults, CD was treated with metronidazole and in children with oral vancomycin. RV infection was treated with fluid/electrolyte replacement. During diarrhea, a significant rise in tacrolimus serum level was noted. All patients cleared CD. One child developed recurrent episodes of RV infection and died from bacterial sepsis; the renal recipient died six months post transplant from myocardial infarction. The remaining six patients are currently alive with well functioning grafts.

Conclusions: Simultaneous infection with CD and RV may lead to severe diarrhea in SORs. Both pathogens should be considered in SOR presenting with diarrhea.

88 Zygomycosis and other rare filamentous fungal infections in solid organ transplant recipients

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Background: Fungi cause severe infections in solid organ recipients. Recently, a shift from Aspergillus spp and Candida spp to other rare yeasts and moulds was noticed.

Methods: In a series of 2875 solid organ transplants (kidney, pancreas, islets, liver, heart, lung, and bowel) performed between January 1995 and December 2006 at the Innsbruck medical university, eleven cases of non-Aspergillus filamentous fungal infections were diagnosed.

Results: The encountered species included Zygomyzetae ($n = 8$), 1 Alternaria alternate, Pseudallescheria boydii, Trichoderma spp. (one each). Five patients died from invasive fungal infection (zygomycosis: $n = 4$, Pseudallerichia boydii: $n = 1$); of these five cases four were diagnosed post mortem. In five cases infection was surgically treated in combination with amphotericin B lipid formulation, itraconazole or posaconazole. Risk factors for these rare filamentous fungal infections were renal failure ($n = 8$, 73%), intensified initial immunosuppression ($n = 8$, 73%) or rejection ($n = 8$, 73%). Two were associated with post transplant lymphoproliferative disorder and one with graft versus host disease. An increase in the incidence of these emerging pathogens was found with only three cases before 2004 and the remaining seven cases during the past three years.

Conclusions: New fungal pathogens are increasingly found in solid organ recipients. If diagnosed in time, the outcome seems to be better than the outcome of invasive asperillosis. Intensified immunosuppression seems to be a major risk factor and surgical therapy may play an important role in these infections.

89 Drugmonitoring bei immunsuppressiver Therapie: Vergleich immunologischer Methoden mit der HPLC

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Grundlagen: Immunsuppressiva haben eine geringe therapeutische Breite. Eine regelmäßige Überwachung der Medika-

mentenspiegel ist daher notwendig. Ziel dieses Berichts war, immunologische Routine-Methoden für Cyclosporin A (CyA), Mycopholate Mofetil (MMF) und Everolimus (ERL) mit den HPLC-Referenzmethoden zu vergleichen.

Methodik: CyA: 248 Proben von 117 Patienten (103 NTX, 78 HTX, 810 LTX, 53 KMTX), MMF:195 Proben von 69 Patienten (32 NTX, 21 HTX, 10 LTX, 6 KMTX), ERL: 165 Proben von 52 Patienten (40 NTX, 12 HTX). Die immunologischen Messungen von CyA und MMF erfolgten am Dimension Xpand bzw. am Cobas Mira S (Enzyme-Multiplied Immunoassay Technique (EMIT); spezifischer monoklonaler Antikörpers). Die Bestimmung von ERL erfolgte am TDxFLx Analyzer (Innofluor Certican Assay System mittels Fluoreszenz-Polarisations-Immuno Assay (FPIA)). Die Konzentrationen aller drei Medikamente wurden auch mittels HPLC bestimmt.

Ergebnisse: Korrelation nach Passing-Bablock: immunologischen Teste vs HPLC Methode: CyA: $r^2 = 0.93$ ($y = 1.27x +$ 32,21); MPA: $r^2 = 0.97$ ($y = 1.079x + 0.287$); ERL: $r^2 = 0.92$ ($y = 1.11x + 0.38$). Auswertung nach Bland und Altman (95% Wahrscheinlichkeit) für CyA Dimension *vs HPLC* ($27.2 \pm$ 54.15), für MPA EMIT *vs. HPLC* (0.466 ± 0.81) and für ERL; TDxFLx *vs. HPLC* (1.19 ± 2.57).

Schlussfolgerungen: Die erhobenen Daten zeigen, dass die grundsätzliche Übereinstimmung zwischen immunologischen Methoden und HPLC zwar sehr gut ist, im Einzelfall jedoch auf Grund von Interferenzen mit Metabolite und Co-Medikamenten ungenaue Ergebnisse der immunologischen Methoden zu problematischen therapeutischen Entscheidungen führen könnten.

90 A modified Ricordi method for the prevention of oxidative stress in the isolation of porcine pancreatic islet cells

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Background: High yields of pure and viable porcine islet cells (PIC) to be used for microencapsulation are crucial for successful xenotransplantation. Mechanical disruption of the pancreas, enzymes used for digestion, digestion temperature and time are among the factors known to cause oxidative stress and to impact on yield, purity and viability of PIC. The aim of our study was to optimize conventional procedures in order to minimize oxidative stress that occurs during the isolation and purification of PIC.

Methods: Porcine pancreata were harvested at a local slaughterhouse and 15 consecutive isolations of PIC were performed with a modified Ricordi method which uses shorter digestion time, lower digestion temperature and minimal mechanical stress. PIC were purified with a Lymphoprep™ density gradient. Purity and viability were assessed immediately after the isolation process and after overnight culture. PIC function was tested in glucose stimulation experiments and insulin concentration was determined by ELISA. Carbonyl proteins (CP) and lipase levels were assessed with chemoluminescence or a colorimetric liquid assay, respectively.

Results: The mean yield of PIC was 3479 ± 524 IEQ/g pancreas, with 96.4% viability and 97.7% purity. There was no significant loss in PIC viability after overnight culture. Insulin secretion in response to glucose was not impaired after isolation and purification. CP and lipase levels did not change significantly during the isolation procedure.

Conclusions: The modifications of the Ricordi method seem to prevent substantial oxidative stress and resulted in high yields of pure and viable PIC.

91 Transplantation and characterisation of 293-Luc cells microencapsulated in Sodium Cellulose Sulfate in a prevascularized site in rats

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Background: Naturally, islet cells are highly vascularised in the pancreas and this physiological vessel structure is damaged during the isolation process. Isolated islet cells are dependent on diffusion of oxygen from the surrounding tissue. After transplantation a lot of isolated islet cells become apoptotic because of hypoxia and insulin independency can not be achieved because of graft dysfunction what is even worse when working with microencapsulated islet cells. Therefore we attempted to create a prevascularized site using a V.A.C.® (Vacuum Assisted Closure) GranuFoam™ that is normally used in wound healing and HBO (hyperbaric oxygenation) to induce angiogenesis.

Methods: Forty Sprague-Dawley rats are divided in 5 groups and the V.A.C.®-GranuFoam™ is implanted in the abdominal subcutaneous tissue. HBO is administered to the different groups at different time-points for at least one week after implantation to a maximum of 2 weeks prior and 2 weeks after implantation. 293-Luc cells are microencapsulated with Sodium Cellulose Sulfate (NaCS) in cooperation with Austrianova, Vienna. Luciferin is administered via the tail vein at different time points and in-vivo bioimaging is used to quantify viable cells in the microcapsules transplanted in the prevasularized site. Szintgraphy is used to measure vascularisation and The V.A.C.®-GranuFoam™ and the microcapsules are explanted and processed for histology and immunohistochemistry.

Results: It is feasible to create a prevascularised site in the subcutaneous fatty tissue of rats using a V.A.C.®-GranuFoam™ and HBO. Angiogenesis is not induced without HBO within one months after implantation of the V.A.C.®-GranuFoam™ but within 2 weeks after implantation of the system and HBO therapy. Vessels are not only distributed in the outer

parts of the V.A.C.®-GranuFoam™, the whole sponge-like V.A.C.®-GranuFoam™ is pervaded by new vessels. It is feasible to microencapsulate 293-Luc cells with NaCS. Transplantation of the microcapsules in the prevascularized site does not impair cell viability as shown by in-vivo bioimaging.

Conclusions: As ischemically damaged islet cells are likely to undergo cell death or loose functionality due to hypoxia, the use of the V.A.C.®-GranuFoam™ and HBO might be a promising method to create a prevascularised site to achieve better results in islet transplantation.

92 Oxidative stress and chemokines in organ donation: the impact on organ quality and transplantation outcome

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Background: The concentration of Carbonyl proteins (CP), a sensitive parameter of oxidative protein modification caused by oxidative stress which occur when radicals or reactive oxygen species generated in the mitochondria react with proteins forming irreversible oxidative modifications of these molecules can have a significant impact on organ quality after transplantation. The aim of this study was to show the impact of carbonyl proteins and nitrotyrosines (NT) as markers for oxidative cell damage, adhesion molecules, cytokines and molecules that are elevated in pro-inflammatory states like sCD40L in the multi-organ donor on organ quality and transplantation outcome.

Methods: Samples of 30 multi-organ donors were collected prior to organ donation and processed for further analysis. Measurement of CP in the serum is performed after derivatization with 2,4-dinitrophenyl-hydrazine (DNPH) by a chemiluminescence technique on a chemiluminescence reader (Lumistar, BMG, Germany) after addition of 200 µL/well Super Signal Maximum Sensitivity substrate (Pierce, Rockford, USA). Monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP), the adhesion molecules soluble platelet vascular cell adhesion molecule (sP-VCAM) and soluble platelet selectin, sCD40L and the interleukins IL-6 and IL-8 are determined using FlowCytomic, a multiple analytic detection method on the flow cytometer. When feasible, organ biopsies are collected and stained for carbonyl proteins and nitrotyrosines immunohistochemically. Data from the organ recipients is collected at different time points and the influence of the parameters measured on organ function and patient survival after transplantation is evaluated.

Results: CP, NT, MCP-1, MIP, sP-VCAM, sP-selectin, sCD40L, IL-6 and IL-8 are significantly elevated in brain death patients as compared to healthy persons. The concentration of the interleukins is dependent on the cause of death and the time on intensive care prior to organ donation. CP and NT are elevated in organ donors with a history of cardiovascular disease and increase significantly with time on intensive care and the time after brain death. The concentration of all the parameters mentioned above seems to have an impact on organ function after whole organ transplantation.

Conclusions: As oxidative stress and activation of interleukins and other pro-inflammatory proteins increased with time on intensive care and are dependent on the cause of death prior to organ donation, time prior to organ donation after brain death should be shortened and methods to minimize oxidative stress in multi-organ donors should be investigated.

93 p38 MAPK and ROS control cell death during ischemia and reperfusion: possible therapeutic implications?

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Ischemia (I) and reperfusion (R) trigger a series of events, which culminate in severe injuries to transplanted organs. Reactive oxygen species (ROS) have long been implicated in IR-associated tissue damage. ROS causes cell death typically by triggering of pro-apoptotic signaling and through effects on intracellular Ca^{2+} trafficking. Various protein kinase activities including mitogen activating protein kinases (MAPKs) modulate the extent of cellular damage. We have previously shown that the activity of MAPKs was significantly altered during IR, suggesting that they may provide a promising target for ameliorating reperfusion injury. Here we probed into the role of ROS and MAPK signaling in the control of IR damage and searched for a link between these modulators.

Experiments were performed using the cardiomyocyte cell line HL-1, primary cardiomyocytes or a murine heart transplant model. MAPK activation was assessed with phosphorylation-specific antibodies. ROS and Ca^{2+} were measured after DCF or Rhod-2 loading of cells using confocal microscopy.

(i) Ten minutes of reoxygenation following 45 minutes of hypoxia resulted in a dramatic and almost synchronous increase in ERK, JNK, and p38 activity. (ii) Late reoxygenation showed a return of MAPK activity to basal levels. (iii) In vitro a significant increase of apoptosis was observed after 48 hours of reperfusion. (iv) Selective inhibition of p38 by SB203580 but not of MEK1,2 or JNK significantly reduced cell death. (v) 45 minutes of hypoxia also lead to a dramatic increase in mitochondrial ROS production and Ca^{2+} levels. (vi) ROS may be involved in cell killing since treatment with the anti-oxidant N-acetyl cysteine (NAC) simultaneously decreased ROS production and apoptosis.

These data demonstrate the suitability of the chosen experimental approaches for the investigation of I/R-associated alterations in intracellular signaling and cellular responses. They show that exposure of HL-1 cells to IR resulted in significant apoptotic cell death, caused in part also through excessive mitochondrial ROS production commonly also observed during IR in vivo. Selective p38 MAPK, but not MEK and JNK inhibition decreased apoptosis. Future work will dissect the possible connection between MAP kinase activation and ROS production.

94 Pharmcokinetics of a prolonged release formulation of tacrolimus (Advagraf)

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Background: Tacrolimus is a calcineurin inhibitor and has been established on the market for more than ten years under the trade name Prograf® and is one of the two cornerstone immunosuppressants used following organ transplantation. A lifelong maintenance therapy with an immunosuppressive agent is necessary to prevent transplant rejection. Currently the oral Prograf formulation is available as 0.5 mg, 1 mg, 5 mg hard capsules. In clinical practice Prograf capsules are administered as a twice-daily dosing regimen.

Poor compliance with dosing has been shown to be one of the factors associated with the incidence of transplant rejection and late graft loss [1–4]. In a prospective cohort study of 278 adult recipients of cadaveric donor renal transplants, Weng et al. [5] demonstrated a statistically significant association for adherence to medication regimen with once daily dosing versus twice daily dosing. Schweizer et al. [6] have suggested that a once daily dosing regimen may help to improve patient compliance. Thus a formulation that permits a once daily dosing regimen may help to improve compliance with dosing and thereby decreasing the risk of late graft rejection and loss.

Tacrolimus Prolonged Release Formulation – Advagraf: Advagraf is a prolonged release formulation of tacrolimus developed to provide once-daily dosing while maintaining similar safety and efficacy profile as for Prograf. Advagraf has been granted marketing authorization by the European Medicines Evaluation Agency for the prophylaxis of transplant rejection in adult kidney or liver allograft recipients and for and for treatment of allograft rejection resistant to treatment with other immunosuppressive drugs in adult patients.

Pharmacokinetic studies in stable adult kidney, liver and heart transplant recipients have shown that following conversion from Prograf to Advagraf on a 1:1 (mg:mg) total daily dose, systemic exposure to tacrolimus over 24 hour (AUC_{0-24}) was within the bioequivalence criteria. There was good correlation between AUC_{0-24} and trough level (C_{24}) of tacrolimus for Advagraf. Furthermore, the relationship between these two parameters for Advagraf is similar to that for Prograf. Efficacy and safety profile of Advagraf was comparable to Prograf.

Conclusions: Advagraf can be administered once daily in the morning on the basis of the same therapeutic drug monitoring concept as Prograf administered twice daily in the morning and evening.

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95 T-cell non-engraftment with imminent graft rejection after allogeneic stem cell transplantation in a patient with severe congenital neutropenia: treatment with repetitive donor lymphocyte infusions resulting in complete donor chimerism

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Background: We report on a boy suffering from severe congenital neutropenia who was treated with complementary medicine since his early childhood. At the age of 9 years he was admitted in life-threatening condition suffering from severe abscess-forming pneumonia. Genetic testing was performed detecting a nonsense mutation on exon 5 (Q208X) coding for neutrophil elastase. The only curative option was allogeneic stem cell transplantation (SCT) from his HLA-matched sister. In order to prevent gvhd the first run of aphereses was CD34+ selected and in order to facilitate engraftment a second run of aphereses was CD3+ /19+ depleted.

Methods: Because of multiple pre-existent infections a reduced-intensity-conditioning (RIC) regimen with fludarabin/melphalan/thiotepa and OKT3 was chosen. The patient received a total of 14×10^6/kg bodyweight CD34+ stem cells together with 8×10^4 kg/bodyweight CD3 T-cells.

Results: On day +4 engraftment was stimulated with pegfilgastrim and darbepoetin alpha. On day +10 white blood cells (WBC) reached 1750/µl, on day +14 platelet count was 143000/µl and haemoglobin 10.0 g/dl respectively, without further need for substitution. During the first two weeks of engraftment chimerism analysis revealed 98.06% of allogeneic WBC, followed by a decline to a minimum of 48.37% on day +43. This decrease was caused by recovery of an increasing autologous T-cell population consisting of CD3+/CD4+ and CD3+/CD8+ phenotype. Allogeneic T-cell proportion rapidly declined to 3% on day +31, therefore donor leukocyte infusions (DLIs) were started. After 9 DLIs allogeneic CD3+ cells had increased to 98.6% on day +105. On day +88, graft-versus-host disease (GVHD) occurred with a maximum of grade 3 of the skin, grade 2 of the liver and grade 3 of the gut. The GVHD of the gut was aggravated by adenovirus gastroenteritis with bloody watery stools. GvHD was controlled by applying multimodal immunosuppressive therapy including methylprednisolone, cyclosporine A, infliximab and daclizumab.

Conclusions: Mixed chimerism is a frequent finding after T-cell depleted SCT going along with a higher risk of graft rejection.

In our case the host showed nearly total donor chimerism two weeks after transplantation, however subtyping the WBC-population showed that especially CD3+ cell engraftment nearly failed showing a minimum of 3% donor Single Nucleotid Polymorphisms (SNPs) on day +14. To our knowledge this is the first case published of nearly failed T-cell engraftment that changed to engraftment after frequently applied DLIs. We suspect that RIC did not eliminate all host T-cells. The autologous CD3+ cell proportion was on a very active level because of the patient's neutropenia since birth and therefore having a nearly permanent stimulation. The transplantation of high amounts of CD56+ NK-cells and T-cell precursors after CD3/19 depletion could not eradicate the autologous T-cell proportion. Eradica-

tion finally could only be achieved by fresh donor lymphocytes, resulting also in GVHD.

96 Inhibition of the mammalian target of rapamycin (mTOR) promotes inflammatory cytokine production through NF-κB in dendritic cells

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Background: The serine/threonine kinase mammalian target of rapamycin (mTOR) has a central role in cell-cycle progression in many cell types including lymphocytes. The mTOR inhibitor rapamycin blocks lymphocyte proliferation and is increasingly employed as alternative treatment to calcineurin inhibitors to block allograft rejection. However, recent observations revealed distinct inflammatory conditions associated with the use of rapamycin in allogeneic transplant recipients such as lymphocytic alveolitis, interstitial pneumonitis, anemia, or even glomerulonephritis. The underlying mechanisms of these inflammatory disease states are not understood.

Methods and results: Here we analyzed whether components of the innate immune system like monocytes and dendritic cells (DC) are affected by rapamycin treatment. We demonstrate that Toll-like receptor (TLR) engagement activated the mTOR signaling pathway in innate immune cells in a rapamycin-sensitive manner. Rapamycin as well as genetic ablation of mTOR promoted production of the proinflammatory cytokines IL-12 along with IL-6 and TNF-α and strongly inhibited the anti-inflammatory cytokine IL-10 in monocytes and also myeloid DCs after TLR2 and TLR4 stimulation through a transcriptional process. As a molecular mechanism rapamycin enhanced activation of NF-κB, a master regulator of proinflammatory responses, via the p65 transactivation domain resulting in enhanced IL-12 and IL-6 production. Transcriptional profiling confirmed that mTOR negatively regulated NF-κB-dependant genes and mediators important for inflammation and immunoregulation. Moreover, inhibition of mTOR in dendritic cells enhanced T cell proliferation and IFN-γ production.

Conclusions: Our findings bear important implications for the understanding of the frequently observed inflammatory incidences observed in transplanted patients receiving rapamycin as an immunosuppressant. Moreover, these results may add a novel dimension to the observed anti-tumor properties of mTOR inhibitors. In conclusion, we have demonstrated that rapamycin promotes proinflammatory reactions in vitro and in vivo and identify mTOR as a novel regulator of innate immune responses.

97 The fibrin-derived peptide B$\beta_{15\text{-}42}$ ameliorates ischemia-reperfusion injury in a rat heart transplant model

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Background: The purpose of this study was to evaluate the protective effect of the fibrin-derived peptide Bβ_{15-42} on ischemia/reperfusion injury in a rat cardiac transplant model. It has already been proved that Bβ_{15-42} reduces leukocyte migration over the endothelium by preventing the transmigration process. Since leukocyte migration plays a crucial role in the ischemia reperfusion injury during cardiac transplantation we hypothesized that the fibrin-derived peptide Bβ_{15-42} could ameliorate myocardial damage after cardiac transplantation.

Methods: Hearts of male Lewis rats (250–300 g) were flushed with chilled (0–1 C) Custodiol preservation solution and either transplanted immediately or stored for 4 or 8 hours in the same solution and then transplanted into syngenic recipients. 1.2 mg of the fibrin-derived peptide Bβ_{15-42} was given i.v. immediately after implantation of the heart or added to the preservation solution prior to harvest. At 24 hours and 10 days hearts were retrieved for morphological evaluation. At time of harvest, serum samples were collected for troponin T level analysis. In the group of 8 hours of cold ischemia and 24 hours of in vivo reperfusion functional evaluation of hearts were assessed in an isolated working heart by measuring pumpfunction curves (pressure-volume).

Results: Hearts transplanted immediately or after 4 hours of cold ischemia showed no morphological damage. In contrast, 8 hours of ischemia resulted in severe myocardial ischemia, associated with an inflammatory response at 24 hours. Lesions further progressed at 10 days. Administration of Bβ_{15-42} resulted in a significant amelioration of myocardial necrosis together with a diminished inflammatory response. A protective effect towards myocyte damage was further underlined by reduced troponin T levels in groups receiving Bβ_{15-42}. In the working heart preparation the administration of Bβ_{15-42} resulted in a significant amelioration of graft function.

Conclusions: The fibrin-derived peptide Bβ_{15-42} is a promising novel therapeutic approach for prevention of ischemia-reperfusion injury in cardiac transplantation.

98 A multicenter RCT of deceased organ donor pre-treatment with corticosteroids for the prevention of postischemic acute renal failure

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Background: This randomized placebo controlled study seeks to elucidate whether pre-treatment of deceased organ donors with corticosteroids before organ retrieval will decrease inflammation in the donor kidney and will reduce the rate or duration of postischemic acute renal transplant failure (ARTF). We have shown previously, that inflammation of the donor kidney prior to transplantation is among the main risk factors for ARTF (Hauser P et al. Lab Invest 2004, Kainz A, AJT 2004).

Methods: The predetermined interim analysis of 210 recipients will be presented. This analysis includes genome-

wide gene expression profiles of donor kidney biopsies with subsequent systems biology approaches such as transcription factors analysis, regulatory networks, and protein–protein interaction data. Trajectories of GFR in the first week after engraftment will be analyzed by mixed linear regression models for longitudinal data, the incidence of ARTF, defined as more than one post-transplant dialysis will be analyzed by Chi-square test.

Results: At the time of abstract submission 15 randomly selected biopsy samples have been analysed. Unsupervised hierarchical clustering of experimental data suggests a distinct molecular signature associated with reduction of inflammation in the steroid group. Selection of twofold differentially upregulated genes in the steroid group yielded 189 significant sequences that can be categorized according to PANTHER ontologies into four main biological processes: monosaccharide metabolism ($p < 0.001$) proteolysis ($p < 0.001$), proteintargeting and localization ($p < 0.003$), coenzyme and prosthetic group metabolism ($p < 0.003$).

Conclusions: These preliminary genomics data suggest that inflammation was reduced by steroid pre-treatment. The effect of this intervention on clinical endpoints of ARTF will be presented in the interim analysis at the meeting.

99 Anti-D alloimmunization after D-mismatched allogeneic stem cell transplantation following reduced-intensity conditioning

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Anti-D alloimmunization develops in up to 20% of RhD-negative patients on chemotherapy following exposure to RhD antigen, but is reported to be rare in recipients of haematopoietic stem cell transplants (HSCT), especially following myeloablative conditioning. After HSCT following reduced-intensity conditioning (RIC) rapid isohemagglutinin production of donor lymphocytes have been observed in the minor ABO-incompatible setting resulting in severe hemolysis. The objective of this study was to evaluate the incidence of anti-D alloimmunization after D-mismatched HSCT following RIC.

From 112 consecutive patients receiving RIC-HSCT between April 1999 and March 2006, 26 patients had a D-mismatched donor. Twelve RhD-positive patients had an RhD-negative donor, 14 RhD-negative patients received a RhD-positive graft. RIC consisted of the Saetle protocol (fludarabine and 2 Gy total body irradiation; TBI) or the FLAMSA protocol (amsacrine, fludarabine, cytarabine, cyclophosphamide, ATG and 4 Gy TBI). For graft-versus-host disease (GvHD) prophylaxis cyclosporin A (CsA) and mycophenolate mofetil (MMF) were given. From the day of HSCT, red blood cell support consisted of donor Rh-type RBCs.

After a median follow-up of 30 months, 16 of 26 patients with a D-mismatch donor were alive. Two RhD-negative patients died within 10 days after HSCT and were not evaluable for anti-D alloimmunization. Eight patients developed acute GvHD between days 7 and 52 and 11 patients chronic GvHD between days 75 and 274 after HSCT, respectively. RhD-positive patients with RhD-negative donors received a median of 11 (range: 0–92) RhD-negative RBC units during a median of 8 months (range: 0–38) after HSCT. RhD-negative patients with RhD-positive donors were transfused with a median of 11 (range: 0–44) RhD-positive RBC units during a median of 2 months (range: 0–54). There was no difference in transfusion requirements between D-mismatched vs. D-matched patients. After a median of 13 months (range: 0–73) in 2 RhD-positive patients with an RhD-negative donor an anti-D antibody was detected. They had sibling male donors who had no transfusion history or irregular antibodies detectable. Both patients never experienced acute or chronic GvHD.

Fludarabine based RIC and CsA with MMF given post transplant prevents anti-D formation in RhD-negative recipients of an RhD-positive graft. However, anti-D developed in RhD-positive recipients of an RhD-negative graft who never were exposed to RhD-positive blood products after HSCT. This maybe caused by the relative high amount of residual RhD-positive RBCs at the time of transplant and an immunosuppressive regimen with CsA and MMF were donor B-cells can escape T-cell control as seen in the minor ABO-incompatible setting. Therefore, patients with an RhD-mismatched donor should routinely be tested for RhD alloimmunization in the post-transplant course.

100 Myeloperoxidase als Serummarker bei CMV-Infektionen und Abstoßungsreaktionen transplantierter Patienten

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Grundlagen: Abstoßungreaktionen und Infektionen sind gängige Probleme nach Organtransplantation. Serumparameter stehen bei Patienten nach Lebertransplantation (LTX) zur Abstoßungsdiagnostik zur Verfügung, bei Patienten nach Herztransplantation (HTX) ist die Endomyokardbiopsie (EMB) Goldstandard, Serumparameter würden helfen dieses invasive Verfahren selektiver einzusetzen. Cytomegalie (CMV) Infektionen können bei transplantierten Patienten zu schwerwiegenden Krankheitsbildern führen und müssen deshalb früh erkannt und therapiert werden. Neben dem CMV-Antigen pp65 stehen derzeit keine ausreichenden diagnostischen Maßnahmen zur Verfügung. Ziel dieser Studie ist es, die Myeloperoxidase (MPO), als Enzym der neutrophilen Granulozyten, als neuen Parameter neben etablierten Serumparametern und der EMB bei Abstoßungen und Infektionen bei Patienten nach LTX und HTX auf ihre Sensitivität zu testen.

Methodik: Aus 246 Blutproben von 28 Patienten (19 LTX und 9 HTX) wurde die MPO im Plasma mittels ELISA (ImmunDiagnositk AG, Germany) bestimmt. CRP, GGT, Leukozytenzahl und CMV pp65 Antigen (Clonab CMV Kit, Biotest, Germany) wurden im Rahmen von Routinekontrollen, EMBs zu definierten Zeitpunkten nach HTX durchgeführt. Deskriptive Statistik, Mann Whitney U-test und Spearman's

Korrelation wurden angewendet, ein $p<0{,}05$ wurde als statistisch signifikant angesehen.

Ergebnisse: Die MPO-Werte bei Patienten mit Abstossungreaktion (7 LTX und 5 HTX) sind gegenüber einer Kontrollgruppe signifikant erhöht ($487 \pm 291{,}9$ vs. $121 \pm 45{,}5$; $p<0{,}001$). Sowohl bei LTX-Patienten ($533{,}8 \pm 352{,}54$ vs. $120{,}9 \pm 45{,}53$; $p<0{,}001$) als auch bei HTX-Patienten ($382{,}2 \pm 137{,}93$ vs. $120{,}9 \pm 45{,}53$; $p<0{,}001$) wurde derselbe signifikante Unterschied gefunden. Die MPO-Werte bei Patienten mit Infektionen (8 LTX und 2 HTX) und positiver CMV ($n=6$) sind gegenüber Patienten ohne Komplikation signifikant erhöht ($p<0{,}001$). Schon vor der klinischen Manifestation der Infektionen bzw. Abstoßungsreaktion kommt es zu einem signifikanten Anstieg der MPO.

Schlussfolgerungen: Die MPO ist im Vergleich zu den anderen untersuchten Parametern bereits vor der klinischen Manifestation einer Infektion oder Abstossungsreaktion erhöht und könnte deshalb als sensitiver Serumparameter zur Früherkennung sowohl einer latenten Infektion als auch Abstossungsreaktion eingesetzt werden.

101 Outcome of umbilical cord blood transplantations in adults with high-risk hematologic malignancies

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Background: Umbilical cord blood (UCB) is increasingly used as an alternative source in adults who need an allogeneic transplantation but lack a suitable donor.

Methods: Between December 2001 and May 2007 thirteen patients (8 AML, 2 ALL, 1 AUL, 1 CML with blast crisis, 1 Hodgkin's disease) with a median age of 34.5 years (range: 21–54) underwent 16 UCB transplantations. All recipients had relapsed or resistant disease. Myeloablative conditioning was performed in seven and reduced intensity conditioning in six patients. GvHD prophylaxis consisted of ATG (5 mg/kg from days -4 to -1), cyclosporine A (starting on day -1 with 3 mg/kg/d iv.) and methotrexate ($10\,mg/m^2$ on days $+1$ and $+3$). All cord blood grafts were mismatched. A second UCB transplantation was performed in two patients because of graft failure (only ATG was given before the 2nd graft) and in a third patient because of chemosensitive relapse. Due to low cell count a double UCB graft was used in one patient. The median nucleated cell dose was 3.8×10^7/kg (range: 2.5–6.3).

Results: Neutrophil recovery occurred at a median of 35 days (range: 25–51). Graft failure was observed in 2 cases. Six patients died because of infections and 4 because of progression or relapse of disease. Five patients developed acute GvHD (grade I, $n=1$; grade II, $n=4$). No chronic GvHD has been observed. In July 2007, three of 13 patients (23%) are disease-free with a follow-up of 66, 12 and 11 months. All of them have a Karnofsky index of 100% and are back at work.

Conclusions: The outcome of these patients with advanced stage of disease treated with UCB transplantations is comparable with published data using a suitable HLA-matched donor. UCB transplantation should be considered if no suitable donor is available in time.

102 First-line therapy in multiple myeloma patients with bortezomib, doxorubicin and dexamethasone followed by autologous stem cell

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Background: Induction chemotherapy followed by high dose melphalane and autologous stem cell transplantation (ASCT) is considered the standard treatment for patients with multiple myeloma. Incorporation of new agents like the proteasome inhibitor bortezomib (PS-341) in combination with doxorubicin and dexamethasone (PAD) increased response rates in induction therapy significantly and may also contribute to improved results after autologous transplantation.

Methods: Stem cell mobilisation after 4 cycles of PAD was performed successfully in 22 patients with newly diagnosed multiple myeloma. Until now, 17/22 patients underwent ASCT after conditioning with melphalane $200\,mg/m^2$. The pre-transplantation remission status (EBMT criteria) of these patients was: 1 CR, 6 nCR, 9 PR and 1 SD. Evaluation of treatment response was performed 3 months after ASCT.

Results: Ten of 17 patients could be evaluated, seven patients did not reach the first evaluation point yet. According to EMBT criteria, 2 patients achieved CR and 5 nCR (total 7/10, 70%). One patient died of pneumonia and two patients showed progressive disease. The median follow-up after ASCT for these 10 patients is 6 months (range: 4–16).

Conclusions: These preliminary data show that improved response rates after PAD induction may translate into improved CR/nCR rates after single ASCT.

103 Efficacy and safety of low-dose cyclosporine with everolimus and steroids in de novo heart transplant patients: results of a multicenter, randomized trial

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Background: The synergistic immunosuppressive mechanisms of everolimus and cyclosporine (CsA) permit a reduction in CsA exposure, ameliorating CNI-associated nephrotoxicity while maintaining efficacy. Prospective data are lacking, however, regarding low-dose CsA exposure in everolimus-treated heart transplant patients.

Methods: In a six-month, multicenter, open-label study, de novo cardiac graft recipients were randomized to standard-exposure (SE) or reduced-exposure (RE) CsA after month 2 post-

transplant, based on CsA C2 target ranges. All patients received concentration-controlled everolimus and steroids, with or without induction. The primary endpoint was renal function (serum creatinine) and the main secondary endpoint was composite efficacy failure, both at six months post-transplant.

Results: 199 patients were randomized (100 SE, 99 RE). Mean serum creatinine was 141.0 ± 53.1 µmol/L in the SE group versus 130.1 ± 53.7 µmol/L in the RE group at month 6 ($p = 0.093$). There were no significant differences in any efficacy endpoint between treatment groups at month 6. The incidence of BPAR >3A at month 6 was 21.0% (21/100) in the SE group and 16.2% (16/99) in the RE group. Nine patients died (3 SE, 6 RE). The type and incidence of adverse events and infections was similar between treatment groups. Cytomegalovirus infection occurred in 10 patients (5.0%).

Conclusions: Concentration-controlled everolimus with CsA C2 monitoring and corticosteroids results in a low incidence of BPAR with good graft survival at six months, combined with good renal function in de novo heart transplant recipients. Reduced CsA exposure with concomitant everolimus does not impair efficacy and benefit renal function.

104 Langzeitprognose von Patienten mit primärer pulmonaler Hypertonie nach Lungentransplantation

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Grundlagen: Durch bedeutende Fortschritte in der konservativen medikamentösen Behandlung der primär pulmonalen Hypertonie (PPH) kann der Verlauf der Erkrankung deutlich verzögert werden. Oft werden PPH Patienten erst in einem sehr späten Stadium zur Lungentransplantation (LuTX) vorgestellt. An unserer Abteilung wurde versucht auch diesen Patienten ein Überleben mittels LuTX zu ermöglichen. Dieses Abstrakt stellt eine Zusammenfassung unserer Ergebnisse dar.

Methodik: Alle in unserem Zentrum transplantierten PPH Patienten wurden retrospektiv analysiert, die Resultate mit den anderen lungentransplantierten Patienten verglichen und mögliche Einflußfaktoren auf die perioperative Mortalität analysiert.

Ergebnisse: Von Jänner 1999 bis November 2006 wurden 42 PPH Patienten (23 w/55%, 19 m/45%; 32 ± 13 a) Doppellungen transplantiert. 24 Patienten (57%) waren in NYHA-Klasse IV, 16 Patienten (38%) Klasse III und nur 2 Patienten (5%) NYHA-Klasse < III. 15 Patienten (36%) hatten eine ausgeprägte Rechtsherzinsuffizienz und 28 Patienten (67%) wurden mit intravenöser Pumpentherapie behandelt. Der unmittelbare postoperative Verlauf bei PPH-Patienten war gezeichnet durch eine hohe perioperative Komplikationsrate (Pneumonien 17%, Wundinfektionen 7%, Hämofiltration 45%, Blutungen 29%, kardiale Komplikationen 21%, neurologische Komplikationen 26%). Eine Analyse von möglichen Einflussfaktoren zeigte keine signifikanten Unterschiede zwischen Überlebenden und Verstorbenen. 30-Tage-, 1- und 5-Jahres-Überleben bei PPH-Patienten war 80%, 66% und 63%; im Vergleich zu allen anderen Lungentransplantierten ($n = 460$ Pat) 93%, 78%, 61% ($p =$ ns). Nach 1, 3 and 5 Jahren hatten 97%, 87%, 63% der PPH-Patienten keine BOS, verglichen zu allen anderen Lungentransplantierten 93%, 81%, 71% ($p =$ ns).

Schlussfolgerungen: Obwohl wir keinen einzelnen Parameter für das Überleben bei PPH-Patienten finden konnten, ist der unmittelbare postoperative Verlauf signifikant schlechter als bei allen anderen lungentransplantierten Patienten. Wir schließen, dass eine frühere Überweisung an ein Transplantzentrum, wenn die Patienten noch in einem besseren Allgemeinzustand sind, das postoperative Überleben und damit auch die Langzeitprognose dieser Patienten bestimmen.

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