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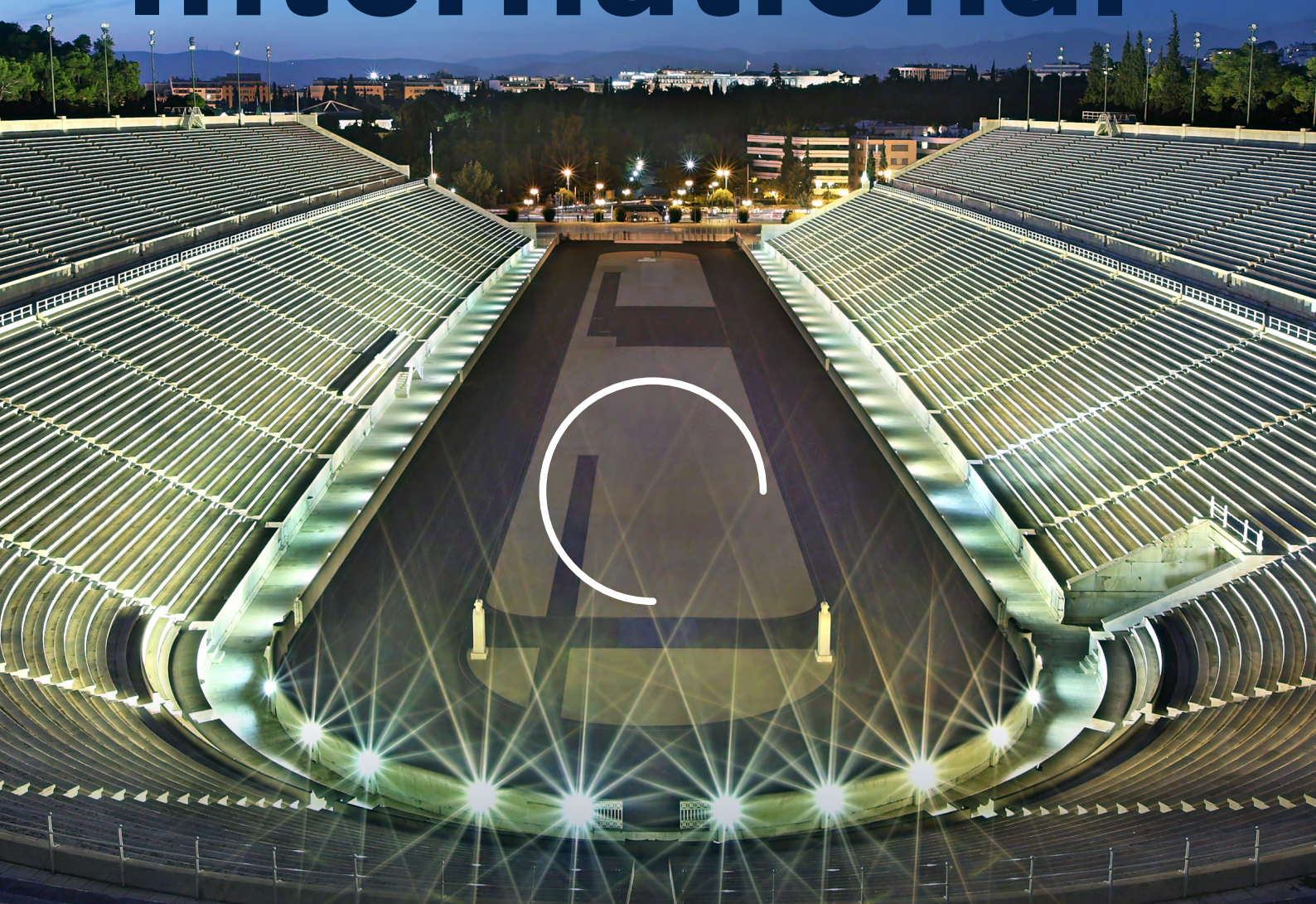

21st Congress of the European Society for Organ Transplantation

17 September - 20 September 2023. Athens, Greece

esotcongress.org

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About the European Society for Organ Transplantation

The European Society for Organ Transplantation (ESOT) was founded nearly 40 years ago and has been dedicated to pursuing excellence in organ transplantation ever since.

Facilitating a wealth of international clinical trials and research collaborations over the years, ESOT remains committed to its primary aim of improving patient outcomes in transplantation.

With a community of transplant professionals from around the world, ESOT is an influential international organisation and the facilitator of the biennial ESOT Congress. ESOT attracts the foremost transplantation experts to work in its committees and sections. It has an impressive track record in supporting research, extensive education and promoting changes in European policy.

Our Mission

To improve outcomes for patients with terminal organ disease through transplantation, organ regeneration and substitution.

Our Vision

To promote sustainable scientific advancement through multidisciplinary communities of healthcare professionals

To deliver first-class education, training and career advancement opportunities to all healthcare professionals, with specific training programmes for low-income countries

To work with partner organisations, professional bodies and competent authorities to improve public and institutional awareness of the latest research in the field

To develop and promote policies for equitable access to transplantation and related therapeutic strategies

Abstracts of the 21st Biennial European Society for Organ Transplantation (ESOT) Congress, Athens, Greece, 17 – 20 September 2023

BRIEF ORALS

Molecular transplant immunology

BOS1_1 17B-ESTRADIOL THERAPY MODULATES MICROGLIA ACTIVATION AFTER ISCHEMIA AND REPERFUSION REPERCUSSIONS

Fernanda Yamamoto Ricardo-Da-Silva[*1], Pedro Luiz Zonta de Freitas*[*1], Marcelo Sousa[1], Lucas Ferreira Da Anunciação[1], Luiz Felipe Pinho Moreira[1], Cristiano Jesus Correia[1], Ana Cristina Breithaupt-Faloppa[1]

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Background: Organ transplantation is adopted as the main therapeutic approach for patients without treatment options. In this context, the process of ischemia and reperfusion of the organ during transplant surgery is inevitable. IR induced systemic inflammation is responsible for several systemic physiological changes, including neurological and cognitive complications; those are directly linked to brain parenchyma inflammation, mainly due to the activation of microglia. Studies indicate 17β-estradiol (E2) as a potential therapy to decrease the IR induced inflammatory process. Therefore, here we investigate the effects of E2 treatment on the brain parenchyma after visceral ischemia and reperfusion.
Methods: Male Wistar rats were divided in 3 groups (n= 6/group): (I) sham, surgically manipulated; (II) VIR, animals subjected to ischemia and reperfusion. (III) E2, animals treated with 17β-estradiol (280 µg/Kg, i.v.) after VIR (1h after reperfusion). The visceral ischemia was induced by insertion of a 2-Fogarty® catheter in the descending aorta (aortic occlusion for 20 min, followed by a reperfusion period of 4h). Immunohistochemistry of anti-Iba-1 also know AIF-1 (Allograft inflammatory factor 1) was performed to assess microglia activation in the brain parenchyma (prefrontal cortex, hippocampus, as well as the thalamus and hypothalamus).
Results: The number of active resident microglia cells had greater amounts in the left side of the brain parenchyma of the VIR group compared to Sham (VIR: 25.3 ± 4.1; Sham: 19.4 ± 2.6 active cells/mm²; *p*= 0,04). There was a reduction of active cells count in the E2 group when compared to the VIR group, on the right (E2: 16.36 ± 2; VIR: 25.31 ± 4 active cells/mm²; *p*= 0.02) and left (E2: 17.39 ± 2; VIR: 25.04 ± 5 active cells/mm²; *p*= 0.05) sides of the brain parenchyma.
Conclusions: Our data showed that the systemic inflammation triggered by visceral ischemia and reperfusion was responsible for activating microglia. However, treatment with E2 proved to be an important therapeutic agent, by effectively controlling microglia activation even after reperfusion is initiated.
Grant 88887.621072/2021-00, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES

BOS1_2 SINGLE CELL RNA SEQUENCING OF DONOR-REACTIVE T CELLS REVEALS ROLE OF APOPTOSIS IN DONOR-SPECIFIC HYPORESPONSIVENESS OF KIDNEY TRANSPLANT RECIPIENTS

Amy van der List[1], Nicolle Litjens*[1], Rutger Brouwer[2], Mariska Klepper[1], Alexander den Dekker[2], Wilfred van Ijcken[2], Michiel Betjes[1]

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Background: After kidney transplantation (KT) donor-specific hyporesponsiveness (DSH) of recipient T cells develops over time. Recently, apoptosis was identified as a possible underlying mechanism.
Methods: In this study, both transcriptomic profiles and complete V(D)J variable regions of TR transcripts from individual alloreactive T cells of kidney transplant recipients were determined with single cell RNA sequencing. Alloreactive T cells were identified by CD137 expression after stimulation of peripheral blood mononuclear cells (PBMCs) obtained from KT recipients (N=7) prior to and 3-5 years after transplantation with CD3-depleted PBMCs of their donor or a third party control. The alloreactive T cells were sorted, sequenced and the transcriptome and T cell receptor profile analysed using unsupervised clustering.
Results: Alloreactive T cells retain a highly polyclonal TRA/TRB repertoire over time. Clustering based on the transcriptome divided the donor-reactive T cells into three main groups; one cluster of cytotoxic CD8+ T cells and two clusters of CD4+ T cells with distinct activation profiles. Differential expression analysis revealed that donor-reactive CD4+ T cells in both clusters had downregulation of genes involved in apoptosis and intracellular signalling pathways post-transplant. Remarkably, no change in the transcriptome of donor-reactive cytotoxic CD8+ T cells was observed over time. Inclusion of third-party controls enabled us to ascertain that the differences we detected post-transplant were truly donor-specific and not due to the influence of immunosuppression.
Conclusions: Single cell expression profiling demonstrated a loss of activated and pro-apoptotic donor-reactive CD4+ T cell clones after transplantation in stable kidney transplant recipients. This supports a role of apoptosis of highly activated alloreactive CD4+ T cells in the development of donor-specific hyporesponsiveness in stable kidney transplant recipients.

BOS1_3 ELDERLY RENAL TRANSPLANT RECIPIENTS HAVE LESS POLYFUNCTIONAL ALLOREACTIVE CD4 T CELLS PRE TRANSPLANT AND LOWER IL-2 MEDIATED T CELL PROLIFERATION

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Background: Elderly kidney transplant recipients have a lower risk for both early and late acute T-cell mediated rejection (TCMR). Recently, we identified alloreactive memory CD4 T cells expressing at least two pro-inflammatory cytokines (poly-alloCD4) as pivotal cells for TCMR. The decline of poly-alloCD4 after transplantation leads to donor-specific hyporesponsiveness (DSH). Therefore, we hypothesized that the frequency and kinetics post-transplantation of poly-alloCD4 in elderly recipients differs from younger recipients.
Methods: Peripheral blood mononuclear cells (PBMCs) of N=16 young (<45 years) and N=14 elderly (>55 years) stable renal transplant recipients were obtained before, at 6 months, 12 months, and at 3-5 years after transplantation. Expression of the co-stimulatory molecule CD137 identified alloreactive T cells following co-culture of recipient PBMCs with CD3-depleted PBMCs from their donor or third-party control. The phenotype and proportions of cytokine producing alloreactive CD137+ T cells as well as the proliferative capacity of T cells was evaluated using flow cytometry. Cytometric bead array was used to measure cytokines in supernatant.
Results: The frequency of poly-alloCD4 expressing three pro-inflammatory cytokines (IFNγ+IL2+TNFα+) was significantly lower in elderly prior to transplantation (p<0.01). T cells of elderly had decreased capacity to proliferate in response to alloantigen pre-transplantation although only significant within the CD8 T cell compartment (p<0.02). Elderly also had lowered IL-2 production which was correlated with the reduced proliferative response. Post-transplantation, a decline in frequencies of poly-alloCD4 was observed in both age groups with a plateau reached after 12 months with no difference in kinetics.
Conclusions: DSH develops with similar kinetics post-transplant in both elderly and young stable renal transplant recipients through a decrease in frequency of poly-alloCD4. However, prior to transplantation, elderly recipients have lower levels of poly-alloCD4 expressing IFNγ+IL2+TNFα+ and decreased capacity to proliferate which is associated with decreased production of IL-2. Together, these factors can explain the lowered risk of acute TCMR in the elderly and them reaching DSH earlier than younger recipients.

BOS1_4 DUAL INHIBITION OF THE COMPLEMENT SYSTEM AND TOLL-LIKE RECEPTORS PREVENTS SYSTEMIC AND LOCAL KIDNEY INFLAMMATION IN MICE EXPERIENCING BRAIN DEATH

Neeltina M Jager[*1], Petra Ottens[2], Camilla Schjalm[3], Susanne Veldhuis[2], Tom Eirik Mollnes[3;4], Henri Leuvenink[2], Søren Pischke[3;5]

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Background: Brain death (BD) induces a potentially harmful systemic inflammation, which may reduce organ quality for transplantation. The complement system (CS) and Toll-like receptors (TLRs) are key for the innate immune system both for recognition and response. The cluster of differentiation 14 (CD14) is a co-receptor for several TLRs, necessary for TLR signaling. We hypothesized that dual inhibition of CS and TLRs by complement protein 5 (C5) and CD14 inhibition will prevent innate immune-mediated inflammation during BD.
Methods: BD was induced with a fluid-filled intracranial balloon in wild-type C57/BL6 mice. Prior to BD, mice were left untreated (n=8), treated with a C5 inhibitor (n=7), a CD14 inhibitor (n=7), or both inhibitors (n=7). Sham mice did not experience BD and were left untreated (n=8). Blood and kidneys were collected three hours after BD. Inflammatory plasma cytokines were analyzed using a 23-plex immunoassay, kidney mRNA expression by qPCR.
Results: In plasma, BD significantly induced expression of interleukin-6 (IL-6), human IL-8 homolog, IL-12, monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein MIP-1α, and MIP-1β compared to sham (all p<0.01). In kidneys, BD significantly induced IL-6, IL-8, TNF, MCP-1, P-Selectin, and VCAM-1 (all p<0.01). C5 and CD14 single inhibition significantly reduced BD-induced activation of all markers in plasma (all p<0.01) and in kidneys (p<0.01, except C5 inhibition for P-Selectin p=0.06). Dual inhibition of C5 and CD14 further reduced all plasma cytokines to levels comparable with sham animals (all p>0.05). In kidneys, double inhibition was comparable to single inhibition.

Conclusions: The innate immune system is crucial for inducing inflammatory reactions during BD. Inhibition of both the CS and TLRs is necessary to efficiently prevent BD-induced systemic inflammation and to reduce local kidney inflammation. CS and TLR inhibitors are clinically available and clinical studies should be performed on deceased BD donors to enhance donor organ quality.

BOS1_5 RELEVANCE OF THE BANFF HUMAN ORGAN TRANSPLANT CONSENSUS GENE PANEL FOR DETECTING ANTIBODY AND T-CELL MEDIATED REJECTION OF KIDNEY ALLOGRAFTS

Alessia Giarraputo[*1;2], **Dina Zielinski[1], Valentin Goutaudier[1], Blaise Robin[1], Olivier Aubert[1], Mark Haas[3], Annalisa Angelini[2], Michael Mengel[4], Carmen Lefaucheur[1], Alexandre Loupy[1]**

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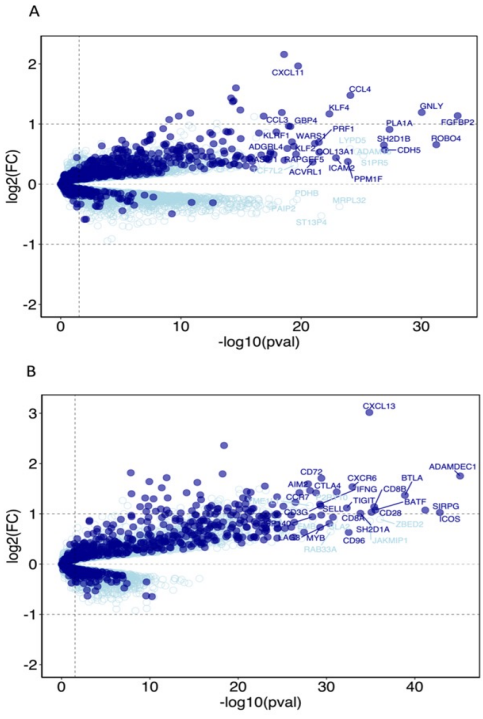
Background: Gene expression studies relying on whole-transcriptome profiling have defined the molecular phenotypes of kidney allograft rejection, but have several barriers limiting its application in clinical practice. The Banff Human Organ Transplant panel (BHOT) was developed to facilitate reproducible gene expression analysis of solid organ allografts, but its relevance to assess antibody-mediated (AMR) and T-cell mediated rejection (TCMR) in kidney allograft biopsies has not been demonstrated.
Methods: We performed *in silico* analysis and projected the BHOT panel on published microarray data of 547 kidney transplant biopsies (AMR, n=129; TCMR, n=77; non-rejection related cases, n=341). We compared expression between whole-transcriptome or BHOT panel genes, and performed differential expression, pathway, and gene network analysis. Finally, we evaluated the performance of BHOT genes to classify AMR and TCMR.
Results: Targeted versus whole-transcriptome analysis demonstrates that the BHOT panel captures the key gene signatures and pathways associated with rejection Figure A (AMR) and Figure B (TCMR). The top significant AMR-associated pathways based on BHOT genes were associated with interferon-gamma and interleukin signaling, toll-like receptor cascade and B-cell activation. For TCMR, the top pathways derived from the BHOT panel were related to PD-1 signal transduction, TLR signaling cascade, phosphorylation of CD3, demonstrating that the panel detects relevant pathophysiological mechanisms. The performance of BHOT-based ensemble classification models for detecting AMR (AUC=0.88; 95% CI=0.85-0.91) and TCMR (AUC=0.85; 95% CI=0.81-0.90) were highly similar to the performance of classifiers based on whole-transcriptome data for AMR (AUC=0.88; 95% CI=0.84-0.91) and TCMR (AUC=0.85; 95% CI=0.80-0.90).
Conclusions: We demonstrate that the BHOT gene panel comprises the relevant genes and pathways associated with AMR and TCMR in kidney allograft tissue. Our findings show that this targeted panel is sufficient and sensitive to serve as a proxy to whole-transcriptome-based analysis for gene expression profiling in kidney allograft biopsies.



BOS1_6 DDPCR DETECTION OF MIRNAS FIBROSIS SIGNATURE IN URINE-EVS FROM KIDNEY TRANSPLANTED PATIENTS: A NON-INVASIVE APPROACH TO DETECT KIDNEY FIBROSIS

Marta Clos-Sansalvador[*1;2], **Javier Paúl-Martínez[1], Paula Rodríguez-Martínez[3], Sergio G. Garcia[1;4], Marta Sanroque-Muñoz[1], Miriam Font-Morón[1], Jordi Bover[1], Anna Vila-Santandreu[1], Marcella Franquesa[1], Javier Juega[1], Francesc E. Borras[1;5]**

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Background: Predicting or diagnosing renal fibrosis (IFTA) to reduce chronic allograft loss is still a major challenge in kidney transplantation. Current analytical standards, such as creatinine, proteinuria or Glomerular Filtration Rate have poor predictability value and the diagnostic still relies on costly and invasive kidney biopsy. Thus, molecular analyses of urinary extracellular vesicles (uEV) have emerged as a possible source of new biomarkers, and as a platform to overcome the risks, costs and sampling limitations of renal biopsy. In this context, in a preliminary study our group isolated uEV by size exclusion chromatography from 6 healthy donors and 11 kidney transplanted patients (kTx) with altered kidney function. RNA sequencing led us to identify a miRNA signature in the kTx patients. Specifically, seven of those miRNAs were found in kTx patients diagnosed with IFTA by renal biopsy. Aiming to explore a possible clinical application, here we validated these results using digital droplet PCR (ddPCR), a technique with higher throughput screening potential.
Methods: miRNAs signature was studied in a new cohort of kTx patients (n=20) using ddPCR. Briefly, the uEV RNA was obtained from 1 mL urine (Urine Exosome RNA Isolation Kit, Norgen Biotek Corp.). After retrotranscription, the ddPCR was performed. For data analysis, patients were divided into non-IFTA (scored as less than 1, 40% of total) and IFTA patients (scored as 1 or more, 60% of total), according to their Renal Biopsy Banff score.
Results: ddPCR results achieved higher technical sensitivity, accuracy and reproducibility compared to real-time quantitative PCR (qPCR). This permitted absolute quantification of miRNAs even in samples with limited target abundance. Furthermore, we mostly validated the previous miRNAs fibrosis signature identified by RNAseq using ddPCR in this new cohort of kTx patients.
Conclusions: ddPCR detection of multiple miRNAs in urine-EVs is a suitable non-invasive approach to monitor fibrosis in kTx patients.

BOS1_7 MEK INHIBITION, A NEW THERAPEUTIC APPROACH IN ORGAN TRANSPLANTATION

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Background: Because graft survival is still impacted by chronic dysfunction related, among other things, to poor control of the alloimmune response, the development of new strategies that would be efficient in preventing rejection and free of toxicity is necessary. As recent studies in mouse models have shown that MEK inhibition is effective in controlling the alloimmune response in experimental GVHD without abrogating anti-tumor and anti-viral immunity, we evaluated the therapeutic potential of MEK pathway inhibition in a preclinical model of allogeneic human skin transplantation in humanized NSG mice.
Methods: Human skin grafted animals, whose immune system was reconstituted with human PBMCs, were treated with trametinib, an anti-MEK molecule already used in the clinic for the treatment of melanoma. Graft survival was evaluated and the effects on the human allogeneic immune response were analyzed.
Results: Experiments performed with 4 human skin donors and 4 human blood donors showed that trametinib-induced MEK inhibition significantly prolonged the survival of allogeneic skin graft compared with untreated controls (30.7 ± 4.3 days, n=17 vs 18.4±3.3 days, n=15, p<0,0001) without interfering in vivo immune reconstitution in the NSG mice. Analysis of PBMCS from these animals showed an increase of the hCD4/hCD8 T cell ratio in trametinib treated mice however we could not detect any difference in the expression of T hCD4 and/or hCD8 inhibitory markers and hCD4 regulatory T cells. To further investigate the effect of trametinib in an unbiased way, we used single cell RNAseq analysis (10X chromium) on spleen hCD45 cells harvested 16 days following reconstitution. Regarding hCD8 T cells, trametinib treated animals presented more early activated cells and less effector cells, suggesting that trametinib inhibited hCD8 T cell differenciation. Regarding hCD4 T cells, trametinib seemed favor hCD4 early proliferation and impede differenciation toward follicular helper T cells and promote TH1.

BOS2_11 EX SITU PORCINE LIVER MACHINE PERFUSION ACTIVATES THE COMPLEMENT SYSTEM AND INCREASES CYTOKINES INDEPENDENT OF PRE-INDUCED LIVER INJURY

Ida Færden*[1;2], Marte Bliksøen[3;4], Camilla Schjalm[3], Olav Liavåg[3;4], Waleed Majeed[3], Morten Hagness[4], Tom Eirik Mollnes[3;5], Søren Pischke[2;3]

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Background: Ischemia-reperfusion injury (IRI) is a key challenge in liver transplantation leading to short- and long-term failure of the transplanted liver. Machine perfusion (MP) has proven to limit the metabolic consequences of IRI and has the potential to rescue discarded livers. However, the effect of MP on the innate inflammatory response is unknown. We aimed to investigate complement activation and downstream effects that may be targeted to reduce IRI and inflammation during MP.
Methods: Porcine livers (n=24) were exposed to either biliary injury (n=8), global liver injury (n=8), or no liver injury (n=8). Ex situ liver MP was performed with 1h hypothermic, 1h rewarming, and 4h normothermic perfusion. Belzer preservation solution was used during the hypothermic phase. Thereafter, heparinized leukocyte- and platelet-depleted homologous blood was used as perfusate. Perfusate and tissue samples were collected at set time points and analysed for terminal complement complex (TCC) and cytokines (TNF, IL6, IL-1β, IL8 and IL10) using ELISA and Multiplex.
Results: During normothermic MP, TCC increased significantly from start (median 11 interquartile range [5.14-17]) to end MP (40 [15-60], p<0.0001). There was no statistical difference between the biliary injury, the global injury, and the no liver injury groups (p>0.5). During normothermic MP cytokines increased significantly in plasma (TNF, IL 6, IL10), in liver tissue (TNF, IL-1β, IL6, IL8) and bile tissue (TNF, IL-1β, IL6, IL8), all p<0.0001 Friedmann test/ Mann Whitney test.
Conclusions: Complement and downstream cytokines are strongly and persistently activated during normothermic MP independently of pre-induced liver injury. Cytokines increase significantly despite a leukocyte-depleted perfusate and are thus probably due to liver-derived cytokine production. Future studies should evaluate the source of cytokine production and if complement inhibition can suppress cytokine production during liver MP. Inhibition of complement activation might be a therapeutic option during MP.

BOS2_12 TETRAHYDRO-BENZOTHIOPHENE ROR GAMMA T INVERSE AGONISTS TO TARGET TH17 IN SENSITIZED SKIN ALLOGRAFT MOUSE MODEL

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Background: Th17 cells play a critical role in acute cellular as well as in chronic antibody mediated allograft rejection. We have recently designed tetrahydro-benzothiophene derivatives as novel inverse agonists of retinoic acid receptor-related orphan receptor gamma t (RORγt) and demonstrated in vitro activity in Th17 polarization assay from PBMCs. The objective of the current study is to determine the effect of tetrahydro-benzothiophene derivatives on the rejection of complete mismatch skin graft in a sensitized murine model.
Methods: C57BL/6 mice were sensitized by administration of 107 Balb/c splenocytes (IP) at day 0, 7 and 14 and transplanted with Balb/c skin grafts at day fifteen. Mice were injected daily with a tetrahydro-benzothiophene RORγt inverse agonist (TF-S14, 1mg/kg, IP), or tacrolimus (0.5mg/kg, IP) or combination. Graft survival was evaluated daily for rejection end point (100% necrosis). Skin grafts were sampled at day five for histology.
Results: Tetrahydro-benzothiophene RORγt inverse agonist prolonged median graft survival from 6 to 13.5 and from 7 to 23 days when combined with tacrolimus, Figure 1. Neutrophilic infiltration of skin-grafts decreased in RORγt inverse agonist, or combination therapy compared to vehicle or tacrolimus treated mice, Figure 2.
Conclusions: The novel tetrahydro-benzothiophene RORγt inhibitor offers a new therapeutic mechanism to treat rejection in highly sensitized patients regardless of degree of donor mismatch.

Figure 1. Sensitized allograft survival of Balb/c skin grafts in C57BL/6 recipient mice of vehicle treated (ctrl), TF-S14 1mg/kg treated (TF-S14), tacrolimus 0.5mg/kg treated (tacro) and TF-S14 1mg/kg + tacrolimus 0.5mg/kg treated (TF-S14+tacro). P-value <0.01, mantel-cox test; median survival for ctrl, tacro, TF-S14 and TF-S14 + tacro is 6, 7 ,13.5 and 23 days, respectively.

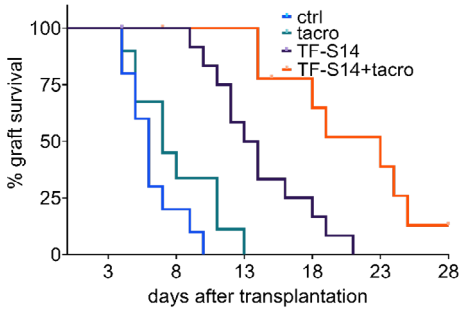
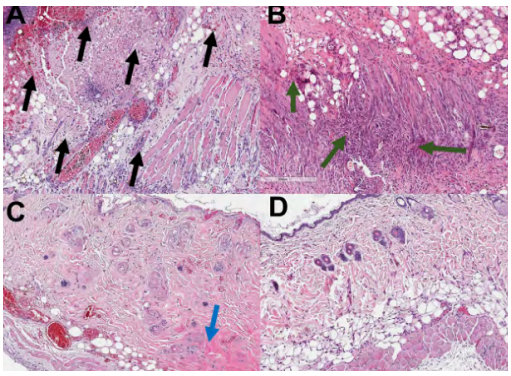


Figure 2. Histology images of skin grafts of at magnification x10; (A) control group showing diffuse inflammation and neutrophilic infiltration (black arrows), (B) tacro group showing neutrophilic infiltration (green arrows); (C) TF-S14 group showing localized inflammation (blue arrow), (D) TF-S14 + tacro group showing intact layers and no neutrophilic infiltration in the graft.



BOS2_13 CD8+CD3- CELLS ARE CRITICAL FOR TREG MEDIATED HUMORAL TOLERANCE

Romy Steiner*[1;2;3], Anna Weijler[3], Moritz Muckenhuber[3], Thomas Wekerle[3], Jonathan Sprent[4], Nina Pilat[1;2;3]

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Background: Recently, our group achieved significant extension of allograft survival in a murine model of skin transplantation by selective *in vivo* expansion and activation of Tregs using interleukin-2 (IL-2) coupled to a specific antibody against IL-2 (IL-2cplx). Here, we aimed to investigate the effect of alloreactive CD8+ cells in Treg-mediated skin graft survival.
Methods: Recipient C57BL/6 mice received IL-2cplx, rapamycin and a short-term treatment of anti-IL-6 mAb along with fully mismatched BALB/c or single MHCII mismatched BM12 (no CD8 T cell alloreactivity) skin grafts. Indicated groups were treated with different anti-CD8 mAbs, depleting either all CD8+ populations (anti-CD8a) or specifically CD8+ T cells (antiCD8b). To dissect the mechanisms of allograft rejection in this model, donor-specific antibody (DSA) development, *in vitro* T cell alloreactivity and graft infiltrating leucocytes were assessed.
Results: IL-2cplx therapy in combination with rapamycin and anti-IL-6 mAb led to prolonged survival of fully mismatched (BALB/c; MST = 30.5d) and MHCII mismatched (BM12; MST = 77.5d) skin allografts. Importantly, IL-2cplx based therapy prevented humoral rejection and development of DSAs. CD8 depletion did significantly extend skin graft survival, however CD8a (but not CD8b) depletion prevented humoral tolerance. Furthermore, CD8a depletion resulted in the increase of donor-responsive Th2 cells as well as graft infiltrating recipient CD4+ effector T cells by POD20. In addition, T follicular helper and T follicular regulatory cell levels were increased within the spleen in the absence of CD8 alloreactivity compared with non-depleted/fully mismatched recipients.
Conclusions: IL-2cplx therapy induces humoral tolerance and prevents the development of DSAs. Depletion of pan-CD8 cells using anti-CD8a mAb does not prolong skin graft survival but leads to donor-specific antibody formation whereas CD8b depletion did not restore humoral alloreactivity, suggesting a critical role of CD8+ non-T cells in the sustained prevention of recipient sensitization. Moreover, proinflammatory processes are thought to be a consequence of CD8a depletion, as increased migration of recipient CD4+ effector cells into skin grafts and elevated donor-responsive Th2 cells were observed.

P357 DESENSITIZATION WITH IMLIFIDASE IN CROSS-MATCH-POSITIVE, HIGHLY SENSITIZED KIDNEY TRANSPLANT RECIPIENTS: A SINGLE-CENTER EXPERIENCE

Stathis Tsiakas*[1], Angeliki Vittoraki[2], Maria Darema[1], Evangelos Mantios[1], George Liapis[3], Smaragdi Marinaki[1], John Boletis[1]

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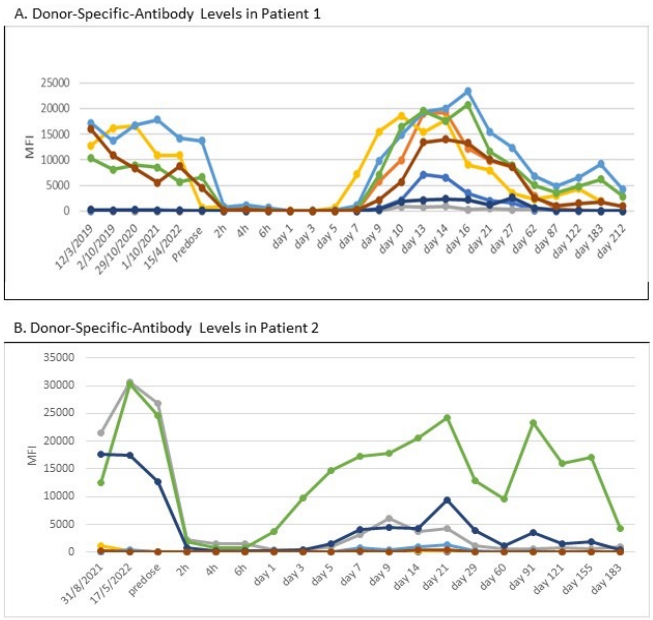
Background: HLA sensitization is a significant immunological barrier in kidney transplantation, resulting in prolonged waiting times and inferior patient survival. Imlifidase acts by eliminating all IgG subclasses, leading to a rapid conversion of a positive crossmatch (XM) to negative. Here, we report the first three cases treated with imlifidase in our centre.
Methods: Three highly sensitized patients (cPRA ≥ 97%) received priority for deceased donor kidney transplant after their HLA incompatible living donor donated a kidney to the national waiting list. Single antigen bead analysis, CDC and Flow XMs were performed pre- and post-imlifidase administration. Immunosuppression regimen included rabbit anti-thymocyte globulin, rituximab, and intravenous immunoglobulin (IVIG) in addition to mycophenolate mofetil, tacrolimus and methylprednisolone.
Results: The first patient was a 49-year-old male with preformed HLA class II donor specific antibodies (DSA) with a cumulative mean fluorescence intensity (cMFI) of 35858 and positive B-Flow XM. At 2 h post-imlifidase dose, the XM was negative. DSAs rebounded on day 9, whereas renal function continued to improve. No kidney biopsy was performed. On day 259, creatinine level was 0.93 mg/dl. The second patient was a 43-year-old female with preformed HLA class I & II DSA (cMFI: 64158) and positive CDC and T/B Flow XMs. Both XMs were negative at 2hours post-imlifidase. The patient developed antibody-mediated rejection on day 4, which was successfully treated with plasma exchange and IVIG. On day 185, creatinine level was 0.78 mg/dl. The third patient was a 24-year-old female with preformed HLA class I & II DSA (cMFI: 73377) and positive CDC and B-Flow XMs. At 2hours post-imlifidase, both XMs were negative. The patient developed hyperacute rejection and the graft was removed during surgery. Renal graft biopsy revealed intense IgM expression on glomerular and peritubular capillaries in the absence of IgG, findings suggestive of IgM-induced hyperacute rejection.
Conclusions: Imlifidase offers a rational therapeutic approach for kidney transplantation to highly sensitized patients. Besides IgG, other antibodies that are not routinely screened prior to transplantation, may have harmful effects on the renal graft.



P358 LUMINAL INTESTINAL PRESERVATION FOLLOWING BRAIN DEATH MAY REDUCE INNATE IMMUNE SYSTEM ACTIVATION

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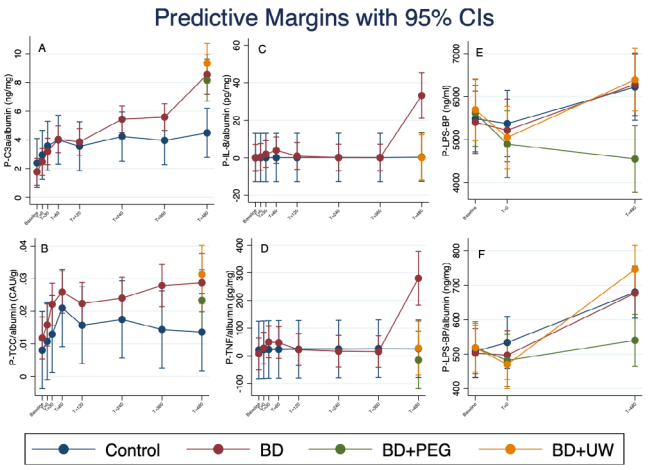
Background: Organs obtained from brain dead (BD) donors often have worse outcomes. Activation of the complement system and translocation of intestinal bacteria could be causative. We aimed to examine activation of the complement system following BD and evaluate the effect of adding luminal intestinal preservation to vascular preservation.
Methods: BD was induced in 30 pigs (four groups: control (n=7), BD alone (n=8), BD + luminal intestinal polyethylene glycol (PEG, n=7) and BD + luminal intestinal University of Wisconsin solution (UW, n=8) using a previously validated method. All animals were observed for 6 hours before organ retrieval. In the PEG and UW groups, 2000 ml of the selected solution was instilled into the duodenum during the organ procurement. Repeated measurements of C3a, Terminal Complement Complex (TCC), IL-8 and TNF were performed in plasma at baseline, BD, 30, 60, 120, 240 and 360 minutes after BD, and following the intestinal intervention (480). Plasma lipopolysaccharide binding protein (LPS-BP) was measured at baseline, BD, and 480 minutes after BD. All were normalised to albumin concentration.
Results: All animals were kept circulatory- and respiratory stable until organ procurement. At 480 minutes, C3a was significantly higher in BD, BD+PEG, and BD+UW groups compared to control group (all p<0.05) (fig. 1A). TCC was significantly higher in the combined BD group compared to control at 360 minutes, at 480 minutes, the BD and BD+UW groups. TCC was significantly higher compared to the control group (all p<0.05) (fig. 1B). IL-8 and TNF were significantly higher in the BD group compared to all other groups at 480 minutes (p=0.003 and p=0.001) (fig. 1C and D). LPS-BP increased following induction of BD in all groups except BD+PEG, which at 480 minutes were significantly lower (p=0.002) (fig. 1E and F) compared with all other groups.
Conclusions: The complement system is activated following BD independently of intestinal and luminal preservation and may lead to inflammation. Luminal intestinal preservation during organ procurement led to reduced cytokine and LPS-BP expression, which may be due to reduced bacterial translocation occurring during surgery independent of BD. Luminal PEG intervention may be combined with early innate immune system inhibition in BD donors to prevent systemic inflammation.



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