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Humoral Rejection

O1-0027 HUMORAL REJECTION AFTER PEDIATRIC SMALL BOWEL TRANSPLANTATION

Laetitia-Marie Petit, Marion Rabant, Daniele Canioni, Nicole Brousse, Sabine Irtan, Christophe Chardot, Virginie Colomb, Olivier Goulet, Florence Lacaille [1]Necker Enfants Malades, Paris, France; [2]Hopital Universitaire de Geneve, Geneve, Switzerland

Humoral - antibody-mediated rejection (AMR) has been recently described after intestinal transplantation (ITx). We report our experience in the diagnosis and the therapeutics engaged.

Since 06/2008 to 07/2012, 28 children (18 boys) were enlisted, 19 (12 boys) transplanted: 11 isolated ITx (SBTx), 5 liver combined ITx (L-SBTx), 1 multivisceral transplantation with kidneys (MVT-k), 1 modified MVT. Median age of transplanted children: 6.9 years (18months-15years). 16/28 had anti-HLA antibodies (DSA) screening pre or post ITx. Immunosuppression associated Tacrolimus, Methylprednisolone (MP) and Basiliximab. When AMR was suspected on DSA over 2000 MFI or pathology or C4d staining, the patient received MP, IVimmunoglobulins (IVIg) and plasmapheresis (PP). Six children were excluded from this study: no AMR was detected for 3, and 3 had early surgical complications.

6/19 patients had pre-transplant anti-HLA antibodies, including 2 re-transplantations. At the end 13/19 patients had DSA over 2000 MFI after Tx (68%), 7 SBTx, 5 L-SBTx- 2 retransplantations, and 1 MVTx-k. In 6/13 patients the finding of DSA was contemporaneous of severe rejection and 3 had early C4d positive staining. They received the whole treatment and because of a very resistant rejection Infliximab (1/6), Rituxumab (2/6) and Eculizimab (1/6) were tried. Three children died, and the graft was removed in one, 2 weaned off PN. In 7/13 the biopsies showed a light acute rejection (2/7) or were normal (5/7) with DSA>2000. All children received IVIg, 2 also received MP and PP. Two children died 10 and 12 months after L-SBTx, due to a sepsis (patient suffering from severe GVH and from exfoliative rejection). 5/7 are weaned off PN with a normal graft function.

AMR is a severe complication after ITx that could lead to graft loss and even death. Mortality rate is high (38%) in this group and graft survival in living patients is 54%, median follow-up 18months. Pathological lesions need to be better identified but C4d staining seems to be an early helpful staining. In order to better define and treat AMR, systematically screening of anti-HLA antibodies and further studies are needed.

O2-0084 C4D STAINING IS AN INDEPENDENT RISK FACTOR OF GRAFT LOSS FOR RENAL ALLOGRAFT FAILURE IN PATIENTS WITH ACUTE ANTIBODY-MEDIATED REJECTION

Anissa Moktefi[1], Marie Matignon[1], Dominique Desvaux[1], Isabelle Brocheriou[2], XY Dubois[3], Philippe Lang[1], Philippe Grimbert[1] [1]Henri Mondor, APHP, Créteil, France; [2]Tenon, APHP, Paris, France; [3]INSERM U702, Paris, France

Background: C4d positive staining as mandatory in acute antibody-mediated rejection (AMR) is controversial. Predictive prognosis value of C4d positive staining in AMR remains incompletely studied. We conducted a retrospective single-center study to better define C4d- entity and its prognosis value.

Methods: Among 1347 renal allograft biopsies performed during 01/2005 and 01/2012 at our center, 35 were included on following criteria: clinically indicated biopsy and first episode of acute AMR defined by DSA detection and morphological changes (Banff 2009). We generated Kaplan-Meier survival curves and performed a multivariable analysis using the Cox proportional hazards regression model to identify risk factors for allograft failure after acute AMR.

Results: Among the 35 patients included, 18 (51%) presented with acute AMR C4d -. Induction therapy included significantly less thymoglobulin in C4d- patients ($P = 0.01$) and they were significantly older ($P = 0.004$) than C4d+ patients. Renal allograft morphological changes were identical in both groups as anti-rejection therapy. Kaplan-Meier survival estimates showed that C4d- is associated with graft survival (49 ± 7 months vs. 32 ± 8 months respectively, $P = 0.04$, Mantel-Cox log-rank test). The Cox proportional hazards regression analysis identified that C4d- (hazard ratio, 0.73 [95% confidence interval, 0.06–0.98]; $P = 0.04$) and estimated glomerular filtration rate (hazard ratio, 0.39 [95% confidence interval, 0.20–0.92]; $P = 0.03$) were independent risk factors for allograft loss.

Conclusion: Our single-center study has elucidated, for the first time at our knowledge, that C4d staining in kidney transplant recipients with acute AMR is an independent risk factor for graft failure. Level of allograft dysfunction at the time of diagnosis was also an independent predictor of graft loss. C4d staining merits to be included in therapeutic decision and these results need to be confirmed on a larger cohort.

O3-0110 C4D IS A GOOD MARKER OF ACUTE HUMORAL REJECTION IN SMALL BOWEL TRANSPLANTATION

Marion Rabant[1], Laetitia-Marie Petit[2], Nicole Brousse[1], Patrick Barbet[1], Florence Lacaille[2], Danielle Canioni[1] [1]Service d'Anatomopathologie, Hôpital Necker, Paris, France; [2]Service de Gastropédiatrie, Hôpital Necker, Paris, France

Background: Diagnosis criteria for acute humoral rejection are well established in kidney and heart transplantation, while no consensus exists for small bowel (SB) transplantation rejection.

Methods: We retrospectively studied intestinal biopsies, obtained from pediatrics SB allograft recipients (May 2009 to August 2011), with ($n = 13$) and without ($n = 3$) Donor Specific Antibodies (DSA). We systematically looked for histological signs for cellular rejection, vascular changes (microcirculation inflammation, thrombosis, arteritis, edema, villous architecture, inflammatory cells in the lamina propria, mucosal ulceration and immunohistochemical C4d staining. C4d was scored by a semi-quantitative evaluation of mucosal capillary staining according to the Banff classification for renal allografts; from 0 to 3 (<1% of capillaries= score–0, 1–10% = score–1, 10–50% = score–2, >50% = score–3).

Results: We examined 203 biopsies from the 13 SB allografts recipients (16 ± 4/patient) with DSA (2 preformed and 11 de novo, developed within the first month) transplanted in this period (SB only, $n = 7$; liver+SB, $n = 4$, multivisceral, $n = 2$). All but 2 patients experienced episodes of cellular rejection (from indeterminate to severe). C4d staining was graded as 0–1 in 4 patients, with no vascular or mucosal changes. They received only Intravenous Immunoglobulin (IVIG) ($n = 3$) or IVIG+plasmapheresis ($n = 1$) and had a good clinical outcome. Nine patients had a C4d score–2 ($n = 5$) or 3 ($n = 4$) on at least one biopsy. Five of these patients showed vascular thrombosis, always associated with mucosal ulceration and 2 others showed mucosal ulceration only. Two patients with C4d score–3 (50%) were explanted for refractory humoral rejection despite intensive therapy including Eculizumab for one. Two other patients died for extra-immunological cause. The 5 others had a good outcome with intensive treatment (Steroids, Plasmapheresis, IVIG and Rituximab). In control group without DSA, C4d was graded as 0 or 1 and no vascular changes or mucosal ulceration were noticed.

Conclusions: C4d score≥2 seems to be a good marker of humoral rejection, associated with vascular changes and mucosal ulceration. It may also be a prognostic marker of refractory rejection episodes.

O4-0092 LONG-TERM ALLOGRAFT TOLERANCE IS CHARACTERIZED BY THE GENERATION OF MEMORY ALLO-SPECIFIC REGULATORY B CELLS ABLE TO TRANSFER TOLERANCE VIA TGF BETA

Justine Durand, Laetitia Le Texier, Claire Usal, Emmanuel Merieau, Michèle Heslan, Maria-Cristina Cuturi, Elise Chiffoleau INSERM U1064 Centre de Recherche en Transplantation et en Immunologie, Nantes, Loire Atlantique, France

Numerous reports have highlighted the central role of regulatory T cells (Treg) in graft tolerance, but few studies have investigated the B cell aspect. We described a model of cardiac allograft tolerance in rat, characterized by the accumulation in the blood of inhibited B cells blocked at the switch recombination process and over-expressing inhibitory receptors.

The functional characterisation of splenic B cells from tolerant recipients demonstrates the presence of regulatory B cells (Breg), able to transfer donor-specific tolerance in a TGF Beta-dependant manner. Moreover, these cells are also able to suppress in vitro a MLR, by inhibiting TNF? and IFN? secretion, suggesting the presence of allo-specific Breg. Indeed, these Breg exhibit a CD5neg CD24neg IgDneg CD27+/– memory phenotype. We observed that these Breg are able, following transfer to a new recipient, to strongly inhibit the allo-specific B and T cell responses. However, these Breg do not generate new Breg, but rather CD4+ Treg also able following transfer to prevent allograft rejection.

These data demonstrate in our model the presence of Breg of memory phenotype, that are able to transfer donor-specific allograft tolerance via TGF Beta. Therefore, these Breg are different from the B10 transitional cells described in the literature. Interestingly, a similar B cell phenotype has been reported in patients operationally tolerant to kidney transplant. Therefore, deciphering the phenotype and the function of these cells is crucial to develop new strategies to modulate the B cell response.

O5-0125 CHRONIC ANTIBODY MEDIATED REJECTION WITH MICROCIRCULATION INFLAMMATION IN KIDNEY TRANSPLANTATION: CHARACTERISTIC AND EVOLUTION

Lila Ghouti-Terki[1], Benedicte Sautenet[1], Christelle Barbet[1], Marie-Christine Machet[2], Charlotte Magdelaine[3], Yvon Lebranchu[1], Jean-Michel Halimi[1], Mathias Buchler[1], Philippe Gatault[1] [1]Service de Néphrologie-Transplantation-Hémodialyse, TOURS, France; [2]Service d'Anatomo-Pathologie, TOURS, France; [3]Laboratoire Français du Sang, TOURS, France

Introduction: Chronic antibody mediated rejection (CAMR) is a major cause of graft loss. In our knowledge, there is no validated treatments that cure CAMR. Histological injuries of microcirculation inflammation (MI) (glomerulitis and capillaritis) can be found in presence of donor specific antigen (DSA). They are associated with CAMR and could be a prognostic factor. In our retrospective study, we described clinical and biological characteristics at diagnosis and the evolution of this pathology (CAMR with MI).

Patients and methods: All patients with CAMR and MI between 2007 and 2012 were screened. Clinical, biological and histological data had been analysed.

Results: 31 patients were selected. CARM was diagnosed with the median range of 5.5 years after the graft. At diagnosis, patients have majoritarily isolated proteinuria. The renal function was stable in 27 patients. HLA class II specific DSA was the most frequent antibody, especially anti-DQ (80.6%). 40% of our patients had peritubular capillary C4d deposition. Patients had poor prognosis regarding renal function. Kidney failure appeared during the first year after the diagnosis and was complicated by graft loss after a median range of 18 months (8–36). Having HLA antibody before transplantation and acute cellular rejection was associated with graft failure. There was no modification of HLA class II specific DSA in our study, whereas HLA class I specific DSA disappeared in 90% of patients. There was no benefit to treat CAMR with high dose intravenous immunoglobulins associated with Rituximab compared to patients without treatment regarding graft survival (P = 0.27) and renal function at one year (P = 0.72). Moreover, this treatment couldn't prevent the increase of transplant glomerulopathy.

Conclusion: CAMR with MI was responsible of ¼ of graft loss at 18 months. Association of rituximab and high dose intravenous immunoglobulins didn't seem to confer a benefice.

O6-0177 BLOOD MIR-142–5P EXPRESSION IN RENAL TRANSPLANT PATIENTS WITH CHRONIC ANTIBODY-MEDIATED REJECTION

Richard Danger[1], Amélie Lavault[1], Yohann Foucher[2], Magali Giral[3], Maxim Durand[2], Nicolas Degauque[1], Annaïck Pallier[1], Stéphanie Castagnet[1], Jean-Paul Van Huyen[4], Michel Delahousse[5], Karine Renaudin[3], Jean-Paul Soulillou[1], Sophie Brouard[1] [1]Institut National de la Santé Et de la Recherche Médicale INSERM U1064 and Institut de Transplantation Urologie Néphrologie (ITUN), Nantes, France; [2]Université de Nantes, Nantes, France; [3]Centre Hospitalier Universitaire, Hôtel Dieu, Nantes, France; [4]Service d'Anatomie Pathologique, Hôpital Européen Georges Pompidou, Paris, France; [5]Service de Néphrologie et Transplantation Rénale, Hôpital Foch, Suresnes, France

The aim of this study was to determine whether microRNA expression patterns may be associated with a diagnosis of chronic antibody mediated rejection (CAMR). We performed expression profiling of 381 miRNAs in peripheral blood mononuclear cells (PBMC) of kidney transplant recipients with CAMR or stable graft function. Among 257 expressed miRNAs, 10 miRNAs associated with CAMR were selected. Among them, miR-142–5p was increased in PBMC and biopsies of patients with CAMR as well as in a rodent model of CAMR but not in PBMC of patients with renal failure, suggesting that its over-expression in CAMR was associated with immunological disorders rather than renal dysfunction. A ROC curve analysis performed on 20 independent samples showed that miR-142–5p is a potential biomarker of CAMR that allow a very good discrimination of the patients from each group (AUC=0.77; $P = 0.041$). Moreover, its expression was decreased in PHA-activated blood cells and was not modulated in PBMC from patients with acute rejection, excluding a non-specific T cell activation expression. Finally, the absence of modulation of this miRNA in immunosuppressed patients suggests that its expression was not influenced by treatment, supporting miR-142–5p as a blood biomarker specific for CAMR.

O7-0090 CHARACTERIZATION OF HLA-SPECIFIC B CELLS USING HLA COATED FLOW-BEADS

Nicolas Degauque[1], Ahmed Akl[1], Romain Crochette[1], Julie Lepourry[1], Annaick Pallier[1], Magali Giral[2], Stéphanie Castagnet[1], Emilie Dugast[1], Cécile Guillot-Gueguen[2], Annie Elong[1], Xavier Saulquin[3], Anne Devys[4], Sophie Brouard[1], Jean-Paul Soulillou[1] [1]INSERM UMR1064, Nantes, France; [2]CHU de Nantes, ITUN, Nantes, France; [3]INSERM UMR892, Nantes, France; [4]Etablissement Français du Sang, Laboratoire HLA, Nantes, France

The appearance of donor-specific HLA antibodies following transplantation is associated with a high rate of graft failure. Nevertheless, nothing is known about the role of B cells exhibiting an anti-HLA receptor in the generation of donor-specific antibodies and on graft outcome. Moreover, no reliable methodology is available to isolate and characterize B cells able to directly bind HLA molecules. Here, we describe a new approach to quantify and characterize B cells engaging physical interaction following the incubation with single or multiplex class I HLA-single antigen beads. Bead-B-lymphocyte Rosettes (BBR) frequency was enumerated and characterized by flow cytometry in blood B cells from anti-HLA sensitized kidney transplant recipients. A significantly higher anti-HLA-A*0201 BBR frequency in HLA-A*0201 sensitized recipients compared to non-sensitized stable patients and healthy volunteers was detected. However, the use of multiplexed beads and competitive inhibition using HLA-A*0201 HLA tetramer shows a significant decrease in specific HLA antigen BBR in sensitized recipients but without precise allelic specificity. Detailed phenotype analysis shows a ten-fold increased ratio of non-switched memory IgD+CD27 + /class-switched memory IgD-CD27 + B cells in BBR (P = 0.03). This method opens a new window for analyzing alloreactive B cells precursors in various clinical settings including bone-marrow and organ transplantation.

O8-0158 PRE-TRANSPLANTATION ANTI-HLA ANTIBODIES ARE ASSOCIATED TO AN INCREASED FREQUENCY OF ACTIVATED NAÏVE CELLS AND A DECREASED FREQUENCY OF MEMORY CELLS

Renaud Snanoudj[1], Sophie Candon[1], Dave Roelen[3], Frans Claas[3], Christophe Legendre[2], Lucienne Chatenoud[1] [1]INSERM 1013, Paris, France; [2]Service de Transplantation, Hôpital Necker, Paris, France; [3]Dept. Immunohematology and Blood Transfusion, Leiden, Netherlands

Introduction: There is no data available about a possible relationship between peripheral B cell phenotype and the presence of anti-HLA antibodies in patients waiting for kidney transplantation. In this study, we compared the B-cell phenotypes at day–0 transplant in patients with or without HLA antibodies.

Methodology: We studied B cell phenotype on whole blood cells by flow cytometry according to the Bm classification, based on IgD/CD38 double staining performed on CD19 + cells, before starting immunosuppression. We included 101 consecutive unselected patients admitted for kidney transplantation in our department. Presence and mean fluorescence intensity (MFI) of class I and II anti-HLA antibodies were analyzed with Luminex single antigen technique.

Results: In 76% of cases, patients received their first transplant. According to the threshold of MFI chosen in Luminex, 83% of patients had at least one anti-HLA antibody with a MFI>1000 and 50% one antibody with a MFI>3000. Naive cells accounted for 69.0 ± 14.2% of circulating B-cells, consisting of 10.6% of virgin Bm1 cells and 58.4% of activated Bm2 cells. We observed 24.4 ± 14.5% of memory Bm5 cells, 4.8 ± 4.0% of transitional Bm2' cells, and 0.4 ± 0.8% of plasmablasts.

In patients with at least one HLA antibody with a MFI > 3000, the percentage of activated naïve Bm2 cells among total CD19+ cells was significantly higher (64.4 ± 15.1 vs. 52.5 ± 19.1, $p < 0.001$) at the expense of virgin naïve and both subsets of memory B cells, compared with patients without HLA antibodies carrying such MFI. With the cut-off of 1000, the same difference was observed but was only significant for the activated naïve B cells (60.2 vs. 49.6%, P = 0.027). We observed a correlation between broadness of HLA immunization assessed through percentage of HLA antibodies (or signle antigen flow beads). No difference was observed in the repartition of day-0 B-cell subsets in patients who experienced or not antibody-mediated rejection during the first year post-transplantation.

Conclusion: Patients with HLA antibodies at a high MFI display a higher proportion of activated naïve cells at the expense of resting memory and naïve cells. Day-0 peripheral B-cell phenotype is not predictive of first-year post-transplantation immunological events.

O9-0058 THREE CASE-REPORTS OF ECULIZUMAB-RESISTANT ACUTE HUMORAL REJECTION

Maren Burbach[1], Nacera Ouali[1], Christophe Ridel[1], Karine Dahan[1], Isabelle Brocheriou[1], Caroline Superbielle[2], Alexandre Hertig[1], Eric Rondeau[1]
[1]Hôpital Tenon, Paris, France; [2]Hôpital Saint Louis, Paris, France

Acute antibody-mediated rejection (AAMR) is responsible for up to 20–30% of acute rejection following kidney transplantation. New therapeutic agents have recently emerged such as eculizmab an inhibitor of terminal complement activation.

Stegall et al. have shown that eculizumab administered at the time of transplantation decreased the incidence of early AMR in 26 sensitized renal transplant recipients (2 cases of AAMR/26). Locke et al. reported a case of successful treatment of severe AMR using eculizumab and plasmapheresis (PP)/intravenous immunoglogulin (IVIg).

Here we report three cases of AAMR, resistant to aggressive immunosuppressive treatment including eculizumab.

The end stage renal diseases of the patients were haemolytic uremic syndrome (HUS), Alport syndrome and polycystic kidney disease. Two of them were highly sensitized with donor specific antibodies (DSA). Deterioration of renal function at 1 year, 1 and 9 months respectively led to renal transplant biopsy and disclosed in all cases AAMR with intense glomerulitis and peritubular capillaritis(g3, cpt3). Interestingly C4d staining on the peritubular capillaries was negative in two cases (glomerular staining was positive) and the DSA of these patients did not bind C1q. The patients were treated with steroids, PP or immunoadsorption, IVIg, rituximab. Eculizumab was administered preventively 3 months after the transplant in the HUS patient (but did not prevent AAMR at one year), immediately at diagnosis of AAMR in the highly sensitized patient and 1 month after failure of conventional treatment in the last case (21, 13 and 3 infusions of 900 to 1200mg respectively). Successive biopsies remained unchanged and renal function did not improve.

This report of three cases of AAMR resistant to eculizumab two of which were C4d negative with C1q- DSA suggests the involvement of a complement-independent mechanism, of poor prognosis. The indication of eculizumab in C4d-/C1q- DSA AAMR warrants further studies.

IMMUNOSUPPRESSION

O10-0047 MINIMIZATION OF MAINTENANCE IMMUNOSUPPRESSION IN RENAL TRANSPLANTATION: 10 YEAR RESULTS OF A RANDOMIZED STUDY COMPARING CYCLOSPORINE A MONOTHERAPY TO BITHERAPY WITH CICLOSPORINE A-MMF OR CICLOSPORINE-AZA

Laure Ecotiere[1], Antoine Thierry[1], Ramzi Abou-Ayache[1], Isabelle Etienne[2], Olivier Toupance[3], Bruno Hurault de Ligny[4], Yann Le Meur[5], Guy Touchard[1] [1]CHU La Milétrie, Poitiers, France; [2]CHU Bois Guillaume, Rouen, France; [3]Hôpital Maison Blanche, Reims, France; [4]Hôtital Côte de Nacre, Caen, France; [5]Hôpital La Cavale Blanche, Brest, France

Background: In renal transplantation, the goal of minimization of immunosuppression is to limit side effects without altering the graft outcome. Although cyclosporine A (CsA) monotherapy constitutes a strategy of minimization, its long term effects on graft outcome remain to be evaluated.
Methods: We conduct a prospective randomized multicenter study that include 207 renal transplant patients at low immunological-risk, after the first year of transplantation, in order to compare CsA monotherapy (group C) to bitherapy CsA-Mycophenolate mofetil (MMF) 1g/day (group B) or CsA-Azathioprine (Aza) 1−2mg/kg/day (group A). The primary endpoint is the incidence of graft dysfunction.
Results: With a mean follow up time of 118 ± 23 months, the incidence of renal graft dysfunction and acute rejection episode are 51.5% and 15% respectively, without difference between the 3 groups. Patient and graft survivals are 100%, 94.6%, 96% and 94.6%, 85%, 91.9% in groups A, B and C respectively (ns). Randomly assigned regimen is maintained in 118 patients (58.7%). Seven patients died with a functional graft mainly because of neoplasia. At the end of the follow-up, serum creatinine level and creatinine clearance levels are similar between the 3 groups. The mean clearance is significantly higher in group A compared to group C ($P = 0.039$) and remain stable over the follow-up period, whereas a decrease of 1.1 and 1.5ml/min/year is observed in groups B and C respectively. The incidence of cancer (6 in bitherapy and 11 in monotherapy) is not different.
Conclusion: In low immunological risk recipients, after steroid withdrawal in the first year post transplantation, CsA monotherapy was similar to minimized bitherapy for renal function, acute rejection episodes and patient and graft survival. These long-term results confirm the interest of an immunosuppressive strategy based on CsA monotherapy.

O11-0152 BK VIRUS LATENT INFECTION IN KIDNEY TRANSPLANTATION: IMPACT OF MINIMIZATION OF THERAPEUTIC IMMUNOSUPPRESSION ON ANTI-BKV SPECIFIC T CELL RESPONSES.

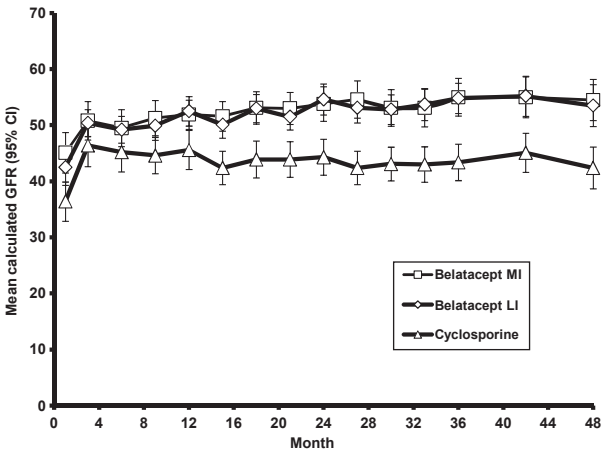
Sophie Candon, Albane Sartorius, Arvish Soorkia, Julien Zuber, Christophe Legendre, Lucienne Chatenoud [1]Hopital Necker - Enfants Malades, Paris, France; [2]Université Paris Descartes, Paris, France

Reactivation of BK virus (BKV) latent infection in kidney transplant recipients is a major cause of graft injury. Minimization of therapeutic of immunosuppression (IS) after diagnosis of BKV reactivation currently constitute the recommended first line strategy for prevention of BKV-associated nephropathy (BKVAN). In our center, such IS reduction is performed according to a step-by-step protocol based on the kinetics of BKV viremia and the presence or absence of histological BKVAN.
The objective: of this study was to evaluate the impact of our IS minimization protocol on BKV-specific T cell immunity.
Methods: Using an ELISPOT-IFN-gamma assay, we analyzed anti-BKV T cell responses in 12 kidney graft recipients with uncomplicated BKV viremia (VIR) and 6 patients with BKVAN during reduction of IS.
Results: Anti-BKV T cell response at baseline (first positive viremia) was similarly low in BKVAN and VIR patients. After IS reduction, the frequency of anti-BKV IFN-gamma producing T cells significantly increased (>2 fold) in all patients but one. This increase was obtained later in BKVAN+ than in VIR+ patients: after 4.2 months (range 0.9−17.7) versus 0.6 months (range 0.6−8.5) respectively and 3 steps (range 2−3) versus 1 step (range 1−3) of IS reduction respectively ($p < 0.05$). Control of BKV reactivation (< 3log BKVcopies/ml plasma) was obtained in 11 patients out of the 13 who completed the protocol. Among these, the time required to observe improved frequencies of BKV-specific T cells correlated significantly with the time needed to obtain control of BKV reactivation. This control was observed after 4 steps (range 4−5) of IS reduction in BKVAN patients versus 2 steps (range 1−3) in VIR patients ($p < 0.05$).
Conclusion: These results suggest that BKVAN patients may benefit from more drastic reduction of IS in order to allow faster recovery of anti-BKV T cell immunity.

O12-0032 LONG-TERM EXTENSION OF THE BELATACEPT BENEFIT-EXT STUDY: RESULTS AT MONTH 48

J. Grinyo[1], S. Florman[2], J. Medina[3], M. Del C. Rial[4], F. Muhlbacher[5], A. Durrbach[6], F. Vincenti[7], M.B. Harler[8], T. Duan[8], L. Rostaing[9] [1]University Hospital Bellvitge, Barcelona, Spain; [2]Mount Sinai Medical Center, New York, NY, United States; [3]Hospital do Rim e Hipertensão, Sao Paulo, Brazil; [4]Instituto de Nefrologia, Buenos Aires, Argentina; [5]5University Klinik für Chirurgie, General Hospital Vienna (AKH), Vienna, Austria; [6]CHU Bicêtre, Le Kremlin Bicêtre, France; [7]UCSF Kidney Transplant Service, San Francisco, United States; [8]Bristol-Myers Squibb, Princeton, NJ, United States; [9]CHU Rangueil, Toulouse, France

Background: Published results from the completed BENEFIT-EXT study reported comparable patient and graft survival and better renal function in belatacept-treated patients versus a cyclosporine-based regimen. Patients who completed this trial and remained on assigned therapy were eligible to enter a long term extension (LTE). This report presents the results of the LTE study at 4 years.
Methods: BENEFIT-EXT was a 3-year, phase III study in recipients of de novo extended criteria donor kidneys who were randomized to a more intensive (MI) or less intensive (LI) belatacept regimen, or cyclosporine (CsA). Primary objective was to assess long-term safety and tolerability of belatacept in the LTE cohort. Other endpoints included patient/graft survival, acute rejection, and calculated GFR (cGFR).
Results: 304/323 patients who completed 3 years of treatment entered the LTE ($n = 104$ MI; $n = 113$ LI; $n = 87$ CsA). 16 patients discontinued the LTE between years 3 and 4 ($n = 7$ MI; $n = 6$ LI; $n = 3$ CsA). 6 patients died during year 4 ($n = 2$ MI; $n = 4$ LI) and 2 experienced graft loss ($n = 1$ LI; $n = 1$ CsA). One belatacept MI patient experienced an acute rejection episode (Grade IIA) during year 4. For the population who entered the LTE, the incidence rate (events/100 pt-years of exposure) of serious infections from randomization through database lock was 23.8 (MI), 15.9 (LI), and 18.7 (CsA), and the incidence rate of overall malignancies was 2.6 (MI), 3.2 (LI), and 2.8 (CsA). 4 cases of post-transplant lymphoproliferative disorder (PTLD) occurred in the LTE population through August 2011 ($n = 3$ LI; $n = 1$ CsA). 2 of 3 PTLD cases in the LI group occurred in patients seronegative for Epstein-Barr virus (EBV) at the time of transplantation. cGFR (mean ± SD) at year 4 was 54.5 ± 18.0 (MI), 53.5 ± 19.1 (LI), and 42.4 ± 16.5 (CsA) ml/min/1.73 m^2 (Figure).



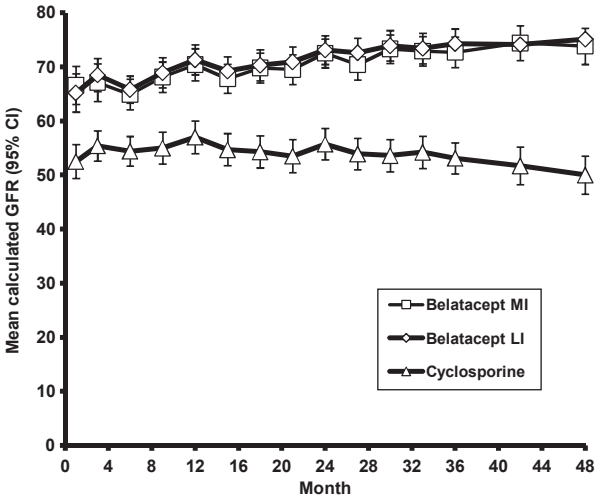
Conclusions: The safety profile of belatacept was consistent over time; no new safety concerns were identified. Two of three new cases of PTLD occurred in EBV negative patients; use of belatacept has since been contraindicated in patients who are EBV negative or have unknown serostatus. A higher cGFR in belatacept-treated versus CsA-treated patients was maintained over time.

0075 LONG-TERM EXTENSION OF THE BELATACEPT BENEFIT STUDY: RESULTS AT MONTH 48

F. Vincenti[1], B. Charpentier[2], L. Rostaing[3], R. Reyes-Acevedo[4], P. Massari[5], S. Vitko[6], J. Alberu[7], M.B. Harler[8], T. Duan[8], C. Larsen[9] [1]UCSF Kidney Transplant Service, San Francisco, CA, United States; [2]CHU Bicêtre, Le Kremlin Bicêtre, France; [3]CHU Rangueil, Toulouse, France; [4]Hospital Miguel Hidalgo De Aguascalientes, Mexico, Mexico; [5]Hospital Privado Centro Medico de Cordoba, Cordoba, Argentina; [6]Institut Klinicke A. Expermentalni Mediciny, Praha, Czech Republic; [7]Instituto Nacional de Ciencias Médicas y Nutricion "Salvador Zubiran", Mexico DF, Mexico; [8]Bristol-Myers Squibb, Princeton, NJ, United States; [9]Bristol-Myers Squibb, Princeton, NJ, United States

Background: Published results of the completed 3-yr BENEFIT study reported comparable patient and graft survival and better renal function in kidney

transplant recipients receiving belatacept versus a cyclosporine-based regimen, despite higher rates/grades of acute rejection. Patients who completed this trial and remained on assigned therapy were able to enter a long term extension (LTE). This report presents the results of the LTE study at 4 years.
Methods: BENEFIT was a randomized, phase III study in adults receiving a kidney transplant from a living or standard criteria deceased donor. Patients were randomized to a more (MI) or less intensive (LI) regimen of belatacept, or CsA. Primary objective was to assess long-term safety of belatacept in the LTE cohort. Other endpoints included patient/graft survival, acute rejection, and calculated GFR (cGFR).
Results: 457/471 patients who completed 3 years of treatment entered the LTE (n = 155/158 MI; n = 166/170 LI; n = 136/143 CsA). 25 patients discontinued the LTE between years 3 and 4 (n = 6 MI; n = 6 LI; n = 13 CsA). 4 patients died during year 4 (n = 1 MI; n = 3 CsA) and 1 experienced graft loss (n = 1 CsA). 2 patients experienced an acute rejection episode (n = 1 LI [Grade IIA]; n = 1 CsA [Grade IA]). For the population who entered the LTE, the incidence rate (events/100 pt-years of exposure) of serious infections from initial randomization through database lock was 10.3 (MI), 10.4 (LI), and 15.7 (CsA), and the incidence rate of overall malignancies was 2.3 (MI), 1.4 (LI), and 3.0 (CsA). No new cases of PTLD were observed, and no new safety signals were identified. cGFR (mean ±SD) at year 4 was 73.8 ± 19.6 (MI), 75.1 ± 17.0 (LI), and 50.0 ± 18.7 (CsA) ml/min/1.73 m^2 (Figure).



Conclusions: A consistent safety profile over time and a low discontinuation rate was observed in patients receiving belatacept in the LTE. A higher cGFR in belatacept-treated versus CsA-treated patients, first observed in the early post-transplant period, was sustained at 4 years.

O13-0034 THREE-YEAR SAFETY AND EFFICACY OUTCOMES IN KIDNEY TRANSPLANT PATIENTS RANDOMIZED TO STEROID AVOIDANCE OR MAINTENANCE STEROIDS WITH EARLY INTENSIFIED DOSING OF ENTERIC-COATED MYCOPHENOLATE SODIUM: THE INFINITY STUDY

Antoine Thierry[1], Georges Mourad[2], Mathias Büchler[3], Lionel Rostaing[4], Florence Villemain[5], Anne-Elisabeth Heng[6], Yann Le Meur[7], Gabriel Choukroun[8], Olivier Toupance[9], Christophe Legendre[10], Patrick Le Pogamp[11], Michelle Kessler[12], Pierre Merville[13], Bruno Moulin[14], Quéré Stéphane[15], Lécuyer Aurélie[15], Di Gambattista Fabienne[15], Touchard Guy[1] [1]CHU La Milétrie, INSERM U1082, Poitiers, France; [2]CHU La Peyronie, Montpellier, France; [3]CHU Bretonneau, Tours, France; [4]Hôpital de Rangueil, Toulouse, France; [5]CHU d'Angers, Angers, France; [6]Hôpital Gabriel Montpied, Clermont-Ferrand, France; [7]Hôpital La Cavale Blanche, Brest, France; [8]Hôpital Sud, Amiens, France; [9]Hôpital Maison Blanche, Reims, France; [10]Hôpital Necker, Paris, France; [11]CHU de Rennes, Rennes, France; [12]Hôpital Brabois Adultes, Vandoeuvre les Nancy, France; [13]Hôpital Pellegrin, Bordeaux, France; [14]Nouvel Hôpital Civil, Strasbourg, France; [15]Novartis Pharma SAS, Rueil-Malmaison, France

Background: Short-term use of intensified enteric-coated mycophenolate sodium (EC-MPS) dosing may facilitate steroid avoidance after kidney transplantation.
Methods: *De novo* kidney transplant recipients at low immunological risk were randomized in a 6-month, multicenter, open-label trial to steroid avoidance (SA) or maintenance steroids (controls), all with intensified EC-MPS (2160mg/day to week 6, 1440mg/day thereafter), cyclosporine and IL-2RA induction. At month 6, patients who completed the study on-treatment could enter a further 30-month observational study.
Results: Of the 131/166 patients (78.9%; 70 steroid avoidance, 61 controls) who completed the 6-month study on-treatment and were eligible for analysis in the follow-up study, 126 completed the 36-month study visit (68 SA, 58 controls). By month 36, 32.4% of SA patients and 51.7% of controls were receiving steroids. At the same time, the proportion of patients treated by cyclosporine plus EC-MPS was 83.6% in the SA group and 70.2% of controls. The primary endpoint, treatment failure (biopsy-proven acute rejection [BPAR], graft loss, death or loss to follow-up) occurred in 21.4% of patients randomized to steroid avoidance vs. 16.4% of controls (P = 0.46) by month 36. The incidence of BPAR was 20% and 11.5%, respectively (P = 0.19). Graft and patient survival were similar between groups. Mean glomerular filtration rate (MDRD) was similar at month 36 (SA 50 ± 19 ml/min/1.73m^2, controls 55 ± 20 ml/min/1.73m^2, P = 0.10). The incidence of adverse events with a suspected relation to steroids was 22.9% in the steroid avoidance group versus 37.1% of controls (P = 0.06) (infections 17.1% vs. 16.1% of which no CMV infection, dyslipidemia 2.9% vs. 6.5%, diabetes 2.9% vs. 4.8%, gastrointestinal complications 0.0% vs. 4.8% and proteinuria 0 vs. 1.6%).
Conclusion: Steroid avoidance is possible with early intensified EC-MPS dosing, calcineurin inhibition and IL-2RA induction in low-risk kidney transplant recipients without compromising efficacy at three years post-transplant, with a numerical reduction in steroid-related adverse events.

O14-0036 MAINTAINING A CALCINEURIN-INHIBITOR AFTER THE DIAGNOSIS OF PTLD IS SAFE AND IMPROVES RENAL GRAFT SURVIVAL

Jean-Emmanuel Serre[1], David Michonneau[2], Laure-Hélène Noël[2], Henri Kreis[2], Christophe Legendre[2], Marie-France Mamzer-Bruneel[2], Emmanuel Morelon[1], Olivier Thaunat[1] [1]Hospices Civils de Lyon, Lyon, France; [2]Hôpital Necker, Paris, France

Background: Post transplant lymphoproliferative disorder (PTLD) corresponds to an uncontrolled proliferation of transformed lymphocytes that is fostered by immunosuppression. Treatment of PTLD therefore includes, in addition to chemotherapy, a reduction of maintenance immunosuppression (RIS). There is currently no consensus on the modality of RIS, in particular regarding the management of calcineurin-inhibitors (CNI). PTLD patients have an increased risk for graft loss suggesting that current RIS strategies need to be optimised with regard to graft outcome.
Method and results: The files of 101 PTLD patients followed in two renal transplantation centers were reviewed. During the follow-up (mean follow-up: 45 ± 44 months) 39 patients died (38.6%) and the rate of death-censored graft loss was 20.8%. Multivariate analysis, established that eGFR<30ml/min/1.73m^2 at the diagnosis of PTLD, biopsy-proven acute rejection episode following RIS, and absence of a CNI in maintenance immunosuppression are the only independent risk factors for allograft loss. Histological analysis of graft biopsies revealed that maintaining a CNI after the diagnosis of PTLD reduces the risk of humoral rejection. Remarkably, CNI maintenance was neither associated with a higher mortality, nor with a worse disease free survival in multivariate analysis.
Conclusion: We conclude that maintaining a CNI at reduced dose after the diagnosis of PTLD is safe and improves renal graft outcome, possibly through a bet.

O15-0017 RANDOMIZED, COMPARATIVE, MULTICENTER STUDY RAD2310 COMPARING EVEROLIMUS AND MMF IN *DE NOVO* HEART TRANSPLANTATION: RESULTS AT 2 YEARS.

Pascale Boisssonnat[1], Eric Epailly[2], Laurent Sebbag[1], Sophie Mattei[3], Romain Guillemain[4], shaida Varnous[5], Iradj Gandjbakhch[5] [1]Hopital Cardiologique Louis Pradel, Lyon, France; [2]Hopital Civil Strasbourg, Strasbourg, France; [3]Hopital Brabois, Nancy, France; [4]Hopital Europeen Georges Pompidou, Paris, France; [5]Hopital Pitié Salpétrière, Paris, France

Objective: The aim of the study was to compare the combination of everolimus (EVR) + CsA reduced dose (rCsA) versus MMF + CsA standard dose (sCsA) in *de novo* heart transplant recipients. At 12 months, non-inferiority was demonstrated for the composite efficacy endpoint (BPAR ISHLT≥ 3A, AR with hemodynamic compromise, death, graft loss/re-transplantation, lost to follow-up). Inferior renal function with EVR was probably due to non-adherence to CsA exposure. A beneficial effect on cardiac allograft vasculopathy assessed by IVUS was also demonstrated at 12 months. The final results at 24 months are presented here.
Methods: 721 patients were randomly allocated to 3 groups to receive either EVR 1.5 mg/d (C0 = 3−8 ng/ml, n = 282) + rCsA, or EVR 3 mg (C0 = 6−12 ng/ml, n = 168) + rCsA, or MMF 3g + sCsA, (n = 271). The EVR 3 mg results are not reported here because the inclusions were prematurely discontinued due to higher mortality.
Results: At M24, the incidence of the composite endpoint (39.4% EVR vs. 41.3% MMF) and of its different components was comparable. The incidence of infections was similar in both groups (EVR 1.5 mg 70% vs. MMF 67%) and CMV infections were significantly lower with EVR (9.3% vs. 23.9%). Kidney function remained stable in both groups between M12 and M24 (EVR: 58.8 vs. 58.8 ml/min/1.73 m^2 and MMF: 64.4 vs. 65.3 ml/min/1.73 m^2) and 8 deaths were recorded under EVR compared to 12 under MMF.
Conclusion: At 2 years, EVR combined with a low dose of CsA maintained a similar efficacy as the control together with a reassuring safety and tolerability profile. The death rate appeared balanced between the 2 groups and the kidney function remained stable beyond the 1st year.

O16-0157 STEROID-FREE REGIMEN AND OPTIMIZATION OF MYCOPHENOLIC ACID (MPA) EXPOSURE IN LIVER TRANSPLANT RECIPIENTS: FINAL RESULTS OF CELLESTE

Faouzi Saliba[1], François Durand[2], Jean Gugenheim[3], Sylvie Radenne[4], Vincent Leroy[5], Martine Neau-Cransac[6], Yvon Calmus[7], Mario Altieri[8], Christophe Duvoux[9], Georges-Philippe Pageaux[10], Sebastien Dharancy[11], Jean Hardwigsen[12], Claire Vanlemmens[13], Pierre Marquet[14], Carole DUR[15], Naïla Taguieva[15], Lionel Rostaing[16] [1]Hôpital Paul Brousse, Villejuif, France; [2]Hôpital Beaujon, Vlichy, France; [3]Hôpital de l'Archet, Nice, France; [4]Hôpital de la croix rousse, Lyon, France; [5]CHRU de Grenoble, Grenoble, France; [6]Hôpital Pellegrin, Bordeaux, France; [7]Hôpital Saint Antoine, Paris, France; [8]CHRU de Caen, Caen, France; [9]Hôpital Henri Mondor, Créteil, France; [10]Hôpital Saint Eloi, Montpellier, France; [11]Hôpital Claude Huriez, Lille, France; [12]Hôpital de la conception, Marseille, France; [13]Hôpital Jean Minjoz, Besançon, France; [14]CHRU de Limoges, Limoges, France; [15]ROCHE, Boulogne-Billancourt, France; [16]Hôpital Rangueil, Toulouse, France

Introduction and Aims: After liver transplantation, minimizing immunosuppressive regimen by avoiding steroids is still a main challenge. We conducted a randomized multicenter open-label study to assess the feasibility and potential benefit of a steroid (CS)-free regimen coupled with mycophenolate mofetil (MMF, CellCept®) therapeutic drug monitoring (TDM) in *de novo* liver transplant recipients receiving MMF and tacrolimus (Tac).

Methods: Adult transplant recipients have been randomized at day 0, to either a concentration-controlled (A arm) or a fixed-dose (B arm) MMF-containing regimens. In A arm, MMF was introduced at 3 g per day and MMF doses were adjusted from day 5 according to a calculated 3 points MPA exposure. B arm patients received a fixed-dose of 2g per day of MMF and a 6-month CS therapy. The primary endpoint was proportion of patients who experienced biopsy-proven and treated acute rejection [BPTAR] during the first year post-transplant. All biopsy samples were assessed locally and reviewed according to a centralised procedure.

Results: 187 patients were randomized (n_A=94; n_B=93). Indications for liver transplantation were: alcoholic cirrhosis 27.8%, HCV related disease14.4%, HCC 45% and others 12.8%. The per-protocol population comprised 174 patients (n_A=87, n_B=87). The primary objective of non-inferiority was met: only 7 patients in arm A (8%) and 8 in arm B (9.2%) experienced a BPTAR in the 1st year] ← , 9.49]. Results were similar in the Intent-To-Treat population. Median MPA AUC was slightly higher in the adjusted dose MMF arm A from D14 to M6 with no statistically significant difference between arms. TAC exposure, MDRD calculated GFR and 12-months graft and patient survival (n_A=90.8% vs. n_B=89.8%) was similar in both arms. Among adverse events, 32 (32.5%) patients experienced a diarrhea as compared to 26 (28.3%) patients in B arm. The incidence of *de novo* diabetes was significantly (P = 0.049) lower in A arm (19.8% vs. 32.6%). Leucopenia < 2000/mm^3 was significantly lower in B arm (2.2% vs. 15.4%; P = 0.002). All other adverse events were comparable between groups.

Conclusion: This study showed that the use of MMF in combination with Tac in de novo liver transplant recipients allows early CS discontinuation (from day 1 post-transplant) with a good tolerability and very low rejection rate. Routine use of TDM to optimize MMF dosage didn't show any additional beneficial effect.

O17-0018 ANTITHYMOCYTES GLOBULINS AND IMMUNE SENESCENCE IN RENAL TRANSPLANT PATIENTS

Jamal Bamoulid[1], Caroline Roubiou[1], Thomas Crépin[1], Béatrice Gaugler[2], Christophe Ferrand[2], Pierre Tiberghien[2], Jean Marc Chalopin[1], Philippe Saas[2], Didier Ducloux[1] [1]CHU Besançon, Besançon, Franche-Comté, France; [2]INSERM U1098, Besançon, Franche-Comté, France

Background: CD4 T cell reconstitution after antithymocytes globulins (ATG) is dependent on pre-transplant thymic function and persistent ATG-induced CD4 T cell lymphopenia is associated with abnormalities close to those observed in immune senescence. We hypothesized that ATG could be responsible of accelerated immune senescence in renal transplant recipients (RTR).

Methods: We analyzed a prospective cohort of 66 incident RTR. Thymic output of recent thymic emigrants (RTE), regulatory T cells (Treg), CD8 + T cell subsets were studied by flow cytometry at transplant and one year after transplantation. Thirty out of 66 patients were also studied for T lymphocyte relative telomere length and telomerase activity at both times. Age, gender, induction therapy (ATG or anti-CD25 monoclonal antibody [mAb]), immunosuppressive regimen, and CMV status were analysed as potential confounding factors.

Results: 46 patients received ATG whereas 20 received monoclonal anti-CD25 mAb. Pre-transplant RTE cell count predicted CD4 + T cell count 1 year after transplantation. RTE cell count significantly decreased and was lower in ATG than in anti-CD25 mAb-treated recipients. Proportion of CD8 + CD28-T cells increased after transplant in ATG patients. This increase was more pronounced in RTR with an inverted CD4/CD8 T-cell ratio and in CMV-positive RTR. Treg were more frequent after transplant in ATG than in anti-CD25 mAb recipients. In ATG recipients, Treg peripheral expansion was related to poor thymic function one year post-transplant. RTL and RTA increased in anti-CD25 mAb but not in ATG recipients one year post-transplant.

Conclusions: ATG is associated with reduced thymic output of naive T cells, increased Treg, lymphocyte phenotype, RTL and RTA evocative of immune senescence. Mechanisms and clinical consequences remain to be studied.

O18-0148 ROLE OF BELATACEPT (CTLA4-IG) ON B CELL PHENOTYPE IN RENAL TRANSPLANT RECIPIENTS

Claire Leibler[1], Philippe Grimbert[1], Marie Matignon[1], Philippe Lang[1], Edgardo Carosella[2], Nathalie Rouas-Freiss[2], Catherine Menier[2] [1]Hôpital Henri Mondor, Service de Néphrologie et Transplantation, Créteil, Franc; [2]UMR CEA UP7, Service de Recherche en Hémato-Immunologie, Hôpital Saint Louis, Paris, France

Introduction: Phase III clinical studies have shown that upon Belatacept (CTLA4-Ig) treatment, renal allograft function and anti-HLA immunization are improved compared to anticalcineurin. Thus, B cell phenotype and immune response may be modulated by Belatacept, which has been the focus of our study.

Method: Twenty three age-matched first kidney transplant patients were included for this study and divided into 3 groups: kidney recipients with stable graft function under Belatacept (n = 9) or anticalcineurin regimen (n = 9), and with chronic humoral rejection (n = 5). The phenotypic characterization of B cell subpopulations was performed on PBMC and sorted B cells by flow cytometry analysis. Mature and transitional B cell sub-populations were identified by the following stainings: CD19/IgD/CD38/CD27 and CD19/CD24/CD38.

Results: B cell frequency was similar in both Belatacept and anticalcineurin groups (2.8(0.4−12) % vs. 2.2(1.3−6) % (P = 0.43)) while significantly lower in patients with chronic humoral rejection despite the lack of recent increase in immunosuppressive therapy (0.4(0.1−2) (P = 0.03 et 0.01)).

The distribution of B cell sub-populations was significantly different between Belatacept and anticalcineurin groups. Notably, in the Belatacept group, transitional B cell frequency was significantly increased as defined by both phenotypes: $CD19^+CD24^{high}CD38^{high}$ (4(0.5−7.3)% vs. 1.25(0−3.7)% (P = 0.003)) and $CD19^+IgD^{high}CD38^{high}CD27^-$ (4.5(1−7)% vs. 1.1(0.2−4)% (P = 0.003)).

The comparative study of B cell survival transcriptional profiles and the analysis of B cell inhibitory functions are currently carried out.

Conclusion: These results show for the first time that Belatacept influences B cell compartments by favoring the occurrence of transitional B lymphocytes which may display regulatory properties. This role may explain the lower allo-immunization rate observed in Belatacept-treated patients.

COMPLICATIONS

O19-0009 ECULIZUMAB FOR ATYPICAL HEMOLYTIC UREMIC SYNDROME RECURRENCE IN RENAL TRANSPLANTATION

Julien Zuber[1], Moglie Le Quintrec[2], Saoussen Krid[1], Caroline Bertoye[1], Victor Gueutin[3], Annie Lahoche[4], Nils Heyne[5], Giuseppe Ardissino[6], Valérie Chatelet[7], Laure-Hélène Noël[1], Maryvonne Hourmant[8], Patrick Niaudet[1], Véronique Frémeaux-Bacchi[9], Eric Rondeau[10], Christophe Legendre[1], Chantal Loirat[11] [1]Necker-Enfants Malades, Paris, France; [2]Foch, Suresnes, France; [3]Pitié-Salpétrière, Paris, France; [4]CHU Lille, Lille, France; [5]University Hospital Tuebingen, Tuebingen, Germany; [6]Center for HUS control, Milan, Italy; [7]CHU Caen, Caen, France; [8]CHU Nantes, Nantes, France; [9]HEGP, Paris, France; [10]Tenon, Paris, France; [11]Robert-Debré, Paris, France

Background: Eculizumab (anti-C5) has been sporadically reported as an efficient therapy for atypical Hemolytic Uremic Syndrome (aHUS). However, the lack of series precludes any firm conclusion about the optimal use of anti-C5 for preventing or treating aHUS posttransplant recurrence.
Patients and Methods: We thoroughly studied 22 renal transplant recipients with aHUS who received off-label therapy with anti-C5.
Results and Discussion: Nine patients, all carrying a complement genetic abnormality associated with a high risk of aHUS recurrence, received prophylactic anti-C5 therapy to prevent post-transplant recurrence. Three patients had already lost 4 previous renal transplants from early recurrence. Eight of them had a successful recurrence-free posttransplant course and achieved a satisfactory graft function, while the remaining patient experienced early arterial thrombosis of the graft. Thirteen renal transplant recipients were given anti-C5 for post-transplant aHUS recurrence. A complete reversal of aHUS activity was obtained in all of them. Importantly, the delay of anti-C5 initiation after the onset of the aHUS episode inversely correlated with the degree of renal function improvement. Three patients in whom anti-C5 was subsequently stopped experienced a relapse. Our cost analysis indicates that the difference in cost between anti-C5 and plasma therapies varies significantly depending on the weight of the individual and the frequency of maintenance plasma therapy.
Conclusion: Altogether these data suggest that long-term eculizumab therapy is highly effective for preventing and treating posttransplant aHUS recurrence. Our study also indicates that anti-C5 should be promptly started if a recurrence occurs.

O20-0077 ECULIZUMAB FOR POST-TRANSPLANTATION RECURRENCES OF FAMILIAL HUS WITH C3 MUTATION

Romain Crochette[1], Bruno Hurault De Ligny[1], Thierry Lobbedez[1], Patrick Henri[1], Maxence Ficheux[1], Cindy Castrale[1], Jean-Philippe Ryckelynck[1], Véronique Fremeaux-Bacchi[2], Valérie Chatelet[1] [1]Centre Hospitalo-Universitaire, Caen, France; [2]Hopitaux universitaires Paris Ouest-HEGP, Paris, France

Eculizumab has proven its effectiveness in the treatment of atypical Hemolytic and Uremic Syndrome (aHUS) and could prevent disease recurrences after renal transplantation.

We report the case of a sister and a brother, with a gain of function mutation (R570Q) in the C3 gene, who respectively presented an early and a late recurrence of aHUS on their renal transplants. Interestingly, the brother had previously undergone two transplants but never experienced disease recurrences. Thus, for a same mutation, there is a heterogeneous clinical presentation of the disease after transplantation, suggesting an important role played by triggering events. Eculizumab was effective in this case but it did not lead to a full recovery of the renal function, probably because of the late administration of the treatment.

Eculizumab showed sustained efficacy and safety after 4 years of treatment of an aHUS recurrence in the sister. The history of the brother teaches us that the absence of aHUS recurrence after one or two anterior transplantations does not exclude a recurrence on a later transplantation.

These data suggest that Eculizumab should be used as a preventive treatment for a renal transplantation in patients with aHUS related to a C3 mutation.

O21-0176 THE SOLUBLE RECEPTOR FOR UROKINASE IS NOT ELIMINATED BY IMMUNOABSORPTION ON PROTEIN A COLUMNS IN THE RECURRENCE OF FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS AFTER TRANSPLANTATION, SUGGESTING THE PRESENCE OF ANOTHER CIRCULATING FACTOR

Severine Beaudreuil, Elie Zhang, Helene Francois, Bernard Charpentier, Hans Lorenzo, Antoine Durrbach INSERM 1014, IFRNT, Hopital Bicetre, Le Kremlin Bicetre, IFRNT, Université Paris Sud, France

Introduction: Focal and segmental glomerulosclerosis (FSGS) is a serious disease whose pathogenesis is unknown. Its recurrence after transplantation (Tx) and its partial remission after treatment with immunoabsorption (Iabs) on protein A column is in favor of the existence of a circulating factor responsible for the disease and able to bind to the protein A column. Recently, the soluble receptor of urokinase (suPAR) was described as the soluble factor responsible for FSGS. We tested the capacity of suPAR to bind to Iabs and to be eliminated by Iabs.
Materials and Methods: SuPAR was measured by ELISA Quantikine Human suPAR (R & D) in the elates of 6 patients with recurrent FSGS after Tx and treated by Iabs (group 3) and in the serum of 11 patients with recurrent FSGS after Tx (group 1) and 11 healthy controls (group 2). In addition, suPAR was immuno-absorbed *in vitro* on protein A Sepharose column C6MB (sigma) from serum of patients from group 1 and 2. suPAR was quantified in the elutes (glycine pH2, 5) and in post column sera.
Results: The concentration of suPAR in serum is identical before and after Iabs on protein A in group 1 and 2 (respectively for group 1: 1715.7 vs. 1537 pg / ml, p = ns. For group 2 2268.2 vs. 2515.7 pg/ml; p ns). SuPAR concentration was low in elutes from protein A columns incubated with serum of patients from group 1 and 2 (respectively 71 and 83.6 pg / ml). *In vivo*: suPAR concentration from the elute obtained after Iabs of patients with recurrent FSGS was 30 pg / ml (group 3).
Conclusion: suPAR does not bind to protein A *in vitro* or *In vivo*. SuPAR does not seem to be the only circulating factor responsible for recurrent FSGS in our population.

O22-0097 NEW ANTICOAGULANT IMPROVES EARLY AND LONG TERM KIDNEY GRAFT FUNCTION

Solenne Tillet[1], Sébastien Giraud[1], William Hébrard[2], Raphael Thuillier[1], Gérard Mauco[1], Maurice Petitou[3], Thierry Hauet[1] [1]Inserm U1082, Université de Poitiers, CHU de Poitiers, Poitiers, France; [2]Plateforme de Chirurgie Expérimentale IbiSA, INRA Le Magnéraud, Surgères, France; [3]Endotis Pharma, Parc Biocitech, Romainville, France

Introduction: Static organ preservation still needs to be improved for an optimal use of marginal donors. Coagulation activation triggers major injuries affecting organs during the transplantation process. In the current work, we tested a new compound with a unique anticoagulant profile (EP217609), inhibiting two coagulation proteases, for its effectiveness in organ preservation.
Methods: EP217609 was evaluated *in vitro*, in endothelial cells cultivated under conditions mimicking organ preservation, and *In vivo* in a porcine model of kidney auto-transplantation. Kidneys were subjected to 60 mn warm ischemia prior to collection then to 24 h cold storage, in UW solution supplemented with standard heparin (UFH) or EP217609. Animals were followed during 3 months after transplantation.
Results: *In vitro*, addition of EP217609 improved cell survival at the end of hypoxia period ($P = 0.06$) *In vivo*, kidneys preserved with UW+UFH showed delayed function recovery, with only 3/6 animals recovering diuresis at day 2, compared to 5/6 of animals on the EP217609 group. The 3 months follow up revealed benefits of EP217609 illustrated by a faster return to normal creatininemia levels obtained as early as day 30 ($p < 0.05$ versus UFH). Fibrosis level as well as epithelio-mesenchymal transition injury were importantly reduced in the EP217609 group. Leucocytes inflammation/coagulation pathway mRNA expression were significantly reduce in EP217609.
Conclusion: In this model, targeting the coagulation pathway, with an inhibitor of two coagulation proteases, during kidney preservation provided benefits on term of graft function recovery, inflammation reduction and chronic outcomes. The unique anticoagulant profile of EP217609 makes it effective in organ preservation, with a high potential for translation to the clinic. Indeed, such a molecule, easily added to a preservation protocol in the clinic, would be invaluable to help facing the decreasing quality of transplanted organs.

O23-0114 NET TIME-DEPENDENT ROC CURVES: A NEW METHOD FOR EVALUATING THE ACCURACY OF A MARKER TO PREDICT MORTALITY RELATED TO END-STAGE RENAL DISEASE IN KIDNEY TRANSPLANT RECIPIENTS

Marine Lorent[1], Magali Giral[2], Yohann Foucher[1] [1]Departement of Biostatistics EA 4275, Clinical Research and Subjective Measures in Health Sciences, University of Nantes, Nantes, France; [2]Transplantation, Urology and Nephrology Institute (ITUN), Nantes Hospital and University, Inserm U1064, Nantes, France

Identifying prognostic markers of mortality in transplantation is essential for determining patients at high-risk of death and optimizing medical management. Nevertheless, an important part of the mortality may not be due directly to end-stage renal disease and/or kidney transplantation. Moreover, it is often impossible to individually determine the death causality, for example a cancer can be related to the chronic disease and immunosuppressive drug exposure or this cancer would have an independent link.

In survival analysis, one solution is to distinguish between the expected mortality of one general population (estimated on the basis of mortality tables) and the excess mortality attributable to the pathology, by using an additive relative survival model. The main objective of such models is to estimate the "net survival", survival which would be observed if the only possible death is related to the transplantation.

We have adapted this concept in order to evaluate the capacity of a marker to predict the mortality attributable specifically to transplantation.

We illustrate this method of net time-dependent ROC curves with the analysis of kidney transplant recipients of the DIVAT (Nantes). From 1230 patients and by using the mortality tables of the general population, we have validated the score of Hernandez et al. (Transplantation 2009) to predict the all-cause mortality for a prognostic time at 10 years post transplantation (AUC=0.68, CI95%= [0.62, 0.74]). However, this score seems to not predict the mortality specifically related to end stage renal disease in the ten years post transplantation (AUC=0.65, IC95%= [0.56, 0.72]). We have modified this score by recalculating each variable weight. This modified score allows to do a better prognostic of the excess mortality (AUC=0.73, IC95%= [0.64, 0.80]).

O24-0165 PREVALENCE AND PREDICTION OF CARDIOVASCULAR EVENT AFTER KIDNEY TRANSPLANTATION

Marianne Delville, Delphine Girard, Laurent Sabbah, Caroline Elie, Christophe Legendre, Rebecca Sberro Soussan APHP, Hopital Necker, Service de Transplantation Rénale Adulte, Paris, France

Introduction: Ischemic heart disease is the first cause of mortality after renal transplantation. Prevention needs a good screening strategy. The purpose of this study is to evaluate our center practice.

Material and method: 244 patients above 50 years old at transplantation waiting list inscription and receiving kidney graft were included. Results of cardiac evaluation done before kidney transplantation were analyzed: clinical evaluation, electrocardiogram, echography, cardiac scintigraphy (n = 188) and coronarography (n = 81). During one year of follow-up, occurrence of cardiovascular event was collected.

Results: 62% of patients had 4 cardiovascular risk factor or more: 45.3% of smokers, 37% of diabetes, 81% of dyslipidemia, 94,7% of high blood pressure andet 21.3% withavec past cardiac clinical history. Cardiac evaluation showed: 18.5% abnormal electrocardiogram (LVH, rythm or repolarization alteration); for cardiac echography: 50.4% of LVH (120/244), 10.2% cardiac kinetic anomalies, 9.2% with EF < 55%; 13.5% pathologic thallium imaging and 10,2% of coronary artery disease at coronarography.

38/283 (13%) of patients experienced a cardiovascular event: troponin increase (n = 14/38) or acute coronary syndrome (n = 7/38) association of electrocardiogram alteration and troponin increase. No patient died.

Significant parameters associated with a cardiovascular events were: a past medical history of cardiovascular disease (OR= 2,06; P = 0,03), Left Ventricular Hypertrophy (OR=2,04; P = 0,037), Left Ventricular Function < 55% (OR=1,46 [1,19;2,09]; P = 0,08) and an abnormal thallium scintigraphy made in pre-transplantation evaluation (OR=2,25 [1,09;5,96]; P = 0,03). Other parameters including other traditional cardiovascular risk factors as BMIIMC, dyslipidemia or diabetes were not significant.

Conclusion: Cardiovascular event are commonly observed after transplantation. Cardiac echography and thallium scintigraphy are key evaluation because they are prognostic factor. More over, they allow asymptomatic patients evaluation (53.8%).

O25-0003 DETERMINATION OF OPTIMAL CHOLECALCIFEROL TREATMENT IN RENAL TRANSPLANT RECIPIENTS USING A POPULATION PHARMACOKINETIC APPROACH

Sihem Benaboud[1], Saik Urien[1], Eric Thervet[3], Dominique Prié[4], Christophe Legendre[5], Jean-Claude Souberbielle[4], Déborah Hirt[2], Gérard Friedlander[4], Jean-Marc Tréluyer[2], Marie Courbebaisse[4] [1]Université Paris Descartes, Sorbonne Paris Cité, EA3620, Unité de Recherche clinique, AP-HP, Hôpital Tarnier, Paris, France; [2]Université Paris Descartes, Sorbonne Paris Cité, EA3620, Unité de Recherche Clinique, AP-HP, Hôpital Tarnier, Paris, France; [3]Service de Néphrologie, Hôpital Européen Georges Pompidou, AP-HP, Université Paris Descartes,, Paris, France; [4]Service d'Explorations Fonctionnelles, Hôpital Necker Enfants Malades, AP-HP, Université Paris Descartes, Paris, France; [5]Service de Transplantation Rénale, Hôpital Necker Enfants Malades, Université Paris Descartes, Paris, France; [6]Service de Pharmacologie Clinique, AP-HP, Groupe Hospitalier Cochin-Broca-Hôtel Dieu, Service de Pharmacologie Clinique, AP-HP, Groupe Hospitalier Cochin-Broca-Hôtel Dieu, Paris, France

Purpose: No information on optimal cholecalciferol dosing in kidney transplant patients is currently available because the time course of serum 25-hydroxy vitamin D (25(OH)D) concentration has never been investigated. Therefore this study was carried out to investigate 25(OH)D pharmacokinetics in renal transplant recipients and to determine the optimal dosage scheme allowing to maintain 25(OH)D concentrations between 30−80 ng/ml during the first year post-transplantation.

Methods: Four months after renal transplantation, 49 patients received four oral doses of 100 000 IU cholecalciferol every two weeks (intensive phase), then every two months until one year after transplantation (maintenance phase). A control group of 47 transplanted patients was not supplemented but underwent blood sampling. In the treated group, seventy-four samples were collected before the first cholecalciferol administration and 119 thereafter. Two blood samples per patient were collected in the control group. Serum 25(OH)D concentrations were analyzed using a population approach. The turnover of 25(OH)D was modeled using a one-compartment-model with first order formation and elimination, and basal concentration.

Results: The mean population parameter estimates and the associated between- subject variability were: formation rate constant (kf) 0.11 d-1, clearance (CL/F) 2.5 L/d (0.42), central volume of distribution (VC/F) 237 L and basal concentration (C0)12.82 ng/ml (0.41). In order to maintain 25(OH)D concentrations between 30−80 ng/ml, cholecalciferol dosing should be: six successive administrations of 100 000 IU at two-week intervals, then 100 000 IU, once a month, until the end of the first year.

Conclusions: We present here the first pharmacokinetic model describing the time course of 25(OH) D. We propose an optimal and practical scheme for treatment of vitamin D insufficiency after renal transplantation. Considering the numerous effects of vitamin D on health, this scheme could help clinicians to improve the care of kidney recipients.

O26-0155 IMPACT OF SARCOPENIA IN MORTALITY AFTER LIVER TRANSPLANTATION IN HIV COINFECTED PATIENTS

Teresa Maria Antonini[1], Oriana Ciacio[1], Petru Bucur[1], Siviu Bivol[2], Audrey Coilly[1], Philippe Ichai[1], Gabriella Pittau[1], Elina Teicher[3], Anne Marie Roque Afonso[4], Antonio Sa Cunha[1], René Adam[1], Denis Castaing[1], Didier Samuel[1], Jean Charles Duclos-Vallée[1], Eric Vibert[1] [1]AP-HP, Centre Hépato-Biliare, Inserm U785, Hôpital Paul Brousse, Villejuif, France; [2]AP-HP, Service de Radiologie, Hôpital Paul Brousse, Villejuif, France; [3]AP-HP, Service Maladies Infectieuses, Hôpital Kremlin Bicetre, Le Kremlin Bicetre, France; [4]AP-HP, Service de Virologie, Hôpital Paul Brousse, Villejuif, France

Long term results of liver transplantation (LT) for viral hepatitis in HIV-positive patients are inferior to those of mono-infected patients, new tools are needed to select candidates.

Aim: To evaluate the impact of sarcopenia on post transplant mortality in HIV coinfected patients.

Patients and methods: Between January 2007 and December 2011, 56 HIV+ patients (mean age 46,8 y [+/− 5], F/M [8/48], with mean follow-up 35 months [+/− 24], were transplanted for HCV, HBV and HBV/HCV/HDV related cirrhosis (n = 44, 6 and 6 respectively) of those 17 patients had HCC.

Cross-sectional areas of the left and the right psoas muscles at the level of the 4th lumbar vertebra were determined in our population. Pre-operative donor and recipient characteristics were analyzed and for continous variables a cut-off has been determined by a ROC curve.

Results: Overall survival at 1 and 3 years was 77% and 55% respectively. In univariate analysis, risk factor were psoas area <1691 mm2, MELD > 17, cirrhosis other than HBV and preoperative urea. In multivariate analysis all those factors but urea, were significantly associated with mortality (P = 0.028, 0.049 and 0.038 respectively). In sarcopenic recipients with MELD score > 17 and non HBV correlated cirrhosis overall survival was 22.2% vs. 66% of remnant population (OR 11.3 P = 0.001).

Conclusion: In HIV coinfected patients, mortality after liver transplantation is strongly associated with sarcopenia (P = 0,028) especially in HCV+ patients with high MELD score (>17).

O27-0080 POST RENAL TRANSPLANT HYPERPARATHYROIDISM: CAUSES AND CONSEQUENCES FOR GRAFT FUNCTION

Thomas Stehlé[1], Marie-Noëlle Peraldi[2], Emmanuelle Vidal-Petiot[1], François Vrtovsnik[3], Martin Flamant[1] [1]Service de Physiologie Explorations fonctionnelles, Hôpital Bichat-Claude Bernard, Paris, France; [2]Service de Néphrologie-Transplantation, Hôpital Saint-Louis, Paris, France; [3]Service de Néphrologie, hôpital Bichat-Claude Bernard, Paris, France

Post kidney transplant tertiary hyperparathyroidism (HPT3) is a common complication in kidney transplant recipients. This metabolic abnormality is characterized by an autonomous pattern of PTH secretion. Potential effects of HPT3 on graft function have never been proved.

The objective of this longitudinal retrospective observational study was to determine the consequences of HPT3 on glomerular filtration rate measured by 51CrEDTA clearance (mGFR), at 3 and 12 months after kidney transplant (KT).

The patients with secondary forms of hyperparathyroidism due to deceased graft function or vitamin-D deficiency were excluded. The diagnosis of HPT3 was based either on the association of elevated PTH level and ionized calcemia (iCa), or of elevated PTH with high normal iCa level, or of hypercalcemia with unadapt PTH level.

At 3 months after KT, 35 patients had HPT3 (HPT3 group), and 39 had normal parathyroid function (Normal group). Risk factors of this disorder were cinacalcet treatment before KT and duration of dialysis of more than 6 months. At 12 months mGFR was 44.4 ± 14.6 ml/min/1.73m^2 in HPT3 group versus 53.8 ± 16.9 ml/min/1.73m^2 in Normal group ($P = 0.01$). In multivariate analysis HPT and immune rejection were the only two factors explaining a GFR <40 ml/min/1.73m^2 at 1 year. Although the correlation between calcium excretion rate and GFR variation between 3 and 12 months did not reach statistical significance, calcium deposits were present in 24% of biopsies in the HPT group against 3% in the Normal group.

Our results demonstrate a strong association between PKT-HPT and kidney graft function. Calcium deposits could mediate the effect of this metabolic disorder on kidney graft function. Our results encourage optimal management of mineral disorders in patients waiting kidney graft, and lowering dialysis duration, in order to prevent tertiary hyperparathyroidism.

HLA/DSA

O28-0005 OUTCOME AFTER KIDNEY TRANSPLANTATION ACCORDING TO THE VIRTUAL, FLOW CYTOMETRIC, AND COMPLEMENT-DEPENDENT CYTOTOXIC CROSSMATCHES

Gabrielle Duneau[1], Rodolphe Thiébaut[3], Thomas Bachelet[2], Pierre Merville[2], Jean-Luc Taupin[2], Lionel Couzi[2] [1]Université Bordeaux 2, Bordeaux, France; [2]Centre Hospitalier Universitaire, Bordeaux, France; [3]INSERM U897, Bordeaux, France

Background: Currently, decision to perform kidney transplantation is still based on complement-dependent cytotoxicity crossmatch (CDC-XM) or flow cytometric crossmatch (FC-XM). But, the decision to perform a CDC-XM or FC-XM is often based on virtual XM (v-XM). In this study we evaluated the concordance of these assays and assessed graft outcome according to these XM results.

Method: 303 kidney transplant recipients were included. v-XM was retrospectively performed on a recent serum using the single antigen bead assays on a Luminex® platform. We used Cohen's Kappa coefficient (CKC) to determine agreement between the assays. Kaplan-Meier analysis was used to construct graft survival curves.

Results: v-XM was found positive for 8.91% of patients, CDC-XM for 5.86% and FC-XM for 17.6%. The agreement between CDC-XM and v-XM was poor (CKC=0.18; 87.9% of concordant XM). The agreement between FC-XM and v-XM was also poor (CKC=0.07; 78.2% of concordant XM). Rejection-free survival was worse in patients with a positive FC-XM/positive v-XM ($P = 0.01$), while no differences were observed between the 3 others groups (negative FC-XM/positive v-XM, positive FC-XM/negative v-XM, negative FC-XM/negative v-XM). Graft survival was similar between the four different FC-XM/v-XM groups ($P = 0.67$). Similar results were observed with the different CDC-XM/v-XM groups.

Conclusion: Poor agreements are observed between the different XM assays. Since graft survival and rejection-free graft survival are similar among negative v-XM patients whatever the XM result, it could be possible to omit XM before kidney transplantation in this sub-group of patients.

O29-0182 RELEVANCE OF PRETRANSPLANT VIRTUAL CROSSMATCHING FOR DONOR SPECIFIC ANTI-HLA DETECTED BY SINGLE ANTIGEN LUMINEX ASSAY ON ACUTE REJECTION IN LUNG TRANSPLANTATION

Chantal Gautreau[1], Caroline Suberbielle[1], Magali Colombat[2], Maryvonnick Carmagnat[1], Marc Busson[3], Dominique Charron[1], Marc Stern[4] [1]AP-HP Hôpital Saint Louis Laboratoire d'Histocompatibilité, Paris, France; [2]Hôpital Foch Service d'Anatomo-Pathologie, Suresnes, France; [3]INSERM UMR940, Paris, France; [4]Hôpital Foch Service de pneumologie et transplantation pulmonaire, Suresnes, France

Due to the short ischemia time allowed between organ harvest and transplantation, it is difficult in lung transplantation to do a prospective Complement dependent cytotoxic crossmatch.and a single antigen luminex (LSA) assay in virtual crossmatch (VC) is thus performed to determine the compatibility between the donor HLA antigens and the recipient antibodies.

The use of LSA-VC to allocate organ is a subject of debate over the relevance of donor-specific anti-HLA antibodies (DSA) detected by this method.

The presence of DSA post-transplant in conjunction with tissue immunostaining for Complement fixation (C4d) have provided evidence that humoral rejection occurs in lung transplantation often in parallel with acute cellular rejection affecting more than a third of lung transplant recipients.

This retrospective study aimed to assess the relevance of DSA detected by LSA-VC in pre-transplant serum samples from lung transplanted patients, on 1) the occurrence of acute rejection (cellular and/or humoral rejection), 2) the overall patients survival at more than 3.5 years post transplant.

Thirty seven patients were consecutively lung transplanted in 2008 in Hôpital Foch transplant unit. For each patient one serum drawn before transplantation was retrospectively tested by LSA (One Lambda). DSA anti-HLA class I and class II identification were considered positive when the mean fluorescence intensity was>1000. LSA-VC was considered negative when no DSA was identified. Diagnosis of acute rejection was supported by clinical and histologic features, C4d staining of lung biopsy and circulating DSA.

Pretransplant LSA-VC was found positive in 27% of patients. Acute rejection episodes occurred in 32% of patients within the first year. Acute rejection occurred in 70% of cases when VC is positive and 21% when VC is negative and difference is significant ($P = 0.0001$) by using a log-rank test. At 3–4 years post-transplant 81% of patients were alive. No significant difference in patient survival was observed between positive and negative LSA-VC.

Our study supports the use of virtual crossmatching by using LSA to allocate lung organ with the aim to lower the occurrence of acute rejection in lung transplantation.

O30-0040 EFFECT OF PROTEASOM INHIBITION WITH BORTEZOMIB ASSOCIATED WITH STEROIDS ON THE DECREASE OF HLA ANTIBODIES BEFORE TRANSPLANTATION

Olivier Aubert[1], Helene Francois[1], Caroline Suberbielle[2], Erika Nnang Obada[1], Bernard Charpentier[1], Antoine Durrbach[1] [1]Le Kremlin Bicetre, IFRNT, Université Paris Sud, Le Kremlin Bicetre, Val de Marne, France; [2]Laboratoire d'Histocompatibilité, Hopital Saint Louis, Paris, France

Introduction: Currently, all proposed treatments for desensitivation are often ineffective or transient (plasmapheresis, intravenous immunoglobulin or rituximab) to control the production of antiHLA antibodies by B or plasma memories cells. Bortezomib (BTZ), a proteasome inhibitor active against multiple myeloma in association with steroids has been proposed to desensitize patients with alloimmunization. Used alone, the results are contradictory. The objective of this study is to investigate the effect of corticosteroids and BTZ on the reduction of anti-HLA antibodies before renal transplantation.

Materials and methods: It is a prospective monocentric study taking place from March 2009 to February 2011. Patients included had a stable immunization against HLA, and were awaiting kidney transplantation.

Treatment consisted of Velcade ® (BTZ) 1.3 mg/m2 on days 1, 3, 7 and 9 associated with 40mg of dexamethasone intravenously. Anti HLA class 1 and 2 were determined by Luminex single antigen at D0, M1, M3 and M6.

Results: Twenty-three patients were included and had an average number of anti-HLA antibodies on day 0 of 49, 31 corresponding to Class 1 and 18 to Class 2. At D0, the MFI (mean fluorescence) was 10,627 for class 1 immunodominant antibody and 7577 class 2. Eleven (47.5%) patients had a decrease of more than 25% of the class 1 immunodominant antibody at M3 1 and 15 (65%) at M6. Seven (32%) patients had a decrease of more than 25% of the Class 2 immunodominant antibody at M3 and 11 (50%) at M6. A M3, 51% of anti-HLA antibodies have declined by more than 25% (MFI) and 31% over 50%. This effect was maintained at M6, 58% of anti-HLA antibodies have declined by more than 25% and 41.5% over 50%.

Including any previously received treatment, no factor were predictive of antibody decline after BTZ. No serious adverse events have been observed. Nine of 23 patients were transplanted successfully thereafter.

Conclusion: Associated with corticosteroids, BTZ is an effective alternative therapy for reducing the rate of class 1 and 2 of anti HLA, regardless of the previous use of other treatments such as Rituximab prior or IVIg.

O31-0154 ANTI-HLA IMMUNIZATION PROFILE AT THE TIME AND AFTER RENAL ALLOGRAFT NEPHRECTOMY

Claire Leibler[1], Marie Matignon[1], Caroline Suberbielle-Boissel[2], Françoise Roudot-Thoraval[1], Laurent Salomon[1], Philippe Lang[1], Philippe Grimbert[1] [1]Hôpital Henri Mondor, Créteil, France; [2]Hôpital Saint Louis, Paris, France

Introduction: Renal allograft nephrectomy is a major risk factor for alloimmunization and can increase time on the waiting-list. Anti-HLA immunization profile, DSA and non-DSA, remains incompletely studied.

Methods: We retrospectively analyzed anti-HLA DSA and non-DSA, using Luminex technology, in 58 patients presented with renal allograft nephrectomy between 2005 and 2010 at our center. For each patient, anti-HLA antibodies were assessed at the time of nephrectomy, 3 and 12 months after. Twenty-one were excluded because of intravenous immunoglobulins treatment after surgery.

Results: We defined three groups of patients according to nephrectomy clinical background: group 1A- late nephrectomy (> 6 months) and intolerance graft syndrome ($n = 32$), group 1B– asymptomatic late nephrectomy ($n = 13$) and group 2 – early nephrectomy (< 6 months) ($n = 13$).

At the time of nephrectomy, class II non-DSA were similar in group 1A and 1B but group 1A has significantly more class I non-DSA (MFI > 1000, > 3000 and > 6000) than group 1B ($P = 0,03$; 0,05 and 0,03 respectively). MFI max class I DSA was significantly higher in group 1A ($P = 0,003$). After 3 and 12 months, median number of class I non-DSA with MFI > 1000 increased significantly in group 1A ($n = 15$) with peak reached after 3 months. After 12 months, group 1B ($n = 12$) showed bimodal evolution: median number of anti-HLA non-DSA with MFI > 3000 increased significantly for 60% of patients and 40% were stables. Class II non-DSA were on steady state before nephrectomy and after 12 months. All patients developed all DSA after 12 months.

Patients in group 2 ($n = 10$) presented with significantly less anti-HLA antibodies at the time of nephrectomy. Median number of anti-HLA non-DSA with MFI > 1000 increased significantly after 3 and 12 months ($P = 0.02$ and 0.04). Sixty six percent of patients developed all DSA after 12 months with MFI max > 6000 for all of them.

Conclusion: Our results confirmed that renal allograft nephrectomy is a major risk factor of DSA and non-DSA HLA immunization. Clinical background at the time of nephrectomy clearly influences anti-HLA antibodies. To optimize strategies to prevent alloimmunization at the time of renal allograft nephrectomy, we need to consider this parameter.

O32-0168 PRETRANSPLANT DONOR HLA-CW-SPECIFIC ANTIBODIES AND ANTIBODY-MEDIATED REJECTION IN RENAL ALLOGRAFT RECIPIENTS

Marie-Cécile Bories[1], Olivier Aubert[1], Caroline Suberbielle[2], Alexandre Loupy[1], Frank Martinez[1], Dany Anglicheau[1], Renaud Snanoudj[1], Dominique Charron[2], Christophe Legendre[1], Rebecca Sberro-Soussan[1] [1]Hôpital Necker, PARIS, France; [2]Hôpital Saint Louis, PARIS, France

Donor-specific HLA alloantibodies (DSA) may cause acute and chronic antibody-mediated rejection (AMR) and significantly compromise allograft survival. The clinical relevance of antibodies directed against some HLA class I antigens, particularly HLA-Cw, is unclear with conflicting reports on their pathogenicity. Their role, in the absence of classic anti-class I and II antibodies, is unknown. We evaluated the clinical relevance of the presence of donor-specific HLA-Cw alloantibodies at day 0 in renal transplant recipients.

Methods: From July 2009 to April 2011, 21 patients were included with HLA-Cw DSA at day 0. Neither class I nor class II DSA were significantly present (Mean Fluorescent Intensity (MFI) < 500, Luminex Single Antigen Method). Immunosuppressive treatment included steroids, mycophenolate mofetil, calcineurin inhibitors, with basiliximab ($n = 7$) or antithymocyte globulin ($n = 14$) at induction. AMR were treated preventively with plasma exchanges (PE $n = 7$), antiCD20 ($n = 5$) and intravenous immunoglobulins (IVIg, $n = 16$). AMR were classified according to 2011 Banff meeting report.

Results: One year graft survival was 95.2% and survival rate was 100%. Five patients (23.8%) experienced AMR. The median MFI was respectively 6130 [978−17941] for those patients and 2696 for those without AMR. They were treated with methylprednisolone, PE, IVIg and anti-CD20. At one year GFR was 48 ml/min/1.73m^2 in these five cases and 52 + /−21.4 ml/min/1.73 m^2 in the 15 controls. The one-year biopsy showed persistant glomerulitis (grade1−2) in all patients. Infectious diseases occured in 13 patients.

O33-0161 IMPACT OF INTRAVENOUS IMMUNOGLOBULINS ON ANTI-HLA IMMUNIZATION AFTER RENAL ALLOGRAFT NEPHRECTOMY

Claire Leibler[1], Marie Matignon[1], Caroline Suberbielle-Boissel[2], Françoise Roudot-Thoraval[1], Laurent Salomon[1], Philippe Lang[1], Philippe Grimbert[1] [1]Hôpital Henri Mondor, Créteil, France; [2]Hôpital Saint Louis, Paris, France

Introduction: Renal allograft nephrectomy is a major risk factor for anti-HLA alloimmunization. At our knowledge, no study evaluated any preventive strategy. We analyzed impact of intravenous immunoglobulins (IVIg) on anti-HLA immunization after renal allograft nephrectomy.

Methods: We retrospectively analyzed anti-HLA DSA and non-DSA, using Luminex technology, in 58 patients presented with renal allograft nephrectomy between 2005 and 2010 at our center. For each patient, anti-HLA antibodies were assessed at the time of nephrectomy, 3 and 12 months after. Twenty-one (36%) were treated with IVIg (1,5g/kg).

Results: Renal allograft nephrectomy was performed for intolerance graft syndrome (IGS) more than 6 months after transplant in 17 (81%) treated patients (TT) and in 15 (41%) non treated (NT) ($P = 0{,}005$). At the time of nephrectomy, class I non-DSA immunization was significantly higher in TT patients compare to NT. After 3 or 12 months, DSA and non-DSA immunization increased significantly in both groups. In patients presented with toxic graft syndrome, TT and NT groups were strictly comparable (TT $n = 17$; NT $n = 15$) at the time of nephrectomy, 3 and 12 months after. Among those patients, immunization was really high even at the time of nephrectomy (DSA/median number of MM = 0.76 (0−1); median number of anti-HLA non-DSA with MFI>1000 = 34 (0−75)).

Patients with no DSA at the time of nephrectomy (TT $n = 7$; NT $n = 12$), presented with same anti-HLA non-DSA characteristics in both groups TT or not. Five (71%) TT patients presented with IGS besides 2 (16%) NT ($P = 0.04$). After 3 and 12 months, class I and class II DSA and non-DSA were significantly higher in NT group compare to TT group. TT group only increased significantly number of class I non-DSA with MFI > 1000. After 3 months, increase of class I non-DSA median number (MFI > 1000) and of non-DSA MFI max were significantly lower in TT group (50% vs. 96% ($P = 0{,}04$); 21% vs. 87% ($P = 0{,}02$) respectively).

Conclusion: IVIg at the time of renal allograft nephrectomy did not seem to have any impact of anti-HLA immunization in presensitized patients. Our study suggested that IVIg can prevent class I alloimmunization in patients with no DSA at the time of nephrectomy. These results need to be considered in processing therapeutic protocols to prevent alloimmunization after renal allograft nephrectomy.

O34-0140 KIDNEY INTRAGRAFT DSA DETECTION IMPLEMENTS THE DEFINITION OF ANTIBODY-MEDIATED LESIONS

Thomas Bachelet, Lionel Couzi, Sebastien Lepreux, Maud Legeret, Guillaume Pariscoat, Gwendaline Guidicelli, Pierre Merville, Jean-Luc Taupin [1]CHU Bordeaux, Bordeaux, France; [2]Université Bordeaux 2, Bordeaux, France; [3]UMR CNRS 5164 CIRID, Bordeaux, France

Introduction: Allograft pathology, interaction of antibody with the tissue as demonstrated by C4d deposition and serologic evidence of DSA are the three cardinal features required for the diagnosis of antibody-mediated lesions (AML) in kidney transplantation. However, discrepancy between histological and serological findings is common, and more reliable diagnostic tools are called for.

Material and Method: Intragraft DSA (gDSA) was assessed in fifty-one graft biopsies, performed for cause (12 acute antibody mediated rejection [AAMR], 4 suspicions of AAMR, 8 chronic antibody mediated rejection [CAMR], 8 suspicions of CAMR, 9 acute cellular mediated rejection [ACMR], 6 with lesions of interstitial fibrosis and tubular atrophy [IFTA], and 3 normals) after acidic elution from the graft tissue, using class I and class II single antigen flow cytometry bead assays on a Luminex platform. Most of the kidney transplant recipients were HLA-sensitized (90%), and 67% displayed circulating sDSA. Histological Banff's items analysis was performed individually and in functional clusters.

Results: Fifteen (29%) patients had gDSA. The gDSA were present in the lesions electively mediated by antibodies (7/12 AAMR, 5/8 CAMR, 1/11 ACMR). gDSA+ biopsies were more frequently associated with AML than gDSA-biopsies, taken both individually [ptc> 0 in 53% of gDSA + ($n = 8$) vs. 26% gDSA- ($n = 9$), $P = 0.05$; g > 0 in 33% of gDSA + ($n = 5$) vs. 6% gDSA- ($n = 2$), $P = 0.01$; cg> 0 in 60% of gDSA + ($n = 9$) vs. 17% gDSA- ($n = 6$), $P = 0.002$] and in clusters [ptc+g+cg> 0 in 93% of gDSA + ($n = 14$) vs. 34% gDSA-($n = 12$), $P = 0.003$, ptc+g > 0 in 60% of gDSA + ($n = 9$) vs. 26% gDSA-($n = 9$), $P = 0.02$ and cg + mm> 0 in 75% of gDSA + ($n = 10$) vs. 23% gDSA-($n = 8$), $P = 0003$]. For sDSA+ biopsies, this association was only found for the cluster of microcirculation lesions [ptc+g+cg> 0 in 61% of sDSA + ($n = 24$) vs. 29% in sDSA-($n = 4$), $P = 0.04$]. The presence of C4d deposits in peritubular capillaries ($n = 26$ among 50 interpretable biopsies) was associated with gDSA ($P = 0.01$) but not with sDSA ($P = 0.3$) and 80% ($n = 12$) of the gDSA+ biopsies also displayed C4d deposition.

Conclusions: The presence of gDSA could represent an alternative and relevant marker of AML.

O35-0087 SIMILAR OUTCOME OF KIDNEY TRANSPLANTATIONS IN PATIENTS WITH «NATURAL» DONOR SPECIFIC ANTIBODIES RECEIVING LOW OR HIGH IMMUNOLOGICAL RISK IMMUNOSUPPRESSIVE PROTOCOL

Antoine Sicard[1], Lucile Amrouche[1], Caroline Suberbielle[3], Michel Delahousse[2], Christophe Legendre[1], Renaud Snanoudj[1] [1]Service de Transplantation Rénale, Hôpital Necker, Paris, Ile de France, France; [2]Service de Néphrologie, Hôpital Foch, Suresnes, Ile de France, France; [3]Service d'Histocompatibilité, Hôpital Saint Louis, Paris, Ile de France, France

Introduction: The development of Single Antigen Flow Beads assays has led to the observation that individuals without history of pregnancy, transfusion or transplantation could have ''natural'' anti-HLA antibodies. Although the detection of ''natural'' Donor Specific Antibodies (nDSA) before kidney transplantation is frequent, their association with an increased risk of antibody-mediated rejection (AMR) has never been studied. We compared a group of patients with preformed nDSA who received a High Immunologic Risk (HIR) immunosuppressive protocol with a historic control group treated with a standard therapy (ST).

Methodology: Patients were retrospectively included and were men with no history of transfusion or transplantation but with nDSA detected the day of transplantation. We compared 23 patients (HIR group) who received induction with thymoglobulin and intravenous immunoglobulins (IvIg) with 20 patients (ST group) who received basiliximab induction.

Results: The groups were similar regarding baseline characteristics. In the HIR group, the mean number of DSA was 1.6 ± 0.7 with a mean fluorescence intensity (MFI) of 1275 ± 1117 (320−3494). It was statistically similar in the ST group: 1.2 ± 0.4 and 1771 ± 2323 (564−8883).

After follow-up (HIR, 24 months; ST, 56 months), graft and patient survival were 100%. The one-year incidence of clinical/subclinical acute AMR was similar: 3/23 (13%) in the HIR group and 2/20 (10%) in the ST one. Cellular rejections were more frequent in the ST group (4/20 (20%) vs. 0/23 (0%), $P = 0.039$). There was no difference between the mean measured GFR at 1 year (HIR: 62 ± 33 vs. ST: 64 ± 30 ml/min/1.73m2). On one-year protocol biopsy, no patient had histological sign of chronic AMR. Mean IFTA and capillaritis scores were low and similar.

Conclusion: Patients receiving a kidney transplant with nDSA at day 0 experienced a one-year favorable outcome, with a low incidence of AMR. Evolution was similar after induction with thymoglobulin and IVIg compared to induction with anti-IL2-Receptor. This observation suggests that heavy immunosuppressive protocol is not mandatory in these patients but needs to be confirmed by further studies.

O36-0145 STUDY OF THE HUMORAL RESPONSE AFTER TRANSPLANTATION OF PORCINE NEUROBLASTS IN THE RAT BRAIN

Elodie Mathieux, Xavier Leveque, Veronique Nerriere-Daguin, Severine Menoret, Ignacio Anegon, Isabelle Neveu, Philippe Naveilhan
INSERM U1064, Nantes, France

Cell therapy is a real hope for neurodegenerative diseases. Interesting results were obtained after the transplantation of human fetal neuroblasts into the brain of Parkinsonian patients but tissue availability and ethical concerns limit this approach. Other cellular sources have therefore to be found. Fetal pig neuroblasts are interesting candidates as cellular source, but intracerebral xenotransplants are systematically rejected, even under systemic immunosuppression. A better understanding of immune mechanisms is still necessary to prevent rejection of intracerebral xenograft. Efforts have been done to inhibit locally the cellular immune reaction but little is known about the necessity of targeting the host humoral response.

We became interested in the humoral response of intracerebral xenograft with porcine neuroblasts in the rat striatum. The antibody production is observed in the sera of rats by immunocytologicals and FACS analysis. First, we don't observe preformed IgG directed against epitopes pigs. It's important to report that IgG don't recognize epitopes rats. We see an increase of IgG production correlated with time post transplant and the stage of rejection. In fact, anti-porcine antibodies appear quite late and maximum of accumulation is observed after the rejection of porcine neurons. Furthermore, a deposit of IgG and complement is found in graft during rejection. Moreover, we demonstrate that IgG recognize neurons, astrocytes and porcine aortic endothelial cells (PAEC).

The question is now to know the balance between humoral response and cellular response in the rejection to determine what may be the best strategy to limit the rejection. For this, the important role of the humoral response in transplant rejection is now studied in rats knock-out to immunoglobulin. Preliminary results show that the rejection seems delayed in KO rats but number of rats per group is not sufficient to conclude.

BIOMARKERS

O37-0112 URINARY CELL MRNA PROFILING PREDICTS THE SUBSEQUENT OCCURRENCE OF ACUTE REJECTION OF THE RENAL ALLOGRAFT: PRELIMINARY RESULTS FROM THE PREFIGUR STUDY

Marion Rabant[3], Raja Bonifay[2], Xavier Lebreton[1], Mélanie Routhier[2], Fabiola Terzi[2], Christophe Legendre[1], Dany Anglicheau[1] [1]Service de Transplantation Rénale Adulte, Hôpital Necker, AP-HP, Paris, France; [2]INSERM U845, Paris, France; [3]Laboratoire d'Anatomie Pathologique, Hôpital Necker, Paris, France

Introduction: Cross-sectional studies have suggested that urinary cell mRNA profiling may diagnose non-invasively acute rejection of the renal allograft. We initiated a single-center study in 01/2010 (Prefigur: Prediction of Allograft Fibrosis using urinary cell mRNAs), including a longitudinal follow-up of urinary cell mRNAs during the first year post-transplantation (Tx). Preliminary results are shown.

Methods: Urine samples were collected at 10 days, 1 Mo and 3 Mo post-Tx. mRNAs involved in alloimmune response and fibrogenesis, and 18S ribosomal RNA were quantified. All patients transplanted in our center in 2010, excluding HIV+ and/or HCV+ patients, were enroled (n = 142). Clinical, biological and histological (protocol biopsies at 3 Mo and 12 Mo and 'for cause' biopsies) data were collected. Our hypothesis was that urinary cell mRNAs are predictive of acute rejection between 3 Mo and 12 Mo.

Results: 136 patients were available (57% male; 50.3 ± 1.4 years). They received a kidney (deceased donor: 75%) of 54.0 ± 1.7 years, which meet the expanded criteria in 49% of cases. The cold ischemia time was 22.9 ± 8.1 h in deceased donor. Delayed graft function occurred in 33% of cases. A protocol biopsy was performed in 123 and 107 patients at 3 Mo and 12 Mo, respectively. 120 'for cause' biopsies were performed in 72 patients. Between 3 Mo and 12 Mo, 26 acute rejections (7 TCMR, 14 AbMR, 5 mixed) were diagnosed (19 subclinical / 7 clinical) in 25 patients. Urinary cell expression profile at 10 days and 1 Mo did not predict the subsequent occurrence of rejection. However, the occurrence of biopsy-proven acute rejection between 3 Mo and 12 Mo was significantly associated with urinary expression of perforin (P = 0.003) and CD25 (P = 0.02) mRNAs at 3 Mo. At 3 Mo, perforin was able to predict the subsequent occurrence of acute rejection with a sensitivity of 76% and a specificity of 72%.

Conclusion: These preliminary results from the Prefigur study suggest that urinary cell mRNA profiling may predict non-invasively the subsequent occurrence of acute rejection of the renal allograft.

O38-0181 DEVELOPMENT OF URINARY CELL MRNA SIGNATURE FOR DISTINGUISHING ACUTE KIDNEY ALLOGRAFT DYSFUNCTION

Marie Matignon[1], Thangamani Muthukumar[2], Catherine Snopkowski[2], Christine Hoang[2], Carol Li[2], Christina Chang[2], Ruchuang Ding[2], Choli Hartono[2], Manikkam Suthanthiran[2] [1]Henri Mondor, Créteil, France; [2]NYP - Weill Cornell, New York, United States

Background: Kidney allograft biopsy remains the gold standard diagnostic tool to differentiate common causes of acute allograft dysfunction; rejection from acute tubular necrosis (ATN) as well as acute antibody mediated from acute cell mediated rejection. Development of molecular biomarkers can help in early diagnosis, personalize therapy and may improve allograft outcome.

Methods: We examined if urinary cell mRNA profiling of kidney transplant recipients with graft dysfunction distinguish acute tubular necrosis (ATN) from acute cellular rejection (ACR) and acute antibody mediated rejection (AMR). We isolated total RNA from urinary cells of 58 kidney transplant recipients at the time of allograft biopsy done to ascertain the cause of graft dysfunction; 19 with ATN; 19 with ACR and 20 with AMR. Biopsies were categorized as per the Banff schema.

We designed oligonucleotide primers and TaqMan Probes for the measurement of a panel of 26 mRNAs using pre-amplification enhanced quantitative real-time PCR assay. The gene panel was based on literature review and included those expressed in B, T, mesangial, endothelial and renal tubular cells as well as those encoding complement components.

Results: Clinical and biochemical characteristics did not distinguish among ATN, ACR and AMR.

The levels of the housekeeping gene 18S rRNA was similar in the three groups. Urinary cell perforin mRNA levels distinguished ATN from acute rejection. Perforin mRNA level was significantly higher in ACR and AMR than in ATN (P = 0.003; post test p < 0.05) and was not different between ACR and AMR.

Five of the 26 mRNAs; CXCL13, FoxP3, OX40, CD3 and CD14 distinguished ACR from AMR. The remaining mRNAs were similar between the two groups suggesting shared pathogenetic pathways driving AMR and ACR.

Conclusion: Our results demonstrate the feasibility of using urinary cell mRNA to distinguish the common causes of allograft dysfunction from one another. Currently an allograft biopsy is performed to diagnose the causes of graft dysfunction. Validation of the molecular signature in a large cohort may obviate the need for invasive allograft biopsy to distinguish common causes of allograft dysfunction.

O39-0104 CLINICAL RISK PROFILE AND POOR TRANSPLANTATION OUTCOME ASSOCIATED WITH ISOLATED INFLAMMATORY INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY (I-IFTA) ON 1 YEAR SURVEILLANCE KIDNEY ALLOGRAFT BIOPSIES

Grégoire Couvrat-Desvergnes, Karine Renaudin, Magali Giral ITUN, Nantes, France

Introduction: Interstitial Fibrosis and Tubular Atrophy (IFTA) with inflammation including the scarred compartment (i-IFTA, ie total-i score) are recognized to be associated with a risk of graft loss or renal dysfunction. In this study, our aims were to identify clinical and biological features within the first year of tranplantation associated with isolated i-IFTA on one year surveillance biopsies and the 3 years outcome.

Patients and methods: This transversal study was performed on 276 patients, who received consecutively a first kidney or combined pancreas and kidney transplantation between 2004 and 2009 at the Transplantation Institute of Nantes University Hospital (France) and who underwent a surveillance kidney biopsy at one year. Histological lesions were analyzed according to the Banff 09 classification and classified into 4 diagnoses: normal histology, isolated IFTA with (i-IFTA ie total i-score) or without inflammation (IFTA), and lesions related to alloimmunity process (humoral or cellular acute or chronic rejection and borderline). Univariate analyses (Kruskal-Wallis, Xhi 2) and multinomial model were realized.

Results: of the multivariate analysis show that i-IFTA presented similar risk factors than IFTA (recipient age > 55, CIT>24h and DGF) but with proportionally more HLA mismatches and acute rejection episodes bringing them closer to the allo-immune group. At one year, patients with IFTA, i-IFTA or allo-immune features had lower eGFR rates (MDRD) compared with the normal group (46.9, 54.3, 48.05 and 63.5 ml/min respectively) (p < 0.0001). However, patients with i-IFTA or allo-immune lesions had a higher one year daily proteinuria (0.36g/d and 0.49g/d respectively) compared with patients with isolated IFTA (0.18g/d) or normal biopsies (0.22d/d) (P = 0.0011). Finally, at 3 years of follow-up patients with i-IFTA and allo-immune lesions had a higher decrease of e-GFR of 12.89% and 12.40% respectively than patients with IFTA (1.07%) and normal histology (4.39%).

Conclusion: We show that whether IFTA and i-IFTA share the same background of risk factors, i-IFTA present superimposed risk profiles (more acute rejection and HLA incompatibilities) and poorer graft outcome closer to allo-immune lesions.

O40-0050 SOLUBLE CASK, A CALCIUM / CALMODULIN SERINE PROTEIN KINASE, IS A FACTOR OF GLOMERULAR PERMEABILITY ASSOCIATED WITH RECURRENCE OF FOCAL SEGMENTAL HYALINO-SCLEROSIS AFTER TRANSPLANTATION

Severine Beaudreuil[1], Elie Zhang[1], Francis Harper[2], Helene Francois[1], Bernard Charpentier[1], Antoine Durrbach[1] [1]INSERM 1014, IFRNT, Hopital Bicetre, Le Kremlin Bicetre, IFRNT, Université Paris Sud, France; [2]CNRS, Institut Gustave Roussy, France

Introduction: Recurrence of nephrotic syndrome associated with focal segmental hyalinosis (FSH) rapidly after renal transplantation suggests the presence in the serum of a circulating factor that destabilizes the glomerular filtration barrier. Plasmapheresis and immuno-absorption on protein A columns have demonstrated their efficiency by eliminating the unknown factor and, therefore, reduce proteinuria. To characterize this factor, we analyzed the products eluted from prot A immune-absorption columns.

Method: Differential analysis by gel electrophoresis and mass spectroscopy eluates of protein A column from patients treated for recurrent FSGS after transplantation or patients treated for the presence of antibodies against factor XI.

Results: After elution of protein with an apparent molecular mass of 85 kDa was identified in patients with recurrent FSGS and not in controls. Analysis by mass spectrometry identified CASK (calcium / calmodulin-dependent serine protein kinase), a membrane-associated guanylate kinase (MAGUK) important for the maintenance of cell polarization in podocytes in patients treated with immuno-absorption for recurrence of FSGS. CASK was found in the serum of patients with recurrent FSGS and not in control sera. CASK produced *in vitro* by subcloning its cDNA induces the relocation of ZO-1 cell junctions and reorganization of actin stress fibers. It induces an increase in the trans-epithelial permeability of a monolayer of podocytes in culture. *In vivo* in mice, a single injection of CASK induced proteinuria. The electron microscopy analysis highlights a fusion of pedicels in podocytes.

Conclusion: Our results suggest that CASK is a soluble factor of glomerular permeability associated with recurrence of FSGS humans.

O41-0078 A PHENOTYPIC, TRANSCRIPTIONAL AND TCR Vβ REPERTOIRE SIGNATURE OF CD8 + T CELLS DEFINE A POPULATION AT-RISK OF LONG-TERM KIDNEY GRAFT DYSFUNCTION

Nicolas Degauque[1], Françoise Boeffard[1], Annaick Pallier[1], Richard Danger[1], Magali Giral[2], Jaques Dantal[2], Yohann Foucher[3], Cécile Guillot-Gueguen[2], Jean-Paul Soulillou[1], Sophie Brouard[1] [1]INSERM UMR1064, Nantes, France; [2]CHU de Nantes, ITUN, Nantes, France; [3]Université de Nantes, Nantes, France

Introduction: The biological mechanisms leading to chronic antibody-mediated rejection(CAMR), a major cause of late graft failure following kidney transplantation, are still poorly defined. Although anti-donor HLA antibodies are commonly associated with poor graft outcome; less attention had been paid to other players of the adaptive immune system.
Aim: We took advantage of a large cohort of 133 selected patients (112 patients remaining stable in time and 21 with kidney dysfunction over 6 years) to question the factors that may influence graft outcome.
Results: We show that T cell monitoring, and especially CD8 TCR repertoire alterations, may allow identifying patients at risk of graft dysfunction. As compared to patients without TCR Vβ repertoire alterations, patients with an altered TCR Vβ repertoire at the inclusion have a 2.1 fold higher risk of graft dysfunction during their follow-up. The Vβ repertoire alteration occurs years before the appearance of de novo anti-HLA antibodies. Moreover, these patients with an altered TCR repertoire exhibit an increase in effector memory $CD45RA^{-}CD197^{-}CD8^{+}$ T cells with an accumulation of differentiated ($CD28^{low}$) CD8 T cells. Finally, a specific CD8 gene expression pattern composed of 92 genes related to CD8 T cell function and phenotype can discriminate these patients from patients with lesions of CAMR.
Conclusions: Monitoring the TCR Vβ repertoire of circulating CD8 T cells may help to improve the identification of at-risk patients before the detection of HLA antibodies. Besides offering a new tool for monitoring patients, our data shed new light on the status of T cell immunity in long-term graft outcome.

O42-0163 A MULTISTATE MODEL TO INVESTIGATE THE RELATIONSHIP BETWEEN PRE-GRAFT LEVEL OF ANGIOTENSIN II TYPE 1 RECEPTOR (AT1R) ANTIBODIES AND KIDNEY TRANSPLANT RECIPIENTS OUTCOME

Florence Gillaizeau[1], Duska Dragun[3], Jean-Paul Soulillou[1], Alexandre Dufay[1], Jean-Paul Duong Van Huyen[1], Karine Renaudin[4], Anne Moreau[4], Magali Giral[1], Yohann Foucher[2] [1]CHU - Institut de Transplantation Urologie Nephrologie - INSERM U1064, Nantes, France; [2]Université de Nantes - EA4275 - Faculté de Médecine et Pharmacie, Nantes, France; [3]Clinic for Nephrology and Intensive Care Medicine, Charité Universitätsmedizin, Berlin, Germany; [4]Service d'Anatomo Pathologie, Nantes, France

Introduction: Antibodies reactive with non-HLA target - angiotensin II type 1 receptor (AT_1R) have been found during acute rejection with vascular involvement in kidney transplants. We developed a multistate model to assess the relationship between the pre-formed non-HLA immunisation (before the transplantation) against AT_1R (AT_1R-Abs) and the transplantation outcome on a large population of kidney recipients from the DIVAT cohort (www.divat.fr)
Patients and Methods: 599 patients who consecutively received kidney transplantations in Nantes University Hospital between 1998 and 2007 and for whom a pre-transplantation serum sample was available were included in the study. Anti-AT_1R-Abs were detected by a quantitative assay using extracts of cell overexpressing the human AT_1R as a solid phase. A threshold of AT_1R-Ab levels was statistically determined at 10 Units based on the time to graft failure. Outcomes were the transition probabilities between 4 states: graft without any acute episode rejection (ARE), graft with at least one ARE, return to dialysis and patient death. We used a parametric Semi-Markov model.
Results: The 599 kidney transplant recipients had a mean follow-up time of 6.9 years (±3.4). At the time of the study, 403(67%) patients had a functional graft without ARE whereas 105 (15%) patients returned to dialysis, 63 (11%) patients had an ARE and 50 (8%) patients died with a functional graft. Results of the multistate semi-Markov model showed that a high pre-graft level of AT1R antibody (>10U) was associated with a higher risk of ARE ($p < 0.05$). In addition, the model showed that a pre-graft level of AT1R-Abs > 10U did not influence the risk of graft failure within the first 3 years following the transplantation ($P = 0.56$), whereas a higher risk of graft failure appeared significantly from 3 years post transplantation onwards ($P = 0.02$). Finally, the association between the pre-graft level of AT1R antibodies and the time to death was not significant.
Conclusion: Our multistate semi-Markov model showed that, a pre-graft level of AT1R-Abs>10 U is significantly associated with the probability of ARE and also with the time-to-return to dialysis by itself, independently to the risk of ARE, but only after three years of follow up.

O43-0025 INTERSTITIAL FIBROSIS/TUBULAR ATROPHY (IF/TA) ACCORDING TO EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROFILE: PRELIMINARY RESULTS OF THE CERTITEM TRIAL

Nassim Kamar[1], Laetitia Albano[2], Dany Anglicheau[3], Antoine Durrbach[4], Olivier Toupance[5], Bruno Moulin[6], Pierre Merville[7], Marc Hazzan[8], Philippe Lang[9], Guy Touchard[10], Bruno Hurault de Ligny[11], Stéphane Quéré[12], Fabienne Di Giambattista[12], Magali Colombat[13], Yi-Chun Dubois[13], Alexandre Hertig[13], Eric Rondeau[13], On behalf of the CERTITEM study group [1]Hôpital de Rangueil, Toulouse, France; [2]Hôpital Pasteur, Nice, France; [3]Université Paris Descartes & Hôpital Necker, Paris, France; [4]Hôpital du Kremlin-Bicêtre, Le Kremlin-Bicêtre, France; [5]Hôpital Maison Blanche, Reims, France; [6]Hôpital Civil, Strasbourg, France; [7]Hôpital Pellegrin, Bordeaux, France; [8]CHU de Lille, Lille, France; [9]CHU Henri Mondor, Créteil, France; [10]Hôpital La Milétrie, Poitiers, France; [11]CHU de Caen, Caen, France; [12]Novartis Pharma SAS, Rueil-Malmaison, France; [13]Hôpital Tenon, Paris, France

Introduction: Tubular expression of vimentin and cytoplasmic translocation of β-catenin, consistent with EMT, may predict IF/TA and help identify patients (pts) at risk of IF/TA who could benefit from conversion to everolimus (EVR).
Methods: CERTITEM is a prospective, multicenter, randomized trial of kidney transplant recipients who received CsA-based therapy to M3 post Tx and subsequently randomized either to continue CsA or start EVR (C0 6−10ng/ml), after stratification by EMT profile. Primary endpoint is to compare progression of IF/TA (protocol biopsy) between M3 and M12 according to treatments in patients EMT+ at M3 (≥10% tubular cells showing vimentin). Descriptive results at month 3 are described here.
Results: One Ninty four points were randomized: 38.7% EMT+ (36 EVR, 39 CsA), 61.3% EMT- (60 EVR, 59 CsA). Mean IF/TA score at M3 was higher in EMT+ vs. EMT- pts (0.7 ± 0.6 vs. 0.2 ± 0.4, $p < 0{,}001$). Similarly IF/TA grades (0/I/II/III) were higher in EMT+ pts: 30/40/4/1 vs. TEM- 101/16/1/0, $p < 0.001$. Mean TEM scores were respectively 2.5 ± 0.6 and 0,5 ± 0,5 for EMT+ and EMT- pts. On multivariate analysis, factors associated with EMT+ status were: female gender (OR 2.67, $P = 0.009$), DGF (OR 4.7, $p < 0.001$) and IF/TA grade at M3 (OR 20.9 grades ≥II vs. 0, OR 2.0 grades ≥II vs. I; $p < 0.001$ overall).
Conclusion: These descriptive results confirm that IF/TA score at M3 is significantly higher in EMT+ patients. This study will evaluate whether conversion from CNI to EVR, 4 months after transplantation, inhibits progression of IF/TA in EMT+ recipients and evaluate the prognostic value of EMT for IF/TA progression.

O44-0167 CRITICAL REAPPRAISAL OF BANFF'S CLASSIFICATION IN A CLINICAL PERSPECTIVE

Thomas Bachelet, Anna Boueilh, Sebastien Lepreux, Lionel Couzi, Pierre Merville [1]CHU Bordeaux, Bordeaux, France; [2]Université Bordeaux 2, Bordeaux, France; [3]UMR CNRS 5164, Bordeaux, France

Introduction: The application of Banff's classification to renal graft biopsies analysis has led to recognize consensus diagnoses associated with the subsequent outcome of the transplants. Besides, it has been suggested by some to apply a critical review of this classification by returning to the elementary lesion as apprehended by the semi-quantitative score of each item. Moreover, performing functional clusters of these items may be relevant to guide therapeutic decisions.
Method: We applied this strategy to a series of biopsies for cause and questioned the importance of each item and the different clusters. Glomerular filtration rate (eGFR in ml/min/1.73m2) at biopsy (eGFRi) at the end of follow-up (eGFRf) and graft survival were collected.
Results: Eighty seven biopsies were included (mean follow-up: 2 ± 1.1 years): 44 with t+i > 0; 23 with ptc+g+cg>0; 6 with ptc+g > 0; 77 with ci+ct> 0 and 32 with ci+ct>2; 47 with cv>0 and 35 with ah> 0. Eighteen patients had DSA at biopsy and 5 display a positive C4d staining. Only t+i > 0, ci+ct>2 and cv> 0 were associated with a poorer DFGi (32 ± 16 vs. 41 ± 20, $P = 0.01$; 29 ± 12 vs. 42 ± 20, $P = 0.001$; 32 ± 17 vs. 41 ± 19 $P = 0.007$, respectively). In contrast, loss of function ([(DFGF-DGFi) / DFGi] * 100) was more severe in the presence of ptc+g+cg>0 lesions (−52% vs. −9 ± 33% ± 39, $P = 0.001$) and ptc+g > 0 lesions (−57 ± 26% vs. 17% ± 42, $P = 0.02$).

Only cg score impacted graft survival in a multivariate analysis including all Banff's items taken as quantitative variable (Odd Ratio=5.7 [1.4−22.7] of graft loss / cg point, $P = 0.01$). Graft survival (62/87 at the end of follow-up) was worse when ptc+g+cg>0 lesions, and ci+ct>2 lesions were found. A ROC curve analysis of the ci+ct+cv+cg scarring score, as predictor of the subsequent graft loss, determined a maximal AUC of 0.77 for a threshold of 5.
Conclusions: Banff's cluster analysis may be relevant in a clinical perspective. Microcirculation lesions were associated with the more severe prognosis. They thus may justify the implementation of immunosuppression, weighting its intensity to the associated scarring lesions.

O45-0061 A MOLECULAR *IN VIVO* SIGNATURE OF CYCLOSPORINE NEPHROTOXICITY

Pierre Galichon[3], Morgane Wetzstein[1], Yi-Chun Xu-Dubois[1], Alexandre Hertig[3], Eric Rondeau[3] [1]INSERM U702, Paris, IdF, France; [2]Service d'Urgences Néphrologiques et Transplantation Rénale, Hôpital Tenon, Paris, IdF, France; [3]Université Pierre et Marie Curie, Paris, IdF, France

Introduction: Although widely used since decades, the mechanisms of cyclosporine action and toxicity are still poorly understood.The renal toxicity of cyclosporine is associated with tubular lesions, resulting from direct epithelial toxicity, or secondary to vascular toxicity and ischemia. The overall changes in tubular gene expression *In vivo* under CSA treatment have never been reported.

Methods: We used a classical model of renal toxicity in salt-depleted rats treated by cyclosporine for 2 weeks. The renal toxicity was demonstrated by a rise in blood urea level, tubular vacuolization and atrophy, with expression of epithelial to mesenchymal transition markers. The cortical tubules where Laser-microdissected for RNA extraction, reverse transcription and amplification followed by microarray gene expression analysis.

Results: The transcriptome revealed the presence of renal tubular (mostly proximal: NPT2a, megalin) markers, with no endothelial (CD31, Tie2) or glomerular (synaptopodin, nephrin) contamination. It was relevant to previous knowledge of genes overexpressed in tubular diseases (KIM1, osteopontine). The pathway analysis revealed a very significant enrichment in genes implicated in protein synthesis ($P = 8{,}9\text{x}10^{-26}$ for ribosome, $P = 7{,}3\text{x}10^{-11}$ for protein processing in endoplasmic reticulum, $P = 4{,}9\text{x}10^{-10}$ for protein export), peroxisome ($P = 3{,}3\text{x}10^{-4}$) and terpenoid backbone synthesis ($P = 1{,}2\text{x}10^{-4}$). These changes in major cellular pathways could be either beneficial or deleterious to the aggressed tubular cells. The most strongly upregulated gene, *nupr1*, is a recently discovered transcription factor, and, although it was not included in the predesigned pathways for enrichment analysis, it is acting in situations of cellular stress to block protein synthesis and apoptosis.

Conclusion: *Nupr1* is a potential effector of the modifications of protein synthesis pathways in response to cyclosporine toxicity.

IMMUNOLOGY

O46-0021 TRANILAST, AN ANALOGUE OF TRYPTOPHAN CATABOLITES, INDUCES ALLOGRAFT SURVIVAL THROUGH CD161+ MYELOID-DERIVED SUPPRESSOR CELLS

Séverine Ménoret[1], Séverine Bézie[2], Xian-Liang Li[2], Claire Usal[2], Lise Caron[2], Ignacio Anegon[2] [1]Platform Transgenic Rats Nantes IBiSA-CNRS-UMR 1064, Nantes, France; [2]UMR 1064-CRTI, Nantes, France

Background: Indoleamine 2, 3-dioxygenase (IDO) converts tryptophan in various catabolites and has been shown to induce immune tolerance in different immune-mediated diseases, including organ transplantation. One of these tolerogenic metabolites is anthranilic acid. Tranilast is a clinically approved, structural and functional analogue of anthranilic acid that has been recently shown to be effective in murine models of multiple sclerosis and rheumatoid arthritis. We examined the effect of tranilast in a rat cardiac allograft model.

Materials/methods: Lewis 1W rat hearts were grafted in MHC-mismatched Lewis 1A rats. The receiver is orally treated with a clinical used dose of 650 mg/kg of tranilast daily for 30 days. Total splenocytes and purified spleen cell subtypes sorted by FACS Aria were transferred to sublethaly irradiated rats by *i.v* injection the day before transplantation.

Results: Graft survival in recipients treated with tranilast were significantly prolonged (65 ± 42.7 days, $n = 15$, $p < 0.0001$) when compared to control group (8.3 ± 2.3 days, $n = 6$) and in 50% of recipients (tranilast induced long-term allograft survival (>100 days). Adoptive transfer of total splenocytes from tolerant tranilast-treated rats to naïve rats resulted in tolerance in all animals ($n = 5$). Moreover, splenocytes from these adoptively transferred tolerant recipients were again capable of transferring tolerance to all naïve recipients ($n = 5$). Tolerant splenocytes depleted of T and B cells ($n = 6$) or depleted of T, B and DCs ($n = 5$) transferred tolerance. Importantly, depletion of $CD161^+$ cells from T, B and DC-depleted splenocytes abrogated tolerance transfer (9.3 ± 0.6 $n = 3$). To confirm these results, we adoptively transferred $CD161^+TCR^-$ cells from tolerant rats which resulted in tolerance (130 ± 91.6 days, $n = 5$, 3/5 recipients >100 days) whereas $CD161^-TCR^-$ cells from the same animals did not (12.7 ± 4.6 days, $n = 3$, $p < 0,005$). In the $CD161^+TCR^-$ cells, we could distinguish 2 cells populations $CD161^{high}$ (NK cells) and $CD161^{low}$ (these cells have the phenotype of myeloid-derived suppressor cells (MDSCs) (CD3-ClassII-CD11b+CD80/86 + myeloid marker rat)). When we transferred $CD161^{high}$ or $CD161^{low}$ from the same animals, we obtained tolerance only with $CD161^{low}$ MDSCs (59 ± 64days, $n = 5$, 2/5 recipients >100 days vs. 18 ± 14 days, $n = 3$).

Discussion: This is the first demonstration that tranilast mediates transplantation tolerance. Tolerance was active and transferable by MDSCs.. Experiments are under way to define by which downstream mechanisms these cell populations mediate tolerance.

O47-0023 INTERLEUKIN-7 RECEPTOR BLOCKADE DECREASES T CELL NUMBERS AND PROLONGS ALLOGRAFT SURVIVAL

Hoa-Le Mai, Francoise Boeffard, Julie Lepourry, Richard Danger, Bernard Martinet, Bernard Vanhove, Sophie Brouard, Jean-Paul Soulillou INSERM UMR1064, Universite de Nantes, Nantes, France

Introduction: T cell depletion therapy, although widely used, is followed by T cell homeostasis leading to an increase in memory T cells which can promote transplant rejection. We demonstrated that IL-7R blockade with or without prior T cell depletion inhibited T cell homeostasis and prolonged allograft survival using murine models.

Methods: Tail skin from C57BL/6 was transplanted to Balb/c mice, divided into 3 groups: group 1 (no treatment), 2 (T cell depletion by combining 2 depleting mAbs: anti-CD4 and anti-CD8 on day -3 and -1), and 3 (same as group 2, followed by a blocking anti-IL-7Rα mAb qod). Islets from C57BL/6 were transplanted to streptozotocin-induced diabetic Balb/c mice, divided into 3 groups: group A (no treatment), B (anti-IL-7Rα mAb qod from day 0 to rejection), and C (anti-IL-7Rα mAb qod from day -21 to PTD90).

Results: Median skin graft survival in group 1, 2, and 3 was 9.5, 30, and 58 days, respectively (group 3 vs. 2, $p < 0.0001$). IL-7R blockade following T cell depletion profoundly inhibited lymphocyte reconstitution. At PTD35, the mean absolute numbers ($x10^5$ cells) of CD3 + , CD3 + CD4 + , CD3 + CD8 + , and B220 + cells in the lymph node, spleen, and peripheral blood of group 3 were reduced by 4- to 20-fold compared to those of group 2 (all $p < 0.05$). Importantly, the mean absolute number of $CD44^{hi}CD62L^{lo}$memory T cells was 4- to 8-fold lower, whereas the percentage of CD4 + FOXP3 + Treg among total CD4 + T cells was doubled in group 3 compared to group 2 (all $p < 0.05$). IL-7R blockade abrogated cellular immune responses as shown by direct IFNγ ELISPOT (41 vs. 194 spots/10^5 T cells, $p < 0.05$) and by MLR-3H thymidine (238 vs. 10510 CPM, $p < 0.05$) and inhibited humoral immune responses as shown by a reduction in DSA (395 vs. 4175 MFI, $p < 0.0001$) (group 3 vs. 2). Median islet graft survival was not significantly prolonged when anti-IL-7Rα was given from day 0 (group B vs. A: 29 vs. 21 days, $P = 0.12$). However, when treatment was started 21 days before islet graft, 5 of 6 recipients had indefinite graft survival >180 days (group C vs. A, $p < 0.005$), associated with an abrogation of DSA (group C vs. A: 260 vs. 976 MFI, $p < 0.01$).

Conclusion: IL-7R blockade may be clinically relevant and may be combined with T cell depletion as a potent induction therapy.

O48-0162 T LYMPHOCYTES SUBPOPULATION INTO CMV INFECTION WITH ANTIVIRAL DRUG RESISTANCE

Hannah Kaminski[1], Lionel Couzi[1], sebastien helou[1], Isabelle Guarigue[4], thomas bachelet[2], Julie Dechanet-Merville[2], Pierre Merville[3] [1]Néphrologie-Transplantation, Centre Hospitalier Universitaire de Bordeaux, Hôpital Pellegrin, Bordeaux, France; [2]Centre National de la Recherche Scientifique (CNRS) – Unité Mixte de Recherche (UMR) 5164, Bordeaux, France; [3]Université Bordeaux Segalen, Bordeaux, France; [4]Laboratoire de Virologie, Centre Hospitalier Universitaire de Bordeaux, Hôpital Pellegrin, Bordeaux, France; [5]CNRS UMR 5234, Bordeaux, France

Introduction: CMV infection remains a major issue in kidney transplant recipients because of its relationship with opportunistic infections, rejection, graft survival, lymphoproliferative disorders, and actually with increased mortality risk.

Developping anti-CMV drug resistance further increases this risk.

Lately, we find that D+R- recipient under preemptive therapy would have a high incidence of CMV infection, of treatment failure, and of anti-CMV drug resistance with a poor prognosis.

On the other hand, our group show that gamma-delta T cell are involve in CMV infection control.

Immune status, CMV infection and drug resistance emergence interplay remain unclear.

In this study, the objective was to assess how the immune status impact on CMV infection and resistance emergence in kidney transplant recipient.

Material and method: We study the day 0, M3, M6, M12 post transplantation subpopulation evolution of lymphocytes which means CD3 + , CD4 + , CD8 + and gamma-delta T cells (LT gd).

According to the prophylactic strategy, 5 groups were analysed:

R+ recipients who received antilymphocyte globulin induction (R+/GAL) with preemptive therapy (n = 56), R+/GAL with prophylactic therapy (n = 9), D+R- preemptive (n = 48) and D+R- preventive (n = 11) groups and without infection (n = 74)

Results: We did not observed any statistic difference between the groups for CD4 +, CD3 + and CD8 + cells.

R+/GAL recipients with preemptive strategy have a prompt infection resolution with a high level of gamma-delta T cells, related with a short-lived viremia ($P = 0.04$).

Into the D+R- group, the infection features seem worst with the preemptive strategy: resistance emergences were more frequent and related with a longer viremia, and a higher CMV load.

In preemptive D+R- recipient with antiviral drug resistance, the gamma-delta T cells at six month post transplantation reach a higher percentage than the D+R- patient without resistance ($P = 0,0033$).

Discussion: Thereby, preemptive therapy seems suit to the R+ kidney transplant recipient with prompt CMV infection resolution related with prompt gamma-delta T cell expansion and no anti-CMV drug resistance occurrence. Gamma-delta T cell is known to be related with CMV infection resolution and an even faster infection resolution when expansion occurs faster. By the way, when antiviral drug resistance happens, gamma-delta T cell is inversely related with the CMV resolution viremia, especially in D+R- preemptive group. So gamma-delta T cell may be useful to predict CMV infection resolution but also to predict antiviral drug resistance for D+R-recipient with persistent viremia.

Couzi L et al, Am J Transplant. 2012; 12: 202–9.

O49-0016 HLA-E-RESTRICTED CROSS-RECOGNITION OF ALLOGENEIC ENDOTHELIAL CELLS BY CMV-COMMITTED CD8 T CELLS

Mathilde Allard[1], Pierre Tonerre[2], Béatrice Charreau[2], Nadine Gervois[1] [1]UMR 892 INSERM / 6299 CNRS / Université Nantes, Nantes, Loire Atlantique, France; [2]UMR 1064 INSERM / Université Nantes, Nantes, Loire Atlantique, France

Persistant viral infections, especialy CMV infections, are well known to adversely affect the outcome of organ transplantation. CMV could account for graft rejection by activating graft endothelial cells (ECs), thereby attracting and activating alloreactive T cells. Another factor of association between viral infections and developement of allograft rejection could be cross-reactivity of viral-specific T cells to allogeneic HLA molecules, but only few examples of such T-cell cross reactivity have been documented so far.

Screening for alloreactive CD8 T cells in kidney transplant patients undergoing herpes virus infections, we isolated a monoclonal HLA-E-restricted population from the PBL of a CMV seropositive recipient. This population shows HLA-E-dependent reactivity against peptides derived from the leader sequences of both various HCMV-UL40 and allogeneic classical HLA-I molecules. Although HLA-E has been shown to behave as a strong

transplantation antigen in rodent models, whether HLA-E could trigger an allogeneic cellular response in humans remains to be studied. As we previously reported that HLA-E protein expression in human nonlymphoid organs is mainly restricted to ECs, we investigated the reactivity of these HLA-E-restricted CD8 T cells towards allogeneic ECs. We show that CMV-Committed HLA-E-restricted CD8 T cells recognized and efficiently killed 6 out of 7 allogeneic ECs culture *in vitro*. In accordance with previous studies, we also demonstrated that HLA-E-restricted T cells are tightly regulated by NK receptors, especially by an inhibitory KIR that strongly prevent their TCR-induced activation through recognition of HLA-C molecules. Consistent with this, allogeneic ECs that express the relevant (protective) HLA-C were not lysed by HLA-E restricted CD8 T cells unless mAb specific for the appropriate KIR was added.

In conclusion, while HLA-E restricted CD8 T cells have potential to contribute to the control of CMV infection, they may also directly mediate graft rejection *In vivo* through cross-recognition of graft ECs. Hence, a better evaluation of the role of HLA-E-restricted T cells in transplantation and of the impact of HLA-genotype, especially HLA-C, on their alloreactivity may determine whether they indeed represent a risk factor following organ transplantation.

O50-0141 UNUSUALLY LONG PEPTIDE RECOGNIZED BY CD8$^+$ TREGS INDUCE PROLONGATION OF CARDIAC ALLOGRAFT SURVIVAL IN RAT

Elodie Picarda[1], Séverine Bézie[1], Vanessa Venturi[2], Karine Renaudin[3], Emmanuel Mérieau[1], Miles Davenport[2], Ignacio Anegon[1], Carole Guillonneau[1] [1]Inserm UMR1064, Nantes, France; [2]Centre for Vascular Research, University of New South Wales, Kensington, Australia; [3]Pathology Laboratory, CHU Hotel Dieu, Nantes, France

We recently reported that in a rat major histocompatibility complex (MHC) mismatched heart allograft model, treatment with CD40Ig, a chimeric molecule that blocks CD40L, leads to indefinite allograft survival mediated by CD8$^+$CD45RC^{low} Tregs. We studied the recognition properties of CD8$^+$ Tregs, characterizing the allogeneic peptide(s) recognized and the TCR usage of the cells.

Allogeneic peptides were derived from polymorphic regions of donor MHC class I and class II molecules. 82 overlapping peptides of 16 amino acids (aa) were tested in a coculture of Tregs with mature syngeneic plasmacytoid dendritic cells (pDCs) (ratio 4:1). For *In vivo* study, peptide was delivered continuously by a mini osmotic pump implanted intraperitoneally in recipients. Moreover, the repertoire of the TCR of CD8$^+$ Tregs was studied by flow cytometry analysis and sequencing the CDR3 region.

After 6 days of culture, 2 peptides in particular led to the activation of Tregs, as shown by the upregulation of the CD25 molecule (from 24.4% to 28.9% of CD25 expression). Continuous administration of one dominant allopeptide by mini osmotic pump, from day -7 to day 21 after transplantation, resulted in indefinite allograft survival in 25% of the recipients. These rats showed higher number of Tregs in spleen compared to untreated ones, an inhibition of alloantibody responses and no sign of chronic rejection. We showed previously that CD8$^+$CD45RC^{low} Tregs expressed a specific altered Vbeta11 repertoire, with the same CDR3? length in all animals (9 aa). This upregulation was confirmed at the protein level, since 19.9 ± 3.7% of Tregs from a CD40Ig-treated animal expressed the Vβ11 chain compared to 6.1 ± 2.3% in naïve ones. Sequencing of about 100 TCR clones of Tregs in 6 long-surviving animals revealed a highly diverse repertoire in 3 rats and a more limited diversity in the 3 others, each displaying a bias toward a particular CDR3β sequence. This heterogeneous repertoire, with no overlap between animals, can be referred to a private repertoire.

This study demonstrated that CD8$^+$CD45RC^{low} Tregs recognize 2 allogeneic epitopes of 16 aa long, one of the peptides inducing prolongation of allograft survival when administered *In vivo*. These results highlight the clinical potential of antigen-specific regulatory T cells.

O51-0178 *MICA A5.1* MUTATION INCREASES MICA EXPRESSION ON GRAFT ENDOTHELIAL CELLS AND ASSOCIATES WITH MICA SENSITIZATION IN KIDNEY TRANSPLANT RECIPIENTS

Pierre Tonnerre[1], Nathalie Gérard[1], Mathias Chatelais[1], Caroline Poli[2], Stéphanie Allard[2], Sylvie Cury[2], Céline Bressollette[3], Anne Cesbron-Gautier[2], Béatrice Charreau[1] [1]ITUN; INSERM UMR1064, Nantes, France; [2]EFS; Laboratoire HLA, Nantes, France; [3]EA4271, Nantes, France

This study investigated the immunological impact of the *A5.1* mutation, related to the common *MICA*008* allele, in renal transplantation from cellular to clinical levels. *MICA A5.1* mutation associates with a premature stop codon and truncated protein but its immunological impact remains unclear. Here, a comparative analysis of homozygous wild type (WT) or *MICA A5.1* endothelial cells (ECs) showed that *A5.1* mutation elicits an endothelial phenotype characterized by elevated MICA mRNA steady state and proteins at cell surface (7−10 fold increase versus WT). Exacerbated MICA expression correlates with the exclusive production of exosomes, expressing high level of MICA, as circulating MICA. We found no change in regulatory miRNAs or *MICAA5.1*-specific SNPs in 3'UTR or 5'UTR regions. Functionally, *A5.1* ECs enhance NKG2D interaction and activity in NK cells, as compared to WT ECs, as well as NKG2D-dependent lysis of ECs. In the Transplantation setting, polyreactive anti-MICA transplant recipient's sera bind preferentially to *MICA A5.1* donor ECs supporting a key role for *MICA*008/A5.1* molecules as major antigenic determinants on graft's ECs. Clinically, we determined the incidence of a *MICA A5.1* mismatch and provide the first evidence of a statistically significant association between *MICA A5.1* on transplant donor and anti-MICA sensitization of kidney recipients. Together, our study identifies the *A5.1* mutation as an immunodominant factor, and as a potential risk factor for transplant survival.

O52-0055 CONTROL OF TRANSPLANT TOLERANCE AND INTRAGRAFT REGULATORY T CELL LOCALIZATION BY CCL5

Nahzli Dilek, Nicolas Poirier, Claire Usal, Bernard Martinet, Gilles Blancho, Bernard Vanhove INSERM UMR1064, Nantes, France

Background: CCL5 (Rantes) is a chemotactic cytokine playing an active role in recruiting leukocytes into inflammatory sites and known to attract Treg cells in solid tumors where they inhibit immune responses.

Material, Methods and Results: In rat recipients of kidney allografts were tolerance was induced by costimulation blockade, we observed a 2-fold decrease of plasma CCL5 levels as compared to control syngeneic grafted animals (0.60±0.17 vs. 1.17±0.48 ng/ml; $p < 0.01$). Myeloid-derived suppressor cells (MDSC), a major cell type producing CCL5 in blood, also presented a reduced capacity to produce CCL5 in these tolerant animals. In contrast, intragraft levels of CCL5 mRNA and protein were higher in tolerated allografts, establishing an increased graft-to-periphery CCL5 gradient possibly contributing to the recruitment of Treg cells into the graft, as in solid tumor, and leading to the establishment and maintenance of tolerance. To test the hypothesis, we broke the gradient by restoring normal plasma concentrations of CCL5 by implantation of osmotic pumps. This induced a strong reduction of intragraft Treg cells (decrease of 24.405.47 fold; $p < 0.05$ by immunohistofluorescence and of Foxp3 mRNA assessed by qRT-PCR), and led to an increase of creatinine and urea concentrations and eventually to kidney graft rejection.

Conclusions: Our data uncover a novel role of CCL5, possibly controlled by MDSC, in this rat model of kidney transplant tolerance: a graft-to-periphery gradient of CCL5 help recruiting Treg cells into the graft where they maintain tolerance.

O53-0053 DIFFERENTIAL REGULATION OF MOTILITY AND IMMUNE SYNAPSES BY CD28/ CTLA-4 COSTIMULATION IN EFFECTOR AND REGULATORY T CELLS

Nahzli Dilek, Nicolas Poirier, Philippe Hulin, Gilles Blancho, Bernard Vanhove INSERM UMR1064, Nantes, France

Background: We have previously shown that antagonist anti-CD28 antibodies block CD28/CD80−86 costimulation without perturbation of the CTLA-4/CD80−86 inhibitory pathway and favor tolerance induction by increasing Treg suppression in a CTLA-4 dependent manner. Since CTLA-4 is transducing signals that block the TCR-STOP signal, described to allow for T cell arrest and formation of immune synapses, we hypothesized that CTLA-4 might play a major role in the mechanism of action of anti-CD28 antibodies by regulating T cell motility and synapses formation.

Materials, methods and results: Here, we generated human CD4+CD25+CD127+ Teff and CD4+CD25highCD127lowFoxp3+ Treg cell lines and analyzed their behavior in contact with cognate APCs by live-cell dynamic microscopy in the presence of CD28 and CTLA-4 antagonists. CD28 blockade prevented formation of stable contacts between Teff and APCs (11.93 ± 1.175 vs 4.167 ± 1.191 min; $p < 0.05$), increased Teff mobility (100.56.032 vs. 204.8 ± 17.54 μm; $p < 0.0001$) and decreased cell activation measured by calcium flux (0.377 ± 0.028 vs. 0.154 ± 0.024 calcium peaks/min; $p < 0.0001$). In contrast, CD28 antagonists enhanced Treg/APC contacts (5.057 ± 0.866 vs. 13.81 ± 1.104 min; $p < 0.0001$) and increased calcium flux (0.486 ± 0.048 vs. 0.677 ± 0.06 calcium peaks/min; $p < 0.05$), resulting in an increase of Treg activation. The simultaneous blockade of CTLA-4 with antibodies or of CD80/86 with CTLA4Ig reversed some of these effects: it restored the STOP signal and reduced motility/velocity in Teff whereas it increased velocity in Treg and abolished Treg/APC contacts.

Conclusion: Our data shed light on the role of CD28 and CTLA-4 that act as a rheostat to differentially control Teff and Treg function and clarify the observations that selective CD28-blockade but not CD80/86 blockade reinforces Treg cell suppression *in vitro*.

O54-0065 **INDUCTION OF ALLOGRAFT TOLERANCE BY MONOCLONAL CD3 ANTIBODIES: A MATTER OF TIMING**

Sylvaine You[1], Julien Zuber[2], Chantal Kuhn[1], Marije Baas[3], Fabrice Valette[1], Virginia Sauvaget[1], Sabine Sarnacki[4], Birgit Sawitzki[5], Jean-François Bach[1], Hans-Dieter Volk[6], Lucienne Chatenoud[1] [1]INSERM U1013, Université Paris Descartes, Paris, Ile de France, France; [2]Service de Transplantation, Hôpital Necker, Paris, Ile de France, France; [3]Service de Néphrologie, AMC, Amsterdam, Netherlands; [4]Service de Chirurgie Pédiatrique, Hôpital Necker, Paris, Ile de France, France; [5]Institute of Medical Immunology, Charite University, Berlin, Germany; [6]Berlin-Brandenburg Center for Regenerative Therapies, Charite University, Berlin, Germany

Despite remarkable progress in organ transplantation through the development of a wealth of immunosuppressive drugs highly effective at controlling acute rejection, two major problems still remain, the loss of transplants due to chronic rejection and the growing number of sensitized recipients due to previous transplants, transfusions or pregnancies. Induction of immune tolerance appears to be the only way to curb this complex situation. We have previously shown in autoimmunity that CD3 antibody therapy promotes tolerance only when applied in the context of a primed immune system. We sought to extend this observation to the transplant setting.

Pancreatic islets from BALB/c mice were grafted under the kidney capsule of diabetic C57BL/6 recipients. Five injections of Fc non-binding CD3 antibody F (ab')$_2$ fragments (50μg/injection/day) were administered at the time (day-1) or after transplantation (day+7 or 11).

We demonstrate that a short-term, low-dose course with CD3 antibodies starting on day +7 or +11 after transplantation induces long-term survival (>100 days) of fully mismatched islet allografts. Importantly, permanent acceptance of second islet grafts from the original but not third party donors proved that antigen-specific tolerance had indeed been induced in these recipients. Mechanistic studies revealed that antigen-specific regulatory and effector T cells are differentially affected by the treatment. CD3 antibody treatment preferentially induces apoptosis of activated alloreactive T cells which is mandatory for tolerance induction. As a consequence, anti-donor CD8 responses are dramatically reduced in tolerant hosts. In contrast, regulatory T cells are relatively spared from CD3 antibody-induced depletion and can transfer antigen-specific tolerance thus arguing for their prominent role in sustaining long-term graft survival.

Our data show that judicious use of CD3 antibodies can induce transplant tolerance to fully mismatched allografts in recipients primed to the alloantigens. From the translational point of view and due to the availability of humanized CD3 antibodies presently developed in autoimmune diabetes, our findings provides further consideration of using CD3 antibody-based therapy in transplantation.

P.O. DENUÉ & B. RAMUS SESSION

Best Communications

O55-0001 RANDOMIZED, MULTICENTER STUDY OF EVEROLIMUS WITH EARLY REDUCTION OR ELIMINATION OF TACROLIMUS IN 719 *DE NOVO* LIVER TRANSPLANT RECIPIENTS: RESULTS AT 12 MONTHS

Faouzi Saliba[1], Christophe Duvoux[2], François Durand[3], Martine Neau-Cransac[4], Jean Hardwigsen[5], Georges Pageaux[6], Emmanuel Boleslawski[7] [1]AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Villejuif, France; [2]AP-HP Hôpital Mondor, Créteil, France; [3]AP-HP Hôpital Beaujon, Clichy, France; [4]Hôpital Pellegrin, Bordeaux, France; [5]Hôpital de la Conception, Marseille, France; [6]Hôpital Saint-Eloi, Montpellier, France; [7]Hôpital Claude Huriez, Lille, France

Objective: Current IS regimens based on calcineurin inhibitors (CNI) are associated with kidney dysfunction affecting the outcome of liver transplantation (LT). The introduction of everolimus (EVR) could be an option to reduce or eliminate CNIs. This concept was evaluated in this study.

Methods: Following a run-in period of 30 ± 5 days under TAC ± MMF, the patients were randomized to receive EVR (C_0 3–8 ng/ml) with reduced TAC (C_0 3–5 ng/ml, EVR+ r-TAC $n = 245$), or EVR (C_0 6–10 ng/ml) with discontinuation of TAC at month 4 (TAC-WD, $n = 231$), or standard TAC (C_0 6 –10 ng/ml, TAC-C $n = 243$). Steroids were administered in the 3 arms. The primary composite efficacy endpoint combined treated biopsy-proven acute rejection (tBPAR), graft loss and death. The main secondary endpoint was kidney function (GFR/MDRD4).

Results: 719 patients were included. Enrolment in the TAC-WD arm was prematurely terminated due to a higher rate of acute rejection. At M12, non-inferiority was demonstrated for the primary efficacy endpoint (–3.0%, CI 97.5% [–8.7; 2.6] in favour of EVR + r-TAC). The incidence of AR (BPAR, tBPAR) was significantly lower (4.1% - 2.9%) with EVR+ r-TAC vs. TAC-C (10.7% - 7%). Renal function was superior with EVR+ r-TAC compared to TAC-C, with a mean difference in the GFR change from randomisation to M12 of 8.5 ± 2.1 ml/min/1.73 m^2 (97.5% CI: [3.74; 13.27], $p < 0.001$). Overall, the incidence of AEs was comparable in the EVR+ r-TAC (94.7%) and TAC-C (95%) groups.

Conclusion: Early introduction of EVR facilitated a substantial TAC reduction and improved kidney function while maintaining efficacy at 12 months post LT.

O56-0089 INTESTINAL TRANSPLANTATION IN CHILDREN: A 17 YEAR EXPERIENCE IN A SINGLE CENTER

Sabine Irtan[1], Laurent Dupic[2], Virginie Colomb[3], Caroline Télion[4], Yves Aigrain[1], Yann Révillon[1], Christophe Chardot[1], Olivier Goulet[3], Florence Lacaille[3] [1]Service de chirurgie viscérale pédiatrique, Hôpital Necker enfants malades, Université Paris Descartes, Paris, France; [2]Service de réanimation pédiatrique, Hôpital Necker enfants malades, Université Paris Descartes, Paris, France; [3]Service d'Hépato-gastroentérologie et nutrition pédiatrique, Hôpital Necker enfants malades, Université Paris Descartes, Paris, France; [4]Service d'anesthésie-réanimation, Hôpital Necker enfants malades, Université Paris Descartes, Paris, France

Aim: To describe median and long term results of intestinal transplantation in children in order to discuss the indications and indicate further researchs.

Material and methods: From 1994, 100 transplantations were performed on 92 children: 57 isolated small bowels, 39 combined Liver-Intestine, 3 multivisceral (2 with kidney), 1 modified multivisceral. Indications were short bowel syndrome ($n = 36$), motility disorders ($n = 31$), mucosal defects ($n = 31$) and others ($n = 2$). Median follow-up was 8 years [4 months – 17 years].

Results: Overall actuarial patient and graft survival at 10 years was respectively 52% and 33%. Patient and graft survival with liver graft was equal at 49% ($P = 0{,}36$), but respectively 52% and 17% without ($P = 0.07$). Mortality rate was 42% with a median delay of 3 months [0–130]. The major causes of death were sepsis (24%) and multi-organ failure (22%). Median delay of graft loss was 9 months [0–115]. The major cause of graft loss was rejection, either acute (23%) or chronic (35%). All Liver-Intestine children surviving more than 10 years had a functional graft whereas 75% of isolated small bowel were lost.

Conclusion: These results are similar to the International Transplant Registry for patient and graft survival. Initial mortality remains important for Liver-Intestine but graft survival is better than for isolated small bowel. Improvement is strongly needed in immunosuppressant regimen in order to decrease rejection related graft loss and infection related patient death.

O57-0026 IMPACT OF APOPTOTIC CELL INJECTION-INDUCED ENGRAFTMENT ON GRAFT-VERSUS-LEUKEMIA EFFECT

Adrien Chauchet, Romain Vallion, Francis Bonnefoy, Anna Daoui, Baptiste Lamarthée, Philippe Saas, Sylvain Perruche INSERM UMR1098, UFC, EFS BFC, Besançon, Doubs/BFC, France

Introduction: Allogeneic hematopoietic cell transplantation (AHCT) is a curative therapy for hematological malignancies. However, such a therapeutic is limited by graft-versus-host-disease (GvHD) occurrence. New strategies are therefore needed and our laboratory showed that the use of apoptotic cell has immunomodulatory properties to favor engraftment and limit GvHD. These effects are TGF-β and regulatory T cell dependent. However, since apoptotic cell injection favors tolerance, the influence of apoptotic cell injection on leukemia development has never been evaluated.

Methods: To examine whether graft-versus-leukemia (GvL) activity of T cells was maintained after AHCT in the presence of apoptotic cells, we developed an experimental mouse model of T cell-depleted bone marrow (TCD BM) transplantation. Leukemic A20 luciferase$^+$ cells were also injected the day of TCD BM transplantation (d0). A delayed allogeneic donor T cell infusion was given on day 6 to induce GvHD. Apoptotic cells were injected either on d0 or d6.

Results: Eradication of leukemic cells was observed in all animals receiving delayed T cells injection. Indeed, all mice not receiving T cells died from leukemia before day 27 post-graft, as attested by tumor growth evaluation by luminescence. Then, we observed that T cell injection prevents tumor growth as attested by absence of luminescence, however all mice developed GvHD (according to Ferrara's score) and only 27% of mice survived at day 27. In sharp contrast, 50% of mice receiving apoptotic cells on d0 or d6 survived at day 27. In addition, such mice receiving apoptotic cells that prevent GvHD occurrence, did not present leukemic growth as attested by the absence of luminescence at day 27 post-graft.

Conclusion: Our data clearly demonstrated that GvHD suppression by apoptotic cell injection does not abrogate GvL activity of adoptively transferred donor T cells. Thus, apoptotic cell injection should be considered in clinic to favor engraftment, prevent GvHD, conserving high GvL therapeutic activity.

O58-0120 THE ACTIVATION OF MTOR PATHWAY, A MARKER OF ENDOTHELIAL CELL INVOLVEMENT, IS CORRELATED WITH ANTIBODY MEDIATED REJECTION

Marion Tible[6], Alexandre Loupy[3], Dewi Vernerey[1], Mandet Chantal[1], Romain Guillemain[4], Catherine Amrein[4], Aurelie Cazes[2], Veronica Pezzella[4], Dominique Nochy[2], Dominique Charron[5], Xavier Jouven[1], Patrick Bruneval[2], Jean-Paul Duong Van Huyen[2] [1]INSERM UMR 970, Biostatistics and Histopathology Platform, PARCC Cardiovascular Research Institute, Paris, France, Paris, France; [2]Laboratoire d'Anatomie Pathologique, Hôpital Europ'een Georges Pompidou, APHP, Paris, F-75015, France, Paris, France; [3]Service de Transplantation R'enale et de Soins Intensifs, Hôpital Necker, APHP, Paris, F-75015, France, Paris, France; [4]Service de Chirurgie Cardiaque, Hôpital Europ'een Georges Pompidou, APHP, Paris, F-75015, France, Paris, France; [5]Laboratoire d'Histocompatibilit 'eHôpital Saint Louis, APHP, Paris, F-75011, France, Paris, France; [6]Université Paris Descartes, Paris, France, Paris, France

Introduction: In cardiac transplantation, the pathological classification of the antibody mediated rejection (AMR) on endomyocardial biopsies (EMB) is based on histology (microvascular inflammation) and immunohistochemistry (deposition of C4d and/or CD68-positive intravascular macrophages). It is ranked in 4 grades, from pAMR0 to pAMR3. pAMR1 which corresponds to a suspicious AMR is divided in two categories: pAMR1(H+) based on histology findings alone and pAMR1(I+) based on immunopathologic findings alone. Previous studies suggested that mTOR pathway activation, detected by in situ microvascular expression of mTOR targets p70 S6 kinase (p70S6K) and phospho-S6 ribosomal protein (pS6RP) could be associated with endothelial cell involvement during AMR.

Aim: To correlate pS6RP and p70S6K expression with other AMR markers (C4d deposition, CD68-positive intravascular macrophages, and DSA); To assess the diagnostic value of immunohistochemical detection of pS6RP and p70S6K in microvessels of EMB.

Materials and Methods: Two Eighty protocol EMB harvested during a 1 year period of time in a single institution were used. 32 EMB with pathological features of AMR were included in the pAMR+ group encompassing 22 pAMR1(H+), 1 pAMR(I+), and 9 pAMR2. 32 EMB were randomly selected among the pAMR0 EMB and were included in the control group. Capillary expression of pS6RP and p70S6K was assessed by immunohistochemistry

and ranked from grade 0 to grade 4. Only grades 3 and 4 were considered as positive. In parallel DSA were assessed using Luminex technique.

Results: In the pAMR+ group, pS6RP and p70S6K were expressed in the endothelial cells of the EMB ranked pAMR2 (4/8 and 7/9 respectively), and also in grade pAMR1(H+) EMB (3/21 and 10/20 respectively). In the control group 1/32 EMB was positive. Microvascular expression of pS6RP and p70S6K was correlated with DSA (P = 0,0190 and P = 0,0076 respectively) and macrovascular inflammation (P = 0,069 and p < 0,0001 respectively).

Conclusion: The endothelial expression of mTOR targets pS6RP et p70S6K, markers of endothelial cell activation, is associated with AMR in both definite AMR grade pAMR2 and suspicious AMR grade pAMR1(H+), negative for C4d and CD68. That latter finding underscores the diagnostic value of pS6RP and p70S6K immunohistochemistry to characterize AMR C4d-negative EMB.

O59-0105 CYCLOSPORINE A DRIVES A TH17 AND TH2-MEDIATED POST-TRANSPLANT OBLITERATIVE AIRWAY DISEASE

Philippe Lemaître[1], Benoît Vokaer[1], Louis-Marie Charbonnier[1], Yoichiro Iwakura[5], Kenneth Field[4], Marc Estenne[2], Michel Goldman[3], Oberdan Leo[1], Myriam Remmelink[2], Alain Le Moine[1] [1]Institute for Medical Immunology, Université Libre de Bruxelles, Gosselies, Belgium; [2]Hopital Erasme, Université Libre de Bruxelles, Bruxelles, Belgium; [3]Innovative Medicines Inititaive, Bruxelles, Belgium; [4]Bucknell University, Lewisburg, United States; [5]Center of Experimental Medecine and Systems Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Lung transplantation is the only treatment for end-stage lung diseases. Introduction of calcineurin inhibitors (CNIs) has significantly reduced acute rejection rates and was anticipated to have implications for long-term prognosis. However, CNI-refractory bronchiolitis obliterans (BO) represents the leading cause of late graft failure after lung transplantation and T helper (Th)2 and Th17 lymphocytes have been associated with BO development. Taking advantage of a fully allogeneic trachea transplantation model, we addressed the pathogenicity of Th cells in obliterative airway disease (OAD) occurring in cyclosporine A (CsA)-treated recipients. We found that CsA prevented CD8 + T cell infiltration into the graft and downregulated the Th1 response but affected neither Th2 nor Th17 responses *In vivo*. The CsA-mediated Th2 and Th17 bias were further demonstrated using *in vitro* polyclonal T cell stimulation. Indeed, CsA efficiently blocked cytokine-driven differentiation of CD62L+ naive CD4 + T cells into Th1 and Th17 but not Th2. When considering memory CD44 + T cells, CsA inhibited Th1 but not Th2 and Th17 cells. As CD4 + depletion efficiently prevented OAD in CsA-treated recipients, we further explored the role of Th2 and Th17 immunity *In vivo*. Although IL-4 and IL-17 deficient untreated animals developed an OAD comparable to wild-type recipients, a single cytokine deficiency afforded significant protection in CsA-treated recipients. In conclusion, CsA treatment critically imbalanced T helper alloreactivity, unravelling Th2 and Th17 as coexisting pathways mediating chronic rejection of tracheal allografts. Their modulation could represent an interesting therapeutic option to improve the long-term survival of lung transplant recipients.

O60-0049 TWENTY-YEAR EXPERIENCE OF ISLET OF LANGERHANS TRANSPLANTATION AT THE UNIVERSITY OF GENEVA

Murielle Mormont[1], Karim Gariani[1], Lionel Badet[2], Philippe Morel[1], Sandrine Demuylder-Mischler[1], Sophie Borot[1], Raphael Meier[1], Geraldine Parnaud[1], Sophie De Seigneux[1], Sylvain Terraz[1], Jacques Philippe[1], Christian Toso[1], Domenico Bosco[1], Thierry Berney[1] [1]Hôpitaux Universitaires de Genève, Genève, Switzerland; [2]Hospices Civils de Lyon, Lyon, France

Introduction: Islet transplantation is a novel treatment for type 1 diabetes, particularly in patients with brittle diabetes or hypoglycemia unawareness. This procedure can be performed alone (ITA), but also simultaneously to (SIK) or after kidney transplantation (IAK), in the presence of terminal renal failure. The University of Geneva started in 1992 one of the longest uninterrupted islet transplantation program worldwide and serves as the islet isolation center of the Swiss/French GRAGIL consortium.

Method: Since 1992, we have performed 27 islet autotransplants and 277 transplants in 160 patients within the GRAGIL network. This is a retrospective study including all 50 recipients of an islet allograft between 1992 and 2011 and transplanted at the University of Geneva. Patients transplanted in the other institutions of the GRAGIL network are not included in the analysis. Patient survival, graft survival and insulin-independence rate were analyzed by Kaplan-Meier. Survival curves were stratified for immunosuppression protocol, transplantation era, and type of procedure (SIK, ITA, IAK).

Results: Patient survival did not significantly differ with the studied variables. Significant improvements were observed in graft survival (C-peptide positivity: 34% to 100% at 3 years; P = 0.004) and insulin-independence (0% to 87% at 3 years; P = 0.0001) according to era. Significant differences were also observed according to immunosuppression protocol both for graft survival (34%-100% at 3 years; P = 0.01) and insulin-independence (0%-80%; P = 0.0003). The use of T-cell-depleting induction was associated with improved graft function. There was a trend toward better graft survival in ITA as compared to SIK and IAK (52%-100% at 3 years; P = 0.08), and significantly better rate of insulin-independence (7%-58% at 3 years; P = 0.0018). However, these differences were mostly due to a higher proportion of ITAs performed in recent eras.

Conclusion: The outcome of the islet transplantation has remarkably improved over the years to approach that of whole pancreas transplantation in most recent times. This is due to improvements in donor selection, islet isolation techniques, peri-transplant recipient management and immunosuppressive regimens. The use of T-cell-depleting induction immunosuppression has been instrumental.

Virus – Cancer

O61-0007 PRETRANSPLANTATION SCREENING FOR MALIGNANCIES IN KIDNEY TRANSPLANT RECIPIENTS

Claire Billault[1], Lamis Debchi[1], Hadjira Benalia[1], Nadia Arzouk[1], Sophie Baggiarini[1], Véronique Léon[1], Barbara Teix[1], Victor Gueutin[1], Nebojsha Zivanovic[1], Saida Ourahma[1], Sabria Hacini[1], Dorota Szumilak[1], Marcia Venditto[1], Jérome Tourret[1], Lucille Mercadal[1], Emmanuel Van Glabeke[2], Karim Fehri[2], Stéphane Bart[3], Christophe Vaessen[1], Benoit Barrou[1] [1]GH Pitié Salpêtrière, Paris, France; [2]CH André Grégoire, Montreuil, France; [3]CH René Dubos, Pontoise, France

Introduction: Malignant tumors arising after organ transplantation are severe pathologies, especially when they occur early after transplantation when immunosuppression is maximum. Early developement of previously undiagnosed non-hematopoietic tumors ("occult tumors") after transplantation occurs in 2.9% of solid organ transplant recipients. Careful pre-transplantation evalutation of recipients could help minimize that risk. We report the results of the cancer-screening policy in potential recipients carried out in our department.
Material and Methods: Since 2004, 1256 potential recipients for kidney transplantation were screened for tumors. Our standard screening includes complete physical examination, serum immunoelectrophoresis, Hemoccult testing after 40 years of age, detection of PSA levels in males and of CA-125 levels in females with a high risk of ovarian cancer, chest X-ray, abdominal and pelvic CT-scan and abdominal ultrasonography, thoracic CT-scan for smokers, ENT and dermatologic examination. Colonoscopy and screening for urothelial tumors are performed in high-risk patients.
Results: Occult tumors were suspected in 4,5% of our 1256 patients, and then confirmed in 75% of cases. Renal tumor (n = 10) and prostate cancer (n = 13) were the two most frequently found pathologies. Tumors were mostly diagnosed at clinical examination or on CT-scans. Among those patients with tumors diagnosed during the pre-transplantation screening, 6 received a transplant and did not exhibit tumor recurrence, 9 are still awaiting a transplant, 10 are on the waiting list with temporary contra-indication to transplantation, 11 were removed from the waiting list, 4 were never put on the waiting list and 2 died. Occult tumors arose in 5 patients earl after transplantation despite screening (1% of patients), and 2 of them died.
Conclusion: Pre-transplant screening revealed malignant tumors in 4% of potential male recipients and 2% of potential female recipients. Early detection of such tumors leads to better treatment of patients and better management of the national organ pool. Tumor screening policy should follow a balance between investigation costs, risks, and efficiency in regards of the frequency of the malignancies.

O62-0057 KIDNEY TRANSPLANTATION AND PTLD: LONG TERM EVOLUTION OF THE RENAL FUNCTION

Nolwenn Rabot[1], Matthias Buchler[1], Thierry Antoine[3], Etienne Morelon[4], Patrick Le Pogamp[5], Charles Legendre[6], Nassim Kamar[7], Michelle Kessler[8], Jacques Dantal[2] [1]Centre Hospitalier Universitaire, Tours (37), France; [2]Centre Hospitalier Universitaire, Nantes (44), France; [3]Center Hospitalier Universitaire, Poitiers (86), France; [4]Centre Hospitalier Universitaire, Lyon (69), France; [5]Centre Hospitalier Universitaire, Rennes (35), France; [6]Centre Hospitalier Universitaire Necker, Paris (75), France; [7]Centre Hospitalier Universitaire, Toulouse, France; [8]Centre Hospitalier Universitaire, Nancy, France

PTLD is pathology with different phenotypes. Its therapeutic approach is not uniform. The first step of PTLD treatment in renal transplantation is immunosuppression reduction. It exposes to a higher rejection risk, and kidney graft failure.

We evaluated in this retrospective multicentric study the long term renal function after immunosuppression reduction because of a PTLD.
Patients and methods: We identified adult patients with a kidney or kidney and pancreas transplantation between 1990 and 2007 in the Universitary Hospital Centers of Nantes, Tours, Poitiers, Lyon, Nancy, Necker, Rennes and Toulouse and who developped a PTLD.

Reduction of immunosuppression, acute rejection occurrence, plasmatic creatinine and creatinine clearance, allograft failure and death with a functional graft were studied.

A multivariate Cox model was built to determine which factors affect kidney allografts and/or patients survival after the PTLD occurrence, in particular the calcineurin inhibitors (CNI) treatment modification.
Results: 109 kidney transplanted patients who developped a PTLD were included.

The cumulative probability of allograft failure or death with a functional graft during the 10 years post PTLD was 60%, the cumulative probability of allograft failure was 40%.

The CNI withdrawal and PTLD staging were two bad prognosis factors for kidney allograft survival in multivariate analysis (P = 0.0425/P = 0.0315). For the composite criteria kidney allograft survival coupled with patient survival, an age superior to 60 years old was also a bad prognosis factor (P = 0.0159).

At 5 years, among the 25 patients alive with a functional graft, 11/109 patients (10%) had a creatinine clearance >60ml/min. At 10 years, 6/109 patients (5,5%) had a functional graft. Among them 3 were treated by corticosteroids monotherapy; 4 had a creatinine clearance >30 ml/min.

Discussion: The renal function prognosis after an immunosuppression reduction because of a PTLD is strongly bad. The evolution is worse in CNI withdrawal cases, which probably correspond to the worst PTLD cases. This study highlights a heterogeneous approach concerning immunosuppression reduction modalities between the different centers.

O63-0144 CAN CMV INDUCE TRANSPLANT VASCULAR SCLEROSIS IN KIDNEY TRANSPLANT RECIPIENTS?

Anna Boueilh[1], Lionel Couzi[1], Karine Moreau[1], Delphine Morel[1], Sébastien Lepreux[2], Pierre Merville[1], Thomas Bachelet[1] [1]Service Néphrologie Hôpital Pellegrin, BORDEAUX 33076, France; [2]Service Anatomopathologie Hôpital Pellegrin, BORDEAUX 33076, France

Introduction: Cytomegalovirus (CMV) infection may impact graft outcome through its direct and indirect effects. Experimental models have demonstrated the importance of CMV interaction with the endothelial interface through the induction of an angiogenic response. In clinical setting, an association has been shown between CMV infection and cardiac vasculopathy. The concept of CMV-induced transplant vascular sclerosis (TVS) has thus emerged and may participate to graft deterioration. However, data from kidney transplant recipients (KTR) are yet still scarce. We studied then the association between CMV infection and fibrous intimal thickening (CV>0) in KTR.
Methodology: Eighty seven KTR who underwent a graft biopsy for cause were retrospectively included. CMV infection, age of the donor (D.age), age of the KTR (R.age), ischemia time, presence of DSA, blood pressure at the biopsy, immunosuppressive regimen, e-GFR and Banff scoring were collected for each patient. Risk factors of fibrous intimal thickening were searched using multivariate analysis.
Results: 22/32 KTR (69%) who had a CMV infection before the biopsy (CMV+) exhibited CV>0 lesions in comparison with 25/55 (45%) in KTR free of infection (CMV-) (P = 0.04). CV>0 lesions were associated with a lower e-GFR at the biopsy (32.2 ± 17.1 vs. 41.4 ± 18.6ml/min/1.73m^2, P = 0.007). Baseline characteristics (including presence af DSA) as other Banff items were not significantly different between CMV+KTR and CMV-KTR. D.age (45.5 ± 17.5 when CV=0 vs. 55 ± 12.1 when CV>0, P = 0.02) overrode the effect of CMV infection on the occurrence of CV>0 lesions. However, when considering only KTR with a D.age<55 (n = 47), CV>0 lesions were still higher in CMV+KTR than in CMV-KTR (67% vs. 34%, P = 0.05). Finally, using univariate then multivariate analysis applied to these patients, D.age (OR 2.1 [1.1–3.8] for each decade, P = 0.02) and past history of CMV infection (OR 5.3 [1.1–26.3], P = 0.04) were the only factors associated with CV>0 lesions.
Conclusion: We report for the first time an association between CMV infection prior biopsy and fibrous intimal thickening in a cohort of KTR who underwent a biopsy for cause, suggesting cv>0 lesions could be one of the picture of TVS in renal allograft.

O64-0119 DETECTION OF BK VIRUS DNA IN RENAL ALLOGRAFT TISSUE IDENTIFIES VIREMIC RENAL TRANSPLANT RECIPIENTS AT HIGH RISK OF OVERT BKVN

Albane Brodin-Sartorius[1], Marianne Leruez[2], Dany Anglicheau[1], Marion Rabant[3], Louise Galmiche[3], Christophe Legendre[1], Julien Zuber[1] [1]Service de Transplantation Rénale adulte Hôpital Necker, Paris, France; [2]Laboratoire de Virologie Hôpital Necker, Paris, France; [3]Service d'Anatomie Pathologique Hôpital Necker, Paris, France

BK virus associated nephropathy (BKVN) is a major cause of interstitial nephritis in kidney transplant recipients (BKN) leading to graft failure in 50% of cases. With one third of false negativity, the lack of sensitivity of transplant biopsy hampers BKVN diagnosis. We hypothesized that *in situ* detection of BK virus by quantitative PCR in kidney tissue improves the performance of the biopsy in predicting overt BKVN.

Sixty nine renal allograft biopsies from 19 KTRs with BK viremia were retrospectively analyzed. In addition to conventional histological analysis and SV40 immunostaining, a BKV PCR was performed using a frozen core of the renal allograft biopsy. BK viral load was expressed in log10 (copies/1,000 000cells).

After immunosuppression minimization, BK viremia resolved in 7 patients (n-BKVN group) but 12 patients developed interstitial nephritis (BKVN group). At first BK viremia, plasma viral load was 3.3 ± 0.7 and 3.9 ± 0.6 log copies/ml in the two groups, respectively (P=NS). Kidney allograft biopsy was systematically performed in both groups 0.4 ± 0.9 and 1.4 ± 3.9 months later. Among the BKVN group, *in situ* BK viral load was highly positive (7.1 ± 1.6 log copies/millions of cells) in the 5/12 biopsies showing typical histological features of BKVN. *In situ* BK viral load was also positive (5.2 ± 1.3 log/million cells) in 5 other biopsies without any sign of BKVN by light microscopy and SV40 immunostaining. In those patients, a repeat biopsy performed 8.0 ± 5.8 months later diagnosed overt BKVN and *in situ* BK viral load remained highly positive with 7.2 ± 3.2 log/million cells. In the two last patients from the BKVN group, a first allograft biopsy had a negative *in situ* PCR and no histological features of BKVN. A repeat biopsy performed 2.3 ± 0.4 months later showed overt BKVN and positive (6.5 ± 2.0 log/ millions cells) *in situ* BKV

PCR. In summary, in the BKVN group, *in situ* BKV PCR improved sensitivity of the biopsy from 42% to 83% in predicting BKVN in patients with BK viremia. In the n-BKVN group, while all patients had a positive BK viremia at biopsy, all *in situ* BK PCR remained negative. In this group, BK viremia resolved with immunosuppressive regimen reduction in 12.3 ± 14.9 months.

In conclusion, *in situ* detection of BK virus DNA in the kidney allograft biopsy may improve the sensitivity to diagnose/predict BKVN in viremic patients.

O65-0137 ACUTE LIVER FAILURE IN PATIENTS INFECTED WITH HIV

Teresa Maria Antonini[1], Carmen Vinaxia[1], Philippe Ichai[1], Mylene Sebagh[4], Stéphanie Boukoza[2], Elina Teicher[3], Audrey Coilly[1], Anne Marie Roque Afonso[2], Bruno Roche[1], Eric Vibert[1], René Adam[1], Denis Castaing[1], Jean Charles Duclos-Vallée[1], Didier Samuel[1] [1]APHP, Centre Hépato-Biliaire, Hôpital Paul Brousse, Inserm U785, Villejuif, France; [2]APHP, Service de Virologie, Hôpital Paul Brousse, Villejuif, France; [3]APHP, Service de Maladies Infectieuses, Hôpital Kremlin Bicêtre, Kremlin Bicêtre, France; [4]APHP, Service d'Anatomie Pathologique, Hôpital Paul Brousse, Villejuif, France

Management of acute liver failure (ALF) and results of liver transplantation (LT) in HIV + patients are poorly known.

Aim: To describe ALF' evolution in HIV + patients and the results of LT for this indication.

Patients and Methods: Between June 2002 and January 2012, 15 HIV + patients (9 males, mean age 40 years [25−53]) were admitted to our ICU for ALF (PT / FV <50%). 11 patients were treated by HAART (3 of them by ddI/d4T) and 5 had medical history of opportunistic disease.

Results: On admission, mean level of total bilirubin, ALT, creatinine, INR, CD4 count were respectively: 233 μmol/L [50−620], 2472 IU/L [208−7929], 222 μmol/L [34−773], 5 [1.4−15], 345 giga/L [50−1200]. HIV viral load was <20 cp/ml in 8 patients and 2, 3 and 5.9 log in 5, 2 and 1 patient respectively. Pathological examination (METAVIR) showed no fibrosis (F0) in 11 patients and presence of chronic liver disease (F2) in 4 patients (HBV $n = 2$, HCV $n = 1$ and alcohol $n = 1$). ALF' causes were: drug-induced hepatitis ($n = 6$), IRIS ($n = 3$), DRESS ($n = 1$), herpetic hepatitis ($n = 1$), HBV reactivation ($n = 1$), unknown ($n = 3$). Evolution was towards spontaneous recovery ($n = 7$) and 1 patient died of right ventricular haemorrhage. Seven patients were listed for emergency LT due to FH: 2 patients died before LT due to cerebral oedema and 5 were transplanted. One patient died 40 days after LT because of sepsis. Post transplantation evolution of 4 pts was characterized by: median survival of 24 months (3−64), no opportunistic infection, last mean CD4 count of 530 giga/L [270−731] and HIV viral load <20 cp/ml on HAART.

Conclusions: In case of ALF, prognosis of HIV+ patients is severe with 50% evolving to FH. Preliminary results of LT for FH in HIV+ patients are encouraging with 80% survival at 24 months.

O66-0159 KIDNEY TRANSPLANTATION IN ADULT EBV SERONEGATIVE RECIPIENTS: EXPERIENCE OF TWO FRENCH CENTERS

Gabriela Vargas[1], Sophie Caillard[1], S Gouarin[2], W el Haggan[2], Valerie Chatelet[2], Bruno Moulin[1], Bruno Hurault de Ligny[2] [1]Hopitaux Universitaires de Strasbourg, Strasbourg, France; [2]Hopitaux Universitaires de Caen, Caen, France

Introduction: Epstein-Barr virus primary infection constitutes a high risk factor of development of post transplant lymphoproliferative disease (PTLD) especially in patients having an EBV mismatch. Little is known about the characteristics and the long term evolution of EBV infection in EBV seronegative adults after kidney transplantation.

Patients and Methods: Thirty eight adult EBV naive renal transplant recipients were monitored for a median time of 9 years post transplantation ((1 month to 24 years post transplantation) in two adult transplant centers in France.

Results: Induction therapy consisted in antithymocyte globulins in 45% and anti-IL2R in 55% of cases. Cyclosporin was used in 57% and tacrolimus in 26% of cases, mycophenolate mofetil in 64% and azathioprine in 23% of cases. Thirty patients (79%) had an EBV primary infection in the first post transplant year and eight patients stayed EBV naive after a mean follow up of 6 years (1 to 13 years post transplantation). Viremia was monitored extensively by EBV plasma PCR in nine patients; viremia appeared between the first month and one year post transplantation with a median of 4 ± 3.7 months. Seroconversion appeared between one month and thirteen months post transplantation with a median of 8.7 ± 4.2 months. Primary infection is rarely symptomatic, only three patients (10%) presented symptoms related to EBV primary infection (two cases of viral meningitis). Persistent viremia was seen in 15 patients (50%). Seven of the eight patients who did not show primary infection had a chemoprophylaxis with valganciclovir or acyclovir whereas eleven patients developed a primary infection under prophylaxis. We observed two cases of fatal cerebral PTLD occurring during the first year post transplantation.

Conclusion: The majority of EBV seronegative kidney transplant recipients developed an EBV asymptomatic primoinfection during the first post transplant year. Nevertheless, 8 patients (21%) under chemoprophylaxis with valganciclovir never showed EBV infection after a mean follow up of 6 years after transplanntation.

Patient and Graft Survival

O67-0002 EVALUATION OF AN INFORMATIVE PROGRAM ON ORGAN DONATION AND TRANSPLANTATION: KNOWLEDGE AND AWARENESS OF STUDENTS

Evelyne Bironneau[1], Jean Paul Jacob[1], Martine Thierry-Forestier[1], François-Xavier Lamy[2], Antoine Trinh[2], Julien Rogier[3], Sylvie Cazalot[1], Claude Pihan[1] [1]Service de Régulation et d'Appui, Bordeaux, France; [2]Agence de la Biomedecine, St Denis, France; [3]CHU Pellegrin, Bordeaux, France

Objectives: The aim of the study was to evaluate the impact of an educational program on the opinions of students concerning organ donation (OD) and their capacity to engage in family discussion.

Methods: The study was set in high schools on west of France by members of coordination or "service de regulation et d'appui"of Agence de la biomédecine. It included information and questionnaire. The questionnaire contained multiple choice and open questions on their knowledge, opinions before and after the information and the family communication.

Results: 19 interventions were done and 1638 questionnaires were retrieved. Only 9% had never heard about OD. After information, favorable opinion increased from 69% to 82% ($p < 0,001$), the youngers being more uncertain than orders. Television was the main source of information, followed by school and parents. 74% of the students had discussed with their parents and 85% of all the students found easier to discuss on stronger OD with their family. 93.6% of these adolescents were favorable being living donor for a member of their family.

Table I. Effect of age on organ donation opinions, before and after interventions.

	opinion	13–15 y	16–17y	p
Before intervention	favorable	61,2	73,2	<0,001
	unfavorable	7,4	4,1	
	uncertain	31,4	22,7	
After intervention	favorable	73	86,8	<0,001
	unfavorable	6	2,4	
	uncertain	21	10,8	

Conclusion: This study showed that the attitude of adolescents toward organ donation was positive and they are willing to engage in family discussion. Educational programs in schools increase awareness of OD and influence their attitudes and belief on OD.

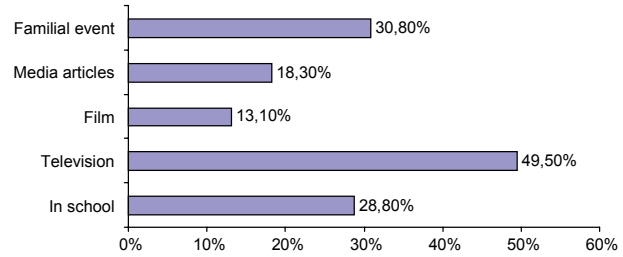


Figure 1 Source of information on organ donation.

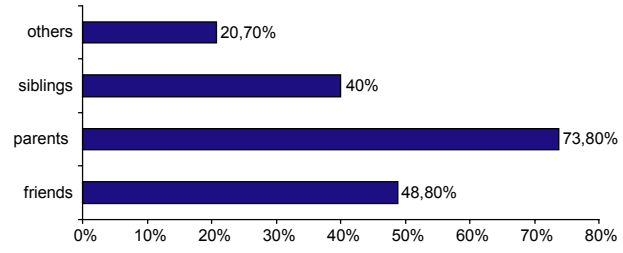


Figure 2 Willingness to discuss.

O68-0132 KIDNEY TRANSPLANTATION PERFORMED IN ADULTS WITH PEDIATRIC EN BLOC GRAFTS FROM UNDER 15 KG DONORS: LYON EXPERIENCE

Ricardo Codas[1], William Hanf[2], Hakim Fassi-Fehri[1], Nadia Abid[1], Sebastien Crouzet[1], Fatiha Mraiagh[2], Palmina Petruzzo[1], Emmanuel Morelon[2], Xavier Martin[1], Lionel Badet[1] [1]Service d'Urologie et Chirurgie de la Transplantation. Hôpital Edouard Herriot, Lyon, France; [2]Service de Néphrologie et Immunologie de la Transplantation. Hôpital Edouard Herriot, Lyon, France

Introduction: Due to kidney graft shortage in France, transplantation teams are reviewing their kidney acceptance criteria. Pediatric transplantation teams often refuse kidneys from under 15 kg donors because they lead to a higher rate of thrombosis, especially in low weight pediatric recipients. However, they can be transplanted as a dual unit in adults. The goal of this study was to evaluate the survival and outcome of six dual unit transplantations in Lyon.

Material and Methods: Between February 2002 and March 2012 six dual unit transplantations were performed with kidneys harvested under 15 kg pediatric donors. All of the donors were under 3 years old. The cause of death was trauma in all cases.

All recipients were non-immunized young adults with a BMI under 25. All patients received the same immunosuppression protocol. During follow-up kidney graft function was estimated by simplified MDRD formula and measured with Inuline and kidney graft size was evaluated by ultrasounds.

Results: After an average follow-up of 34,6 months all grafts were functional. No thrombosis occurred. There was no delay graft function. In one case there was a pelvic hematoma without any repercussion on kidney function. All patients showed signs of hyperfiltration followed by compensating hypertrophy. After 36 months average inuline clearance was 95ml/min.

Conclusion: Our results are very encouraging. Kidneys harvested from under 15 kg pediatric donors should be proposed for dual unit adult transplantation if they are refused by pediatrics teams as long term function is excellent and the rate of surgical complications is acceptable.

O69-0039 DOES LIVER IMPAIRMENT AS RENAL IMPAIRMENT CORRELATE WITH EARLY MORTALITY IN HEART TRANSPLANT PATIENTS?

Pascal Lebray[1], Shaida Varnous[2], Alina Pascale[4], Pascal Leprince[2], Daniel Eyraud[3], Geraldine Rousseau[5], Raluca Macri[3], Salima Ouldamar[2], Raluca Pais[1], Dominique Thabut[1], Jean Christophe Vaillant[5], Jean Chastre[3], Alain Pavie[2] [1]Hepatogastroenterology unit, Paris VI-Pitié-Salpêtrière Hospital, Paris, France; [2]Cardiothoracic surgical unit, Paris VI university- Pitié Salpêtrière Hospital, Paris, France; [3]Anaesthesia department, Paris VI University- Pitié Salpêtrière Hospital, Paris, France; [4]Hepatology unit, Paris VI university - Saint Antoine Hospital, Paris, France; [5]transplantation unit, Paris VI University- Pitié Salpêtrière Hospital, Paris, France

Background: Almost 25% of patients will die prematurely following heart transplantation (HT). As renal failure, liver impairment could influence post-operative survival following cardiac non-transplant surgery. No data are available about liver impairment in non cirrhotic patients previously to HT.

AIM: We analyzed at listing the prevalence of liver impairment and its influence on early heart transplantation failure.

Patients & Method: Data at listing in candidates for HT between 2004 and 2011 were retrospectively analyzed. Exclusion criteria included combined transplantation ($n = 4$) and histological cirrhosis ($n = 6$). Uni- and multivariate analysis with logistic regression evaluated risks factors for early death (3-months death). Data expressed as median or%.

Results: We analyzed 385 patients: 77.6% male, 49 ± 0.7 years-old, 49% UNOS I, 35% redux, 24% with ventricular assisted device (VAD). Four patients underwent dialysis. Causes were: dilated cardiomyopathy (47%), coronaropathy (29%), hypertrophic or restrictive cardiomyopathy (5%), valvulopathy (4%), congenital or retransplantation (2% each), other (10%).

11.8% ($n = 44$) patients died during waiting time. Among the 323 HT patients, 98 (30%) died before month 3.

In univariate analysis, M3 death was associated with: MELD score (combining INR, bilirubine and creatinine;16,1 vs. 11,7, $p<10^{-6}$), creatininemia (129,5 vs.101,5 μmol/l, $P = 0,0007$), total bilirubine (27,5 vs. 17 μmol/l, $P = 0,001$), PAL (113,5 UI/l vs. 88, $P = 0,002$), clinical ascitis (47 vs. 27, $P = 0,006$), Child B/C (39 vs.20, $P = 0,008$), AST (37 vs.33 UI/l, $P = 0,04$), right ventricular failure (36 vs. 19, $P = 0,03$), ARA II (43 vs. 25, $P = 0,03$) and negative Rhesus group (35,6 vs.12,5, $P = 0,02$). No association was found with sex, blood group, age at listing, INR, CRP, redux, invasive ventilation, VAD or vasopressive drugs. Logistic regression analysis found only ascitis (OR = 0.26, $P = 0.04$) and MELD score (OR=0.86, $P = 0.02$) as independent variables. These variables were not associated with waiting list mortality or 1 year post-transplant survival.

A specific score with an AUROC curve of 0.78 was able to correctly predict 3-months death in 79% of cases.

Conclusion: In heart transplant candidates, MELD score and ascitis are independently associated with early post transplant mortality.

O70-0035 LIVER TRANSPLANTATION NORMALIZES SERUM HEPCIDIN LEVEL AND CURES IRON METABOLISM ALTERATION IN HFE P.CYS282TYR HEMOCHROMATOSIS

Edouard Bardou-Jacquet[1], Marianne Latournerie[1], Pauline Houssel[1], Olivier Loréal[1], Karim Boudjema[2], Pierre Brissot[1] [1]Service des Maladies du Foie, CHU Pontchaillou, Rennes, France; [2]Service de Chirurgie Hépato-Biliaire et Digestive, CHU Pontchaillou, Rennes, France

Background & Aim: Iron overload in HFE related hemochromatosis (HH) is induced by a deficient hepatic secretion of hepcidin. Liver transplantation (LT) is a key treatment of potential complications of HH like Hepatocellular Carcinoma (HCC). Thus, the outcome of hepcidin secretion and iron burden after LT is a unique model to study HFE related hemochromatosis physiopathology. Our aim was to describe the long term outcome of hepcidin secretion and iron metabolism parameters in a cohort of transplanted patients.

Methods: All p.Cys282Tyr HFE homozygotes patients who had LT for complications of hemochromatosis between 1999 and 2008 were retrospectively included in the study. Files were reviewed for mortality and causes of death. Determination of biological serum iron parameters, serum hepcidin level and hepatic iron concentration (MRI) were done at the end of follow-up.

Results: Eighteen transplanted patients were included (Aged 56.2 ± 7 years). The median follow up time was 57 (IQR: 12−93) months. Indication for LT were HCC (16 patients), liver failure (1 patient), and biliary hamartomas (1 patient). 16 patients were Child-Pugh A5. Survival at one year was 83.3%, and 66.6% at 5 years. Causes of death were sepsis in the 3 months post-LT (3 patients), HCC recurrence (1 patient), lung cancer (1) and stroke (1). Before LT, serum hepcidin levels were evaluated in 11 patients and found very low in 9 patients and at the lower limit of normal in 2 patients, ii) after LT: 11 patients had iron parameters evaluation at end of follow-up, none of them had iron depletion therapy since LT. Mean serum ferritin was 185 (±99)μg/L, and all patients had normal transferrin saturation. MRI showed no iron overload in 9 patients, one patient had mild iron overload (70 μmol/g), likely favored by metabolic syndrome and excessive alcohol consumption, one patient had high iron overload (180 μmol/g), but was also exhibiting an hereditary spherocytosis. At the end of follow-up serum hepcidin was normal in 10 patients and low in one patient which also had iron deficiency anemia.

Conclusion: This study demonstrates, in patients exhibiting HFE related hemochromatosis, that liver transplantation normalizes hepcidin secretion in the long term, thus preventing recurrence of hepatic iron overload in those patients.

O71-0064 THE IMPACT OF GRAFT IMPLANTATION ORDER ON SHORT- AND LONG-TERM GRAFT SURVIVAL IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTS

Nadja Niclauss, Benoit Bédat, Axel Andres, Christian Toso, Philippe Morel, Thierry Berney Service de Chirurgie Viscérale et Transplantation, Département de Chirurgie, Hôpitaux Universitaires de Genève, Genève, Switzerland

Introduction: The preferred order of revascularization of pancreas and kidney grafts in simultaneous pancreas-kidney transplants has not yet been established. Increased preservation time might have a negative impact on graft function. In particular increased cold ischemia time is associated with a higher risk of technical failure in pancreas grafts. In this study, we investigate the influence of graft implantation order in simultaneous pancreas-kidney transplants on short- and long-term graft survival.

Methods: 12,700 simultaneous pancreas-kidney transplants from the Scientific Registry of Transplant Recipients were analyzed. Graft implantation order was determined based on the ischemia times of pancreas and kidney transplants, respectively. Pancreas and kidney graft survival were analyzed depending on graft implantation order at 3 months, 6 months and 5 years using Kaplan-Meier plots. Significance was tested with logrank test.

Results: In 8,454 transplants the pancreas was implanted first (pancreas before kidney, PBK) and in 4,246 transplants the kidney was implanted first (kidney before pancreas, KBP). Mean follow up was 6.6 and 6.3 years for PBK and KBP, respectively. Pancreas graft survival at 3 months was significantly higher in the PBK group (90.6 vs. 89.3%, $P = 0.024$). When kidney graft implantation was delayed by >2 h from pancreas implantation, difference in graft survival increased to 2.3% (90.1 vs. 87.8% pour PAR et RAP, $P = 0.009$). Pancreas graft survival at 6 months and 5 years as well as kidney graft survival were similar in both groups.

Conclusion: Pancreas graft implantation first in simultaneous pancreas-kidney transplants increases short-term pancreas graft survival. Graft implantation order does not affect long-term pancreas and kidney graft survival.

O72-0068 THE IMPACT OF RECIPIENT BODY MASS INDEX ON SHORT AND LONG TERM SURVIVAL OF THE PANCREAS GRAFT

Benoît Bédat, Nadja Niclauss, Axel Andres, Anne-Sophie Jannot, Christian Toso, Philippe Morel, Thierry Berney Hôpitaux Universitaires de Genève, Genève, Switzerland

Introduction: The prevalence of obesity/overweight continues to rise in our surgical population. Obesity and patient body fat distribution are thought to increase the risk of surgical complications, including in pancreas surgery. In pancreas transplantation, the detrimental effect of high donor body mass index (BMI) has long been recognized, but the impact of recipient BMI on short and long term pancreas graft survival has not been studied.

Methods: We analyzed data from all individuals who underwent a first pancreas transplantation from October 1, 1987 to August 30, 2011 and recorded in the Scientific Registry of Transplant Recipients (SRTR). Recipients and donors were categorized into BMI classes: underweight (< 18.5), normal (18.5−25), overweight (25−30), obese (30−35), very obese (>35). Patient and graft survival were analyzed by Kaplan-Meier analysis. Univariate analysis was done with the logrank test, and multivariate analysis was done with a Cox proportional hazard regression. Stepwise-forward multivariable Cox PH analysis was used to examine the independent effect on survival of selected variables, controlling for possible confounders.

Results: 21,075 individuals were included in the analysis. Median follow-up was 4 years. Short-term graft survival decreased progressively as recipient BMI increased ($p < 0.001$). There is a significant association between recipient BMI and patient survival especially for obese class II patients ($p < 0.001$). In the long term, the association is significant but depends only on short term graft survival except for underweight patients where there is a striking decreased of graft survival due to a higher mortality ($p < 0.001$).

Conclusion: Increased BMI is associated with an increased risk of early graft loss and early mortality. Increased BMI is not associated with increased incidence of post transplant diabetes. Finally, very low BMI is associated with increased risk of death.

Ischemia-reperfusion; Non-Heart Beating Donors

O73-0106 RELEVANCE OF UBIQUITIN PROTEASOME SYSTEM AND ADENOSINE MONOPHOSPHATE PROTEIN KINASE IN FATTY LIVER PRESERVATION: BORTEZOMIB AS A PROMISING ADDITIVE IN PRESERVATION SOLUTION

Mohamed Bejaoui[1], Mohamed Amine Zaouali[1], Eirini Pantazi[1], Alexandre Lopez[1], Teresa Carbonell[2], Emma Folch[1], Hassen Ben Abdennebi[3], Joan Roselló Catafau[1] [1]Laboratoire de Pathologie Expérimentale, CSIC, Barcelone, Spain; [2]Laboratoire de Physiologie, Faculté de Biologie, Université de Barcelone, Barcelone, Spain; [3]Laboratoire de Physiologie Humaine, Faculté de Pharmacie, Université de Monastir, Monastir, Tunisia

Introduction: Hepatic steatosis presents a major challenge in liver transplantation. Steatotic livers show an increased susceptibility to cold ischemia and reperfusion (CIR) injury and the pathophysiologiocal mechanisms are not fully understood. Recently it has been reported that ubiquitin proteasome system (UPS), the main non lysosomal proteolytic system, is activated during cold storage which enhances graft injury. In this communication we evaluated the addition of reversible proteasome inhibitor Bortezomib (Brz) at non toxic low dose to the IGL-1 solution. Its relationship with cytoprotective factors involved in fatty liver preservation as adenosine monophosphate protein kinase (AMPK) was investigated.

Experimental: Steatotic livers were preserved for 24h (4°C) in IGL-1 solution with and without Brz (100 nM) or, pretreated with AMPK inhibitor Adenine 9-β-D-arabinofuranoside (Ara A) and then preserved in IGL+Brz. Following, the livers were perfused for 2 h at 37ªC. Liver injury (ALT/AST) and function (bile production and vascular resistane) were measured. Endoplasmic reticulum stress (GRP78, CHOP), pAMPK and Akt/GSK3beta were also determined by western blot. Proteasome activity was assessed after ischemia and reperfusion.

Results: We show that the UPS activation during cold storage was significantly higher for steatotic livers than compared to lean ones. Brz addition to IGL-1 solution significantly reduced liver injury; ameliorated graft function and decreased reticulum stress (GRP78, CHOP).These benefits were reversed by the pretreatment of obese rats with Ara A. Western blot analyses showed a significant increase in pAMPK after reperfusion but not after cold storage. We also observed in IGL+Bz group, a significant Akt phosphorylation which in turn induces the phosphorylation and thus inhibition of its direct substrate, GSK3beta.

Conclusion: Brz at low non toxic concentration is a promising additive to IGL-1 solution for ameliorating steatotic liver preservation. Its protective effects are due, in part, to the prevention of AMPK degradation during the early phase of reperfusion and the activation of Akt and the subsequent GSK3beta inhibition.

O74-0073 STUDY OF HEMODYNAMICS, COAGULATION AND INFLAMMATION IN THE USE OF NORMOTHERMIC REGIONAL CIRCULATION (NCR) IN A PORCINE MODEL OF NON BEATING HEART DONOR (NBHD)

Thomas Kerforne[4], Sébastien Giraud[2], Géraldine Alain[1], Christophe Jayle[1], William Hebrard[3], Michel Pinsard[4], Thierry Hauet[2] [1]Inserm U1082/ Université de Poitiers; CHU Poitiers Service de chirurgie cardio-thoracique, Poitiers, Poitou-Charente, France; [2]Inserm U1082/ Université de Poitiers; CHU Poitiers Service de Biochimie, Poitiers, Poitou-Charente, France; [3]INRA le Magneraud, Laboratoire de Chirurgie Expérimentale, Surgères, Poitou-Charente, France; [4]Inserm U1082/ Université de Poitiers; CHU Poitiers Département d'Anesthésie-Réanimation, Poitiers, Poitou-Charente, France

Introduction: NBHD are a means to increase grafts pool. NRC is a new modality of treatment of these grafts. NRC is re-oxygenated donor blood to be infuse at 37°C into intra abdominal organs (IAO). We studied the characteristics of a porcine model of NBHD supported by NRC on 3 aspects: hemodynamics, coagulation and inflammation.

Methods: NCR was placed surgically in 6 pigs (*Large white*, 40−45 kg) after 30 min of cardiac arrest. IAO were infused by NCR during 4 h. To assess hemodynamics parameters we measured NRC infusion output (IO), arterial oxygen saturation (SaO_2), veinous oxygen saturation (SVO_2) and hemoglobin concentration (Hb). From these parameters we calculated IAO oxygen extraction (EO_2). We studied coagulation by 2 thrombelastometry (TEM) parameters evolution, clotting formation time (CFT) and maximum clot firmness (MCF) and the kinetic of platelets count. We measured serum level evolution of Tumor Necrosis Factor alpha (TNFα) in samples taken on NCR infusion line.

Results: IO was constant at 2,7 l/min on NCR, SaO_2 (T0 = 98,4 ± 3,0%; T4h= 99,8 ± 0,5%; NS), Hb (T0 = 9,2 ± 1,3 g/dl;T4h=8,5 ± 1,5 g/dl; NS), SVO_2 (T0 = 72,0 ± 6,7%; T4h= 67,2 ± 5,6%; NS) and EO_2 (T0 = 29,8 ± 4,0%; T4h= 34,8 ± 5,9%; NS) did not change significantly during the procedure. TEM parameters did not change significantly during the procedure (CFT :T0 = 49,2 ± 11,5 sec;T4h=82,8 ± 37,4 sec; NS; MCF: T0 = 71,5 ± 4,6 mm;T4h=63,7 ± 12,7 sec; NS), although platelet count decreased significantly without thrombocytopenia criteria (T0 = 307,7 ± 42,9 x 10^3/mm^3; T4h= 171,7 ± 92,8 x 10^3/mm^3; P < 0,05). TNFα serum level did not change significantly during the procedure (T0 = 158,3 ± 99,2 pg/ml; T4h= 105,0 ± 59,7 pg/ml; NS).

Conclusion: Porcine model of NBHD supported by NCR that we have developed presentes hemodynamic stability, the overall coagulation and inflammation.

O75-0063 PREVENTION OF ISCHEMIA-REPERFUSION LUNG INJURY BY SUPPLEMENTATION OF THE PRESERVATION SOLUTION WITH AN OXYGEN CARRIER IN PORCINE LUNG TRANSPLANT MODEL

Matthieu Glorion[1], Edouard Sage[1], Thierry Hauet[2], Elie Fadel[3] [1]Hôpital Foch, Suresnes, France; [2]INSERM U927, Poitiers, France; [3]Hôpital Marie Lannelongue, Plessis Robinson, France

Background: M101 is a new oxygen carrier extracted from arenicola marina with high-oxygen affinity and the ability to function at low temperature. This study assessed the effect of M101 in adjonction to static preservation solution on primary graft dysfonction after lung transplantation.

Methods: A porcine single left lung allotransplantation was performed in 2 experimental groups after 24 h of cold storage. Donor Lungs were flushed and preserved with low-potassium dextran (LPD) in group 1 whereas M101 (2g/l) was added to LPD in group 2. Control animals underwent a sham operation. The HIF-1 protein level was evaluated during cold storage on sequential right lung parenchymal samples. After left lung transplantation, the right pulmonary artery was clamped to evaluate graft function. During 5 h of reperfusion, hemodynamics, oxygenation and dynamic compliance were monitored and compared with controls. HMG-B1, TNF alpha, LDH, and IL-8 were measured in serum. After 5 h of reperfusion, TNF-alpha and LDH were measured in bronchoalveolar lavage.

Results: During the cold ischemia the HIF-1 protein level remains unchanged. After 5 h of reperfusion, group 2 led to a significant reduction of graft vascular resistance (1217 + /- 104 vs. 1627 + /- 211 dynes/s/cm^5, p < 0.05), graft oxygenation ratio was significantly higher (436 + /−10 vs. 324 + /- 32 mm Hg, p < 0.05) and alveolo arterial gradient values tended to be lower (221 vs. 321 mmHg, *P* = 0.06). Expression of HMG B1 in serum tended to be lower (2.1 + /- 0.8 vs. 4.6 + /−1.5 *P* = 0,07) compared with group 1. The TNF alpha, LDH and IL-8 serum levels remained unchanged during reperfusion. However TNF-alpha and IL-8 in bronchoalveolar lavage were significantly higher in the 2 experimental groups compared to control (group 1: 164 + /−18 pg/ml, group 2: 151 + /- 20 pg/ml vs. sham 69 + /−18 pg/ml, p < 0.05 and group 1: 1.14 + /- 0.21pg/ml, group 2: 1.12 + /−0.26 pg/ml vs. sham 0.5 + /- 0.2pg/ml, p < 0.05 respectively).

Conclusions: In this preliminary study, adjunction of a new oxygen carrier M101 in lung preservation solution improves early graft function after prolonged cold ischemia.

O76-0079 EARLY INSTITUTION OF HYPOTHERMIC TOTAL LIQUID VENTILATION AFTER PROLONGED CARDIAC ARREST PROTECTS AGAINST KIDNEY ISCHEMIC ALTERATIONS

Renaud Tissier[1], Sebastien Giraud[2], Nathalie Quellard[2], Beatrice Fernandez[2], Fanny Lidouren[1], Matthias Kohlhauer[1], Lys Darbera[1], Mourad Chenoune[1], Patrick Bruneval[3], Bijan Ghaleb[1], Alain Berdeaux[1], Thierry Hauet[2] [1]Université Paris-Est Créteil, INSERM U955, Créteil, France; [2]Inserm U1082, Université de Poitiers, CHU de Poitiers, Poitiers, France; [3]INSERM U970, Paris, France

Introduction: Warm ischemia before organ removal is a major cause of kidney alterations in transplants from non-heart-beating donors (NHBD). In a rabbit model of cardiac arrest (CA), we showed that rapid cooling using total liquid ventilation (TLV) can improve survival and protect heart and brain after resuscitation. We hypothesized that TLV with perfluorocarbons can also limits kidney alterations after CA, with potential applications for controlled organ from NHBD donors.

Methods: Anesthetized rabbits were submitted to 15 min of untreated ventricular fibrillation. After resuscitation and resumption of spontaneous circulation (ROSC), rabbits underwent Control life support or hypothermia (32−33°C) induced by TLV and maintained with external blankets under mechanical ventilation. Life support was prolonged during 6 h before kidney removal and analysis. Other rabbits were submitted to similar follow-up with no CA (Sham group).

Results: In each group, 8 animals completed the protocol. Despite similar delays before ROSC (~4 min), epinephrine doses required to maintain blood pressure throughout follow-up were lower in TLV versus Control (361 ± 23 vs. 990 ± 179 μg/kg, respectively). This was accompanied by a decrease in creatinine blood levels after CA (12 ± 1 vs. 16 ± 2 mg/l), while still altered when compared to Sham values (8 ± 1 mg/l). Importantly, histological kidney injury were also attenuated in TLV group (p < 0.05 vs. Control). The nephroprotective effect of TLV was not related to differences in delayed inflammatory or immune renal responses since transcriptions of, *e.g.*, IFN-γ, TNF-α, IL-1β, MCP-1, IL18, ICAM-1, E-selectin were similarly altered in TLV and Control versus Sham. An immediate anti-ischemic effect of rapid cooling with TLV might accordingly be involved, as could be explained by mRNA expression of EPO and HO1 (Heme Oxygenase 1) (not significant).

Conclusion: Ultra-fast cooling with TLV limits kidney alterations after prolonged experimental warm ischemia in case of NHBD donors.

O77-0186 LIVER TRANSPLANTATION FROM MAASTRICHT II DONOR. FIRST 10 FRENCH CASES

Eric Savier[1], Federica Dondero[4], Daniel Eyraud[2], Claire Francoz[5], Géraldine Rousseau[1], Pascal Lebray[6], Bruno Riou[3], Jean-Christophe Vaillant[1], Jacques Belguiti[4], Laurent Hannoun[1] [1]Service de Chirurgie Hépato-Bilio-Pancréatique Transplantation Hépatique. Groupe Hospitalier Pitié-Salpêtrière. AP-HP. Universtié P.M. Curie (Paris VI), Paris, France; [2]Département d'Anesthésie-Réanimation. Groupe Hospitalier Pitié-Salpêtrière. AP-HP. Universtié P.M. Curie (Paris VI), Paris, France; [3]Service d'Accueil des Urgences. Groupe Hospitalier Pitié-Salpêtrière. AP-HP. Universtié P.M. Curie (Paris VI), Paris, France; [4]Chirurgie Hépatobiliaire et Transplantation Hépatique, Hôpital Beaujon, Clichy, France; [5]Hépatologie, Réanimation Hépato-digestive. Hôpital Beaujon, Clichy, France; [6]Service d'Hépato-Gastro-Entérologie (Pr T. Poynard). Groupe Hospitalier Pitié-Salpêtrière. AP-HP. Universtié P.M. Curie (Paris VI), Paris, France

Introduction: Organ donation after unexpected cardiac death (DCD) is allowed in France under national protocoled rules, according to Fondevila et al. When death is established, the use of a normothermic extra-corporeal membrane oxygenator (NECMO) allows to perfuse and to oxygenate the abdominal organs and consequently procurement of the liver as a potential graft. Donor's criteria allowing liver harvesting were as follow: age < 55 years, no-flow < 15 min., low-flow < 120 min., efficiency of the NECMO and duration < 240 min., ALT<200 IU/L at 2 h interval after starting the NECMO. Such grafts were proposed to consented patients of less than 65 years old, on the waiting list of liver transplantation (LT) for a liver cancer a main indication and without hepatic insufficiency (MELD < 20). Aim of the study was to reported results of the 10 first LT from unexpected DCD donors.

Methodology: Retrospective bicentric study with monocentric biological comparison between 5 LT from DCD donor and 30 LT from donation after brain death (DBD) donor (SCOT15 as preservation solution).

Results: Ten LT were performed within 2 hospitals (5 LT each) and both experienced a primary non-function (PNF) with subsequent retransplantation. The one-year graft and patient survivals were 80%, without arterial or biliary complication. Biology: During the first 6 h following the reperfusion, the maximal value for AST was 2006 ± 439 IU/L for the DCD group and 2044 ± 361 IU/L for the DBD group ($n = 5$ and 30, NS) and for ALT 1962 ± 507 IU/L and 1150 ± 213 IU/J, respectively (NS). After 6 h the decrease of transaminases was similar in both groups, except in the case of PNF. At day 1, the mean factor V values was 40 ± 9% for the DCD group and 58 ± 3% ($p < 0.05$) for the DBD group and after that identical in both groups as was total bilirubin concentration and gGT release.

Conclusions: Within strict selection liver graft from unexpected DCD donor is transplantable with acceptable morbidity.

O78-0008 KIDNEY TRANSPLANTATION FROM NON-HEAT BEATING DONORS: 3-YEAR RESULTS

Claire Billault[1], Rodolphe Thuret[2], Emmanuel Van Glabeke[3], Karim Fehri[3], Stéphane Bart[4], Frédéric Thibaut[1], Nadia Arzouk[1], Victor Gueutin[1], Jérome Tourret[1], Saida Ourahma[1], Armelle Nicolas-Robin[1], Benoit Barrou[1] [1]GH Pitié Salpêtrière, Paris, France; [2]CHU Lapeyronie, Montpellier, France; [3]CH André Grégoire, Montreuil, France; [4]CH René Dubos, Pontoise, France

Introduction: Transplantation of kidneys from non-heart beating donors (NHBD) in France was first started in 2006. Several questions arose: how to minimize the primary non-function (PNF) rate, what would be the delayed graft function (DGF) rate, how well would these kidneys perform, what would be their long-term survival. Several measures were put in place to tackle these questions, targeting donor selection, procurement and transplantation timeframes, organ perfusion and preservation. These are the results after 3 years of NHBD kidney transplantations in our center.

Material and Methods: Between 2008 and 2011, 205 transplantations from heart-beating deceased donors (HBD) and 53 NHBD transplantations were performed at our institution. Criteria for NHBD selection were established by the Agence de la Biomédecine. We used normothermic recirculation (NRC) over double-lumen catheter (DLC) perfusion prior to procurement whenever possible. Kidneys were preserved using the Lifeport® perfusion machine and transplanted within the shortest possible timeframe.

Results: NRC was used in 33 and DLC in 20 cases. We did not observe any case of PNF; 3 grafts were lost to veinous thrombosis. Graft survival was 91% and patient survival 96%. After a 2-year follow-up, there was no significant difference in renal function estimated by the MDRD equation between NHBD kidneys and HBD-standard criteria (HBD-SC) kidneys (50 ± 13 ml/min vs. 56 ± 25 ml/min, $P = 0.16$); however NHBD kidneys performed significantly better than HBD-extended criteria (HBD-EC) kidneys (50 ± 13 ml/min vs. 38 ± 17 ml/min, $P = 0{,}007$). DGF does not seem to have a significant impact on graft survival in the NHBD group, as opposed to the HBD group. Use of NRC over DLC is associated with a significantly better initial outcome of grafts.

Conclusion: Our results indicate that NHBD grafts can be utilised with comparable results to those of HBD-SC grafts, and better results than HBD-EC grafts. Use of NRC over DLC is associated with better outcome during the first post-transplant month.