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ESOT LEONARDO DA VINCI TRANSPLANT RESEARCH AND INNOVATION AWARD

18F-FDG PET/CT IMAGING AT 3 MONTHS POST TRANSPLANTATION DISPROVES SUBCLINICAL REJECTION IN KIDNEY TRANSPLANT RECIPIENTS

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Subclinical kidney allograft acute rejection (SCR) corresponds to "the histological documentation of unexpected evidence of acute rejection (AR) in a stable patient". SCR detection relies on surveillance biopsy. Still, non-invasive approaches may help avoid biopsy-associated complications and limitations. Positron emission tomography (PET) coupled with computed tomography (CT) after injection of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) may be an option. From 11/2015 to 01/2018, we prospectively performed ^{18}F-FDG-PET/CT in adult kidney transplant recipients (KTR) who underwent surveillance transplant biopsy at ~3 months *post* transplantation. Banff-2017 classification was used. Mean standard uptake value (mSUV) of kidney cortex was measured. Statistics were done via Python library SciPy. Our 95-patient cohort was categorized into 3 groups upon Banff-based histology: normal ($n = 70$); borderline ($n = 16$); AR ($n = 6$). Three cases were excluded for PCR-proven BK nephropathy ($n = 2$) or uninterpretable histology ($n = 1$). No clinical or biological difference was observed between groups. mSUV reached 1.49 ± 0.32, 1.64 ± 0.34 and 1.77 ± 0.35 in normal, borderline and AR groups, respectively. A significant difference of mSUV was found among groups (ANOVA, $p = 0.05$). Furthermore, mSUV was significantly higher in AR *versus* normal groups (t-test, $p = 0.04$). The area under the ROC curve was 0.71, with 66% sensitivity and 62% specificity using mSUV threshold at 1.6. mSUV positively correlated with total inflammation score ($r2 = 0.06$, $p = 0.02$) and with acute composite Banff (g+i+t+v+ptc) score ($r2 = 0.05$, $p = 0.03$). In conclusion, our pilot study suggests that ^{18}F-FDG-PET/CT helps non-invasively detect SCR, with a negative predictive value of 96% using 1.6 as mSUV threshold.

EX VIVO-EXPANDED HUMAN CD19$^+$ TIM-1$^+$ B REGULATORY CELLS CAN PROLONG HUMAN ALLOGRAFT SURVIVAL IN A HUMANISED MOUSE MODEL OF SKIN TRANSPLANTATION AND CAN INDUCE HUMAN CD4$^+$ CD25$^+$ CD127^{LO} TREG

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Background: Transplantation is the gold standard therapy for many end-stage organ diseases, but chronic allograft dysfunction is a leading cause of organ loss. Mouse B regulatory cells can prevent allograft rejection *in vivo* by T regulatory cell (Treg) induction. We report the *in vivo* function of *ex vivo*-expanded human CD19$^+$ TIM-1$^+$ expBreg in a humanised mouse model of skin transplantation and the association with human Treg. We also describe the combined suppressive function of human expBreg and Treg therapy *in vitro*.
Methods: Human CD19$^+$ B cells and CD4$^+$ CD25$^+$ CD127^{lo} Treg were isolated from human donors: CD19$^+$ TIM-1$^+$ expbreg were generated by culture with CD154$^+$ CHO cells and IL-2, IL-4, IL-10; CD4$^+$ CD25$^+$ CD127^{lo} Treg were expanded with anti CD3/CD28 beads and IL-2. Combined suppressive potency of expBreg and Treg was investigated by *in vitro* assays. *In vivo* function of human expBreg was examined by adoptive transfer in a humanised mouse model of skin transplantation.
Results: *In vivo*, expBreg were able to prolong human skin allograft survival in the humanised mouse model and were also associated with an increased percentage of human CD4$^+$ CD25$^+$ CD127^{lo} Treg within human skin allograft. Human expBreg induced potently suppressive human CD4$^+$ CD25$^+$ CD127^{lo} Treg in a TIM-1-dependent manner. Combination therapy of human expBreg and Treg resulted in increased suppressive potency when compared to human Treg alone or to Treg and activated B cells. Treg demonstrated lower proliferative capacity but greater phenotypic stability and suppressive function in the presence of expBreg.
Conclusion: *Ex vivo*-expanded human CD19$^+$ TIM-1$^+$ expbreg can prolong graft survival in a complex biological environment and can induce Treg. Importantly, expBreg can augment the suppressive potency of human Treg and may stabilise the Treg phenotype. Human expBreg may represent a novel cellular therapy that could be used alone or as an adjunct to other cellular therapy regimens to prolong allograft survival in transplantation.

HUMAN EXTRA HEPATIC BILE DUCT-DERIVED ORGANOIDS FOR STEM CELL CHARACTERIZATION AND DISEASE MODELING

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Integrity of the biliary tree is imperative for liver function. Though evidence suggests that peribiliary glands (PBG) harbor progenitor cells that contribute to bile duct homeostasis and repair during disease or injury, these are not well characterized. Therefore, we aim to expand and characterize biliary progenitors using 3D organoid cultures from extrahepatic bile ducts (EBD). For this, extra-hepatic bile ducts and paired liver biopsies ($n = 40$) were collected from donor grafts at time of liver transplantation. EBD organoid cultures were initiated using similar conditions as described for human liver biopsies and propagated by weekly passaging. The EBD-derived organoids were characterized and compared to liver-derived organoids by phenotypic (EM, light sheet microscopy), genomic (q-PCR, RNAseq), proteomic (MassSpec, immunohistochemistry) and functional analysis. Organoids were efficiently grown from the common bile duct for >9 months and stained positive for biliary cell markers (CK19, Epcam and MUC1. RNA analysis demonstrated expression of stem cell markers LGR5, PDX1, Sox9). Functional transporter channel function of CFTR and AE2 was demonstrated using Ussing chamber technology and Forskolin Induced Swelling (FIS) assays. Hepatocyte and cholangiocyte differentiation potential was studied and although PGB-organoids could differentiate towards cholangiocytes, they were less prone to differentiate into hepatocytes when compared to their liver-counterparts. To demonstrate their use as a disease model, EBD organoids were successfully initiated from a cystic fibrosis patient.

This study demonstrates the presence of LGR5-positive stem/progenitor cells in human EBD. These organoids can be propagated long-term, express hepato-biliary genes and proteins, and show functional transporter channel activity. EBD organoids could potentially be used to model biliary disease, and biliary strictures after liver transplantation, and for tissue engineering applications.

RECONDITIONING MARGINAL HUMAN KIDNEYS USING MULTISTEM™ CELL THERAPY DELIVERED DURING NORMOTHERMIC MACHINE PERFUSION

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Introduction: *Ex-vivo* normothermic machine perfusion (NMP) of donor kidneys prior to transplantation provides a platform for direct delivery of advanced therapeutics to revive, optimise and restore organ quality prior to transplantation. Multipotent Adult Progenitor Cells™ (MAPCs) possess potent immunomodulatory properties which could prove beneficial in the treatment of ischaemia reperfusion injury. We investigated the potential reconditioning capability of MAPC cells in donor kidney NMP.
Methods: Paired human kidneys from the same donor were simultaneously perfused for 7 hours (5 pairs). The right or left kidney was randomly allocated to receive MAPC cell treatment. Serial samples of perfusate, urine and tissue biopsies were taken for comparison with the control paired kidney.
Results: MAPC treated kidneys demonstrated improved urine output, $p < 0.01$, decreased expression of the kidney injury biomarker NGAL $p < 0.01$, improved microvascular perfusion on contrast enhanced ultrasound (cortex $p < 0.05$, medulla $p < 0.01$), downregulation of IL-1β ($p < 0.05$) and upregulation IL-10 ($p < 0.05$) & Indolamine-2, 3-dioxygenase ($p < 0.05$). A mouse model of intraperitoneal chemotaxis demonstrated decreased neutrophil recruitment when stimulated with perfusate from MAPC treated kindeys ($p < 0.01$). Immunofluorescence revealed labelled MAPCs became resident within perivascular niche surronding the peritubular capillaries and glomerulus of the perfused kidneys. MAPC therapy was not associated with any detrimental physiological or embolic events.
Conclusion: We report the first successful delivery of a cellular therapy to a human kidney during NMP. Kidneys treated with MAPCs during NMP demonstrate improvement in clinically relevant functional parameters and injury biomarkers. The anti-inflammatory MAPC perfusate secretome reduced

neutrophil chemotaxis. This novel method of cell therapy delivery provides an exciting opportunity to recondition organs prior to clinical transplantation.

PREDICTING RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER LIVER TRANSPLANTATION USING A MODEL THAT INCORPORATES TUMOUR- AND DONOR-RELATED FACTORS

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Background: The aim of this study was to develop a prognostic score that combines donor and tumor characteristics, to predict the risk of recurrence of hepatocellular carcinoma (HCC) after liver transplantation.
Methods: Within the American Scientific Registry of Transplant Recipients, we identified patients with HCC who received a liver transplantation between 2004 and 2014 (training set, $n = 10,887$), and we calculated post-transplant HCC recurrence. We fitted a multivariable competing-risk regression model incorporating information on the recipient, the tumor and the donor. We then developed a prognostic scorem, which was internally validated in a distinct subset of the population ($n = 3,627$).
Results: In the training set ($n = 10,887$), after allowing for competing events, we found that total tumor diameter (adjusted hazard ratio [aHR] 1.52 (95% CI 1.28–1.81) $p < 0.001$), alpha-feto protein (aHR 1.27 (95% CI 1.23–1.32) $p < 0.0001$), recipient male gender (aHR 1.43 (95% CI 1.18–1.74) $p < 0.001$), elevated donor body mass index (aHR 1.26 (95% CI 1.01–1.58) $p = 0.037$), and graft allocation policy (aHR 1.22 (95% CI 1.03–1.44) $p = 0.020$) were independently associated with post-transplant HCC recurrence. Based on the coefficients of the multivariable model, we developed a prognostic score, and tested it in the validation set ($n = 3627$), where it allowed discriminating several categories of patients in terms of risk of HCC recurrence.
Conclusion: This is the first score predicting HCC recurrence after liver transplantation that incorporates both donor and tumor characteristics. It is based on readily available variables, and it could help transplant teams identifying, at the time of waitlist inscription, hazardous combinations between the recipient and the donor.