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Abstract Book

ORAL PRESENTATIONS

OP01

TARGETED DELIVERY OF GALUNISERTIB SUPPRESSES FIBROGENESIS IN AN INTEGRATED EX VIVO RENAL TRANSPLANT AND FIBROSIS MODEL

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Background:
Normothermic machine perfusion (NMP) is an emerging preservation technique for kidney allografts to reduce post-transplant complications, including interstitial fibrosis and tubular atrophy. This technique could be improved by adding antifibrotic molecules to perfusion solutions. We introduce a novel therapeutic approach, tested in a newly developed fibrosis model, to suppress fibrogenesis. Our approach involves *ex vivo* perfusion with a blood based perfusate spiked with transforming growth factor beta (TGF-β) — one of the most important cytokines involved in fibrogenesis—and added galunisertib—a potent inhibitor of the TGF-β signalling pathway.

Methods: Porcine kidneys were subjected to 30min of warm ischemia, 24h of oxygenated hypothermic machine perfusion (HMP), and 6h of NMP with treatment (control, TFG-β, galunisertib, or TGF-β+galunisertib; n=8). To determine whether effects persisted upon ceasing treatment, precision-cut kidney slices (PCKS) were prepared from respective kidneys and incubated for 48h with treatment continued and discontinued (Figure 1a).

Results:
Galunisertib supplementation improved the general viability, characterized by an increased oxygen consumption, elevated ATP levels and attenuated tubular dilation and necrosis. No significant differences in renal function, oxidative stress levels, or injury markers were observed. Galunisertib altered inflammation markers by causing a significant increase in gene expression of *TNF-α*, and a significant decrease of *IL-6* after 6h NMP. This was supported by IL-6 protein expression. Continued TGF-β supplementation promoted fibrogenesis as shown by significantly increased mRNA expression of *ACTA2, COL1A2, FN-1, SERPINE1, SERPINH1,* and *TGF-β* after 48h of incubation. Continued treatment with galunisertib, however, clearly attenuated the expression of all tested fibrosis-related genes after 48h incubation (Figure 1b-g).

Conclusions:
Our findings suggest that galunisertib positively affected mitochondrial activity, tissue integrity and expression of fibrogenesis-related genes, and therefore appears to be a promising drug for further research. These findings illustrate the value of targeted drug delivery using isolated organ perfusion for reducing post-transplant complications

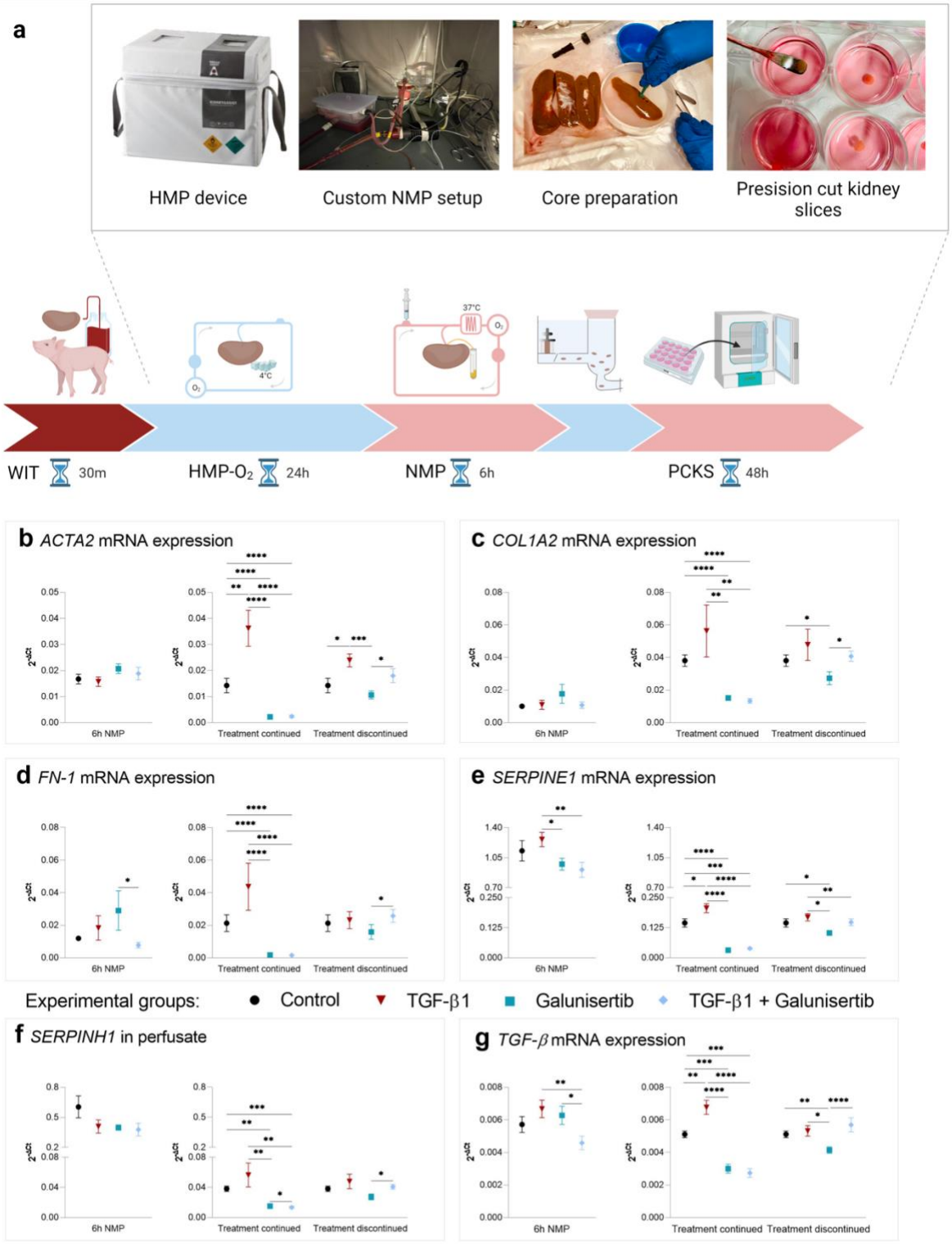

Figure 1: a) Schematic overview of workflow. b) *ACTA2* mRNA expression, c) *COL1A2* mRNA expression, d) *FN-1* mRNA expression e) *SERPINE1* mRNA expression, f) *SERPINH1* mRNA expression, and g) *TGF-β* mRNA expression after 6 hours of normothermic machine perfusion and after 48 hours of PCKS incubation with treatment continued and without treatment continued . *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are expressed as means (± SEM), n=8. TGF-ß1: transforming growth factor beta 1.

OP02

IMPLICATION OF CD8+ CELLS IN TREG MEDIATED SKIN GRAFT SURVIVAL

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Background: Recently our group succeeded in prolonging fully mismatched skin graft survival by in vivo expanding and activating regulatory T cells via administration of interleukin-2 (IL-2) coupled to a specific antibody against IL-2 (IL-2 cplx) in combination with Rapamycin and an IL-6 neutralizing antibody. Here we investigate whether depletion of alloreactive CD8+ T cells has a positive impact on Treg mediated skin graft survival.

Methods: Recipient C57BL/6 mice were transplanted with either fully mismatched BALB/c or single MHCII mismatched BM12 skin grafts in combination with IL-2 cplx, Rapamycin and a short term treatment of anti-IL-6 mAb. To analyze the role of CD8+ T cells, experimental settings devoid of alloreactive CD8+ T cells were created by using a depleting anti-CD8 antibody or a single MHCII mismatched mouse model. To study the mechanisms of skin graft rejection, development of donor-specific antibodies, formation of T cell memory as well as graft infiltrating leucocytes were investigated.

Results: The combination of IL-2 cplx with Rapamycin and an IL-6 neutralizing antibody leads to extended prolongation of single MHCII mismatched skin grafts to a median survival time of 77.5 days versus 30.5 days for fully mismatched skin grafts. Notably, analysis of sera showed a significant decrease of donor reactive IgG ($p<0.05$) in the fully mismatched settings. Depletion of CD8+ cells, however, did not lead to further prolongation of fully mismatched skin graft survival (MST=33.5 days) but a significant increase of donor specific IgG1 after skin graft rejection. Furthermore, analysis of T-cell dependent immune responses in CD8+ cell deficient mice revealed an increase of donor-reactive Th2 cells and higher recipient CD4+ effector T-cell infiltration into the skin grafts by day 20 post transplantation. In addition, analysis of Tfh as well as Tfr revealed increased frequencies in mice devoid of CD8+ alloreactivity.

Conclusions: Combined treatment with IL-2 cplx, Rapamycin and anti-IL-6 leads to significantly prolonged skin allograft survival. Remarkably, humoral response and sensitization are impeded in this setting. Depletion of CD8+ cells results in - albeit delayed - formation of donor-reactive antibodies suggesting that a CD8+ cell population is needed for sustainable prevention of sensitization.

OP03

RENAL-RESIDENT INNATE LYMPHOID CELLS TYPE 2 ARE LOST FOLLOWING RENAL TRANSPLANTATION AND ARE NOT RESTORED AFTER THERAPEUTIC IL-33 TREATMENT

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Background:
Innate lymphoid cells (ILCs) do not express antigen-specific receptors, persist mainly in solid tissues and play a critical role in regulating inflammation and tissue homeostasis. Especially the induction of ILC type 2 cells (ILC2s) by application of IL-33 has been shown to mediate reno-protective processes upon ischemia-reperfusion injury (IRI). However, their precise role for homeostatic regulation and improved graft function following kidney transplantation (KTx) still needs to be defined.

Methods:
Herein, we analysed the intra-renal ILC1, ILC2 and ILC3 subsets before and after experimental kidney transplantation in mice by flow cytometry.

Results:
We established ILC2s as a major population in the naïve murine kidney. Following KTx, an overall decrease of ILC frequencies with a particular depletion of ILC2s in the BALB/c to C57BL/6 allogenic graft compared to the C57BL/6 to C57BL/6 syngeneic graft was noted on day 7 post KTx. In contrast, an increase of ILC2 frequencies in liver, lung, spleen and intestine of recipients was detected. Although recipient-derived ILC2s infiltrated the graft already within 24 hours post KTx, these cells did not replenish the decreasing donor ILC2 pool and did not persist in the graft until day 7 post KTx. Donor pre-treatment with recombinant IL-33 (300 ng/day) for 5 consecutive days prior to transplantation resulted in a significant expansion of intra-renal ILC2s, but failed to sustain the endogenous ILC2 pool following KTx. Moreover, donor pre-treatment resulted in accelerated inflammation and graft deterioration

reflected by elevated creatinine levels compared to controls. On the contrary, recipient treatment with IL-33 resulted in higher ILC2 numbers than control-treated recipients, but again, ILC2s were not retained in the graft and kidney function was not improved.

Conclusions:
In summary, our data demonstrate a strong redistribution of ILC subsets in various organs after experimental kidney transplantation. Although being a prominent ILC subtype in the naïve kidney, ILC2s are lost during the allogeneic immune response following KTx. Both, donor and recipient treatment with IL-33 fails to induce an ILC2-mediated reno-protective effect. Thus, alternative strategies for expansion and preservation of renal-resident ILC2s need to be considered.

OP04

REGULATORY T CELLS SUPPRESS MEMORY IL-17A PRODUCTION IN HIGHLY SENSITISED PATIENTS WITH END-STAGE RENAL DISEASE

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Background:
Highly sensitised patients exhibit poorer long-term outcomes following renal transplantation compared to those without donor-specific antibodies (DSA). IL17-A has been associated with acute rejection. Presence of regulatory T cells (Tregs) has been associated with regulation in patients with chronic rejection. Therapy using autologous expanded Tregs has been demonstrated to be safe. This project aims to test the hypothesis that Tregs could be used in sensitised patients to suppress memory IL-17A responses.

Methods:
We prospectively recruited highly-sensitised patients on haemodialysis, isolated their Tregs and expanded them using established protocols (Interleukin-2 + Rapamycin). IL-17A production by CD8-depleted peripheral blood mononuclear cells (PBMC)(+/- additional depletion of CD25hi cells) in response to Human Leucocyte Antigen (HLA) proteins (PureProt®) was tested in FluoroSpot to assess the memory immune alloresponses at baseline and when expanded autologous Tregs were also added.

Results:
Of the patients included, 10/16 were male, 10/16 were sensitised by previous renal transplant and of those 4/10 had had nephrectomy. 4/10 (40%) of patients post-transplantation were on immunosuppression. We managed to expand Tregs for 11/16 (69%) patients. Five/11 (45%) patients had spontaneous IL-17A production in CD8-depleted PBMCs challenged with an HLA protein that they had been sensitised to (Figure 1). In 4/5 patients (80%), IL-17A production was reduced when autologous *ex vivo* expanded Tregs were added. Moreover, in 4/11 (36%) patients, CD8-CD25hi- PBMCs stimulated with pure HLA protein were associated with IL-17A production, which was suppressed in 3/4 when expanded autologous Tregs were also added.

Conclusions:
Results showed that 45% of highly sensitised patients made IL-17A when challenged by an HLA protein they have made an HLA antibody. In most of these patients, IL-17A could be inhibited by autologous *ex vivo* expanded Tregs. The Phase IIa trial GAMECHANgER-1 will test whether these findings are reproducible *in vivo* in highly sensitised patients awaiting renal transplantation.

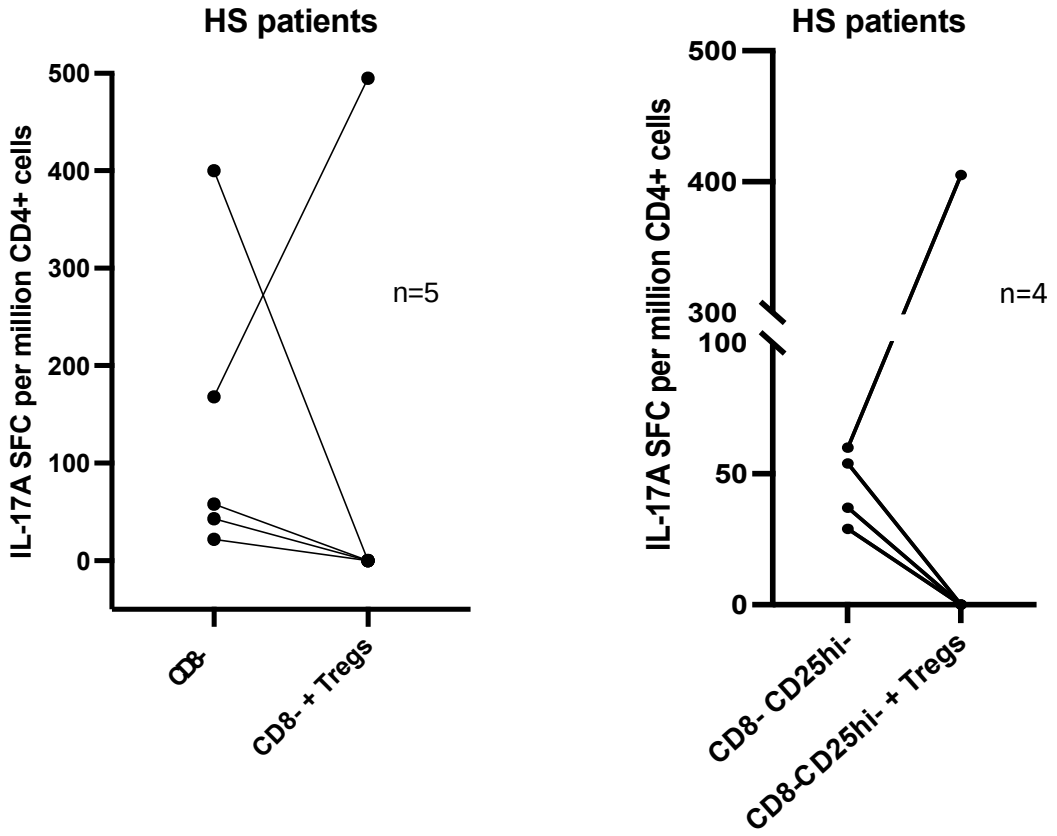

Figure 1: Patterns of anti-donor FluoroSpot reactivity representing suppression by autologous expanded Tregs in highly sensitized patients (HS). IL-17A production by CD8-depleted (A) and CD8+CD25depleted PBMC (B), is associated with a reduction of spots counts when autologous expanded Tregs are also added.

OP05

THE ROLE OF CHIMERIC ANTIGEN RECEPTOR (CAR) VERSUS ENDOGENOUS CO-STIMULATION IN THE FUNCTION OF ALLOANTIGEN-SPECIFIC TREGS

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Background

Cell therapy with regulatory T cells (Tregs) can diminish rejection and their efficacy can be improved by expression of an alloantigen-specific CAR. In pursuit of optimized CARs for use in Tregs, we and others explored the effect of CAR-encoded co-stimulatory domains, finding that inclusion of a CD28 co-stimulatory domain is optimal for human CAR-Treg suppression in vivo using a model of xenogeneic graft versus host disease. However, a limitation was the use of immunodeficient mice which lack a full complement of antigen presenting cells. Here we aimed to re-visit the question of optimal CAR design in an immunocompetent Bl/6-based mouse model of skin transplantation.

Methods

CARs specific for HLA-A2 encoding CD3z with or without intracellular domains from CD28, PD1, ICOS, GITR, OX40 or 41BB were generated and expressed in Bl/6 Tregs. In vitro proliferation and cytokine production was assayed by co-culturing with an HLA-A2$^+$ cell line. In vitro CAR-Treg function was determined on the basis of reduced costimulatory and activation molecules expression on dendritic cells (DC), and in vivo effect using a model of A2+ skin transplantation. To determine the effect of exogenous co-stimulation, CAR-Tregs were cultured together with CD86 positive or negative HLA-A2$^+$ cells.

Results

All CAR variants were expressed in mouse Tregs and effects on proliferation, cytokine production and suppression revealed superior effects of the CD28-encoding CAR, confirming data from human CAR-Tregs. Surprisingly in vivo, we found no significant difference between the ability of Tregs encoding the CD28 and CD3zeta alone CARs to extend allograft survival. Moreover, we found that CD28, PD1, GITR and CD3zeta CAR-Tregs were equal in their ability to suppress expression of CD86 and CD80 on A2+ splenic DCs. Hypothesizing that co-stimulation from the DCs could influence CAR-Treg function, we tested effects of CD86+ or CD86- cells, finding that CD86-mediated stimulation through endogenous CD28 can partially replace the requirement for a CAR-encoded CD28 domain.

Conclusions

CAR-Tregs require co-stimulation via CD28, which can be delivered via the CAR or via DC-CAR-Treg interactions. Optimization of CAR-design should consider signals mediated by physiologically-relevant cell-cell interactions and use fully immunocompetent models.

OP06

TRANSITIONAL-1 B CELL IL-10/TNFA RATIO RISK-STRATIFIES RENAL TRANSPLANT PATIENTS WITH EARLY BORDERLINE CHANGE

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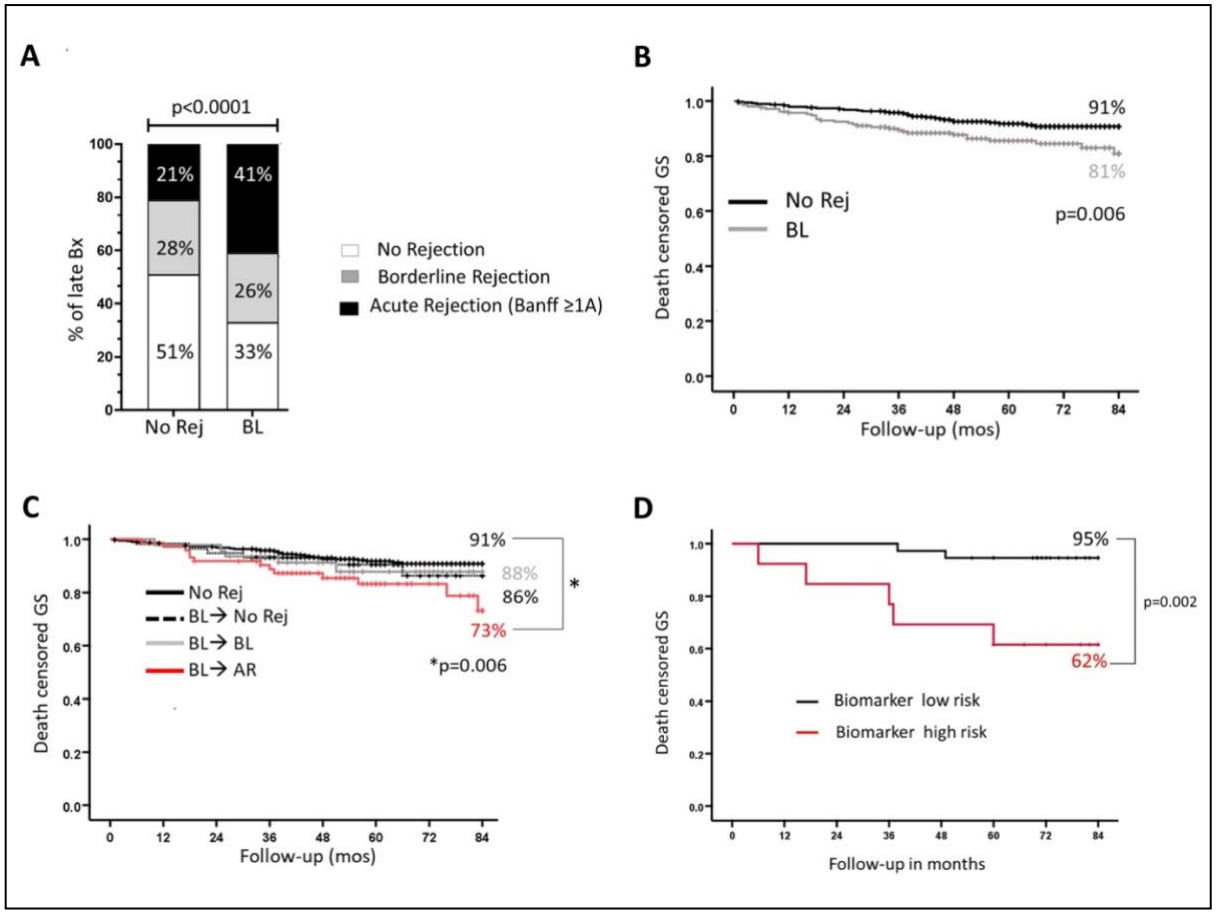
Background: Although borderline change (BLC) on renal allograft biopsies (Bx) associates with decreased graft survival, many patients with BLC maintain stable graft function. Identification of patients with BLC at risk for subsequent poor outcomes would allow for targeted therapeutic interventions to improve outcomes.

Methods: We prospectively examined clinical outcomes in patients with BLC in the 1st 4 mos post-renal transplantation. The availability of 2 protocol Bx (3mos & 1yr) and any for-cause Bx allowed us to determine the natural history of histological progression of early BLC. We determined whether a peripheral blood B cell biomarker based on the transitional-1 B cell (T1B) IL-10/TNFα ratio (determined by flow cytometry), could predict outcomes in patients with BLC.

Results: 851/1187 patients transplanted between 2013-18 underwent early Bx (0-4mos). Of these, 217 (25%) had BLC and were compared to 387 with no significant inflammation. 79% of BLC was diagnosed on pBx and categorized as subclinical (scBLC). Both clinical (cBLC) and scBLC had comparable t and i scores. A significantly higher proportion of patients with early BLC progressed to ≥1A AR between 5-12 mos (Fig1A). This association was independent of potential confounders (OR: 2.5, 95% CI 1.6-3.8,

p<0.001). The rate of progression to AR was similar in both cBLC and scBLC (38% vs. 41%, p=NS). Early BLC was associated with worse 7yr-graft survival (Fig 1B), but this occurred only in those who progressed to AR (Fig 1C). Importantly, graft survival was comparable in patients with early BLC that did or did not receive treatment (steroids or increased IS; 81% vs. 80% p=NS). Thus, patients with early BLC that progress to late AR represent a high-risk group not responsive to conventional treatment. BLC patients who progressed to AR had a significantly lower T1B IL-10/TNFα ratio at 3 mos than patients who did not progress. Thus, T1B cytokine ratio serves as a biomarker for BLC progression to AR (ROC AUC 0.87, Sens 95%, Spec 80%). Further, this biomarker identified patients with early BLC at risk for poor 7yr-graft survival (OR 8.8, 95% CI 1.7-45.7, p=0.006) beyond that predicted by progression of BLC→AR (Fig 1C vs. 1D).

Conclusions: The T1B IL-10/TNFα ratio identifies patients with early BLC that progresses to AR and graft loss and might benefit from newer IS strategies.



OP07

CHANGES OF IMMUNOGLOBULIN E-RECEPTOR POSITIVE CELL SUBSETS AFTER CARDIAC ALLOGRAFT REJECTION IN MICE

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Background: Previously, our group demonstrated the production of donor-specific IgE upon allograft rejection in mice and humans. While the role of IgE in allergy as well as other Th2 type diseases is already well described, the mechanism of MHC-specific IgE in transplant rejection remains to be delineated.

Methods: Untreated CCR5KO recipients received fully mismatched BALB/c cardiac allografts as model of acute antibody-mediated rejection (ABMR). Wild type C57BL/6 mice (WT) recipients were used as controls. Donor-specific IgE and IgG1 antibodies were measured using a customized ELISA employing recombinant MHC class I and II monomers. Before and weekly after transplantation, levels of IgE effector cells, such as basophils and eosinophils, and B cells were measured in peripheral blood using flow cytometry. Formation of MHC-specific IgE immune complexes was determined using a modified IgE-facilitated antigen binding (FAB) assay deploying isolated naïve B cells and fluorophore-labelled MHC tetramers.

Results: Donor-specific IgE developed at slightly higher levels in CCR5KO recipients compared to controls upon acute humoral rejection. Basophil (CD49b+ FceRI+ IgE+) levels were significantly elevated at the time of rejection in CCR5KO mice in the periphery. An increase in basophil-bound IgE was seen in CCR5KO and WT recipients upon graft rejection. Notably, significantly higher levels of IgE+ CD23+ B cells were present in CCR5KO compared to WT recipients after cardiac allograft rejection. Using a modified IgE FAB assay, we demonstrated *in vitro* the formation of IgE-MHC complexes in recipient serum and their binding to naïve B cells.

Conclusions: The formation of MHC-specific IgE was demonstrated upon allograft rejection in a murine model of acute ABMR. Elevated levels of basophils as well as basophil-bound IgE in peripheral blood indicate a role of MHC-specific IgE in combination with its effector cells in the immune response of allograft rejection. We showed for the first time an increase of IgE+ CD23+ B cells *in vivo* after allograft rejection and demonstrated the formation of MHC-IgE complexes and their binding to B cells *in vitro*. As IgE+ CD23+ B cells and IgE complex formation are connected with an increase in T cell activation, this finding suggests a pathomechanism of MHC-specific IgE in solid organ rejection.

OP08

A NOVEL IMMUNOSUPPRESSIVE COMPOUND (79-6) THAT TARGETS BCL6 PREVENTS THE HUMORAL ALLORESPONSE

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Background:
BCL6, is a transcription factor involved in B cell activation and differentiation. BCL6-expressing B cells play a crucial role in the development and maintenance of germinal centers, which are essential for the development of a humoral response. Targeting BCL6-mediated responses has the potential to prevent humoral alloreactivity. Here, a small molecule BCL6 inhibitor named 79-6 was tested *in vitro* and its effect on plasma blast formation and IgG production was investigated.

Methods:
The following experiments were performed in the presence and absence of the small molecule BCL-6 inhibitor 76-9 (range 25-100 µg/mL): (1) Polyclonally-activated B cells (anti-IgM/anti-CD40 and IL-21) from healthy controls were studied for differentiation, plasma cell formation and IgG-production. (2) To study 79-6's inhibitory effect on B cell differentiation stages, circulating TfH cells and B cells were stimulated with alloantigen, and 79-6 was added at different time points (day 0, 3, and 7).

Results:
After polyclonal stimulation, a median of 7.4% of the B cells differentiated into plasmablasts. In the presence of 79-6, plasmablast formation was significantly inhibited by 91% and the proportion of class switched memory B cells dropped by 22%, both $p<0.01$). Production of IgGs was measured in culture supernatants (median of 600 ng/ml), After inhibition by 79-6,

IgG-concentrations were significantly reduced (91%, p <0.01).
After stimulation with alloantigen, B cells successfully differentiated into plasma blasts (median 9.8%). Early addition of 79-6 (day 0, day 3) resulted in inhibition of plasma blast formation (median inhibition: 97% and 73%, respectively), while addition of 79-7 at day 7, when B cells have differentiated into plasmablast, did not result in significant inhibition of plasma blast formation.

Conclusions:
79-6 effectively inhibits differentiation of B lymphocytes into immunoglobulin-producing plasmablasts, whereas it does not inhibit Ig production once plasmablast formation is established. This implies that the timing of 79-6 administration in clinical practice is crucial.

OP09

CIRCULATING AND INTRAGRAFT DONOR(HLA)-SPECIFIC B CELLS DRIVE ALLOGRAFT REJECTION AFTER KIDNEY TRANSPLANTATION.

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Background: Humoral alloimmune memory is the main barrier for successful transplantation and is generated by a complex compartmentalized B-cell immune response. Besides donor-specific antibodies (DSA), circulating donor(HLA)-specific memory B cells (mBc) have been show to play an active role predicting and during antibody-mediated rejection (ABMR) in kidney transplant patients. Here we aimed to characterize the role of donor(HLA)-specific B cells in different immune compartments including peripheral blood and bone marrow, as well as alloreactive B cells with donor(HLA)-antigen specificity within cellular infiltrates of rejecting grafts.

Methods: In order to characterize the presence of the humoral alloimmune response of distinct B-cell counterparts, we evaluated B-cell subsets in a donor(HLA)-specific manner in main lymphoid compartments including bone marrow and peripheral blood, as well as in kidney allograft biopsies at the time of rejection. Analyses of circulating DSA, donor(HLA)-specific IgG-secreting mBc in peripheral blood as well as long-lived plasma cell responses in bone marrow were tracked using solid phase assays and HLA-specific B-cell fluorospot assay. Intragraft donor(HLA)-specific B cells were assessed in OCT-embedded frozen biopsies using a novel (HLA)monomer-based immune technology.

Results: High frequencies of donor(HLA)-specific IgG-secreting long-lived plasma cells and mBCs were detected in the BM and peripheral blood, respectively. Notably, mBCs showed higher donor(HLA)-specific B-cell specificities than circulating DSA. Interestingly, B-cell graft infiltrates were observed in the majority of ABMR samples, which included donor(HLA)-specific B cells harboring the same HLA repertoire as those found in the periphery.

Conclusions: Our study highlights the ubiquity of donor(HLA)-specific B cells during allograft rejection in kidney transplant patients, which seem to be key to orchestrate graft rejection.

OP10

DE NOVO SIX2 ACTIVATION IN HUMAN KIDNEYS TREATED WITH NEONATAL KIDNEY STEM/PROGENITOR CELLS DURING NORMOTHERMIC MACHINE PERFUSION

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Background: The unique SIX2+ stem cell population gives rise to the formation of all nephron structures in the developing kidney and is exhausted before the 36th week of gestation, when after, no new nephrons are formed. We have previously described a unique non-invasive strategy to isolate and expand the native SIX2+ kidney stem cells from the urine of preterm neonates, named neonatal kidney stem/progenitor cells (nKSPC).

Methods: We analysed the mechanism of immunosuppression of nKSPC using mixed lymphocyte reactions and HPLC-MS. Then, we administered nKSPC into human kidneys discarded for transplantation during 6h of normothermic machine perfusion. We analysed the immunomodulatory and regenerative potential of nKSPC therapy by differential gene

and protein expression in the tissue and perfusate solution.

Results: nKSPC administration in donor kidneys had an immunosuppressive potential, by reducing inflammatory cytokine levels via the tryptophan-IDO-kynurenine pathway, and significantly lowering the levels of kidney injury biomarkers. We could track nKSPC in the kidney tissue and most impressively, we showed that nKSPC treatment induces the *de novo* expression of SIX2 in proliferating proximal tubular cells, which suggests the induction of an endogenous regenerative process. This is the first time that the latter phenomenon has been reported.

Conclusions: nKSPC might be the ideal cell type to be applied in kidney-targeted cell therapy, providing immunomodulation and inducing an endogenous regenerative process.

OP11

ADMINISTRATION OF MESENCHYMAL STROMAL CELLS TO REJECTED DONOR LUNGS RESULTS IN REGENERATION AND REDUCTION OF PRIMARY GRAFT DYSFUNCTION

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Background: As lung transplantation remains the choice treatment for a number of irreversible pathologies, the burden caused by a lack of available donor organs is a pressing matter in need of innovative solution. The development of a treatment by which a previously rejected donor lung could be reconditioned for use in a recipient would present a solution to the current dilemma of critical shortages of donor lungs. To address this issue, term amniotic fluid-derived mesenchymal stromal cells (TAF-MSCs) were hypothesized to not only improve damaged lungs when administered during ex vivo lung perfusion (EVLP), but also reduce the incidence of primary graft dysfunction (PGD).

Methods: *E.coli*-derived lipopolysaccharide was used to induce acute lung injury in donor pigs which was confirmed with blood gas values and histology. After lung harvest from the donors, the lungs were placed on EVLP for 4 hours, the left lung was transplanted, and the recipient was then monitored for 3 days. A subsequent right pneumonectomy allowed for evaluation of the transplanted left lung. Lungs were divided between the non-treated and treated groups, which underwent the same EVLP and transplantation protocol. The treatment group, however, received doses of TAF-MSCs through intravascular administration during EVLP and 2 timepoints after transplantation.

Results: Recipients were assessed through comparison of leukocyte counts, histological assessment, and lung functionality. The treated group demonstrated reduced counts of lymphocytes in the first 24 hours of transplantation despite comparable levels between groups at the induction of ARDS. Histological scoring by blinded pathologists showed less injury in treated ones relative to both their non-treated counterparts as well as earlier biopsies taken at the time of confirmed lung injury. Furthermore, all recipients from the treated group were found to have grade 0 PGD by the third day of follow-up with significantly higher PaO2/FiO2 ratios. The non-treated group consisted of one PGD grade 2 recipients with the remainder of the group experiencing PGD grade 3.

Conclusions: Previously damaged lungs with acute lung injury can be recovered for transplantation through the use of intravascular TAF-MSCs with the finding of reduced primary graft dysfunction in the transplanted recipient.

OP13

17B-ESTRADIOL AND METHYLPREDNISOLONE ASSOCIATION AMELIORATES LUNG INFLAMMATION AFTER BRAIN DEATH IN FEMALE RATS

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Background: Brain death (BD) leads to systemic alterations that compromise organ viability. After BD, lungs from female donors present higher inflammation in comparison to male donors, which was associated with the acute

reduction of female hormones, especially estradiol (E2). The aim of this study is to evaluate the associated treatment of E2 and methylprednisolone (MP) in female rats after BD.

Methods: Female Wistar rats were submitted to BD by rapid inflation of an intracranial balloon catheter and maintained for 6h. Rats received MP (MP, 4 mg/ml i.v–2 ml/h) or MP and E2 (E2/MP; E2-50 ug/ml; MP-4 mg/ml i.v–2 ml/h) after 3h of BD until the end of experiment. Sham-operated (S) rats were used as controls. After 6h, cellular infiltration was analyzed in bronchoalveolar lavage (BAL). Lung samples were collected for homogenate, culture (explant) and relative gene expression analyzes. IL-1β, IL-6 and CINC-1 were quantified in homogenate and explant. In parallel, IL-1β and IL-6 gene expression was also evaluated.

Results: After BD, there was an increase in total leukocyte infiltration in BAL, that was prevented by treatments (p=0,002). In the differential count, granulocyte infiltrate was reduced only in the MP/E2 group (p=0,047). In lung tissue, BD increased IL-6 after 6h and the MP/E2 treatment was able to reduce this cytokine (p=0,028). Also, both treatments were capable of reducing IL-1β (p=0,005). There were no differences among groups in CINC1. In lung culture, MP/E2 treatment reduced IL1β (p=0,029) and CINC1 (p=0,007) and both treatments reduced IL-6 (p=0,002). Also, lung relative gene expression of IL-1β was reduced with both treatments (p=0,020). There were no differences in IL-6.

Conclusions: Our data showed that the associated treatment of females rats with MP and E2 modulates lung inflammation by reducing the release and expression of interleukins and chemokines, such as IL-1β, IL-6 and CINC1 and controlling the leukocyte mobilization to the airways, especially granulocytes. These data point to a potential positive effect of the association of corticotherapy and estradiol in improving lung quality in female donors.

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OP14

OCCURRENCE OF VIRAL INFECTIONS MAY BE PREDICTED BY LONGITUDINAL MONITORING OF TORQUE-TENO VIRUS IN PEDIATRIC KIDNEY TRANSPLANT PATIENTS.

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Background: Rejection prevails as dominant cause of late graft failure in kidney transplantation (KTX). Balance of immunosuppression (IS) is essential to minimize the risk for infectious disease. Torque-Teno virus (TTV) plasma load is a proposed marker of immune status in patients with solid-organ transplantation and correlates with dosage of IS in pediatric KTX. We aimed to investigate prediction and association of TTV loads with the occurrence of viral infections, e.g., Epstein-Barr virus (EBV), cytomegaly virus (CMV), BK polyomavirus (BKV), and JC polyomavirus (JCV) in pediatric patients with KTX.

Methods: All pediatric KTX patients at the Medical University of Vienna with a post-transplant time >3 months were included in this study. All viral loads, including TTV, were routinely measured monthly to bimonthly. Log10 TTV loads were analyzed for association with log10 EBV, CMV, BKV, and JCV loads on the same or the next visit measured by quantitative PCR and analyzed with a generalized poisson mixed model with random slopes and intercepts for each patient over time accounting for temporal autocorrelation. Analysis of TTV log10 transformed TTV loads with relevant cut-off values for infection with EBV, CMV, BKV and JCV was conducted with mixed effects logistic regression.

Results: N=72 pediatric KTX patients were included in this study. Baseline data is displayed in Table 1. Significantly higher loads of CMV (p=0.008), plasma BKV (p=0.02), urine BKV (p=0.01) and JCV (p=0.000002) were associated with higher TTV loads on the same visit. Higher TTV loads predicted significantly higher loads of CMV (p=0.0005), plasma BKV (p=0.03), urine BKV (p=0.005) and JCV (p=0.02) on the next visit. Associations with EBV were not significant. Association and prediction of TTV loads with relevant cut-offs for viral infection are displayed in Figure 1.

Conclusions: This is the first study to investigate associations between TTV loads and other clinically relevant viral infections in pediatric KTX. TTV levels can significantly predict higher levels of CMV, BKV, and JCV as well as infection above clinically relevant cut-offs on the same or the next visit.

Figure 1. Mixed effects logistic regression for log10 TTV and opportunistic viral infections

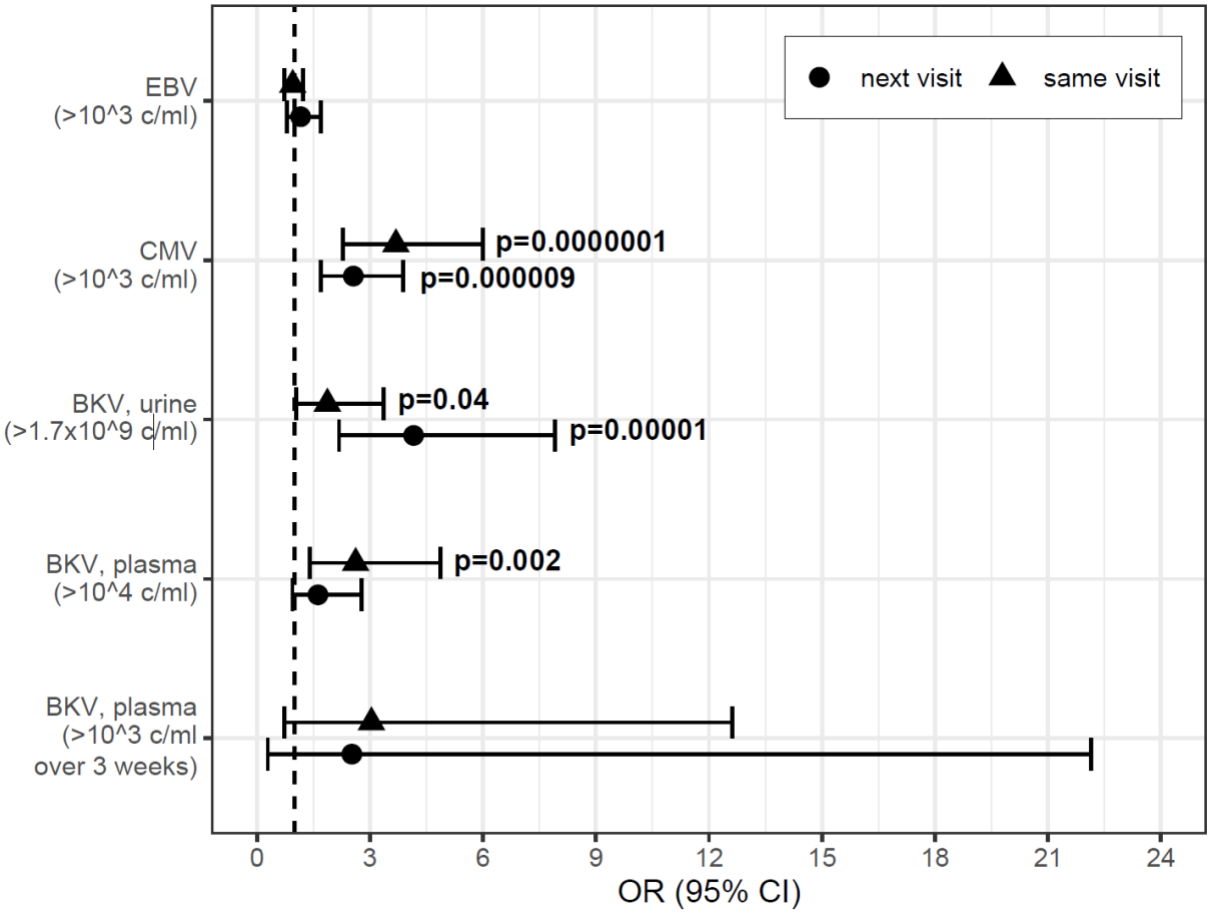

Table 1. Primary kidney disorders and baseline kidney transplantation characteristics

Primary kidney disorders	N	%
CAKUT	35	49
Glomerular disorders	15	21
Polycystic kidney disease	9	13
Congenital nephrotic syndrome	9	13
Metabolic disorders	2	3
Other	2	3
Baseline KTX characteristics	**N**	**%**
Male	47	65
Living Donors	45	63
Basiliximab induction	72	100
Tacrolimus	64	89
Cyclosporin A	4	6
Sirolimus	3	4
w/o calcineurin- or mTOR-inhibitor	1	1
Mycophenolate mofetil	61	85
Azathioprine	5	7
w/o antiproliferative substance	6	8
Steroids	71	99
	Median	**IQR**
Age (years)	12.2	8.0-15.8
Age at KTX (months)	8.1	3.4-13.0
Time post KTX (months)	19	3.3-63
HLA mismatch (n)	3	2-3
Creatinine (mg/dl)	0.89	0.54-1.31
eGFR (ml/min/1.73m²)	96.1	75.9-134.3
Study period (years)	3.5	0.7-6.1
Follow-up time (years)	6.6	0.9-19

CAKUT = congenital anomalies of the kidney and urinary tract, eGFR = estimated glomerular filtration rate

OP15

COMPLEMENT SYSTEM ACTIVATION IN BRAIN DEATH AND THE EFFECT OF LUMINAL INTESTINAL PRESERVATION.

Marc Weiss[1;2;3], Anne Marye de Jong[4], Helene Seegert[1;3], Niels Moeslund[3;5], Hanno Maassen[6;7], Camilla Schjalm[8;9], Eline de Boer[8;9], Marina Sokolova[8;9], Søren Pischke[8;9], Henri Leuvenink[7], Anna Krarup Keller[2;3], Marco Eijken[1;3], Bente Jespersen[1;3]

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Background:
Donor organs obtained from brain dead donors often have worse outcomes compared with organs from living donors. Activation of the complement system and intestinal derived immune system activation have been suggested as causative.
Methods:
30 pigs were included; control group undergoing sham surgery (n=7), brain death group alone (n=8) and brain death with polyethylene glycol (PEG)(n=7) or University of Wisconsin solution (UW)(n=8) instilled into small bowel during organ procurement. Prior to organ procurement, all brain death groups were identical and reported as one. Brain death was induced by inflation of a balloon catheter in the epidural space. All animals were observed for 360 minutes following confirmation of brain

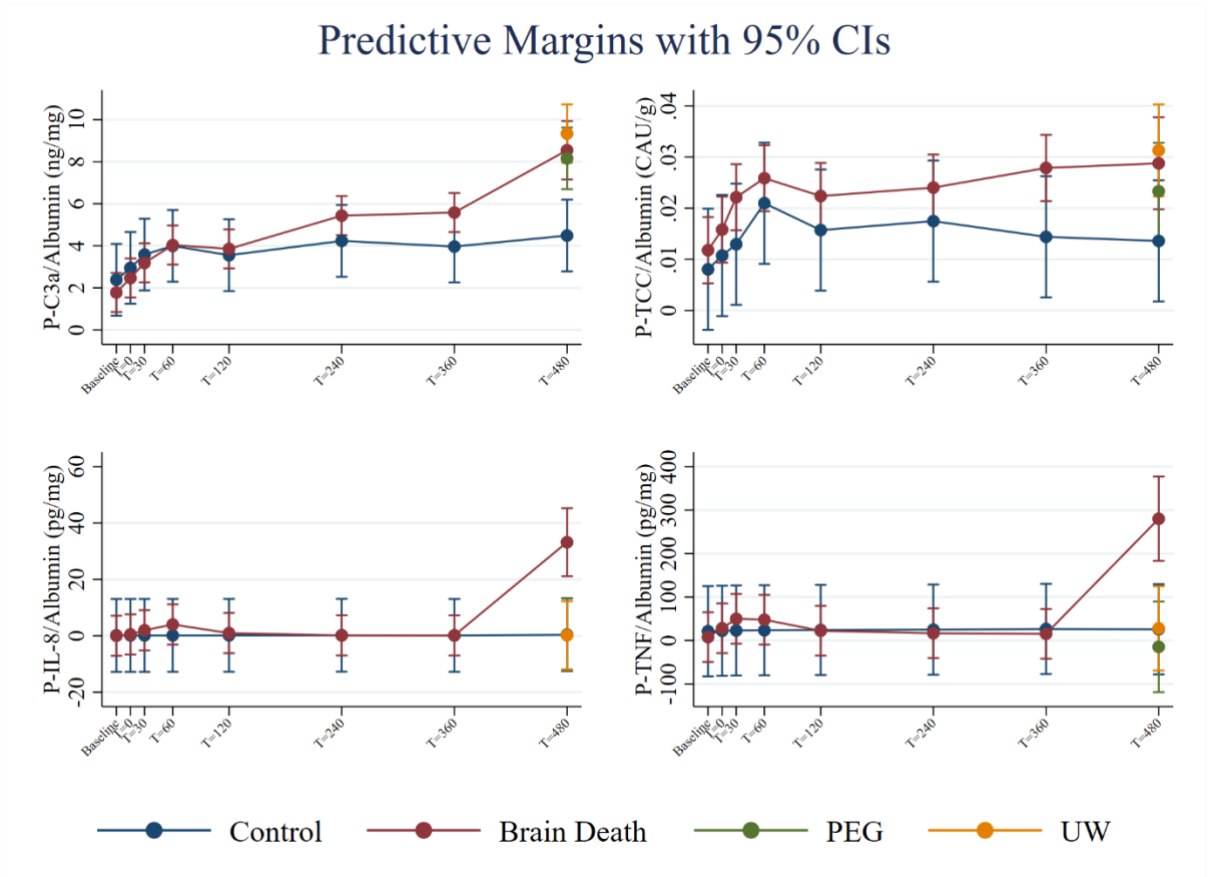
death before organ retrieval. C3a, Terminal Complement Complex (TCC), IL-8 and TNF were measured in plasma collected throughout the experiment and following small bowel preservation (T=480). Biopsies were taken from all transplantable organs at time of removal, and additionally from the small bowel during 24 hours of static cold storage.

Results:

There were no significant differences prior to induction of brain death for any measured variable and all pigs remained circulatory and respiratory stable throughout the observation period. At 480 minutes, C3a was significantly higher in the brain death alone group (mean (95% CI)) (8.15 (6.54;9.75)), PEG (8.15 (6.45;9.85)) and UW (9.73 (8.14;11.33)) compared to control (4.50 (2.77;6.24) ng/mg). TCC was significantly higher in the combined brain death group (0.027 (0.016;0.038)) compared to control (0.015 (0.0023;0.026)) at 360 minutes. At 480 minutes, the brain death alone (0.027 (0.016;0.038)) and UW (0.030 (0.019;0.041)) groups were significantly higher compared to the control group (0.014 (0.0015;0.026) CAU/ml). IL-8 (33.09 (20.64;45.55) pg/mg) and TNF (280.13 (180.61;379.66) pg/mg) were significantly higher in the brain death alone group compared to all other groups at 480 minutes. Histology and immunohistochemistry results are pending.

Conclusions:

This study shows that complement system activation at the level of C3 occurs following brain death. Additionally, the significantly lower levels of IL-8 and TNF in animals treated with small bowel preservation suggest that this treatment might prevent cytokine release.



OP16

EXTRACELLULAR VESICLE SUBSETS RELEASED DURING NORMOTHERMIC MACHINE PERFUSION ARE ASSOCIATED WITH HUMAN KIDNEY CHARACTERISTICS

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Background:

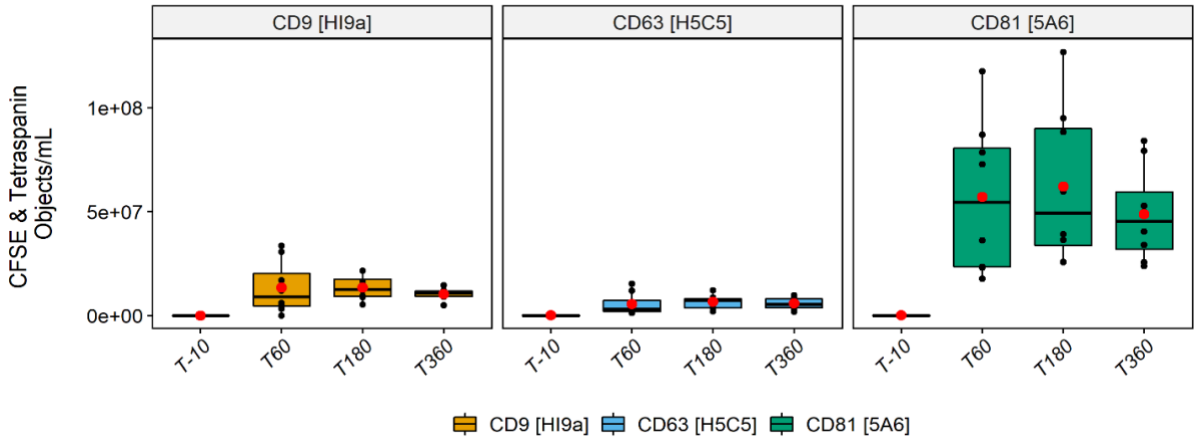
Extracellular Vesicles (EVs) represent stable, tissue specific nano-sized particles that reflect the conditional state of their tissue of origin. Normothermic Machine Perfusion (NMP),

aimed at restoration of cellular metabolism and function to organs, offers the possibility to assess graft status prior to transplantation through analysis of biomarkers in the perfusion fluids. Here, the dynamic release and phenotype of kidney EVs released during NMP were analyzed to examine whether EVs could function as a potential biomarker for assessing kidney quality before transplantation.

Methods:
Eight discarded kidneys (~13 ± 5 hours of cold ischemia, age 68 ± 7 (mean ± standard deviation), all male) were perfused in a closed system at 37 ^{0}C for 6 hours. Perfusates were taken before and at 1, 3 and 6 hours and examined with Nanoparticle Tracking Analysis (NTA) and Imaging Flow Cytometry (IFCM). For IFCM, perfusates were stained with the tetraspanin EV markers CD9, CD63 or CD81, or a mix of the three markers in combination with CFSE to identify, quantify and characterize EVs.

Results:
Analysis of perfusates with NTA revealed that the majority of nanoparticles present in the perfusates are <300 nm. Using IFCM, we selectively studied these small nanoparticles. For CFSE and the mix of tetraspanin double-positive EVs, we observed a ~700 / 740 / 560 fold increase compared to EV levels before perfusion at 1, 3 and 6 hours of NMP, respectively. Especially after 1 hour of NMP, double-positive EV levels were found to be positively correlated with donor age whilst negative correlations were found for cold ischemia time. Furthermore, tetraspanin CD81 was found to represent the majority (~70%) of the excreted double-positive EV (CD9: ~15% / CD63 <10%) (Figure).

Conclusions:
EVs are excreted during NMP with highest excretion levels during the first hour of perfusion. Tetraspanin CD81 is predominantly present on these EVs. The characterization of the excreted EVs as well as their correlation with clinical parameters provides a starting point to study their role as potential biomarkers of kidney quality.



OP17

LIVER NORMOTHERMIC MACHINE PERFUSION SUPPORTS TRANSLATION AND MITOCHONDRIAL FUNCTION AND REDUCES DEGRADATION AND METABOLIC IMBALANCE

Maria Letizia Lo Faro[1], Adam M. Thorne[2], Honglei Huang[1], Philip Charles[3], Maria Kaisar[1;4], Simon Davis[3], Fungai Dengu[1], Sadr Shaheed[1], John Mulvey[1], Roman Fischer[3], David Nasralla[5], Benedikt Kessler[3], Henri Leuvenink[2], Peter Friend[1], Rutger Ploeg[1]
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Background: Liver preservation by normothermic machine perfusion (NMP) involves perfusion of the graft with oxygenated blood and nutrients. The NMP Liver trial by the Consortium for Organ Preservation in Europe (COPE), has shown that NMP is associated with a reduction in graft injury and increased organ utilisation when compared to static cold storage (SCS). The aim of the present study is to provide insight into the molecular

mechanisms involved in NMP liver preservation by proteomics analysis.

Methods: Biopsies from DBD and DCD livers preserved using SCS or NMP were collected as part of the COPE Liver trial at time of retrieval (LT1), at the end of preservation (LT2) and 1 hour after reperfusion in the recipient (LT3). A total of n=437 samples were analysed for this study. Proteins were extracted, digested and analysed by quantitative label-free proteomics (LFQ LC-MS/MS). Protein expression was analysed over time by 1-way ANOVA with permutation-based FDR (1%).

Results: Longitudinal analysis of NMP samples (LT1 vs LT2 vs LT3) identified n=588 proteins with significantly different expressions ($p < 0.05$, FDR 1%, Figure 1). Biopsies at the end of NMP presented upregulated protein translation and increased mitochondrial function and ATP synthesis, alongside downregulation in glycolysis and fatty acid and protein degradation. Similar analysis on SCS samples showed no changes in protein expression between retrieval and end of preservation biopsies, with, in contrast to NMP, a significant downregulation in mRNA processing, mitochondrial electron transport and ATP production post-reperfusion.

Conclusions: The proteomics analysis highlights significant protein changes over the donation-preservation and reperfusion process, with NMP supporting protein translation and mitochondrial function while reducing protein degradation and metabolic imbalance, as opposed to SCS. These findings represent the first large set of proteomics data from the COPE NMP Liver trial and might help to further our understanding of the molecular mechanisms involved in NMP.

OP18

REMOVAL OF NEUTROPHIL EXTRA-CELLULAR TRAPS DURING EX VIVO LUNG PERFUSION SHOWS IMPROVE-MENT OF LUNG FUNCTION IN A PORCINE LUNG INJURY MODEL

Margareta Mittendorfer[1;2;3;4], Leif Pierre[2;3;4], Andrew Aswani[5;6], Dmitry D. Genkin[6], Kirill Surkov[6], Gunilla Kjellberg[7], Franziska Olm[2;3;4], Sandra Lindstedt[1;2;3;4]

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Background: Due to a critical shortage of donor lungs for transplantation, options to increase the donor pool are urgently needed. Many potential donor lungs are discarded due to gastric content aspiration induced acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Regeneration of such rejected lungs would increase the donor pool. Neutrophil extracellular traps (NETs) are implicated in the inflammation profile of ALI. Reduction of NETs could be used as a therapeutic option with the arrival of devices specific to their removal. NucleoCapture devices (Santersus AG) selectively remove NETs from the blood by utilizing human histone H1.3 protein conjugated to polymer beads. This study investigated the impact of the NETs capture device during *ex vivo* lung perfusion (EVLP) on aspiration damaged lungs.

Methods: Healthy pigs (n=12) were stratified into two groups, treated (n=6) and not treated (n=6). All animals received 4ml/kg gastric content distributed equally between the different lung segments using a bronchoscopy to induce ALI. Mild to moderate ARDS was developed over 6 hours and confirmed via blood gas values, chest x-ray imaging and histological examination. Lungs were subsequently explanted *en bloc* and placed on EVLP for 4 hours. Treated lungs were placed in line with a NucleoCapture device connected to the EVLP circuit and the non-treated group underwent the same EVLP protocol without the device.

Results: ARDS was induced in all subjects as confirmed by infiltration on chest x-ray, histopathological examination and by PaO_2/FiO_2 ratio. Following treatment with the NucleoCapture device during 4 hours of EVLP, the PaO_2/FiO_2 ratio was significantly increased compared to non-treated lungs and was found to surpass the threshold values suitable for transplantation. Furthermore, macroscopic evaluation of the treated lungs demonstrated improvement relative to both the initial injury as well as comparison with the non-treated cohort.

Conclusions: Treatment of aspiration injured lungs with a NucleoCapture device to remove NETs from the EVLP circuit resulted in macroscopic and oxygenation improvement of otherwise damaged donor lungs. The amelioration of lung function by removing NETs shows potential clinical use of the device to

increase the donor lung pool for lung transplantation.

OP19

GPR109A ACTIVATION IN HUMAN REGULATORY MACROPHAGES ENHANCES INDUCED TREG GENERATION

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Background: Graft-infiltrating regulatory macrophages (Mregs) of recipient origin are indispensable for allograft acceptance in immunosuppressed and tolerant recipients. Mregs likely contribute to restoration of immune homeostasis of transplanted organs in many ways, but their defining feature is induction of a specialised subset of TIGIT+ Tregs (Riquelme, Nat Comms, 2018). Drugs to enhance Mreg-mediated Treg (miTreg) generation could be valuable in reducing chronic immunological injury to transplants. Here, using an in vitro screening assay, we find G-protein coupled receptor 109A (GPR109A) enhances human miTreg generation.

Methods: Human monocyte-derived cell types were generated as previously described (Riquelme, Protocol, 2018). Expression of GPR109A and miTreg generation were assessed by flow cytometry.

Results: Transcriptomic profiling of a panel of human monocyte-derived macrophages identified GPR109A as significantly up-regulated in Mregs. Flow cytometry confirmed higher expression of GPR109A by Mregs compared to other macrophages. GPR109A was undetectable in human T cells. Mepenzolate bromide, a GPR109A antagonist, caused a dose-dependent suppression of Mreg-mediated Treg induction in cocultures. iTreg generation was also suppressed by a polyclonal antibody against GPR109A in our assay (p=0.0227). However, nicotinic acid and MK-1903, agonists of GPR109A, did not enhance iTreg generation over a range of concentrations. We discovered that Mreg-induced Treg generation was enhanced when cocultures were performed in CTS medium compared to RPMI-based medium. Experiments using mixtures of these media implied the presence of a GPR109A agonist in CTS medium, which filtered through a 5-KDa cut-off membrane and was not chloroform soluble.
Conclusions: Stimulation of human Mregs by a presently unknown small molecule via GPR109A enhances their Treg-inducing capacity in vitro. Experimentally, neuroinflammation and inflammatory bowel disease can be suppressed by GPR109A activation. Notably, dimethyl fumarate (Tecfidera) used in treatment of multiple sclerosis, is a precursor of monomethyl fumarate that acts as a GPR109A ligand. We propose that DMF or alternative GPR109A agonists could be repurposed in solid transplantation to support the Mreg-T cell intragraft regulatory axis.

OP20

INTRINSIC TISSUES FACTORS PREDISPOSE SUSCEPTIBILITY OF ISCHEMIA-REPERFUSION INJURY BY INFLUENCING THE RECRUITMENT OF TREGS INTO KIDNEY ALLOGRAFTS

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Background: Ischemia/reperfusion (I/R) injury following kidney transplantation (KTx) frequently leads to delayed graft function (DGF) and is detrimental to graft longevity. We have previously demonstrated that donor kidney-intrinsic innate immunity and endoplasmic reticulum (ER) stress pathways controls the severity of delayed graft function (DGF) in kidney transplantation and potentiates long kidney allograft survival.

Methods: Kidneys from C57BL/6 (B6) or BALB/c mice were harvested and stored in cold UW solution for 3 hours, and then transplanted into full MHC-mismatched binephrectomized C3H mice (B6 allografts vs BALB/s allografts), respectively. Graft functions were evaluated at post-transplant day (POD) 1, 7 and 14 by renal function panels. Histology, RNA sequencing (RNAseq) and immunophenotyping were performed at the endpoints (POD2 or POD14).

Results: We observed that transplant of kidneys from BALB/c mice with prolonged cold ischemia time had significantly reduced tissue inflammation and improved renal function, compared to kidney allografts from B6 mice, not only in the initial ischemia and reperfusion (I/R) phase (POD1-2) of transplantation but also in acute rejection phase (POD7-14). Interestingly, Immunophenotypic analysis at POD14 showed that compared with B6 kidney allografts, BALB/c kidney allografts exhibited significantly higher percentage and more absolute cell

number of Tregs both in spleen and grafts than those received B6 grafts, which coincided with increased frequencies of myeloid cells that expressed higher levels of MHCII, MerTK and PD-L2. Results from RNAseq revealed that expression of genes, including cytokines/chemokines (IL-10Ra,GzmB, Icos, CXCR4) and transcription factors (satb1, ETS1, batf and Foxo3) that are associated with Treg differentiation/function/survival was significantly enriched in the BALB/c allografts, as opposed to B6 allografts.

Conclusions: Genetic background of donor kidney dictates expression of gene signatures that regulates differentiation and accumulation of regulatory T cells in the kidney allografts, which in turn predisposes susceptibility of ischemia/reperfusion injury and long-term transplant outcome. These findings underscore the importance of immunomodulating donor organs in improving transplant outcome.

OP21

THREE-DOSE COURSE OF MRNA-1273 COVID-19 VACCINE IN KIDNEY TRANSPLANT RECIPIENTS: EVOLUTION OF CELLULAR AND HUMORAL IMMUNITY

David Cucchiari[1], Natalia Egri[1], Diana Rodriguez-Espinosa[1], Enrique Montagud-Marrahi[1], Pedro Ventura-Aguiar[1], Joaquim Casals[1], Jimena Del Risco[1], Frederic Cofan[1], Jordi Rovira[1], Maria José Ramírez-Bajo[1], Elisenda Bañón-Maneus[1], Federico Oppenheimer[1], Ignacio Revuelta[1], Beatriu Bayés[1], Fritz Diekmann[1]

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Background: In kidney transplant recipients (KTRs), there is discordance between the development of cellular and humoral response after vaccination against SARS-CoV-2. We sought to determine the interplay between the two arms of adaptive immunity in a three-dose course of mRNA-1273 100μg (Moderna) vaccine over an eight-month period.

Methods: Final population included 129 KTRs studied at four time-points: at baseline before the 1st dose, after the 2nd dose (median 42 days) and before (203 days) and after (232 days) the 3rd dose. In all the time-points, IgG and IgM were assessed as well as N- and S-protein specific ELISpot. The main outcome was seroconversion after the 3rd dose.
Results: After the 2nd dose, 26.7% of naïve cases experienced seroconversion. Before the 3rd dose and in the absence of clinically-evident COVID-19 this percentage increased to 61.9%. After the 3rd dose, seroconversion was observed in 80.0% of patients. S-ELISpot positivity after the 2nd dose was significantly associated with final seroconversion (OR[95%CI] 3.14 [1.10–8.96], P=0.032), while transplantation <1 year and previous kidney transplant were negatively associated with (OR[95%CI] 0.23 [0.07-0.80], P=0.021 and OR[95%CI] 0.22 [0.06-0.78], P=0.020, respectively) at the multivariate logistic regression analysis. IgG after 3rd dose were significantly higher (P<0.001) in patients who maintained S-ELISpot positivity throughout the study (34.3%) and were correlated with S-spots after the 2nd dose (r=0.344, P<0.001).

Conclusions: A substantial proportion of KTRs vaccinated with mRNA-1273 develops a late seroconversion after two doses and only a fifth remained seronegative after a third. Cellular immunity seems to play a major role in the development of a final strong humoral response.

OP22

B CELL COMPOSITION IS ALTERED AFTER KIDNEY TRANSPLANTATION AND TRANSITIONAL B CELLS CORRELATE WITH SARS-COV-2 VACCINATION RESPONSE

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Background:
The COVID-19 pandemic has major implications on kidney transplant recipients (KTRs) since they show increased mortality due to impaired immune responses to SARS-CoV-2 infection and a reduced efficacy of SARS-CoV-2 vaccination. Surprisingly, dialysis patients have shown superior seroconversion rates after vaccination compared to KTRs. Therefore, we investigated peripheral blood B cell (BC) composition before and after kidney transplantation (KT) and aimed to screen the BC compartment to explain impaired antibody generation.

Methods:
A total of 105 patients were recruited, and multicolor flow cytometric phenotyping of peripheral venous blood BC subpopulations was performed before and one year after KT. Complete follow-up was available for 71 individuals. Anti-SARS-CoV-2 antibodies were collected retrospectively and were available for 40 subjects, who had received two doses of an mRNA-based vaccine (BNT162b2 or mRNA-1273).

Results:
Overall, relative BC frequencies within lymphocytes decreased, and their absolute counts trended in the same direction one year after KT as compared to CKD G5 patients. Frequencies and absolute numbers of naïve BCs remained stable. Frequencies of double negative BCs, a heterogeneous subpopulation of antigen experienced BCs lacking CD27 expression, were increased in after KT, yet their absolute counts were similar at both time points. Transitional BCs (TrBCs) and plasmablasts were significantly reduced after KT in absolute and relative terms. Memory BCs were affected differently since class-switched and IgM-only subsets decreased after KT, but unswitched and IgD-only memory BCs remained unchanged. CD86+ and CD5+ expression on BCs was downregulated after KT. Correlational analysis revealed that TrBCs were the only subset to correlate with titer levels after SARS-CoV-2 vaccination. Responders showed higher TrBCs, both absolute and relative, than non-responders.

Conclusions:
Together, after one year, KTRs showed persistent and profound compositional changes within the BC compartment. Low TrBCs, one year after KT, may account for the low serological response to SARS-CoV-2 vaccination in KTRs compared to dialysis patients. Our findings need confirmation in further studies as they may guide vaccination strategies.

OP23

ASSESSING SARS-COV-2-SPECIFIC ADAPTIVE IMMUNITY TO PREDICT SEROCONVERSION AFTER MRNA-BASED VACCINATION IN SOT

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Background: mRNA vaccines are proving highly efficacious against SARS-CoV-2 and its effect on adaptive immunity has been reported among immunocompetent (IC) individuals, although triggering significantly lower immune responses in solid organ transplant (SOT) patients. Notably, scarce data is available regarding the impact of the different immunosuppressive treatments in the quality and longevity of this response.

Methods: SARS-CoV-2-specific adaptive immune memory was assessed at different compartments (serological, memory B cells [mBC] and cytokine [Th1: IFN-γ, IL-2, IFN-γ/IL-2 and Th2: IL-21 and IL-5] producing T cells) by ELISA and FluoroSpot-based assays, respectively, in 40 SOT receiving either TAC/MMF or TAC/mTor-i-based immunosuppression and 20 IC with a mRNA-based vaccine at different time-points before and after 3-dose vaccination (T1=pre-2nd dose; T2=2 months post-2nd dose; T3=pre-3rd dose). Prediction of subsequent seroconversion was investigated.

Results: Significant differences were observed between patients receiving distinct immunosuppressive treatment. More robust responses in all immune compartments specific to Spike antigen were observed in patients receiving TAC/mTOR-i IS than patients on TAC/MMF. After 2nd vaccine dose, seroconversion rates (55.08% vs. 84%, p=0.006) and IgG-producing mBc frequencies (33.33% vs. 92.31%, p=0.001) were significantly lower in TAC/MMF patients than TAC/mTORi, respectively. Similarly, Th1 and Th2 frequencies were significantly higher among mTORi patients after the second dose (p=0.005 and p<0.001, respectively). To note, the detectable frequencies of mBc and Th1 and Th2 responses prior the second and third doses, predicted those patients seroconverting after each vaccine. Most importantly, these data was also true for those seronegative patients prior to vaccination but showing detectable B and T-cell frequencies specific against Spike protein.

Conclusions: Patients receiving immunosuppression based on TAC/mTor-I display significantly higher capacity to respond to mRNA-based SARS-CoV-2 vaccines as compared to patients on standard of care therapy based on TAC/MMF. Assessing SARS-CoV-2-specific memory B and T-cell responses may allow identifying those patients more likely

to seroconvert after subsequent booster vaccines

E-POSTER PRESENTATIONS

POS03

EXPLORING IMMUNE RISK STRATIFIERS TO PREDICT ANTIBODY-MEDIATED REJECTION AFTER HLA INCOMPATIBLE TRANSPLANTATION

Robert Carroll[1], Massimo Mangiola[2], Ashley Drabik[3], Angela Maldonado[4], Anna Runström[4], Kristoffer Sjoholm[4], Christian Kjellman[4], Jan Tollemar[4], Annette Jackson[3]

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Background:

Donor specific HLA antibody (DSA) risk assessments in crossmatch (XM) positive transplants remains challenging despite new desensitization strategies. Solid phase assays and HLA eplet analysis are superseding physical XM in immunological risk assessments at some transplant centers. We assessed the utility of multiple immune risk stratifiers to predict early antibody mediated rejection (ABMR) in patients desensitized with imlifidase.

Methods:

High resolution HLA typing was imputed for 12 of 22 recipient/donor pairs using Haplostats. Repeated HLA antigen or eplet mismatches (RMM) with the original immunizer, number of DSA, sum MFI of DSA, HLA eplet sharing between donor and immunodominant DSA (immunizer), and total antibody verified HLA eplet mismatches were assessed using FUSION (version 4.6). The endpoint was biopsy proven ABMR. Crossmatch strength was classified as strong if CDC was positive or FCXM MCS >100 above the positive threshold for either T or B cells, otherwise it was classified as weak.

Results:

22 patients with a median (range) cPRA 99.7% (60-100%) fulfilled all criteria. 5/22 (23%) experienced ABMR within 14 days of transplant, median 10 days (range: 3-13). Median of the summed Class I/II HLA DSA MFI were 31000 (range: 2300-92000) for those who experienced early ABMR versus 11000 (range: 1700-96000) in the absence of ABMR. HLA eplet load between the ABMR and non-ABMR groups were similar at Class I (A,B, and C loci) and for Class II (DRB1, DRB3,4,5 DQB1 and DQA1 loci) or as a total eplet mismatch between donor and recipient. Median HLA eplet sharing between donor and immunizer were 5(4-36) in the early ABMR group and 4(0-19) in the absence of ABMR. All 5/5 (100%) patients with ABMR had a strong XM whereas 10/17 (59%) with no early ABMR had a strong XM.

Conclusions:

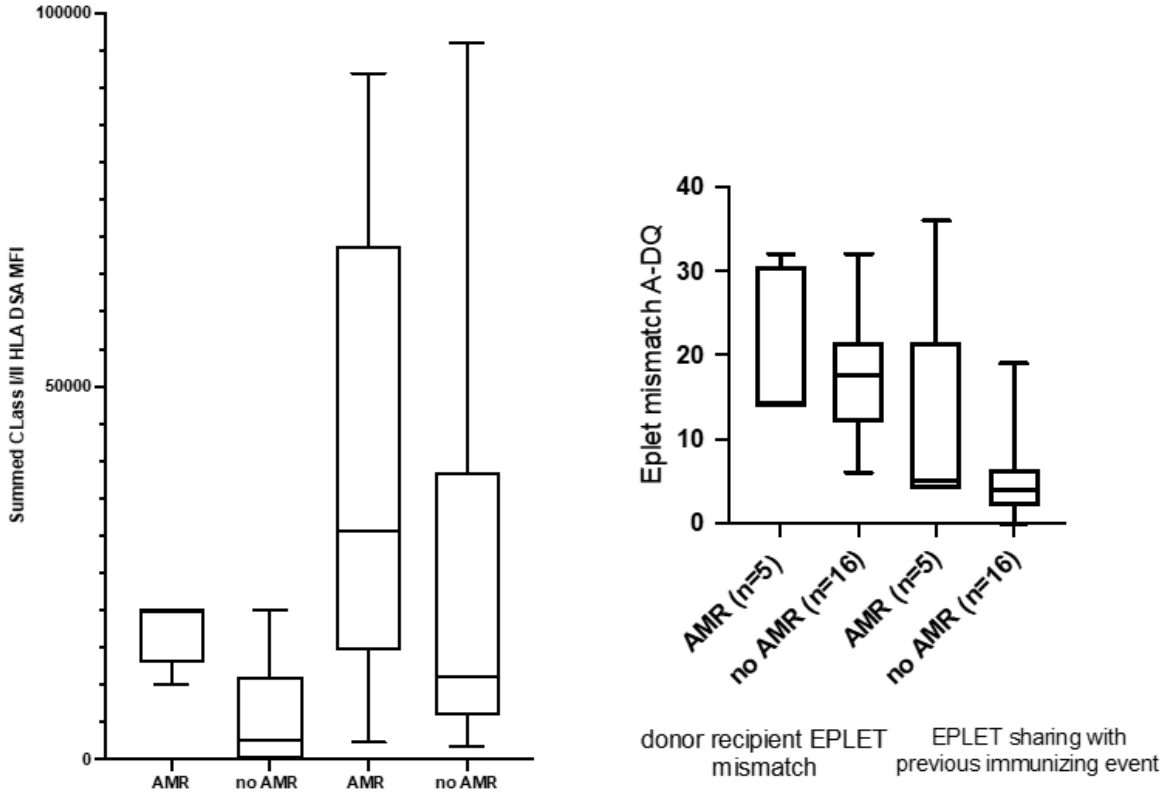
RMM, number of DSA, or total sum DSA MFI did not stratify which patients desensitized with imlifidase were likely to experience early ABMR. Donor/recipient HLA eplet mismatch or eplet sharing between donor and previous immunizer did not reach statistical significance. However, all ABMR patients had a positive CDC and or >100 MCS FCXM. Further stratification with a larger dataset and improved HLA eplet analysis to include immunogenicity may improve solid phase-based risk stratification.

Table 1. Summary of Immunologic Characteristics

*Patient	Early ABMR	Positive Physical XM	[a]RMM	MFI Sum DSA	#DSA	[b]Donor Eplets Shared w/ Immunizer	Donor/Recip class I eplet mismatch	Donor/Recip class II eplet mismatch	ABMR score	C4d
1	YES	Strong	YES	92,460	9	36	17	15	g2, ptc2	3
2	YES	Strong	YES	45,667	6	4	9	5	g1, ptc1	3
3	YES	Strong	YES	26,809	4	5	9	5	g1, ptc2	0
4	YES	Strong	YES	30,650	3	4	11	3	g1, ptc2	0
5	YES	Strong	YES	*2,308	2	7	6	1	g3, ptc3	2
6	NO	Strong	YES	96,061	8	5	9	12	-	-
7	NO	Strong	YES	41,277	2	16	13	14	-	-
8	NO	Strong	YES	44,230	6	4	4	8	-	-
9	NO	Strong	YES	30,155	7	15	9	14	-	-
10	NO	Strong	YES	22,257	1	7	7	4	-	-
11	NO	Strong	no	18,114	4	6	10	4	-	-
12	NO	Strong	no	11,046	2	1	0	6	-	-
13	NO	Strong	YES	9,911	4	2	9	9	-	-
14	NO	Strong	no	*2,876	2	2	17	0	-	-
15	NO	Strong	no	*1,765	2	0	5	7	-	-
16	NO	Weak	YES	46,771	6	4	8	0	-	-
17	NO	Weak	no	35,742	7	2	4	14	-	-
18	NO	Weak	no	10,758	2	3	9	6	-	-
19	NO	Weak	YES	9,020	2	1	17	2	-	-
20	NO	Weak	YES	8,161	2	4	4	14	-	-
21	NO	Weak	no	4,687	2	19	8	21	-	-
22	NO	Weak	YES	3,140	1	4	9	13	-	-

[*] Imlifidase treated patients from HMedIdeS 02, 03, 04, and 06 studies; [a] Donor HLA matches strongest HLA antibody OR shares Antibody Verified eplet with strongest HLA antibody; [b] Donor eplets shared with Strongest HLA antibody (immunizer).

Figure 1. Incidence of ABMR Stratified by Summed Class HLA DSA MFI and Eplet Mismatch and Sharing



POS04

HUMORAL AND CELLULAR IMMUNITY AFTER HOMOLOGOUS AND HETEROLOGOUS COVID-19 VACCINATION REGIMENS IN KIDNEY TRANSPLANT PATIENTS

Viktor Lehner[1], Verena Kappler[1], Louise Platen[1], Christopher Holzmann-Littig[1], Matthias Braunisch[1], Gesa Wilkens[2], Nina Körber[2], Hrvoje Mijocevic[3], Christoph Schmaderer[1], Tanja Bauer[2;4], Ulrike Protzer[2;3;4], Lutz Renders[1;4]

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Background:

Solid organ transplant patients are at higher risk for poor COVID-19-related outcomes due to immunosuppression and have been included as a priority group in Covid-19 vaccination recommendations. We examined the humoral and cellular immunity in kidney transplant (KTx) recipients under immunosupression after COVID-19 vaccination using either a homologous or a heterologous vaccination regimen.

Methods:

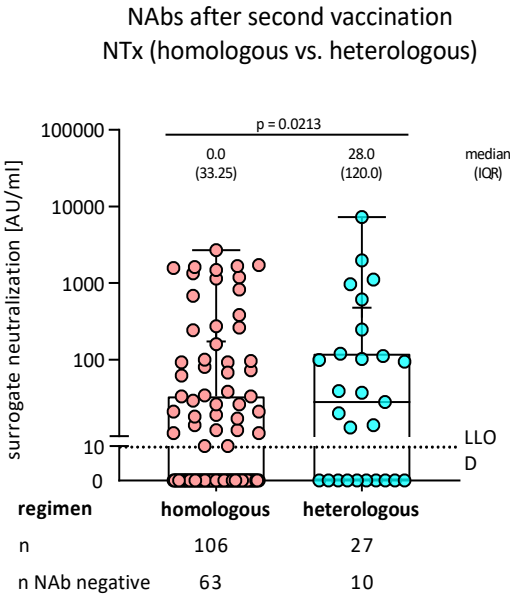
133 SARS-CoV-2-negative KTx recipients (median age 58 years, 60% males, 40% females) are enrolled in the study. For homologous vaccination, patients received two doses of either BNT162b2 mRNA (Comirnaty®), mRNA-1273 (Spikevax®) or ChAdOx1 nCoV-19 (Vaxzevria®). For heterologous vaccination, one dose of ChAdOx1 nCoV-19 was followed by one dose of either of the two mRNA vaccines. Surrogate neutralization activity in serum was determined two weeks after the second vaccination using a neutralizing antibody (NAb) assay. SARS-CoV-2 spike-reactive T cells were determined in a subcohort of KTx recipients (n=54) two weeks after second vaccination using a dual-colour FluoroSpot assay detecting IFN-γ and/or IL-2 secreting cells.

Results:

Only 45.1% (60/133) of the KTx recipients showed a surrogate neutralization activity two weeks after the second vaccination. However, the heterologous vaccination scheme led to significantly higher surrogate neutralization activity (p=0.0213) and also significantly higher numbers of spike (S1 and S2 peptide pool)-reactive cytokine secreting cells (p=0.0004 and p=0.0003, respectively) compared to the homologous vaccination scheme. All of the heterologously vaccinated patients with or without (w/o) SARS-CoV-2 NAb (8/8) had detectable spike-reactive T cells compared to only 71.4% (15/21) of KTx recipients w/o SARS-CoV-2 NAb after homologous vaccination.

Conclusions:

From our data we conclude that a heterologous vaccination regimen is more immunogenic than homologous vaccination in KTx recipients. COVID-19 vaccine efficacy should be controlled in theses patient groups by determining antibody titers to identify non-responders. Combined analysis of humoral and cellular immunity improves the identification of vaccine responders among immunocompromised patients.



POS05

MESENCHYMAL STROMAL CELL-INDUCED REGULATORY B CELLS (IBREGS) AS A PLATFORM TO STUDY SPECIFIC BREG BIOMARKERS

Sergio Garcia Garcia[1;2], Noelia Sandoval Hellín[1], Inés Perezpayá[1;3], Marta Clos Sansalvador[1;2], Miriam Moron Font[1], Francesc E. Borras[1;3], Laura Cañas[1;3], Marcella Franquesa[1;3]

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Background: Regulatory B cells (Breg) are postulated as major mediators of tolerance in the context of kidney transplantation. However, the lack of specific biomarkers of Breg setbacks their application in clinical settings. Mesenchymal stromal cell (MSC) induces Breg (iBregs) while abrogating inflammatory and memory phenotypes. In this work we propose that MSC-B cell in vitro coculture may constitute an optimal platform for the discovery of Breg biomarkers.

Methods: Human B cells were isolated from tonsils and cultured with adipose tissue derived human MSC and a T cell like activating cocktail for 7 days. Cytokine secretion and expression were measured by ELISA and intracellular staining, and cell surface markers by Flow cytometry. B cell regulatory potency was assessed in T cell proliferation assays. 3 independent experiments were performed for every previous method. For biomarker discovery, transcriptomic (RNAseq) and proteomic analysis were performed. For RNA sequencing, MSC cocultured B cells were sorted based on IL-10 production to compare differentially expressed genes among IL-10+ and IL-10- fractions (N=12). For cell sorting, cytokine production was boosted by a 4h stimulation with PMA and ionomycin. For proteomic analysis, differentially expressed proteins were identified among MSC cocultured B cells and activated B cells (N=16).

Results: B cells cultures with MSC become enriched in regulatory "subsets" (iBregs) which might be explained by upregulated transitional B cell phenotype, increased expression and secretion of IL-10 and no TNFa induction. Compared to activated B cells, iBreg modulate T cell proliferation by 40% at 2 to 1 cell ratios. However, when we boost the iBregs with PMA/iono to induce IL-10 secretion and sort IL10+ and IL10- subsets, we observe no regulatory traits in the IL-10+ fraction, while IL-10- B cells are enriched in immune regulation and extracellular matrix organization. Proteomic analysis identifies over 250 proteins upregulated in MSC cocultured B cells compared to activated B cells with some interesting candidates.

Conclusions: MSC constitute an efficient in vitro Breg induction system. In addition, omics analysis of iBregs efficiently identifies potential biomarkers applicable to downstream applications, which might not be associated to the expression of IL-10.

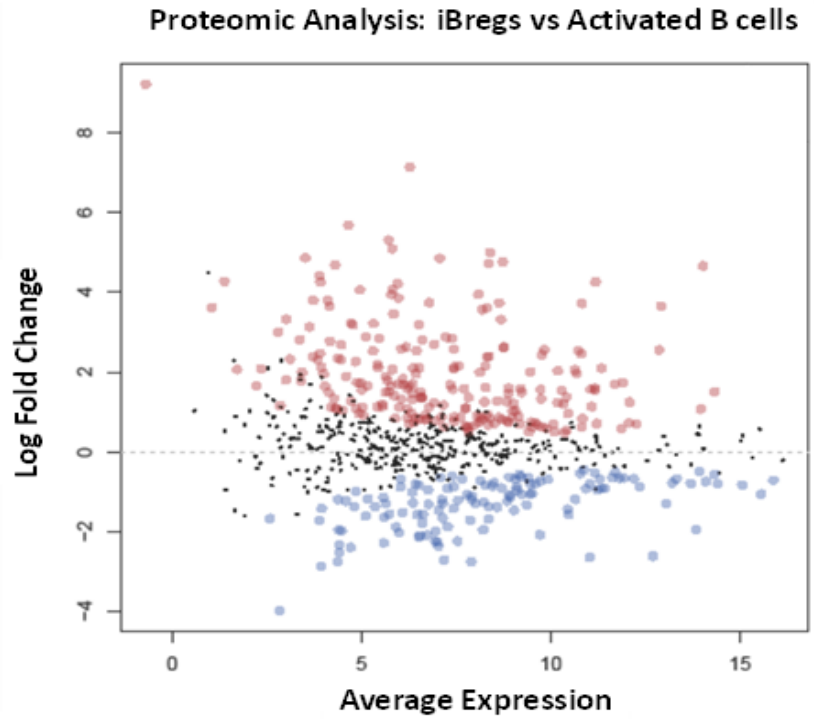


***Figure* 1**: Summarized results *from proteomic analysis (N=16) comparing MSC cocultured B cells to activated b cells*. *MA*-plot represents each protein with a dot. The x axis is the average expression over all samples, the y axis *is* the log fold change of normalized count. Differentially expressed proteins with an adjusted p-value under 0.05 are represented in red and blue for each group.

POS06

REAL LIFE INCIDENCE OF SEVERE COVID-19 IN VACCINATED KIDNEY TRANSPLANT RECIPIENTS

Diana Rodríguez Espinosa[1], Enrique Montagud-Marrahi[1], Judit Cacho[1], Carolt Arana[1], Evelyn Hermida[1], Jimena Del Risco[1], Joaquim Casals[1], Anney Rosario[1], Elena Cuadrado[1], Nestor Rodríguez[1], Aleix Cases[1], Fritz Diekmann[1], José Jesús Broseta[1], David Cucchiari[1]

[1]Hospital Clínic of Barcelona, Spain

Background: The deficient humoral response of kidney transplant recipients (KTR) to mRNA vaccination has been widely reported. However, real life data on the clinical outcomes (hospital admission and mortality) of SARS-CoV-2 infection in this population is scarce, a gap that this study aims to fill.

Methods: This is an observational prospective study where 1032 KTR and 302 haemodialysis patients from three dialysis units affiliated to Hospital Clínic of Barcelona, Spain, had received two doses of mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNTech) SARS-CoV-2 mRNA vaccines. Patients were followed since two weeks after receiving the second vaccine dose and until September, 2021. We evaluated incidence of SARS-CoV-2 infection defined by a positive RT-PCR, hospital admissions derived from infection, and a severe COVID-19 composite outcome, of either intensive ICU, mechanical ventilation, or death.

Results: A total of 44 out of 1032 (4.3%) of KTR were infected with SARS-CoV-2, where 26 (2.5%) were admitted to the hospital (2.5%), 11 (1.1%) had severe COVID-19 and 4 (0.4%) died. In contrast, despite hemodialysis patients had a non-significant higher incidence of infection (18/302; 6%), only 4 patients required hospital admission (1.3%), where just one (0.3%) presented a case of severe COVID-19, and none of them died. In the multivariable analysis, KTR had a significantly higher risk of being hospitalized than HD patients (HR 3.37, P= 0.03), and this risk increased with age and male sex (HR 4.74, P= 0.02).

Conclusions: Patients on immunosuppressive treatment are at significantly increased risk of severe COVID-19, even in comparison to another high risk population such as hemodialysis patients. These results emphasize the need for more frequent boosters in KTR than in the general population.

Table legend:
Number of detected SARS-CoV-2 infection cases and their clinical outcomes in kidney transplant recipients before and after complete mRNA vaccination. *Montagud-Marrahi E, et al. *Am J Transplant*. 2020;20(10):2958-2959.

	Kidney transplant recipients	
	Pre-vaccination (1st wave)*	Post-vaccination (5th wave)
SARS-CoV-2 infection	33/1043 (3.2%)	44/1032 (4.3%)
Hospital admissions	26/33 (78.8%)	26/44 (59.1%)
Severe COVID-19	13/33 (39.4%)	11/44 (25%)
Deaths	2/33 (6%)	4/44 (9.1%)

POS07

REGULATORY B CELLS AS A USEFUL BIOMARKER OF IMPROVED GRAFT OUTCOME IN KIDNEY TRANSPLANT PATIENTS

Inés Perezpayá[1], Sergio Garcia Garcia[2], Noelia Sandoval Hellín[2], Francesc E. Borras[2], Anna Vila[1], Marcella Franquesa[2], Laura Cañas[1]

[1]Hospital Germans Trias i Pujol, Nephrology Department, Spain, *[2]REMAR-IVECAT, Germans Trias i Pujol Health Sciences Research Institute (IGTP, Spain*

Background: In kidney transplantation, Regulatory B cells (Bregs) have been associated to patients with longer duration of graft survival and fewer rejection episodes, suggesting that Bregs might play a role as a biomarker of improved graft outcomes.

Methods: 33 kidney transplant recipients were followed up for 12 months (m) after transplantation. Pre- transplantation and at 7 days, 3 ,6 and 12m post-transplant, clinical and laboratory data were collected and peripheral blood lymphocyte populations were analyzed by flow cytometry.

We assessed total counts of Bregs (CD19+ CD24hi CD38hi), Memory B cells (CD27+ CD19+), Naive B cells (CD27- IgD+ CD19+), Total B (CD19+), and T cells (CD3+) and analyzed its association with graft outcome.

Results: The median age was 61 years-old and most were male on dialysis. The main cause of chronic kidney disease was unknown, 48.5% were HLA-sensitized before transplantation,

only one patient developed de novo HLA antibodies post-transplantation. Most patients received transplant from brain-dead donor and had more than 4 HLA mismatch incompatibility. Induction therapy received was basiliximab (51.5%) or thymoglobulin. 6 patients were on immunosuppressive treatment before kidney transplant, with lower number of Bregs prior to transplantation. The most common maintenance immunosuppressive therapy was glucocorticoids, tacrolimus and mycophenolate mofetil. Within the first 2 weeks after transplantation 27.3% presented infectious complications, with no difference on Bregs counts. 1 patient was COVID+ during the follow up**.** Kidney allograft biopsy was performed in 12 patients, only 2 had a biopsy-proven acute cellular rejection. A negative correlation between Bregs counts at 3 and 12m and creatinine at 12m was seen.

Patients who underwent biopsy per clinical indication had higher serum creatinine and proteinuria at 3, 6 and 12m compared with non-biopsied patients. There were statistically significant differences on Bregs counts at 3 and 12m, being higher in non-biopsied patients, and also on Naïve B cells at 12m. No differences were found in other subsets.

Conclusions: Bregs counts were higher in non-biopsied patients at 3 and 12m and were predictive of creatinine levels at 12m, suggesting that Bregs counts might be useful as a biomarker of improved graft outcome

	BIPOSY (n = 12)	NO BIOPSY (n=21)	P value
Age	60.5 (46-65.75	61 (54-69)	NS
Male	8 (66.7)	12 (57.1)	NS
IS treatment preTR	3	3	NS
CKD etiology			NS
Unknown	4 (33.3)	12 (57.1)	
PKD	1 (8.3)	4 (19)	
NIgA	1	1	
ND	2 (16.7)	1	
Other glomerulonephritis	4	2 (9.6)	
Other	0	1	
Dialysis	9 (75)	16 (76.2)	NS
Month in dialysis	9 (2 – 22)	9 (0.5-14.5)	NS
Type of Donor			NS
After brain death	7 (58.3)	11 (52.4)	
After circulatory death	3 (25)	8 (38.1)	
Live donor	2 (16.7)	2 (9.5)	
CIT (min)	970 (306-1321)	990 (425-1300)	NS
MM			NS
0-3	5 (41.7)	3 (14.3)	
4-6	7 (58.3)	18 (85.7)	
DGF	5 (41.7)	6 (28.6)	NS
HLA-sensitized	6 (50)	10 (47.6)	NS
Induction ttm			NS
Basiliximab	6 (50)	11 (52.4)	
Thymoglobuline	6	10	
Maintenance ttm			NS
P+FK+MMF	11 (91.7)	18 (85.7)	
P+FK+ i MTOR.	1 (8.3)	3 (14.3)	
Infect.complications < 2w	5 (41.7)	4 (19)	NS
Creatinine (mg/dL)			
1w	4,4	3,1	NS
3m	2,4	1,34	0.030
6m	1,9	1,39	0.024
12m	1,87	1,35	0.01
Proteinuria (mg/g)			
3m	912,20	231,09	0.044
6m	792,71	204,20	0.048
12m	586,45	194,73	NS
Bregs count (Nº/ uL)			
pre	3,69	6,81	NS
1w	1,96	4,39	NS
3m	0,75	5,62	0.006
6m	1,54	2,67	NS
12m	0,56	3,69	0.036

Data are presented as percentages or medians (interquartile ranges) All categorical variables were compared using the Chi-squared test and Fisher's exact testing. Continuous variables are analyzed using Student T-test if comparing 2 groups or ANOVA with Tukey post hoc test for multiple comparisons if normally distributed and the Mann-Whitney test for variables with skewed distribution.

POS08

RELEVANCE OF PROTEINURIA AND DONOR SPECIFIC ANTIBODY IN KIDNEY TRANSPLANTED RECIPIENTS AND ALLOGRAFT OUTCOMES

Amna Hashmi[1], Nóra Klenk[1], László Bidiga[2], László Kardos[3], Balázs Nemes[4], József Balla[5], Réka P.Szabó[5]

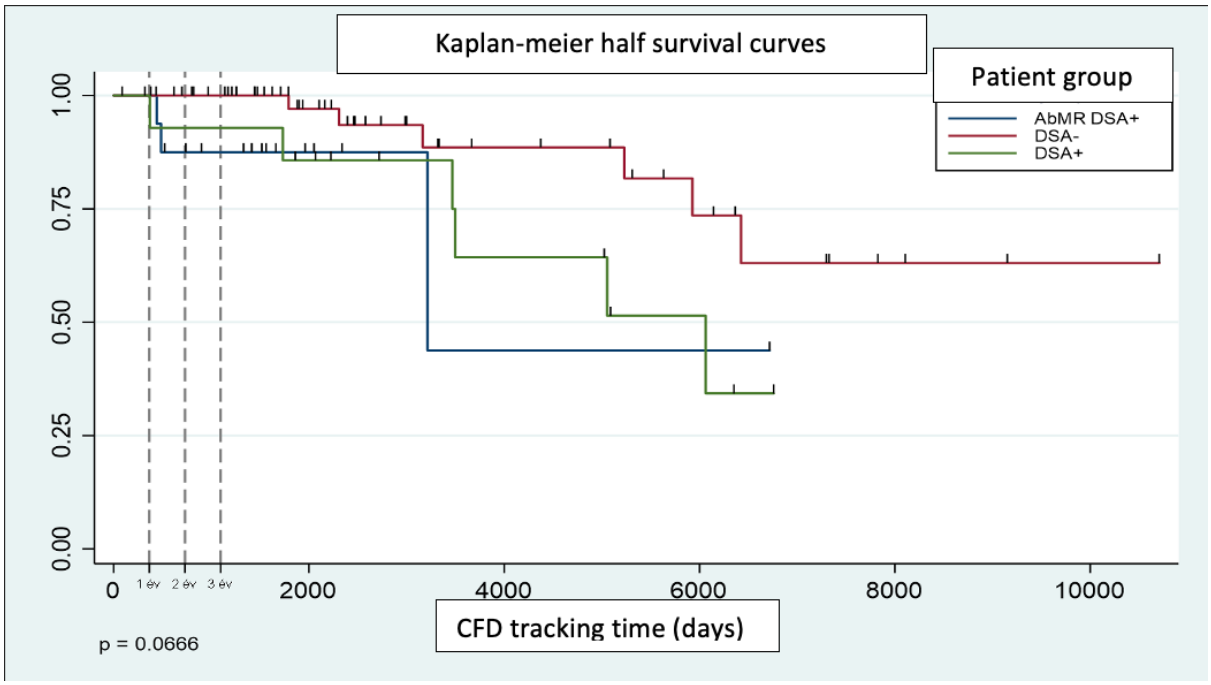
[1]University of Debrecen Medical School, Debrecen, Hungary, *[2]University of Debrecen Medical School, Institute of Pathology, Debrecen, Hungary*, *[3]University of Debrecen Medical School, Institute of Infectology, Debrecen, Hungary*, *[4]University of Debrecen Medical School, Institute of Surgery, Department of Transplantation, Debrecen, Hungary*, *[5]University of Debrecen Medical School, Institute of Internal Medicine, Department of Nephrology, Debrecen, Hungary*

Background: The onset of proteinuria in renal allograft recipients is a frequent complication that may be associated with an increased risk of graft failure and mortality. Our aim was to research if proteinuria effects is independent from donor specific antibody (DSA) for transplant graft survival and the changes of proteinuria response to therapy in ABMR group

Methods: 85 transplanted patients were enrolled in our study and followed up till 31.10.2020 or till death, or date of return to dialysis. We created 3 groups: ABMR group (n=19, biopsy proven antibody mediated rejection), $ABMR_{DSApos}$ positive group (n=14) $ABMR_{DSAneg}$ (n=5), DSA negative group with stabile kidney function without rejection as a reference (n=52). Differences in patient, donor and transplant characteristics between DSA positive and negative groups were assessed by Fishers exact test for categorical variables. Death censored graft loss was assessed by Kaplan Meier analysis with log risk statistics.

Results: Proteinuria decrease after treatment in ABMR group (p=0,0009). Graft failure's frequency increase every 10 mg/mmol elevation of proteinuria means 7 % elevation (hazard ration is 1,07%). Before treatment nephrotic proteinuria was found in group $AMBR_{DSApoz}$ 21 %, 14,29% in group ABMR $_{DSAneg}$ 1,92% in reference group. Estimated 3-year graft survival was 87, 5% in ABMR group, 93 % in DSA pos group, and 100 % in DSA negative group (log-rank probe p=0,0666).

Conclusions: The presence of DSA increases graft loss but it is independent to proteinuria. Therapy refractory proteinuria state represents worse graft survival



POS09

CYP3A5 POLYMORPHISM AND TACROLIMUS PHARMACOKINETICS IN PATIENTS UNDERGOING RENAL TRANSPLANT

Amit Pasari[1], Manish Balwani[1;2]

[1]sarswati Kidney Care center, Department of nephrology, Nagpur, India, *[2]JNMC , Department of nephrology, Wardha, India*

Background: Tacrolimus is essential part of management of renal transplant recipients.

CYP3A5 polymorphism affects tacrolimus metabolism. We assessed CYP3A5 polymorphisms in patients undergoing renal transplantations.

Methods: In a retrospective analysis, we assessed patients for CYP3A5 polymorphisms. We considered tacrolimus trough levels of 7 to 10 ng/ml at one-month post-transplant as normal. All patients had received rabbit anti-thymocyte globulin for induction therapy and were on standard triple immunosuppression.

Results: In this retrospective, observational study, we identified 11 (84.6%) of 13 cases having positive polymorphisms. Out of 11 patients, three did not receive tacrolimus. In the remaining eight patients, 4 (50.0%) each had homozygous and heterozygous mutation. In the two groups, therapeutic tacrolimus level was achieved with a mean tacrolimus dose of 0.041 mg/kg and 0.037 mg/kg, respectively.

Conclusions: In our geographical area, prevalence of CYP3A5 polymorphism is substantially high. All patients undergoing renal transplant should be screened for this polymorphism as it will help determine the optimal tacrolimus dose.

POS10

EFFECTS OF REGULATED CELL DEATH ON EARLY HEPATIC ISCHEMIA REPERFUSION INJURY IN STEATOTIC DONOR ORGANS

Amoon Kasi[1], Alicia Goreth[1], Miriam Banas[1], Simone Reichelt-Wurm[1], Bettina Proneth[2], Alexander Kroemer[3], Hans Schlitt[1], Edward K. Geissler[1], Elke Eggenhofer[1]

[1]University Hospital Regensburg, Regensburg, Germany, *[2]Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), München, Germany*, *[3]MedStar Washington Hospital Center, Washington, United States*

Background:

Up to 7 % of transplanted livers develop graft dysfunction. One of the underlying mechanisms is the ischemia reperfusion injury (IRI). During the process of IRI, signalling pathways of regulated cell death (RCD) are activated. These include the caspase-dependent pathway apoptosis and the caspase-independent pathways necroptosis and ferroptosis.
Due to growing discrepancy in the number of organ donors and recipients, steatotic livers are transplanted more frequent. These marginal organs may show increased susceptibility to IRI. Therefore, this study investigates the influence RCDs on early IRI in steatotic donor organs .
Methods:

In this study an in vitro model of IRI in marginal organs was established. Fatty and non-fatty Human HepaRG™ cells underwent hypoxia for 48 h to simulate ischemia. Subsequently, a reperfusion simulation was performed. In addition, these cells were co-cultured before hypoxia treatment with inhibitors of the mentioned pathways of RCD.
Specific proteins for each pathway were measured using Western Blot, their gene expressions was analysed by PCR. Accumulation of Reactive Oxygen Species (ROS) was visualized by Flow Cytometry.

Results:

Significant differences in the expression of the RCD markers and in the accumulation of ROS were detected between steatotic and non-steatotic liver cells. These results were validated for apoptosis, necroptosis and ferroptosis in Western Blot, PCR and Flow Cytometry.

Conclusions:

The results show that all analysed pathways of RCD are activated during IRI, however none of them dominates. Presumably all pathways interact in a complex way. Marginal organs were affected more by IRI and RCD which could lead to a higher risk of organ failure. This opens up new therapeutic options to improve liver transplantation results concerning especially steatotic livers.

POS11

NOVEL USE OF MESENCHYMAL STROMAL CELLS IN THE TREATMENT OF PRECISION CUT LUNG SLICES

Anna Niroomand[1], Franziska Olm[2], Sandra Lindstedt[2;3]

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Background: Despite a high demand for available organs, many donor lungs are rejected for transplantation due to underlying injury. Acute respiratory distress syndrome (ARDS) and acute lung injury are major causes of the bottleneck on donor lung availability. Given limited options, new therapies to render rejected donor lungs viable must be explored.

Mesenchymal stromal cells (MSCs) are immunomodulatory and possess a tissue regenerative capacity. They have also been specifically shown to improve the function of diseased lungs. It is still difficult, however, to study the exact mechanisms of MSCs interaction with the target tissue. An in vitro model of ARDS to study MSCs would therefore be a great advancement. Precision cut lung slices (PCLS) have previously been implemented as an *ex vivo* method to study chronic diseases as they preserve native lung architecture and facilitate the study of the cells in their native environment. This study hypothesizes that PCLS can be used to study MSCs as a treatment for tissue damaged by ARDS.

Methods: Lung tissue was derived from healthy tissue and from a porcine model of ARDS induced by *E.coli* lipopolysaccharide as confirmed with blood gas values and histological examination. Tissue was filled with agarose (healthy and ARDS control groups) or with agarose mixed with MSCs (treatment group). MSCs derived from bone marrow were cultured in basal medium with 10% pooled platelet lysate and then immunofluorescently labelled before addition at a concentration of $10x10^6$ cells per 50 mL of agarose. 4 mm punches were generated with a vibratome with a thickness of 500 mm and kept in culture for 5 days.

Results: The cells were found to be homogenously distributed throughout the punches and could be identified over the entire 5-day period with confocal microscopy. Furthermore, the punches showed metabolic activity comparable to healthy controls. Blinded histological scoring for lung injury of the MSC-treated group unveiled significantly improved histopathology relative to ARDS control individuals.

Conclusions: The interaction of MSCs to ARDS-damaged lung tissue can be studied using the in vitro technique of PCLS. This facilitates the study of the mechanism of action of this potential therapy for ARDS and would allow for a deeper understanding of how they may treat patients with severe lung injury.

POS12

A NOVEL BI-SPECIFIC FUSION PROTEIN WITH CTLA4IG/PDL2 AS A NOVEL IMMUNOSUPPRESSANT MOLECULE MODULATING T-CELL ALLOIMMUNE RESPONSES

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Background:
Both the CD28-CD80/86 costimulatory and the PD-1-PD-L1/L2 coinhibitory pathways have shown to control adaptive immune responses modulating effector T cells activation and Tfh and mBC survival in solid organ transplantation.

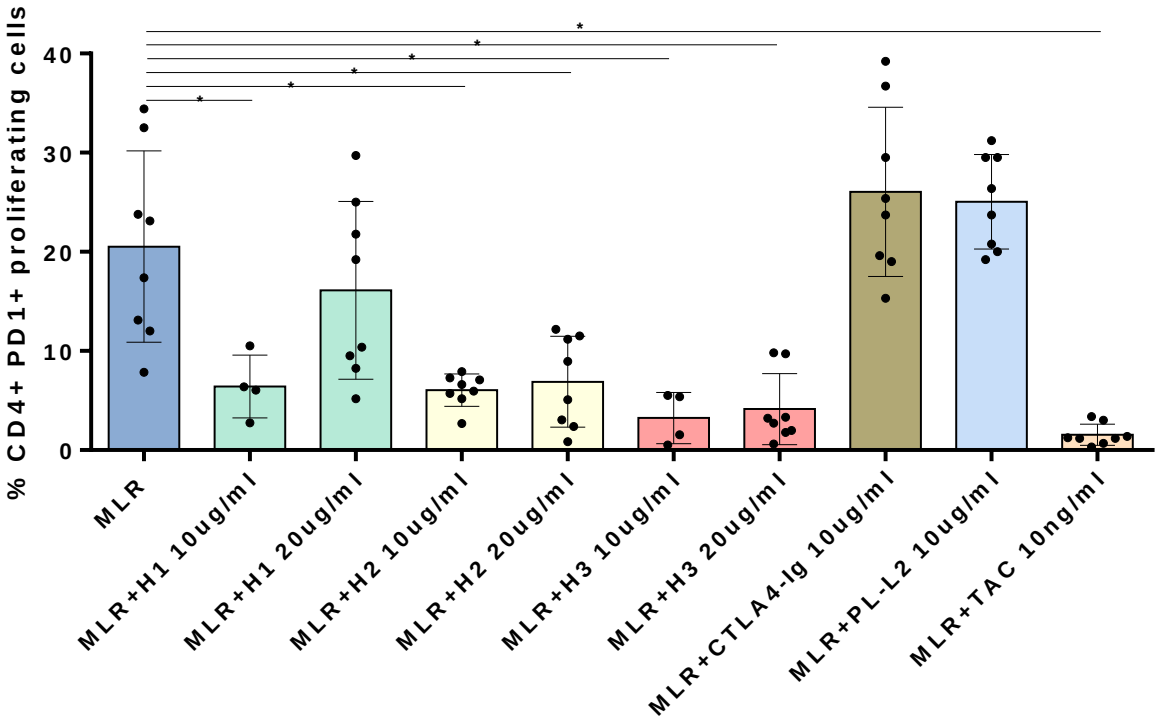
Methods:
We developed a hybrid recombinant bi-specific fusion protein (Hybri) that concomitantly blocks the CD80/86 pathway with a human CTLA4-Ig extracellular domain and stimulates PD-1 with a human PD-L2 extracellular domain (EP19382017.2).
Three different constructs of Hybri2 using different length and flexibility linkers (H1, long flexible; H2, short rigid; H3, long rigid) were evaluated for their binding capacity to each respective ligand in Surface Plasmon Resonance (SPR) studies and their capacity to inhibit alloimmune responses in vitro in mixed lymphocyte reactions (MLR) (n=8) by Flow Cytometry as compared to calcineurin inhibitors (Tacrolimus, TAC 10ng/ml), the costimulatory blockade Belatacept and PD-L2 fusion protein.

Results:
SPR analyses confirmed that all Hybri variants displayed high avidity of each protein (CTLA4-Ig and PDL2) for their respective ligands by low dissociation constants (KD).
In vitro, proliferation of CD3+ T cells was significantly inhibited by H1, H2 and H3 when used at different concentrations. H3 outperformed the capacity of T cell inhibition than other Hybri and immunosuppressants [6.185 (5.17, 8.01) vs 1.345 (1.08, 1.58) vs 1.490 (1.25, 1.94) % of CD3+ proliferating T cells in MLR, MLR+H3 10ug/ml and MLR+H3 20ug/ml, p=0.007 and p=0.001, respectively] and [3.595 (2.81, 4.29) and 0.815 (0.59, 1.28) % of CD3+ proliferating T cells for CTLA4-Ig and TAC, p=0.005 and p= 0.001 when compared to untreated MLR, respectively], whereas PD-L2 did not inhibit the MLR. Notably, when evaluating their effect on proliferating CD4+ PD1+ cells, H3 showed the highest inhibitory capacity [20.250 (12.82, 25.97) vs 3.465 (1.29, 5.41) and 2.950 (1.92, 4.91) % of CD4+ PD1+ proliferating T cells in MLR, MLR+H3 10ug/ml and MLR+H3 20ug/ml, p=0.007 and p=0.002, respectively] whereas

CTLA4-Ig failed to show any inhibitory effect in this T cell subpopulation (Fig 1).

Conclusions:

A novel dual co-stimulation blockade (CTLA4Ig/PDL2) targeting both CD28-CD80/86 and PD1-PDL1/PDL2 signals shows high potential to effectively abrogate cellular alloimmune responses.



POS13

ADVANCED MALIGNANCIES TREATED WITH CTLA-4 ANTAGONIST AND PD-L1 INHIBITORS INTRANSPLANT PATIENTS: THE GRAFT OUTCOME. A SYSTEMATIC REVIEW OF LITERATURE.

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Background: A major cause of morbidity and mortality in transplanted population is cancer; the overall risk is increased two to three times compared to the general population of the same age and gender; it is inversely related to age, with younger recipients experiencing a much greater relative increase in risk compared to older recipients. Moreover, the prognosis for recipients diagnosed with cancer is worse compared to the general population, due to the immunosuppressive drugs. The aim of this study, part of the project “The dynamics of Cancer Staminal Cells and Circulating Tumor Cells in a chimera subject: the solid organ transplant recipient who develops a neoplastic lesion”, is to establish a correlation between use of CTLA-4 antagonist and PD-L1 inhibitors in transplanted patients with de novo neoplasm and the risks for the grafts.

Methods: From an analysis of the literature on the use of anti PD-1 and CTLA-4 antagonists in transplanted patients with advanced cancer, a database of 52 patients was populated. The analyzed variables were: age, sex, transplanted solid organ, immunosuppressive induction and maintenance therapy performed, type of neoplasm developed, developmental latency time, anticancer drugs administered, possible rejection and related therapy administered, patient outcome.

Results: with a statistical analysis it was demonstrated that the use of PD-L1 inhibitors

was not significantly associated with a risk of development of graft rejection. However, the same analysis has shown that there is a marginally advantage in terms of rejection risk with the use of CTLA-4 antagonists (OR = 4.00, p = 0.06). Female sex was found to be a protective factor against cancer. The other variables did not show a statistically significant correlation.

Conclusions: The available data suggest that CTLA-4 antagonists are safer in transplant patients and that PD-1 inhibitors are associated with a higher risk of allograft rejection. A factor responsible for different outcomes between patients treated with PD-L1 inhibitors and CTLA-4 antagonists has not been identified. In conclusion, checkpoint inhibitors have proved to be valid for treatment of various malignant tumors, but their safety in transplanted cancer patients remains unclear.
Undoubtedly, more studies are needed in this specific population of patients.

POS14

DEFINITION OF RENAL INFLAMM-AGEING – IMPLICATIONS FOR ALLOGRAFT QUALITY AND SURVIVAL

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Background:
Donor age is a major risk factor for allograft outcome in kidney transplantation. The underlying cellular mechanisms and the recipient´s immune response within an aged allograft have not yet been analyzed.

Methods:
Herein, we performed a comprehensive immunophenotyping of naïve and transplanted young C57BL/6 versus aged C57BL/6 kidneys by flow cytometry.

Results:
Naïve aged murine kidneys harbor significantly higher frequencies of effector/memory T cells, whereas regulatory T cells were reduced. Aged kidney-derived CD8+ T cells produced more IFNγ than their young counterparts. Senescent renal CD8+ T and NK cells upregulated the cytotoxicity receptor NKG2D and the enrichment of memory-like CD49a+CXCR6+ NK cells was documented in aged grafts. In the C57BL/6 to BALB/c kidney transplantation model, recipient-derived T cells infiltrating an aged graft produced significantly more IFNγ, granzyme B and perforin on day 7 post-transplantation, indicating an enhanced inflammatory, cytotoxic response towards the graft. Pre-treatment of aged kidney donors with the senolytic drug ABT-263 changed the recipient-derived effector molecule profile to significantly reduced levels of IFNγ and IL-10 compared to controls. Graft function after ABT-263 pre-treatment was significantly improved 28 days post kidney transplantation.

Conclusions:
In conclusion, renal senescence also occurs at the immunological level (inflamm-aging) and aged organs provoke an altered recipient-dominated immune response in the graft

POS15

DIRECTING TRYPTOPHAN METABOLISM MITIGATES MURINE HEPATIC ISCHEMIA REPERFUSION INJURY

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Background: Hepatic ischemia/reperfusion injury (IRI) is the leading cause of early graft dysfunction and contributes to the shortage of donor liver grafts. However, despite its clinical importance, therapies to prevent or treat this condition remain elusive. Indoleamine 2,3-dioxygenase (IDO) catalyzes the catabolism of the essential amino acid tryptophan to the product kynurenine and is well known for inducing a powerful immunosuppressive metabolic programming and promoting the restoration of homeostasis. To date, studies have mostly focused on prolonging graft survival by overexpressing IDO in the transplanted tissue. Here we focus on evaluating the efficacy of systemic IDO therapy to control the local hepatic inflammatory response of mouse IRI by administering PEGylated IDO (PEG-IDO) as a means of reducing its immunogenicity and extending its circulation time.

Methods: Male 8-12 week old Balb/c mice were separated into 3 cohorts subject to different intravenous treatment; PEGylated-IDO, IDO, and phosphate buffered saline (PBS). 48 hours

after administration mice were either subject to a sham operation or a well-established model of partial (70%) hepatic IRI with 90 minutes of ischemia and 6 hours of IRI.

Results: PEGylated-IDO significantly improves hepatic IRI. Plasma levels of aspartate alanine aminotransferase and alanine aminotransferase at 6 hours after reperfusion were significantly lower in the PEG-IDO group, when compared with those in PBS and IDO treatment groups. PEG-IDO treated livers showed significantly less histological evidence of congestion, necrosis, and vacuolization. Systemic PEG-IDO therapy also decreased the local hepatic infiltration of the inflammatory CD3+ T cells, Ly-6G+ neutrophils, and CD68+ macrophages, and reduced the expression of proinflammatory IL-6, TNF-α, IL-1β *and* IFN-γ. Furthermore, as measured by the number of TUNEL+ hepatocytes, apoptosis was also suppressed in PEG-IDO treated mice.

Conclusions: The results in this study indicate that redirecting tryptophan immunometabolism via systemic PEG-IDO therapy protects livers from hepatic IR induced damage. This metabolic immune-modulatory strategy represents a new class of anti-inflammatory/immunosuppressive biologic drug for the treatment of liver inflammatory disorders.

POS16

DRUG RESISTANT CYTOMEGALO-VIRUS: A CASE REPORT OF RARE MUTATION IN EARLY PERIOD AFTER KIDNEY TRANSPLANTATION

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Background: Cytomegalovirus (CMV) infection can be refractory to antiviral treatment and represents a challenge for the management of kidney transplant patients. We present a case report of a rare CMV gene mutation in the early period after kidney transplantation.
Case Report**:** On July 13, 2021, a 27-year-old patient (CMV IgG negative) received a kidney from a deceased donor (CMV IgG positive). Immunosuppression consisted of basiliximab induction, tacrolimus, mycophenolate, and methylprednisolone (discontinued on day 5). Standard prophylaxis with valganciclovir was initiated immediately after transplantation.
Two months later (October 15, 2021), mild leukopenia (3.4) with lymphopenia (0.96) occurred for the first time. On October 29, 2021, he became ill with fever, malaise, and diarrhoea. At that time, leukocytes were 1.4, neutrophils 0.52, and lymphocytes 0.67; PCR DNA CMV copies were 125,000 IU/ml, with CMV QuantiFERON reactive 0.22 IU/ml (cut off 0.2 IU/ml). Genotypic resistance to antiviral drug was positive in the UL54 gene, and a T503I mutation was detected (resistance to cidofovir and ganciclovir has been described in the literature for this mutation).
In addition to bone marrow involvement, invasive tissue infection (CMV gastritis and colitis) was detected. The patient was treated with a granulocyte stimulator (corresponding to the number of neutrophils), intravenous ganciclovir in full dose 5 mg/kg per body weight twice daily and received anti-CMV immunoglobulins. Mycophenolate was discontinued and everolimus was introduced. After 4 weeks of therapy, the clinical condition improved completely, and plasma CMV copies decreased to 593 IU/ml. At discharge, letermovir 480 mg once daily was prescribed instead of valganciclovir. The patient is currently in good clinical condition and continues to take letermovir. On December 13, PCR DNA CMV was 488 IU/ml, and 63 IU/ml on January 13, 2022.
Conclusions: Despite adequate prophylaxis with valganciclovir, early CMV infection with a rare gene mutation was recently observed after kidney transplantation. The combination of ganciclovir, anti-CMV immunoglobulins, and switching to everolimus and letermovir helped to successfully manage the infection. Factors contributing to drug resistance remain to be determined.

POS17

EFFECT OF BRAIN-DEAD DONOR BLOOD COMPONENTS ON GENERATION OF DONOR SPECIFIC IMMUNOMODULATORY CELLS

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Background: Peripheral tolerance induction by adoptive transfer of ex-vivo generated donor specific immunomodulatory cells (DSIMC) has shown promise in liver transplantation from living donors. Application of this strategy in brain-dead donor (BDD) liver transplantation is currently being explored. Brain death is associated with a systemic release of inflammatory cytokines. Therefore, plasma and cells from deceased donors may negatively

affect the generation of DSIMC. In this study we examined how BDD blood components affect cell culture viability and generation of DSIMC, compared to components sourced from healthy controls (HC).

Methods: Baseline plasma cytokine profiles were examined using a multiplex immunoassay. Peripheral blood mononuclear cells (PBMC) were cultured with 1-10% plasma from BDDs or from HCs and the viability and cytokine production was subsequently examined. The viability of BDD-PBMC and HC-PBMC was also compared during culture. Separately, the effect of BDD and HC plasma on generation of DSIMC was compared by co-culturing recipient and irradiated donor PBMC in the presence of belatacept and 1% plasma. After two weeks the supernatant and generated cells were characterized using immunoassay and flow cytometry.

Results: IL-6, IL-8 and MCP-1 were increased in BDD plasma. PBMCs cultured with 5% BDD plasma had significantly lower cell numbers, viability, and capacity to produce IFN-γ compared to those cultured with HC plasma ($p < 0.0001$, n=30 in each group). Addition of BD-plasma suppressed cell proliferation in mixed lymphocyte culture in a dose dependent manner. After two hours, BDD-PBMC numbers had reduced significantly more (84% loss in BDD-PBMC vs 17% loss in HC-PBMC) and exhibited lower IFN-γ production upon activation. The culture supernatants of DSIMCs generated with BDD plasma expressed lower levels of IFN-γ compared to controls, though no significant differences were observed in the yield and viability, or frequencies of regulatory T-cells.

Conclusions: BDD plasma exhibited a cytotoxic effect and BDD-PBMC had inferior viability and function compared to HC-PBMC. BDD sourced blood components interferes with immune response, which may compromise the generation of DSIMC.

POS18

IMMUNE MECHANISMS CONTROLLING ALLOREACTIVE CD8+ T CELL AVIDITY MATURATION DURING SOLID ORGAN ALLOGRAFT REJECTION

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Background:
In a mouse model of cardiac transplantation tolerance induced by costimulation blockade (anti-CD154) and donor-splenocyte transfer, we have previously reported that alloreactive T cells fail to expand their high avidity clones at the population level, in stark contrast to the expansion of high avidity alloreactive T cells during acute rejection. We termed the latter phenomenon avidity maturation. The functional consequences and cellular mechanisms that mediate avidity maturation at the T cell population level remain unknown. Understanding the discrete events that promote the expansion of high affinity T cells during graft rejection may help devise better strategies to control alloimmunity in the clinic.

Methods:
To investigate the role of T cell populations with different affinities for alloantigen, we employed adoptive cell transfer studies using $CD8^+$ TCR-transgenic T cells with high (OT-1) and low affinity (OT-3) specific for the same model peptide within ovalbumin (OVA) in a mouse model of OVA-expressing skin transplantation. TCR-Tg cells were reisolated for immunophenotyping by flow cytometry or functional studies ex vivo.

Results:
High affinity OT-1 T cells were more potent than low affinity OT-3 T cells at rejecting skin grafts in lymphoreplete mice. Moreover, high affinity OT-1 T cells preferentially expanded in a competitive environment created by the addition of low affinity OT-3 T cells. This effect was confirmed in immunocompetent mice, as adoptive transfer of OT-3 T cells failed to significantly alter the avidity maturation of the endogenous OVA-alloresponse. High affinity $CD8^+$ T cells expressed more CD25 compared to their low affinity counterparts, raising the hypothesis that they may better compete for IL-2 making them more poised to expand. Finally, we show that Tregs preferentially suppress lower affinity T cells, suggesting that Tregs may indirectly help promote the expansion of high affinity clones.

Conclusions:
During graft rejection, T cell clones with high affinity for alloantigen preferentially expand and may be helped by their lower affinity counterparts through competition for autocrine growth factors. Tregs preferentially constrain low affinity clones, implying that they are important for promoting avidity maturation in vivo.

POS21

NANODIAMOND-DOXORUBICIN COMPLEXES IMPROVE HEPATIC IISCHEMIA REPERFUSION INJURY

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Background: Oxidative stress is a major mediator of hepatic ischemia reperfusion injury (IRI) in liver transplantation (LT). Doxorubicin (DOX) can mitigate oxidative stress, but its clinical use is hampered by significant systemic toxicity. Nanodiamonds (ND) are carbon nanoparticles with potential to be high-affinity carriers for the selective delivery of anthracyclines, such as DOX, and thus reduce the negative effects of systemic delivery. Here we aim to characterize ND-adsorbed DOX (NDX) uptake and evaluate its efficacy in a mouse model of hepatic IRI.

Methods: ND solution was mixed with aqueous DOX and altered to produce NDX complexes. Uptake of DOX, NDFITC, and NDX were tested on THLE-3 hepatocytes. Balb/c mice were divided in to 6 groups and treated *i.v.* with high (1mg/kg) and low (0.5mg/kg) DOX, high (eq. 0.5 mg/kg DOX) and low (eq. 1mg/kg DOX) NDX, ND and PBS for 48 hours prior to our model of 90min partial (70%) warm hepatic IRI.

Results: ND/NDX uptake in hepatocytes was localized to the cytoplasm without reducing cell viability. In contrast, DOX located to the nucleus of hepatocytes and significantly reduced cell viability. NDX therapy mitigated hepatic IRI with only half the required dose of DOX. Plasma ALT levels after 6h of IRI were significantly lower in mice treated with high DOX, low NDX and high NDX when compared to mice treated with low DOX, ND and PBS. NDX therapy reduced the extensive liver damage, the upregulated inflammatory cytokine expression and the increased leukocyte infiltration present in livers of ND, PBS and low DOX treated mice. Finally, an echocardiogram analysis of mouse hearts revealed improved features of cardiotoxicity in ND and NDX treated mice when compared to DOX treated mice.
Conclusions: We establish that NDs form efficient NDX complexes with DOX that are readily taken up by hepatocytes with minimal toxicity. Moreover, NDX complexes enhance the physiological impact of DOX, protecting mice from hepatic IRI with half the DOX, providing a rationale for more effective use of DOX in LT.

POS22

NEOPLASTIC RISK IN SOLID ORGAN TRANSPLANT RECIPIENTS: MONOCENTRIC POPULATION ANALYSIS

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Background: Kidney transplantation is the gold standard treatment to manage end-stage renal disease (ESRD) but long-term exposure to immunosuppressive drugs, has been shown to negatively affect the antiviral and antitumor immunosurveillance capacity and enhance the carcinogenic effect of some risk factors. Transplanted patients have an overall risk of cancer increased two to three times compared to the general population of the same age and gender and the risk of post-transplant cancer is increased about 40% for those with previous cancer. Immunosuppression can also promote uncontrolled viral replication, related to oncogenesis for some viruses.

Methods: A sample of 462 patients from a single Northern Italy Transplant Center was considered, all transplanted between 1 January 2010 and 24 December 2020. Data were collected from clinical records relating to transplantation and post-transplant outpatient visits.
To calculate the neoplastic risk, we allowed only the first diagnoses of cancer, classified using the International Classification of Disease vers. 10 (ICD-10). Control group was straightened out from the population of the province of Varese of the same age and sex.

Results: Patients that underwent kidney transplantation showed an overall risk of cancer that was about three times that (SIR 2.8; 95% CI 1.8-4.3) compared to control group, with an overall risk for males of about double (SIR 2.3; 95% CI 1.3-4.0) and for females over triple (SIR 3.7; 95% CI 1.8-7.5). Significantly increased risks in both sex were observed for Kaposi's Sarcoma (KS) (SIR 195), Lymphomas and Leukemias (SIR 6.8). The incidence rates for KS and Lymphomas were

largely attributable to transplantation, while for solid tumors the incidence attributable to transplantation was just over one third of the overall incidence.
In conclusion, 99% of KS cases and 85% of Lymphomas cases were attributable to transplantation, instead only 39% of solid tumors were attributable to transplantation.

Conclusions: The results of our study validate the concept that an association between immunosuppression and cancer might exist. Knowing the incidence and prevalence of oncological diseases in the transplanted population could allow an early diagnosis and guarantee the best treatments when neoplastic diseases are more susceptible to hit.

POS23

NOVEL IN SITU HYBRIDIZATION AND MULTIPLEX IMMUNOFLUORESCENCE TECHNOLOGY COMBINED WITH WHOLE-SLIDE DIGITAL IMAGE ANALYSIS IN LIVER TISSUE

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Background: Assessing immunological processes in situ within single cells would represent a step forward to improve tissue diagnostics and treatment. Here, we present a proof-of-concept study using in situ hybridization in combination with multiplexed immunofluorescence (mIFISH) to detect low-abundance cytokines and perform single cell phenotyping in formalin-fixed paraffin-embedded (FFPE) human liver tissue. The aim of the study was to validate novel mIFISH technology versus standard assays including immunohistochemistry (IHC), chromogenic ISH (cISH/RNAscope) and qPCR and applied it to liver allografts to explore leukocyte activation, cytokine expression and periportal fibrosis.

Methods: FFPE tissue of NASH livers (n=3), non-NASH livers (n=3) and tonsils (n=3) were assessed for $CD45^+$ leucocyte infiltration and CXCL9 mRNA expression levels with mIFISH, IHC, cISH and qPCR. Liver allograft biopsies from six acute cellular rejection (rejection activity index (RAI) = 6-9), nine subclinical inflammation (RAI = 1-2) and six normal cases (RAI = 0) were labelled as follows: CXCL9.CD45.GZMB and TGF-β_1.CD68.αSMA. Quantitative analysis of histology was performed with an automatic machine-learning algorithm on whole-slide sections. Cell density was expressed as cells/mm^2; mRNA expression levels using ISH were expressed as the dot (each representing one copy) area in $\mu m^2/mm^2$.

Results: mIFISH analysis strongly correlated with IHC for CD45 ($r = 0.99$, $p < 0.001$), with cISH for CXCL9 ($r = 0.99$, $p < 0.001$) and with mRNA expression levels detected by qPCR (CD45 $r = 0.78$, $p = 0.014$; CXCL9 $r = 0.82$, $p = 0.007$). Preliminary semi-quantitative analysis of mIFISH technology in subclinical inflammation showed similar CXCL9 expression levels as in acute cellular rejection. Expression of TGF-β_1 and periportal fibrosis was higher in subclinical inflammation than in normal cases (TGF-β_1 $p = 0.001$, fibrosis $p = 0{,}016$). GZMB showed no difference between subclinical inflammation and normal cases ($p = 0.187$).

Conclusion: The mIFISH assay is reliable compared to gold standard assays such as IHC or qPCR, with the distinct advantage of being able to assess multiple immunological parameters within single cells in the liver. It may be a powerful tool to better understand subclinical allograft rejection.

POS25

REGULATORY T CELLS SUPRESS MEMORY IFN-GAMMA PRODUCTION IN HIGHLY SENSITISED PATIENTS

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Background:
Highly sensitized patients (HS) present worse long-term outcome after transplantation compared to those without donor-specific antibodies (DSA). It has been suggested that desensitisation using anti-CD19 depleting agents do not have satisfying long term outcomes, as they remove B cells with a regulatory phenotype (“Bregs”). *In vitro* anti-donor IFNγ production correlates with progression of graft dysfunction and fewer

regulatory Tcells (Tregs) in patients with chronic rejection. This project aims to understand B cells/Tregs interactions in HS patients.

Methods:
We prospectively recruited HS patients on dialysis, isolated their Tregs and expanded these cells using established protocols (Interleukin-2 + Rapamycin). IFNγ production by CD8-depleted PBMC (+/- additional depletion of CD19+ cells) in response to HLA proteins (PureProt®) was tested in Fluorospot to assess the memory immune alloresponse at baseline and when Tregs were added.

Results:
Out of 16 patients recruited, 10/16 patients (63%) had a background of transplantation with a nephrectomy in 4/10 and 4/10 (25%) were still receiving immunosuppressive drugs. We managed to expand Tregs from 11 patients. Three patients had IFNγ production in CD8-depleted PBMCs challenged with an HLA protein they had been sensitised to (HLA Specific Reactivity group = "HLA SR"). Autologous *ex vivo* expanded Tregs regulated IFNγ production in 1/3 patients (Figure 1A). When CD19- were depleted, 5/10 patients presented an increase of IFNγ production (4 of those with no response from CD8-depleted PBMC) (Figure 1B). Interestingly, autologous *ex vivo* expanded Tregs managed to regulate IFNγ production in 5/5 (100%) of these patients.

Conclusions:

Autologous *ex vivo* expanded Tregs are able to regulate IFNγ production in HS patients when the B cells are depleted, but not when the B cells are present. This demonstrates complex interactions between B cells, Tregs and Teffectors. The determinants of these molecular relationships are currently under investigation.

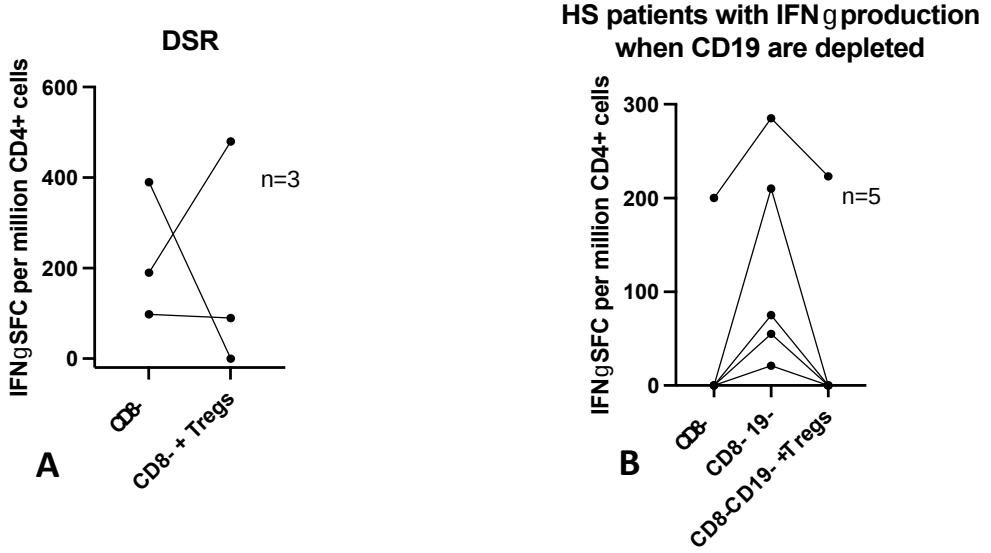

Figure 1: IFN-gamma production by PBMCs from HS patients in response to stimulation with HLA proteins they have been sensitized to. In (A), there is a reduction of IFN-gamma production in 1/3 patients when autologous Tregs are added. In (B), there is an increased of IFN-gamma production when CD19- are depleted, which is reversed when autologous Tregs are added.

POS26

SINGLE CELL ALLOREACTIVE TCR REPERTOIRE PROFILING

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Background: We identified >40 K^b-peptide epitopes directly recognised by alloreactive CD8 T cells from B10.BR mice (H-2^k). Here, we integrated two approaches to profile the alloreactive T cell repertoire (Fig 1a).

Methods: B10.BR were primed with a K^b-expressing skin graft and boosted by inoculation with AAV-K^b. The first approach used index-sorting of single dextramer-positive

cells, nested PCR and Sanger sequencing of paired TCR; in the second, BD Rhapsody library preparation from captured T cells was combined with Illumina sequencing for paired TCR and targeted transcriptome analysis.

Results: Repertoires for 3 dominant epitopes (K^b-SNY, K^b-RTY and K^b-VGP) were determined (Fig 1b). ab diversity scored significantly less for epitope-specific T cells (K^b-SNY 47.3, K^b-RTY 69.5, K^b-VGP 279.5), than for bystander cells (78730). Repertoires comprised both private TCR clones and public meta-clonotypes. Some CDR3b combined with several CDR3a, and cross-reactive TCRs were present (Table).

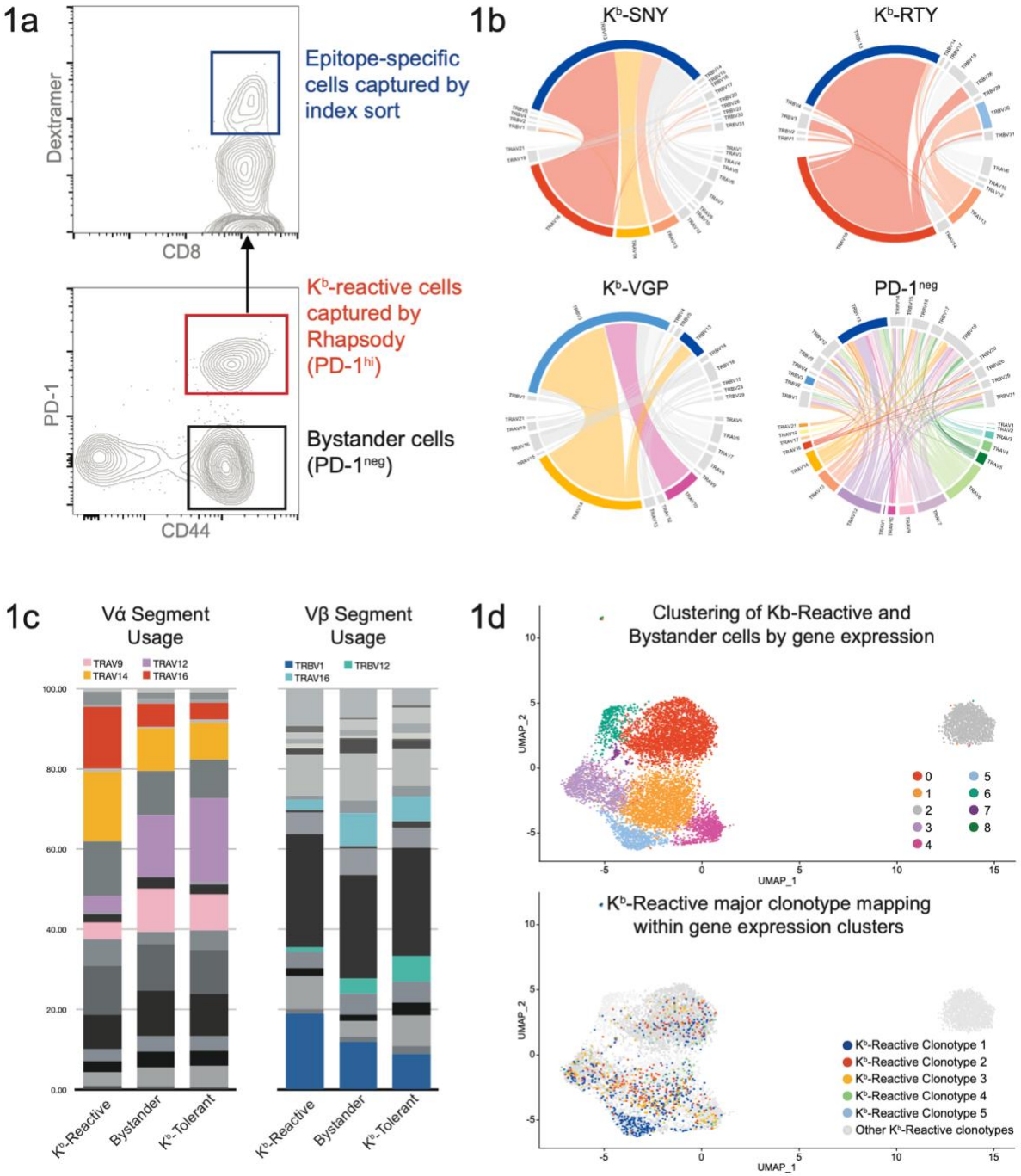
Tabl

TRAV	TRAJ	CDR3a	TRBV
TRAV16D/DV11*03	TRAJ49	AMREANTGYQNFY	TRBV13-2
TRAV16D/DV11*03	TRAJ49	AMREGNTGYQNFY	TRBV13-2
TRAV16D/DV11*03	TRAJ49	AMRESNTGYQNFY	TRBV13-2
TRAV16D/DV11*01	TRAJ49	AMREANTGYQNFY	TRBV13-2
TRAV16D/DV11*01	TRAJ11	AMRVHDSGYNKLT	TRBV13-2
TRAV16D/DV11*01	TRAJ21	AMRESNYNVLY	TRBV13-2
TRAV16D/DV11*01	TRAJ31	AMREGNSNNRIF	TRBV13-1

We analysed K^b-reactive (PD-1^{hi}) or bystander (PD-1^{neg}) cells from B10.BR and self-tolerant cells from K^b-transgenic mice. TRAV14 and TRAV16 were over-represented among the PD-1^{hi} cells compared to PD-1^{neg} or K^b-tolerant cells while cells expressing TRAV9 and TRAV12 were less frequent (Fig 1c). For PD-1^{hi} A, 4506 productive TCR pairs comprised 1165 different clonotypes, of which the top 10 clonotypes made up 30%, for PD-1^{hi} B these figures were 3518, 1159 and 20%. Kb-SNY/RTY metaclonotypes were common to the PD-1^{hi} TCR sequences. No clonal expansions were observed in the PD-1^{neg} or K^b-tolerant populations, with negligible overlap between TCR sequences from PD-1^{hi} T cells and these populations.

PD-1^{hi} cells segregated into clusters corresponding to central, resident, or peripheral memory cells, or precursor exhausted cells (Fig 1d). Individual clonotypes showed distinctive gene expression patterns following activation.

Conclusions: Alloreactive T cell repertoires comprise public and private clonotypes. Identification of pMHC-specific metaclonotypes may enable prediction of the specificity of additional TCRs aligning with the metaclonotype. Even closely-related alloreactive clones differ in gene expression following activation; whether this corresponds to subsequent T cell fate remains to be determined.



POS27

SKIN ALLOGRAFT ACCEPTANCE UNDER LOW DOSE IMMUNOSUPPRESSION DEPENDS ON INTRAGRAFT REGULATION IN NON-TOLERANT MICE

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Background: We previously developed a model of immune regulation-dependent, immunosuppression-dependent allograft acceptance by combining a weak regulation induction protocol with low dose oral tacrolimus in fully mismatched BALB/c-to-C57BL/6 mouse skin transplantation. Here, we used our model to investigate the relative contribution of newly primed effector T cell responses versus loss of Treg-mediated regulation to the onset of delayed acute rejection.

Methods: Recipient mice received anti-CD154 (0.5 mg on d0,1,3,6), donor specific transfusion ($5x10^6$ splenocytes on d0), and tacrolimus mixed in food pellets (75 mg Tac per food-Kg, from d7 onwards).

Results: Graft survival in this model (76±4 days) reveals a synergistic interaction between weak regulation (40±6 days) and low-dose tacrolimus (8±1 days). This graft protection is sustained by graft-intrinsic mechanisms that rely upon tissue-infiltrating regulatory T cells and regulatory macrophages. However, in this model grafts are lost to delayed acute rejection when regulation is invariably overcome by systemically primed alloreactive T cells, unless they are controlled by stronger immunosuppression or clonal deletion. Common experimental strategies to reinforce allospecific regulation, including Treg transfer, at d50 after transplantation did not convert recipients with stable grafts to tolerance.

Conclusions: The model of regulation-dependent immunosuppression shows that a semi-stable balance can be established between regulatory and effector responses directed against an allograft. Low-dose tacrolimus therapy supports this marginal state by preferentially suppressing effector T cells. This model suggests that delayed graft rejection is not primarily a failure of regulation and therefore, future therapies aimed at tolerising stably immunosuppressed recipients should both deplete allospecific T cells and strengthen intragraft regulation.

POS28

SMALL MOLECULE BCL6 INHIBITORS: A NEW DRUG CLASS OF IMMUNOSUP-PRESSION FOR TRANSPLANTATION?

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Background: The transcription factor Bcl6 is critical for humoral immunity and the generation of memory CD4+ T cells in mice. The aim of this study was to assess the potential of novel Bcl6 inhibitors as immunosuppressive agents on allogeneic activation of human CD4+ T cells.

Methods: We performed mixed lymphocyte reactions (MLR) using allogeneic healthy donor PBMC in vitro in the presence of Bcl6 inhibitors 79.6 or FX1, the Bcl6 protein degrader BI-3802, or the solvent DMSO. Briefly, proliferation dye-labeled responder PBMC, pure CD4+CD45RO-CD62L+ (naïve, Tn) or CD4+CD45RO+CD62L- (effector memory, Tem) and CD4+CD45RO+CD62L+ (central memory, Tcm) T cells were cocultured with irradiated stimulator PBMC. After 5 days, CD4+CXCR5- and CD4+CXCR5+ T cell proliferative response and expression of memory markers CD45RO and CD62L were measured by flow cytometry. Concentrations of IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-17a, IL-17f, IL-22, IFN-γ and TNF-a were measured in MLR supernatants by Luminex beads.

Results: Proliferation of CD4+CXCR5- T cells was reduced in a dose-dependent manner and by 95.3% in the presence of FX1 12.5 µg and by 69.9% in the presence of 79.6 200 µg (n=6). Proliferation of CD4+CXCR5+ T cells was reduced by 99.9% in the presence of FX1 (FX1 12.5 µg vs. DMSO, p=0.01) and by 85.8% in the presence of 79.6 (79.6 200 µg vs. DMSO, p=0.01). The inhibitory effects on proliferation were comparable to those of tacrolimus (80% for CD4+CXCR5- and 88.8% for CD4+CXCR5+ T cells). The Bcl6 protein degrader BI-3802 also affected T cell proliferation. FX1 reduced the proportion of CD45RO+ CD62L+ (Tcm) and CD45RO+CD62L- (Tem) T cells after allogeneic stimulation and significantly reduced the proliferation of allogeneically stimulated sorted Tn, Tcm and Tem. FX1 also significantly reduced IL-2, IFN-γ, IL-9, IL-13 and IL-6 in MLR supernatants suggesting that this class of immunosuppressive agents inhibits the production of cytokines involved in various biological processes including T cell and B cell proliferation and differentiation.

Conclusions: Novel small molecule Bcl-6 inhibitors inhibit the proliferation and cytokine production of alloantigen-activated CD4+ T cells. Thus, Bcl6 inhibition may present a novel immunosuppressive mechanism in solid organ transplantation and warrants further investigation.

POS29

TETRAHYDROBIOPTERIN MOBILIZES MAST CELLS AND REGULATORY T-CELLS AND INDUCES A CYTOKINE MODULATION IN A MURINE HEART TRANSPLANT MODEL

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Background:
The vitamin-like compound tetrahydrobiopterin (BH4) has been shown to attenuate acute cellular rejection in a murine model of heart transplantation independently from its cofactor activity on nitric oxide synthases. The underlying mechanisms are still unknown. Herein, we wanted to shed more light on the immunosuppressive property of BH4.

Methods:
A fully MHC mismatched (C3H/He to C57BL/6) mouse heart transplantation model was used. Recipients were treated with BH4 (50mg/kg b.w.) or Cyclosporine A (CsA, 15mg/kg b.w.) for six days. Syngeneic transplants and untreated allograft recipients served as controls. Six days post transplantation the graft function was assessed. The degree of acute rejection was assessed by histopathological analysis according to the ISHLT score and splenocytes were analysed by flow cytometry. Cytokine production was estimated on the level of protein and RNA in sera and transplanted grafts.

Results:
The median graft functioning score at day six did not show significant differences between the four groups. Histopathological analyses showed consistently severe rejection in untreated allografts and mild and no rejection in CsA treated ($p < 0.01$) and syngeneic grafts ($p < 0.03$), respectively. BH4 treated grafts ranged from mild to severe lymphocytic infiltrates (p = ns). In the secondary lymphoid organs of control CsA treated and BH4 treated animals, T cells were characterized by comparable frequencies (p = ns), but regulatory T cells showed a substantial increase in BH4 treated animals compared to control and CsA treated animals ($p < 0.05$). Furthermore, mast cells showed significantly higher frequencies compared to control and CsA treated animals ($p < 0.01$), whereas dendritic cells display a decrease in frequency in BH4 treated animals ($p < 0.05$) compared to control. Cytokine production of BH4 treated animals compared to control and CsA treated animals reveal a modulation of the balance between TH1 and TH2 cytokines, indicated by a significant increase in IL-10, IL-4 and IL-5 production in sera and transcription factor Gata-3 in the grafts.

Conclusions:
The immunosuppressive role of BH4 seems to rely on influencing the interaction between the innate and the adaptive immune system and on inducing a modulation of TH1 and TH2 cytokines.

POS31

CELL CLOUD CLASSIFICATION (CCC): A MODULAR FRAMEWORK FOR NEURAL NETWORKS THAT ENABLES CLINICAL DECISION-MAKING FROM FLOW CYTOMETRY DATA.

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Background:
We have developed an innovative machine-learning approach that uses raw flow cytometry data to group patients into classes associated with given clinical outcomes, such as response to therapy, which enables computational decisions about treatments. Specifically, our approach applies neural networks to classify samples without prior cell subpopulation identification or any *a priori* assumptions about disease-related changes in cell type distribution or characteristics.

Methods:
Flow cytometry characterizes individual cells from patient blood by size, structure and marker expression. Data can be represented in a matrix for each sample with an unordered number of rows corresponding to cells and a defined number of cell-associated features as columns. Here, we present the FeatureLearner, Pooler and Predictor (FPP) concept as a general, modular layout for neural networks for analyzing point cloud data (Figure 1A). Further, we propose a decision tree-based approach to explain the output of our neural networks in an immunologically interpretable way.

Results:
We present our algorithm as the Cell Cloud Classification (CCC) package in Python. We show successful application of CCC to classification problems in multiple datasets (including prediction of CMV infection) and provide examples of the decision tree based interpretation (Figure 1B).

Conclusions:
Our neural network approach makes use of information from all cells and their measured features captured in a flow cytometry data matrix without imposing limits by first clustering them into subpopulations. This innovation

means that CCC has the potential to outperform existing manual and automated sample-classification approaches in classification tasks.

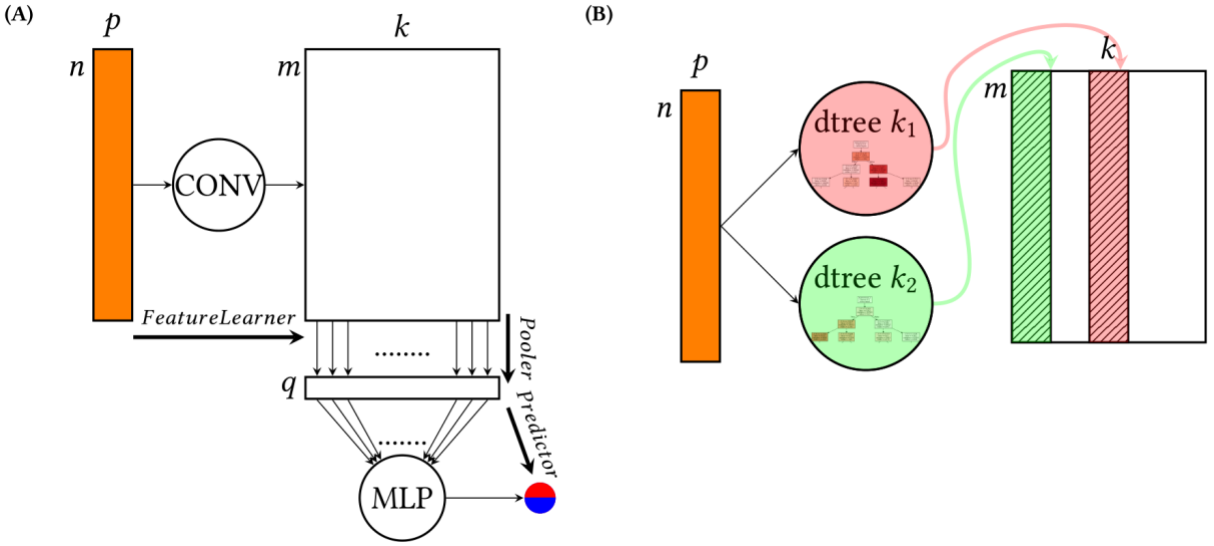

Figure 1. (A) The FPP concept: A matrix with n cells as rows and p cell-features as columns is the input to the *FeatureLearner* (CONV) which transforms it into a second matrix. The *Pooler* then aggregates all rows for each column using q order-invariant functions. The *Predictor* (MLP) uses these pooled values to predict the final class. (B) The final *FeatureLearner* is approximated using multiple decision trees enabling interpretation of the targeted cells and therefore cell subpopulations.

POS33

DONOR-SPECIFIC HYPORESPONSIVENESS FOLLOWING KIDNEY TRANSPLANTATION EXPLAINED BY LOSS OF DONOR-REACTIVE POLYFUNCTIONAL CD4+ EFFECTOR MEMORY T CELLS

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Background: After kidney transplantation, a lowered response of alloreactive T cells to donor antigen develops termed donor-specific hyporesponsiveness (DSH). This study measures changes in phenotype and function of donor-reactive T cells after transplantation to better understand DSH development and guide lowering of immunosuppressive medication.

Methods: This study integrates multiparameter flow cytometry-based assays to characterize phenotype and function of circulating donor-reactive (vs third party-reactive) CD4+ and CD8+ T cells over time. Paired samples were taken from stable kidney transplant recipients (N=46) before, at 3-5 years and more than 5 years after transplantation. Donor-reactive T cells were identified by CD137 expression and data on T cell differentiation status and transcription factor expression evaluated by unsupervised clustering. Proportions of polyfunctional donor-reactive CD137+ T cells capable of producing multiple pro-inflammatory cytokines were characterized as well as proliferation towards donor-antigen.

Results: The data point to progressive and specific loss of activated donor-reactive T cells capable of producing pro-inflammatory cytokines after transplantation. The number of circulating CD4+ donor-reactive T cells declined within the first 3-5 years after transplantation and became virtually undetectable in the years thereafter. The decrease in donor-reactive CD4+ T cells was primarily within the effector memory subset and within T cells capable of producing two or more pro-inflammatory cytokines (TNF-α, IFN-γ and IL-2). This reduction in polyfunctional donor-reactive CD4+ T cells was strongly correlated with the reduced proliferation of CD4+ T cells following kidney transplantation. The frequency of third party-reactive T cells did not alter after transplantation indicative of a donor-specific effect and not an aspecific effect of immunosuppressive medication.

Conclusions: This study detected a decline in highly active donor-reactive T cells capable of producing multiple pro-inflammatory cytokines from the circulation in a time-after transplantation dependent fashion. The loss of polyfunctional donor-reactive effector memory CD4+ T cells likely plays an important role in the development of DSH in kidney transplant recipients.

POS34

DYNAMIC OF DONOR DERIVED CELL-FREE DNA AFTER PANCREAS TRANSPLANTATION

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[1]Fundacio Clinic - IDIBAPS, Spain, *[2]Eurofins Megalab, Spain*, *[3]Hospital Clinic, Spain*

Background: Donor-derived cell-free DNA (ddcfDNA) is a noninvasive test that had demonstrated high predictive performance for acute rejection in solid organ transplant. Studies on monitoring ddcfDNA dynamic during the early periods after transplantation and their correlation with the outcome of the patients are scarce. We aimed at evaluating the dynamic of ddcfDNA test following pancreas transplantation.

Methods: Prospective longitudinal study including all pancreas transplant recipients from January 2017 to December 2018. Plasma samples were collected in PAXgene tubes before transplant (D0), and at 1h, 24h and 7 days (D7) post-transplant, and at time of pancreas biopsy – either per protocol at 3 weeks (B3) and 12 months (B12), or for clinical indication. The ddcfDNA percentage (%) was assessed using Eurofins Genoma AlloNext® utilizing Next Generation Sequencing to determine risk of rejection (either T-cell mediated rejection (TCMR) or antibody mediated rejection ABMR).

Results: A total of 77 patients were included in the analysis (SPK n=65 and PAK n=12). ddcfDNA increased significantly at 1h and 24h after transplantation compared to baseline (D0), most likely reflecting ischemia/reperfusion damage. No correlation with either donor demographics nor cold ischemia time was identified (p>0.05). In 52 cases biopsy-related sample was obtained (Banff criteria: No rejection n=33; Indeterminate n=5; TCMR grade 1-3 n=15; ABMR n=4). In patients with stable graft function (absence of rejection during the first 12months) ddcfDNA decreased by D7 and remained low at B3 and B12 (D0 (0.27±0.14), 1h (4.30±2.53), 24h (2.41±1.79), D7 (0.65±0.47), B3 (0.47±0.28) and B12 (0.61±1.31)) Figure 1. However, in patients with biopsy proven acute rejection (TCMR; 1.74±2.08 or ABMR; 4.42±2.93; p<0.01) % ddcfDNA was increased compared with no proven acute rejection biopsies (N-BPAR; 0.45±0.77). A 1% threshold had a positive predictive value to detect rejection of 86%, whereas the negative predictive value was of 69%. The study is currently ongoing.

Conclusions: We describe the first longitudinal analysis of the dynamic of dd-cfDNA in a cohort of SPK, validating its application to predict graft function in clinical practice.

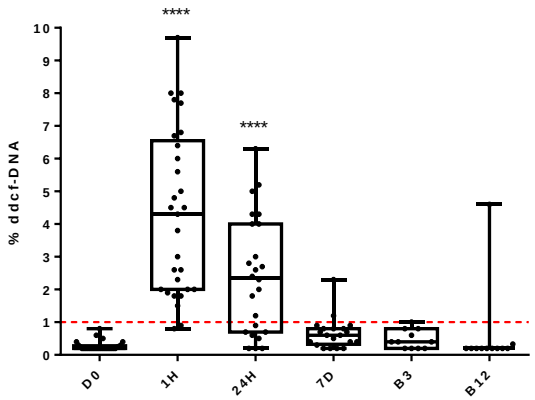

Figure 1: Dynamic of % dd-cfDNA after pancreas transplantation in patients with stable graft function.

POS35

EPIDEMIOLOGICAL AND PREDICTIVE TOOLS IN KIDNEY TRANSPLANT QUALITY PROCESS: CAN THEY ENABLE A DATA REVOLUTION?

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[1]Hepato-Bilio-Pancreatic, Minimally Invasive and Robotic Surgery Unit General and Transplant Surgery Unit, Italy, *[2]Public Health Department, Federico II University Hospital - Naples, Italy*, *[3]Renato Caccioppoli Department , Federico II University Naples, Italy*, *[4]Federico II University Hospital Direction - Naples (Italy), Italy*

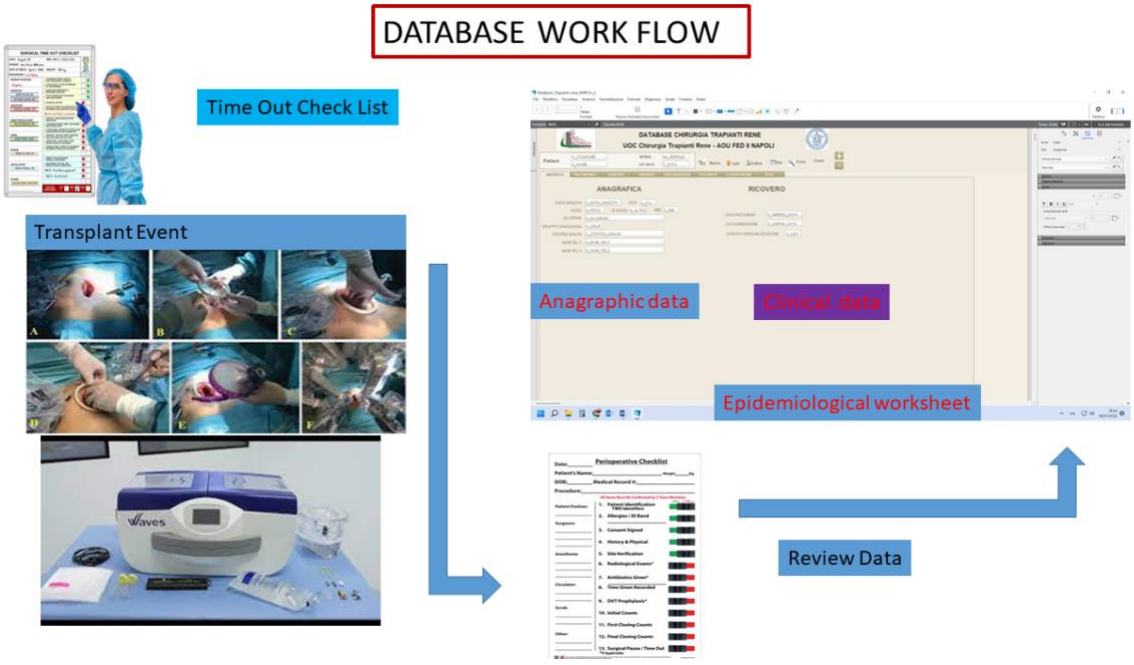
Background: Epidemiological tools can hold some advancements and sound insight into Kidney transplant programs. We are in the era of development of large-scale data-driven models where supervised machine learning may be proposed as class of algorithms that “learn” from existing input–output pairs, for the prediction of long-term allograft survival in kidney transplant recipients. Evidence is not conclusive about their superiority versus the

Bayesian methodology, dealing with eventually “unstructured” data, and with a burden of “noise”. However appropriate data collection may allow Predictive diagnostic estimates improving turning points in post-transplant steps as to enable early detection or exclusion of structural kidney damage.

Methods: A prospective database was projected and integrated in the quality system. Data were collected from the procurement and transplant workflow. Two collecting form were standardized: A Time out check list for the pre surgical steps and a form for the transplant variables collection. A methodology to quantify Donor-derived cell-free DNA (dd-cfDNA), through quantitative digital PCR, by using the collected samples as training set, will be built in order to investigate it as a candidate biomarker with potential for enabling comprehensive monitoring of allograft injury.

Results: Data corresponding to the last year activity and including 31 kidneys transplants were inserted to test the database. (Fig 1).

Conclusions: Accurate data collection may contribute to handle complex relationships between features and outcomes, leading to efficacious estimates and local thresholds definitions and to improved precision and accuracy of predicted outcomes. This capability might therefore facilitate personalized clinical management of kidney transplant recipients, and the quality of the clinical pathway. The potential of a characterized dataset may represent a starting point to design a digital PCR based assay to improve the prediction power in this landscape. Epidemiological founding steps as data collection and management of data favors accurate quantitative synthesis of the data: this aim may enable either nested logistic analysis models or innovative different algorithms, allowing a more robust comparison of the different methodologies forecasts or projections based



POS39

NON-INVASIVE METABOLITE-BASED URINE SIGNATURE DETECTS OVER-IMMUNOSUPPRESSION IN RENAL TRANSPLANT RECIPIENTS.

Srihari Raghavendra Rao[1], Chandra Honrao[1], Leo Rodrigues[1], Chen Dong[1], Kanchan Sonkar[1], Josie Wolf[1], Elizabeth O'day[1], Dirk Kuypers[2]

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Background: Managing complications related to overimmunosuppression represents a challenge in posttransplant care, with infections accounting for the 2nd leading cause of death with functioning graft in renal transplant recipients (RTRs) within the first year. At present there are no clinically validated biomarkers to detect overimmunosuppression. Polyomavirus-associated nephropathy (PVAN) is an opportunistic infection specifically indicative of overimmunosuppression that

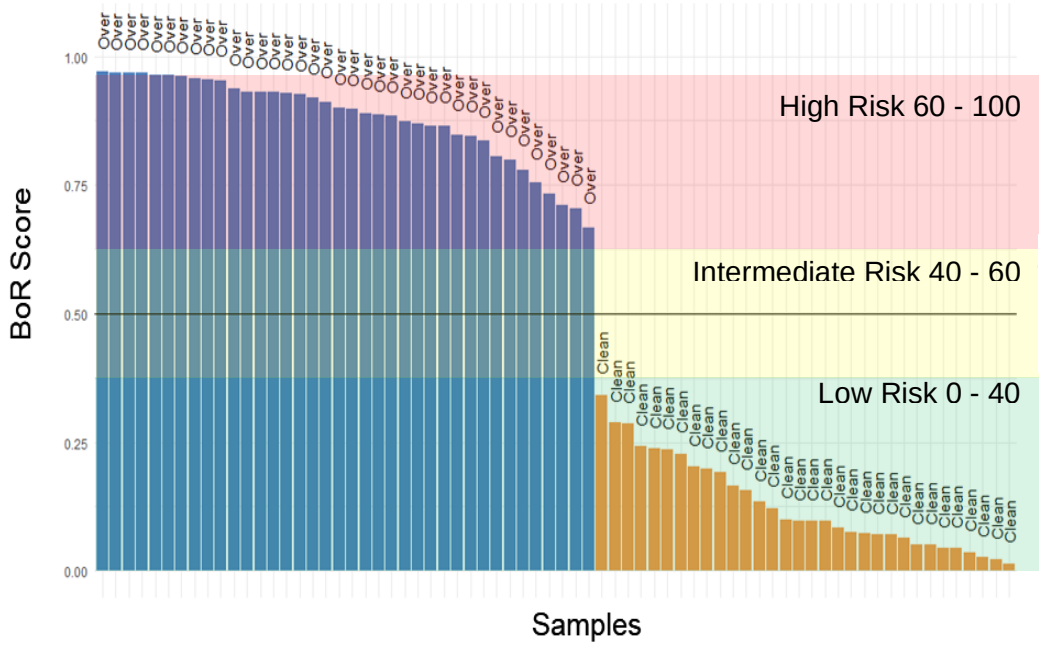
occurs in 5-10% RTRs leading to graft dysfunction/loss. We leveraged metabolic profiling and machine learning to develop a metabolite-based signature to detect overimmunosuppression in RTRs prior to a PVAN event.

Methods: Urine metabolites were extracted and analyzed via NMR spectroscopy from 71 RTRs collected 3 months posttransplant including N=39 with a biopsy-proven PVAN (labeled "over") and N=32 with stable graft function, no histological signs of rejection or indications of overimmunosuppression for 2 years (labeled as "clean"). Differential metabolites between over and clean samples were used to generate cross-validated machine learning models.

Results: Using a Kruskal-Wallis test we identified 24 differential metabolite resonances between clean and over samples with a fold-change greater than 1.5. The differential resonances were matched by chemical shift to reference libraries for metabolite annotation. A total of 8 machine learning models were built from combinations of features including known metabolites, known and unknown metabolite features, and clinical data. The champion model led to a scoring system (BoR Score 0-1.0) with 3 zones, such that 82.3% of patients with a score higher than 0.6 developed PVAN while 89.4% of patients with a score lower than 0.4 had no adverse events related to under- or overimmunosuppression for two years. Patients who scored between 0.4-0.6 (17/71) had indeterminant prediction power.

Conclusions: The metabolite-based urine signature was able to classify overimmunosuppressed RTRs with PVAN from those with a stable graft with high accuracy. Larger validation studies are underway, which could lead to a powerful new biomarker for posttransplant monitoring.

Waterfall plot of Biomarker of Response score in RTRs with and w/o PVAN



POS40

NOVEL AVENUE OF ALLOGRAFT MONITORING: DIRECT MEASUREMENT OF DONOR-DERIVED EXTRACELLULAR VESICLES IN HUMAN PLASMA

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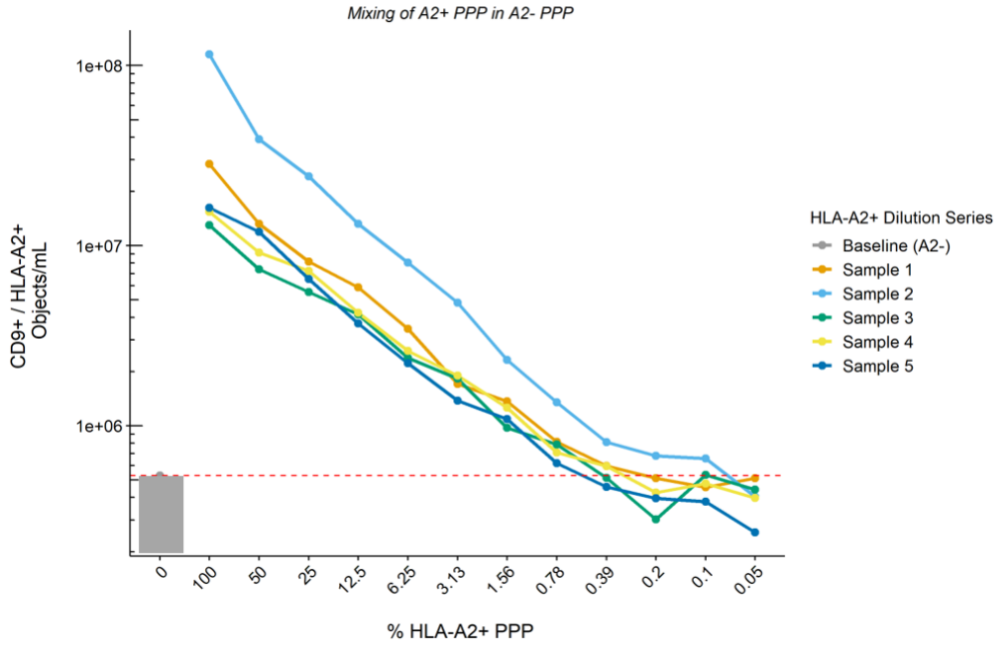
[1]Erasmus MC Transplant Institute, Department of Internal Medicine, Rotterdam, Netherlands

Background:
Extracellular Vesicles (EV) - regarded as "snapshots" of their cell of origin - represent promising liquid biomarkers to monitor allograft function post transplantation. Recently, we developed an imaging flow cytometry (IFCM) based protocol to identify and characterize EV ≤ 400 nm in molecularly complex samples such as human plasma *without* prior isolation of EV. Using this protocol, we measure allograft derived EV based on HLA phenotype as a first step to detect allograft specific EV in the circulation of kidney transplant (KTx) recipients.

Methods:
EDTA blood samples from kidney transplant donors (HLA-A2+, n=21) and recipients (HLA-A2-, n=33) were collected before transplantation as well as 3 days, 7 days, 6 months and during 'for-cause' biopsies (recipients only) after transplantation. Platelet-poor plasma (PPP) was stained with a donor-specific HLA antibody (HLA-A2) in combination with a common EV marker (tetraspanin CD9) and measured using standardized IFCM.

Results:
Quantification and comparison of CD9+/HLA-A2+ double-positive EV showed $1.1E^7 \pm 8.9E^6$ vs $3.5E^5 \pm 2.5E^5$ objects/mL for donor and recipient (pre-KTx) EV respectively, with recipients A2- EV concentrations representing background level of the machine. CV values for inter- and intra-assay variability were 16% and 11%, respectively. Serial dilution of A2+ PPP in A2- PPP (n=5) showed a linear reduction in the numbers of CD9+/HLA-A2+ EV according to the dilution rate whilst total CD9+ EV levels remained unchanged. The lower limit of detection of our protocol was defined as the dilution at which point CD9+/HLA-A2+ EV dropped below baseline (A2- PPP), and was determined to be ~1% of the concentrations measured in undiluted A2+ PPP (Figure). Measurement of longitudinally collected recipient samples revealed the detection of allograft derived EV as soon as 3 days – but up to at least 6 months – after KTx.

Conclusions:
Here we demonstrate for the first time the detection of allograft derived EV in the circulation of KTx recipients in unprocessed human plasma samples. Identification, quantification and characterization of these EV opens up the possibility to monitor these EV over time after transplantation, and may prove to be a minimally-invasive biomarker.



POS42

RENIN, ERYTHROPOIETIN AND VITAMIN D RELEASE FROM HUMAN DONOR KIDNEYS DURING NORMOTHERMIC MACHINE PERFUSION: PREDICTOR OF POST-TRANSPLANTATION OUTCOME?

Hui Lin[1;1], Zhaoyu Du[2], Dafsy Bouari[3], Elsaline Rijkse[3], A.H.Jan Danser[1], Robert Minnee[3], Martin Hoogduijn[2]
[1]University Medical Center Rotterdam, Vascular Medicine and Pharmacology, Department of Internal Medicine, Netherlands, [2]University Medical Center Rotterdam, Erasmus MC Transplant Institute, Department of Internal Medicine, Netherlands, [3]University Medical Center Rotterdam, Erasmus MC Transplant Institute, Department of Surgery, division of HPB & Transplant Surgery, Netherlands

Background: Normothermic (37°C) machine perfusion (NMP) is a potential alternative to currently used hypothermic (4°C) machine perfusion (HMP) for donor kidney preservation before transplantation. NMP allows for metabolic activity and for functional assessment

of donor kidneys. The kidneys are key producers of hormones and we investigated the release of prorenin/renin, erythropoietin (EPO), and vitamin D by kidneys on machine perfusion.

Methods: Ten donor kidneys were subjected to HMP followed by 2h of oxygenated NMP before transplantation. NMP perfusate was collected at three time points (0h, 1h, 2h). Ten HMP perfusate samples were collected for the same measurements.

Results: Median release rates of prorenin (196 [Interquartile Range (IQR) 28-266] ng/hour) and renin (228 [IQR 94-302] ng/hour) in the first hour of NMP were 82- and 32-fold higher than that in HMP perfusates respectively (p=0.0007 and p<0.0001). Median renin release rate showed a 3.2-fold downregulation during the second hour of NMP compared to the first hour. EPO was secreted by kidneys on both HMP (10 [IQR 5-26] mIU/min) and NMP (1st hour: 14 [IQR 8-48] mIU/min; 2nd hour: 15 [IQR 6-92] mIU/min) without significant differences. Active vitamin D was undetectable in HMP perfusate samples, while the median secretion rates of vitamin D were 56 (IQR 30-83) and 28 (IQR 8-50) pmol/hour in the first and second hour of NMP respectively (p=0.0001 and p=0.003). We then investigated whether there were correlations between the hormone releasing capacity and donor kidney status. Prorenin release rate in the second hour of NMP was lower as pre-NMP cold ischemia time (CIT) increased (*r*=-0.605; p=0.049). EPO release rate showed no correlation with donor age or CIT. Donor age and vitamin D release rate during two hours of NMP are highly correlated (*r*=0.652; p=0.034). Interestingly, donation after brain death (DBD) kidneys significantly released more vitamin D than donation after circulatory death (DCD) kidneys during the first and second hour of NMP (p=0.024 and p=0.012).

Conclusions: These data show that NMP increases the hormone release capacity of transplant kidneys. Hormone release may represent a tool to assess kidney function during NMP.

POS43

THE LONG-NON CODING RNAS H19, UCA1 AND NEAT1 PLAY A ROLE IN THE REGULATION OF HYPOXIA IN HEPARG CELLS

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Background: Long non-coding RNAs (lncRNAs) have been reported to be involved in a series of hepatocyte metabolism. Several studies have demonstrated that lncRNA H19 has a fundamental role in promoting hepatic lipogenesis, inflammation, epithelial-mesenchymal transition. Urothelial cancer-associated 1(UCA1) acts as a suppressor gene affecting cell proliferation as well as migration, and nuclear enriched abundant transcript 1(NEAT1) may play an indispensable role in certain cellular processes such as cell differentiation, viral infection, and stress responses. Recent studies have identified that many lncRNAs participated in the hypoxia regulation process of hepatocytes. However, the roles of lncRNA H19, UCA1, and NEAT1 in the regulation of hypoxia in hepatocytes have not been reported, yet.

Methods: RT2-PCR screening assay was used to detect significantly up- or downregulated lncRNAs in hypoxia treated HepaRG cells ($HepaRG^{hyp}$) compared with HepaRG cells kept under normoxic conditions ($HepaRG^{norm}$). After verifying the array results via rtPCR, interesting lncRNA candidates were silenced by siRNA in order to see the effect on response to hypoxic treatment. Influence on the expression of hypoxia-inducible factor 1-α (HIF1-α) was proven by Western blotting.

Results: We found that the levels of UCA1 and H19 were significantly upregulated in the $HepaRG^{hyp}$ cells, while the level of NEAT1 was significantly downregulated in the $HepaRG^{hyp}$ compared to $HepaRG^{norm}$. Silencing of these lncRNAs with siRNA resulted in a loss of response to hypoxic treatment.

Conclusions: LncRNAs UCA1, H19, and NEAT1 participate in the process of hypoxia in HepaRG cells by regulating hypoxia-inducible factor 1-α (HIF1-α) and might therefore be potential targets for the detection and maybe treatment of ischemia reperfusion injury.

POS45

TISSUE BIOENERGETICS DURING THE EARLY PHASE OF NORMOTHERMIC MACHINE PERFUSION OF THE LIVER PREDICTS CLINICAL OUTCOME AFTER TRANSPLANTATION

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Background:
The growing demand for liver grafts promotes the use of extended criteria organs. To assure optimal clinical outcome, pre-transplantational evaluation of organ quality is needed. Bioenergetic performance during normothermic machine perfusion (NMP) may correlate with organ function after liver transplantation (LT). Thus, we aimed at determining mitochondrial function (MF) after cold ischemia and during NMP in DBD and DCD grafts to investigate its use for organ quality assessment for outcome prediction.

Methods:
71 liver allografts (DBD: n = 52, DCD: n = 19) were enrolled in a prospective clinical study and underwent NMP (OrganOx metra) for up to 24 h, of which 47 livers (DBD: n = 38, DCD: n = 9) were transplanted. Biopsy and perfusate samples were collected at the end of cold storage, at 1 h, 6 h and end of NMP, and at 1 h after transplantation. Serial histology and real-time confocal imaging were performed in biopsies. MF was characterized in tissue homogenates by high-resolution respirometry (HRR; O2k, Oroboros Instruments) and was correlated with clinical outcome (L-GrAFT score). Succinate-linked coupling control was assessed, and the damage of the outer mitochondrial membrane was monitored by cytochrome *c* addition.

Results:
We observed a considerable variability in mitochondrial respiration between grafts during cold storage. Furthermore, OXPHOS capacities were significantly higher in DCD grafts as compared to DBD organs before start of NMP, but this difference disappeared during NMP. Most mitochondrial parameters, e.g. *P-L* control efficiency (efficacy of ATP production) of the whole cohort remained unchanged, although MF showed a time dependence in single livers. In the multivariate analysis, area-under-the-curve (AUC) values of LEAK respiration, cytochrome *c* control efficiency (outer mitochondrial membrane, OMM, damage), and the efficacy of the mitochondrial ATP production during the first 6 h of NMP correlated with L-GrAFT.

Conclusions:
During NMP, mitochondrial respiration in DCD livers align with the DBD group. AUC values for markers of OMM damage, ATP synthesis efficiency and dissipative respiration (LEAK) during the first 6 hours of NMP predict clinical outcome upon LT. Mitochondrial respiration assessed by HRR is therefore a promising tool to select optimal grafts.

POS46

A PIG CROSS-CIRCULATORY PLATFORM FOR MONITORING AND MODULATING THE INNATE RESPONSES IN LUNG GRAFTS.

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Background:
Lung transplantation (LT) is the sole treatment of end-stage lung disease. Its results are burdened by primary and/or chronic dysfunctions related to perioperative inflammatory responses induced by ischemia-reperfusion and allogeneic innate recognition. Methods to monitor and control these complex innate responses would improve LT outcome.

Methods:
We evaluated a cross-circulatory platform in the pig model to monitor and modulate the recipient immune cell subset recruitment and activation in an ex-situ donor lung by coupling the extracorporeal blood perfusion system to cell mapping with a fluorescent marker.

Results:
Corticosteroids, used for a proof of concept, potently dampened the MHC class II and CD80/86 upregulation on recipient recruited monocytes and increased their *IL-10:TNFA*

gene expression ratio (particularly in CD16+CD163+ cells), without modifying these features on donor alveolar macrophages. Conversely corticosteroids did not reduce the recruitment of any myeloid and lymphoid subsets in the lung. Furthermore lung explants from corticosteroid-treated cross-circulatory platforms displayed a strong reduction of TNFa gene expression upon reoxygenation and LPS stimulation.

Conclusions:
The cross-circulation coupled to cell mapping is a potent method to investigate the mechanism of immunomodulatory treatments on the innate perioperative response over extended periods of time, in a relevant preclinical model. It also opens the way to possibly precondition donor lungs with immunomodulated recipient cells before LT.

POS48

EARLY EXPERIENCE OF 48-HOUR NORMOTHERMIC MACHINE PERFUSION IN HUMAN KIDNEYS APPLYING URINE RECIRCULATION

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Background:
Normothermic machine perfusion (NMP) of the kidney has been studied extensively during the past decade. Short-term kidney NMP has demonstrated promising results, however currently transplant logistics cannot be improved and for organ treatment longer preservation periods might be necessary. As a proof of principle, we aimed to achieve 48-hour NMP by applying urine recirculation (UR) with a commercially available perfusion device.

Methods:
Discarded human kidneys were normothermically perfused on the XVIVO Kidney Assist perfusion device. The perfusate comprised packed red cells and 5% albumin. For volume management UR was applied. Air (21% O2) and CO2 were used for oxygenation of the circuit and monitored with an in-line blood gas analyzer. Perfusate and urine samples as well as hemodynamics were regularly assessed.

Results:
Five discarded human kidneys underwent kidney NMP following hypothermic machine perfusion (HMP) and static cold storage (SCS). All but one kidneys were DBD organs. Median donor age (range) was 62 (41-68) years. Median (IQR) CIT and HMP were 19.9 (12.1) and 5 (7.2) hrs. An NMP duration of 48 hrs could be achieved in all kidneys. All kidneys were urinating throughout with a median (IQR) output of 22.5 (30.5) ml/h. Overall median arterial flow (IQR) was 695 (383) ml/min. Median (IQR) pH was 7.2 (0.2). Overall median (IQR) perfusate sodium, chloride and potassium were 161 (14.7) mmol/l, 124.5 (11.5) mmol/l, and 6.5 (2.7) mmol/l. Median (IQR) perfusate lactate over time was 109 (55.2) mg/dl. Median perfusate sodium and chloride were significantly higher than corresponding urine values; 130 (27) mmol sodium and 120.5 (11.8) chloride over time, p=0.02 and 0.04. Median arterial flow over time was significantly higher in NMP kidneys with lower perfusate sodium levels; p<0.001, correlations coefficient (Spearman's rho) -0.461.

Conclusions:
This early experience underlines the feasibility of extended ex-situ kidney NMP by applying UR. Hemodynamic stability and urine excretion were achieved for 48 hours.

POS49

EXTENT OF GLYCOCALYX DAMAGE MEASURED DURING HYPOTHERMIC EX VIVO LIVER PERFUSION CORRELATES WITH EARLY ALLOGRAFT DYSFUNCTION

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Background: Alterations of the endothelial glycocalyx have been investigated within different aspects of organ damage and more recently in the context of liver transplantation. Syndecan-1 (Sdc-1) is the main component of endothelial glycocalyx and is released into human serum upon glycocalyx degradation where it can be measured. Hypothermic oxygenated machine perfusion (HOPE) enables transplantation of organs with a higher risk profile, but reliable markers for organ assessment during machine perfusion are

missing. We aimed to assess glycocalyx damage during hypothermic liver perfusion.

Methods: 40 livers were subjected to HOPE with the Organ Assist® perfusion system prior to organ transplantation. Sdc-1 was measured in perfusate, effluent and serum by ELISA as indicator for glycocalyx destruction. Sdc-1 levels were correlated with clinical parameters and compared in patients who did and did not develop early allograft dysfunction (EAD) using Mann-Whitney U test, Pearson correlation and receiver operating characteristics (ROC).

Results: Sdc-1 concentrations in perfusate were elevated in 13 patients that developed EAD compared to non-EAD patients after 0 min [598 (±526) vs. 276 (±150) ng/ml; p=0.076] and 60 min [1099 (±739) vs. 521 (±382) ng/ml; p=0.016] of perfusion, as well as in the effluent [2074 (±1273) vs. 443 (±226) ng/ml; p=0.001 (n=15, 4 with EAD)]. Sdc-1 levels in perfusate and effluent correlated with EAD: 0 min (R=0.433, p=0.006), 60 min (R=0.471, p=0.003) and effluent (R=0.769, p<0.001, n=15, 4 with EAD). Additionally, ROCs showed an association between Sdc-1 concentrations and EAD: perfusate 60 minutes (AUC=0.704 and p=0.018) and effluent (AUC=1 and p=0.004 (n=15, 4 with EAD)). Sdc-1 was not associated with graft survival (p=0.339) in cox regression analysis, however, patients who developed EAD showed a significantly reduced graft survival (log-rank=0.009) compared to those without EAD.

Conclusions: Our results show that the extent of glycocalyx degradation measured during hypothermic liver perfusion is indicating transplantation outcome regarding EAD. Therefore, Sdc-1 could be a useful biomarker for organ assessment during HOPE.

POS50

H2S-ENRICHED FLUSH-OUT IN DBD AND NON-DBD PORCINE KIDNEYS

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Background:
Kidney extraction time has a detrimental effect on post-transplantation outcome. The hypothesis is that this results from a temperature increase of the kidney when extraction takes longer. This study aims to improve the flush-out and potentially decrease ischemic injury by addition of hydrogen sulphide (H_2S) to the flush medium in kidneys from both brain dead and non-brain dead pigs. H_2S is a gasotransmitter capable of inducing a hypometabolic state and its addition during abdominal flush could possibly reduce organ injury.

Methods:
22 porcine kidneys were extracted during organ recovery surgery. Prior to donation, pigs underwent brain death induction or a sham operation. The kidneys were stratified in 4 groups: donation after brain death (DBD) control, DBD H_2S, non-DBD control and non-DBD H_2S. Directly after the abdominal flush, kidneys were extracted and flushed with or without H_2S. Next, all kidneys were subjected to 90 min of ischemia at 21°C before the kidneys were preserved via static cold storage (SCS) for 13 h. The next day, kidneys were tested using normothermic machine perfusion (NMP) to evaluate metabolism, renal function, injury markers and histology.

Results:
The non-DBD kidneys show superiority in renal function (creatinine clearance and FEna) compared to the DBD control group (p = 0.03 and p=0.004). Oxygen consumption was significantly higher in the H_2S treated DBD kidneys compared to the DBD control group (p=0.03). Significant higher complement activation (C3a) was seen after SCS in the DBD control group compared to the H_2S treated DBD group (p=0.03). Pro-inflammatory cytokines IL-1b and IL-8 were significantly lower in H_2S treated DBD kidneys during NMP (p=0.03). NGAL was significantly higher in the non-DBD control group compared to the DBD control group (p=0.03). No difference was seen between all four groups in perfusion parameters, injury markers (lactate, LDH, ASAT, MDA, BAX/BCL2 ratio and histological appearance).

Conclusions:
We found an overall trend of better renal function in the non-DBD kidneys compared to the DBD kidneys. The addition of H_2S showed a reduction of pro-inflammatory cytokines during NMP and lower C3a activation after SCS, however without affecting renal function or injury markers. Further studies are needed to determine the effect of H_2S on transplant outcome.

POS51

HYPOTERMIC MACHINE PERFUSION AND INDOCIANINE GREEN ANGIOGRAPHY: A PILOT STUDY FOR NEW METHODS TO APPRAISE EXTENDED CRITERIA DONORS' GRAFT QUALITY

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Background: Kidney transplantation is the treatment of choice for end stage renal disease patients.
Currently, there is a growing gap between organs available and patients on dialysis so, to expand the donor pool, since 2000's worldwide it has been started to perform transplantations also with marginal organs.
Moreover, it is necessary to find out new methods to prove the quality of the graft before the transplantation, in order to prevent any complications related to donor's history or to the cause of death.
Our propose is to test indocyanine green angiography for determination of microcirculation patency improvement after Hypothermic Perfusion Machine (HMP) re-conditioning, with the aim to establish graft quality. To do that, we had compared the results with pretransplant biopsy findings and renal resistive index assessed at the end of hypothermic perfusion.

Methods: We had submitted to indocyanine green fluorescent (ICG) angiography all the kidneys assigned to our Transplant Unit that belonged to Extended Criteria Donors (ECDs) or Donors After Cardiac death (DCDs). We had performed the ICG angiography before and after HMP, during bench surgery. It has been calculated the fluorescence index and related to the histological score and final renal resistive index.

Results: 5 grafts had been recruited between June 2020 and July 2021, all of these come from DCD and HMP has been performed. In all perfused kidneys the fluorescence intensity increased after the reconditioning, in four out of five the initial index was doubled as we can see in table 1 and figure 1. Through statistical analysis we find out a moderate correlation between initial fluorescent intensity and the resistance index after HMP treatment (r = 0.397279).

Conclusions:
Fluorescent angiography with ICG can be a useful method to verify the improvement of microcirculation thanks to HMP re-conditioning, and if joined by histology and renal resistive index assessment, could establish a new technique for better assessment of graft quality before transplantation. The results we are presenting comes from a pilot study, indeed further studies, hopefully with bigger populations, are needed to standardize the procedure.

POS52

IMPACT OF PROLONGED NORMO-THERMIC MACHINE PERFUSION ON THE BIOENERGETIC FUNCTION OF PORCINE KIDNEYS

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Background
Lower-quality organs of marginal donors accompany greater risk of malfunction after transplantation, which is propelled by ischemia and reperfusion injury (IRI). Emerging technologies like normothermic machine perfusion (NMP) offer the possibility to restore metabolism under near-to-physiologic conditions and thereby assess grafts prior to transplantation. Detailed analysis of mitochondrial respiration may constitute direct and readily available information on organ function. We herein aimed at assessing bioenergetic function in porcine kidney NMP using high-resolution respirometry (HRR).

Methods
Porcine kidneys underwent NMP for up to 24 h. Tissue integrity and cell viability were assessed in biopsies using real-time confocal microscopy with a scoring system (RTCA) ranging from +3 (100% viable) to -3 (100% non-viable). Mitochondrial respiration was analysed in tissue using HRR for the succinate-linked pathway to analyse oxidative phosphorylation (OXPHOS). Outer membrane damage (cytochrome *c* control) and ATP production *(P-L* control) efficiencies were calculated.

Results
Eight porcine kidneys were perfused for 21.69 ± 4.47 hours (mean ± SD). Intrarenal resistance declined significantly from start (0.32 ± 0.13 mmHg/mL/min) towards end of perfusion (0.17 ± 0.09 mmHg/mL/min), $p = 0.0039$. Renal plasma flow doubled from 217.4 ± 86.6 mL/min to 447.8 ± 201.9 mL/min ($p = 0.0056$) during NMP. No significant changes were observed in lactate levels and plasma creatinine. RTCA score indicated a decrease in cell viability (from 1.6 ± 1.5 to 0.6 ± 2.3). Mass-specific OXPHOS capacity of kidney mitochondria respiring on succinate decreased ($p = 0.0003$). Cytochrome *c* control efficiency increased concomitantly ($p = 0.0082$), representing a damage to the outer mitochondrial membrane (OMM). However, no change in the *P-L* control efficiency could be detected, which indicated a sustained efficacy of ATP production.

Conclusions
HRR is a precise and reproducible method to monitor the bioenergetics in the kidney. During prolonged NMP, a decrease in OXPHOS capacity and damage to the OMM, but stable ATP production efficiency was observed. Clearly visible trends in our data underline the suitability of this method and its application in further studies.

POS53

LONG-TERM NORMOTHERMIC PERFUSION OF HUMAN LIVERS: IN-DEPTH ASSESSMENT OF BIOENERGETIC FUNCTION

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Background:
Recovery of livers currently declined for transplantation may help to overcome organ shortage. *Ex vivo* normothermic machine perfusion (NMP) can serve as the platform to facilitate this goal, but an in-depth knowledge of molecular mechanisms during NMP is required. Since mitochondria play a key role in tissue bioenergetics in this regard, we herein perform an in-depth analysis of mitochondrial respiration during prolonged liver NMP.

Methods:
12 human liver allografts declined for transplantation were exposed to NMP for up to 5 days or until lactate reached 200 mg/dL. Perfusate samples were taken every 6 h for blood gas analysis and biochemistry. Wedge biopsies were collected every 24 h and analyzed for bioenergetic function by high-resolution respirometry (HRR) including oxidative phosphorylation (OXPHOS), resting respiration (LEAK), and electron transfer (ET) capacity. Coupling control ratios (*P-L* and *E-P* control efficiencies) were calculated. To analyze cell viability and tissue integrity, real-time confocal microscopy (RTCM) was applied.

Results:
NMP time increased with improved management progression of the study and reached 5 days in 4 grafts. Lactate levels were maintained within the physiological range (16.11 ± 6.60 mg/dL) in all livers for a minimum of 72 h. OXPHOS capacity deteriorated during this period, but *P-L* control efficiency remained stable, indicating a decline in mass-specific respiration, but not efficacy. In line with this observation, we recorded a decrease in cell viability (RTCM score pre: 0.4 ± 0.8, 72 h: 1.6 ± 0.7). In the majority of livers perfused beyond 96 h, the proportion of LEAK respiration doubled, probably due to oxidative damage to the mitochondrial inner membrane. In line with this, the integrity of the outer mitochondrial membrane deteriorated, resulting in significantly elevated cytochrome *c* control factor ($p < 0.0001$). ET excess capacity halved ($p < 0.0001$) as a result of damage to the ET machinery. On the contrary, 4 livers displayed sustained efficacy of mitochondria over 5 days of perfusion.

Conclusions:
Bioenergetic function was successfully preserved in livers with lower pre-existing damage for 5 days of human liver NMP. Our data indicate that coupling control in

mitochondria may serve as an effective measure for organ function.

POS54

MARKERS OF COAGULATION AND FIBRINOLYSIS DURING NORMOTHERMIC LIVER PERFUSION OFFER INSIGHTS INTO PATHOPHYSIOLOGICAL ASPECTS OF PRESERVATION INJURY

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Background:
Perfusate used for normothermic machine perfusion (NMP) consists of red packed blood cells and colloid solution and is therefore free from thrombocytes and coagulation factors. Our aim was to monitor changes of the composition of coagulation factors during NMP and to investigate their association with liver function during NMP and after transplantation.

Methods:
NMP has been performed on 16 grafts including 12 extended criteria donor livers. D-dimer, plasminogen-activator-inhibitor (PAI-1), thrombocyte count, van Willebrand factor activity (vWF), factor V activity (FV) and factor XIII activity (FXIII) in perfusate were assessed. Liver and bile duct biopsies were taken before and after perfusion and after reperfusion. Median follow-up after transplantation was 8.9 months.

Results:
From the 16 livers that were assessed, 10 livers were successfully transplanted. Main reasons for decline were low bile quality, non-lactate clearing or extensive fibrosis in the frozen section.
D-dimer was increased in two livers during perfusion (8.7 µg/mL and 12.3 µg/mL vs. mean peak D-dimer of 3.7 µg/mL SD: 3.1). One liver was transplanted, histological analysis of the bile duct biopsies showed sclerosing cholangitis. Additionally, vWF activity during perfusion was increased (13 % vs. mean peak vWF activity of 6.4% SD: 2.5). The patient later developed an anastomotic stricture. The second liver with elevated D-dimer was severely steatotic and the only liver out of the study group that did not produce bile and was therefore declined. The graft additionally showed high levels of PAI-1 (299 IU/mL vs. mean peak PAI-1 of 186 IU/mL SD: 155) and FXIII activity in perfusate (158 % vs. mean peak FXIII activity 45% SD: 49). PAI-1 was elevated in three other livers during perfusion, one was discarded due to increasing lactate after initial clearing and high grade of steatosis (peak PAI-1 605 IU/mL). The other two livers were successfully transplanted, of which one later developed an anastomotic stricture. Each case, coincided with peaks in either thrombocyte count, bilirubin or FV activity.

Conclusions:
Factors of coagulation offer interesting new insights in pathophysiological aspects and might allow for additional assessment during longer courses of NMP. Prospective studies are warranted to validate these initial findings.

POS55

MITOCHONDRIAL DAMAGE AND KIDNEY FUNCTION IN DCD PORCINE KIDNEYS USING DIFFERENT FLUSH OUT SOLUTIONS

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Background:
During organ retrieval a systemic cold flush is performed to cool down organs. After the initial vascular flush and cooling of the kidneys, the temperature of the kidney increases again. Absence of oxygen and nutrients, this results in warm ischemic injury. We hypothesize that the use of prolonged, oxygenated, and/or nutrient enriched flush out solutions could improve organ quality during prolonged extraction times.

Methods:
Twelve porcine kidneys, donated after cardiac death (DCD), obtained from a local abattoir were subjected to 30 min warm ischemic time and were stratified in 3 different groups (n=4). Group 1: flush with UW-CS. Kidneys were kept on ice. Group 2: flush with 500ml UW-CS. Kidneys were rewarmed 15 min after the start of the flush. Group 3: flush with 1000ml of oxygenated UW-MPS. Kidneys were also rewarmed. The rewarming was performed in a temperature-controlled box to mimic a prolonged extraction of the organs with subsequent increase in temperature. After 60 minutes of ischemia all kidneys underwent 4h of oxygenated hypothermic (HMP) and

subsequently 4h of normothermic machine perfusion (NMP). Mitochondria were isolated from the cortical kidney tissue to measure mitochondrial activity, quality and injury using multiple analyses. Endothelial cells were isolated to evaluate endothelial injury and the kidneys were tested using NMP to evaluate metabolism, renal function, injury markers, histology and renal cortical microperfusion using laser speckle contrast imaging (LSCI).

Results:
No differences in mitochondrial respiration was observed between all 3 groups during the flush, after the flush, HMP or NMP. Mitochondrial membrane potential was similar between all 3 groups. Oxygen consumption during NMP showed similar results, indicating that all 3 groups had a similar metabolic activity. Mitochondrial injury, endothelial injury, renal function and injury and LSCI measurements are currently being performed.

Conclusions:
The first results show a similar mitochondrial respiration and function between all 3 groups over time. Further analysis will reveal whether mitochondrial injury, endothelial injury and renal function are affected by a different flush-outs at the beginning of a DCD donation setting and/or benefit from early oxygen and nutrient supply during the flush out.

POS56

MITOCHONDRIAL INTEGRITY AND FUNCTION IS MAINTAINED DURING LONG-TERM NORMOTHERMIC PERFUSION OF THE LIVER

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Background: Long-term normothermic machine perfusion (NMP) of the liver could represent a novel platform with the potential for organ modification, regeneration and repair. As a prerequisite, detailed insights into cellular metabolism and bioenergetic processes during the preservation are required.

Methods: Attempting to delineate the consequences of long-term organ procurement on mitochondrial integrity and function, a porcine model of 7-day liver NMP was applied. Biopsies from 7 livers were obtained before the initiation of perfusion, as well as on day 1, 5, 6 and 7, and analyzed by high-resolution respirometry (HRR; O2k, Oroboros Instruments, Innsbruck, Austria). Tissue biopsies were homogenized and the succinate-linked respiration was measured at 37°C in MiR05-Kit medium in the absence and presence of ADP. Membrane integrity was assessed by cytochrome *c* addition.

Results: Comparison of mitochondrial efficacy of ATP production before initiation of NMP and after 24 hours revealed an initial decline (*P-L* coupling efficiency on day 0: 0.84±0.05 vs. day 1: 0.75±0.10; p=0.016). Importantly, ATP production efficiency recovered on day 5 (0.81±0.03; p=0.188) remaining stable on day 6 (0.80±0.04; p=0.125) and 7 (0.82±0.04; p=0.437). Potential damage to the outer mitochondrial membrane was assessed by analysis of the cytochrome *c* control factor. A discrete elevation was observed after 24 hours (day 0: 0.21±0.11 vs. day 1: 0.33±0.06; p<0.05). However, recovery from this initial damage was indicated by lowered levels after day 5 (0.19±0.07; p=0.625) of perfusion, remaining unaltered until day seven (day 7: 0.20±0.07; p=0.594).

Conclusions: Our data indicate that both, mitochondrial integrity and function, can be maintained stable during NMP over several days. Importantly, a discrete impairment after 24 hours reveals to recover in the long-term perfusion setting.

POS57

REAL-TIME VISUALIZATION OF RENAL MICROPERFUSION USING LASER SPECKLE CONTRAST IMAGING

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Background:

Intraoperative parameters of renal cortical microperfusion (RCM) have been associated with postoperative ischemia/reperfusion injury. Laser speckle contrast imaging (LSCI) could provide valuable information in this regard with the advantage over the current standard of care of being a non-contact and full-field imaging technique. Our study aims to validate the use of LSCI for the visualization of RCM on ex vivo perfused human-sized porcine kidneys in various models of hemodynamic changes.

Methods:
A comparison was made between three renal perfusion measures: LSCI, the total arterial renal blood flow (RBF), and sidestream dark-field (SDF) imaging in different settings of ischemia/reperfusion. First, kidneys were reperfusion for 1 hour, resulting in increasing renal blood flow until stabilization. Next, a flow altering experiment was performed, changing the flow by steps of 50 ml/min. Finally, the kidneys endured ischemia by blockade of one of the two bifurcations of the renal artery by inflation of a intra-arterial balloon.

Results:
LSCI showed a good correlation with RBF for the reperfusion experiment (0.94 ± 0.02; $p < 0.0001$) and short- and long-lasting local ischemia (0.90 ± 0.03; $p < 0.0001$ and 0.81 ± 0.08; $p < 0.0001$, respectively). The correlation decreased for low flow situations due to RBF redistribution. The correlation between LSCI and SDF (0.81 ± 0.10; $p < 0.0001$) showed superiority over RBF (0.54 ± 0.22; $p < 0.0001$).

Conclusions:
LSCI is capable of imaging RCM with high spatial and temporal resolutions. It can instantaneously detect local perfusion deficits, which is not possible with the current standard of care. Further development of LSCI in transplant surgery could help with clinical decision making.

POS58

SGLT2 INHIBITORS IN KIDNEY TRANSPLANTATION: A MULTICENTER STUDY

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Background: SGLT2 inhibitors (SGLT2i) are the first line therapy in patients with diabetes and CKD and are related to better renocardiovascular outcomes. Regarding to its mechanisms of action, they could be also beneficial in kidney transplantion (KT). Despite a lack in published data about SGLT2i use in KT, their use seems to be common. Our aim is to investigate if SGLT2i are safe in KT recipients, as well as to analyse clinical and laboratory data in a KT cohort at 6 months after SGLT2i initiation.

Methods: Multicenter observational study in KT recipients with diabetes under SGLT2i treatment.

Results: 323 patients were included (284 6 months follow-up). Mean age 61.2±10.3 years-old, 74.4% men, 41% DM2. Previous antidiabetic therapies: insulin (48.1%), DPP4 inhibitors (36.1%), metformin (33.1%). Empagliflozin was started in 55.8%, dapagliflozin in 23.2%, and canagliflozin in 20.6%. After SGLT2i, a body weight reduction was observed, an improve in blood pressure and glycemic control, higher hemoglobin levels, and a decrease in cholesterol levels. A mild decrease in eGFR was detected while albuminuria decreased (table 1). In 10.5%SGLT2i was stopped: 2.78% urinary tract infection (UTI), 1.5% balanitis, and 1.2% eGFR reduction. During the study period, 6 graft losses (1 related to SLGT2), and 6 deaths (not related to SGLT2i).

Conclusions: SGLT2i have a safe profile in KT and are related to a better cardiovascular risk factors profile. Thus, they should be prescribed in KT recipients with diabetes and adequate eGFR. UTI surveillance is essential.

	Before SGLT2i	6 months after SGLT2i	p
Weight (Kg)	84.9 ± 17.3	82.9 ± 17.0	<0.001
Systolic BP (mm Hg)	137.3 ± 16.3	132.9 ± 15.9	<0.001
Diastolic BP (mm Hg)	76.9 ± 10	74.7 ± 10.5	<0.001
Hb (g/dl)	13.5 ± 1.5	14.0 ± 1.6	<0.001
eGFR (CKD-EPI)	59.1 ± 19.9	56.3 ± 20.6	<0.001
Glucose mg/dl	148.5 ± 43.3	135.2 ± 38.3	<0.001
Hb A1c (%)	7.50 ± 1.07	7.15 ± 1.05	<0.001
Mg (mg/dl)	1.63 ± 0.23	1.80 ± 0.24	<0.001
Uric acid (mg/dl)	6.36 ± 1.46	5.97 ± 1.39	<0.001
Cholesterol mg/dl	157.8 ± 36.7	153.6 ± 35.7	0.036
Urinary albumin/creatinine ratio (mg/g)	81 (23-240)	50 (12-172)	<0.001
Urinary protein/creatinine ratio(mg/g)	136 ± 68-380)	130 ± 70-270)	<0.001
Use of erythropoiesis stimulators (%)	10.9%	9.7%	0.453

POS60

THE EFFECT OF DIFFERENT NUTRIENTS ON MITOCHONDRIAL FUNCTION DURING LONG TERM INCUBATION OF PRECISION-CUT KIDNEY SLICES

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Background

Marginal donor kidneys are more susceptible to ischemia-reperfusion injury, which causes the mitochondria to produce reactive oxygen species (ROS) after reperfusion. To diminish production of ROS, mitochondria should be preserved optimally before transplantation. Normothermic machine perfusion (NMP) is increasingly explored to improve quality during preservation. An important question is how to provide metabolic support during normothermia and which nutrients are best to support mitochondrial function. The aim of this study is to investigate the effect of different nutrients on mitochondrial function during incubation of precision-cut kidney slices.

Methods

Porcine and human kidneys were studied. Pig kidneys were procured at the slaughterhouse after 30 minutes of warm ischemia followed by 3 hours oxygenated hypothermic machine perfusion. For the human kidney, a cortical piece was taken from kidneys retrieved after a nephrectomy and put on cold storage until arrival at the lab. Thereafter, precision-cut kidney slices (PCKS) were made and incubated in different media. The basic incubation medium was Dulbecco's Modified Eagle Medium (DMEM) without glucose and pyruvate. Then, either glucose, glutamine and/or fatty acids were added to the medium of the groups (table 1). At zero, 24 and 48 hours, mitochondrial respiration, using the Oxygraph-2k, was assessed, in which the glycolysis (pyruvate and glutamate) and the fatty acids beta-oxidation (C16-carnitine) were stimulated. Furthermore, mitochondrial energy status and injury markers will be analysed.

Results

Mitochondrial respiration, in terms of the respiratory control ratio (RCR) (state3/state4), after HMP is best when only glutamate is used to stimulate. Mitochondria were better preserved after 48 hours with the addition of glucose, glutamine and fatty acids compared to no nutrients. No differences were seen between glycolysis or fatty acid stimulation. Four groups showed a significant greater spare respiratory capacity (SRC) (state uncoupled/state3) than the RCR. Human PCKS did show the same trend as porcine PCKS after 24 hours, however no significant differences were seen.

Conclusions

Nutrient composition impacts mitochondrial function of PCKS. These data pave the way to optimize the perfusion solution for NMP of marginal kidneys.

Table 1. Overview nutrients per group

	Control *Pig & human*	Control 2 *Pig & human*	Experimental group 1 *Pig & human*	Experimental group 2 *Pig*	Experimental group 3 *Pig*	Experimental group 4 *Pig*	Experimental group 5 *Pig*
Glucose (2mg/mL)	-	+	+	-	-	-	+
Glutamine (2mM)	-	+	+	-	+	+	-
Fatty acids (1,5 mg/mL)	-	+	-	+	+	-	-

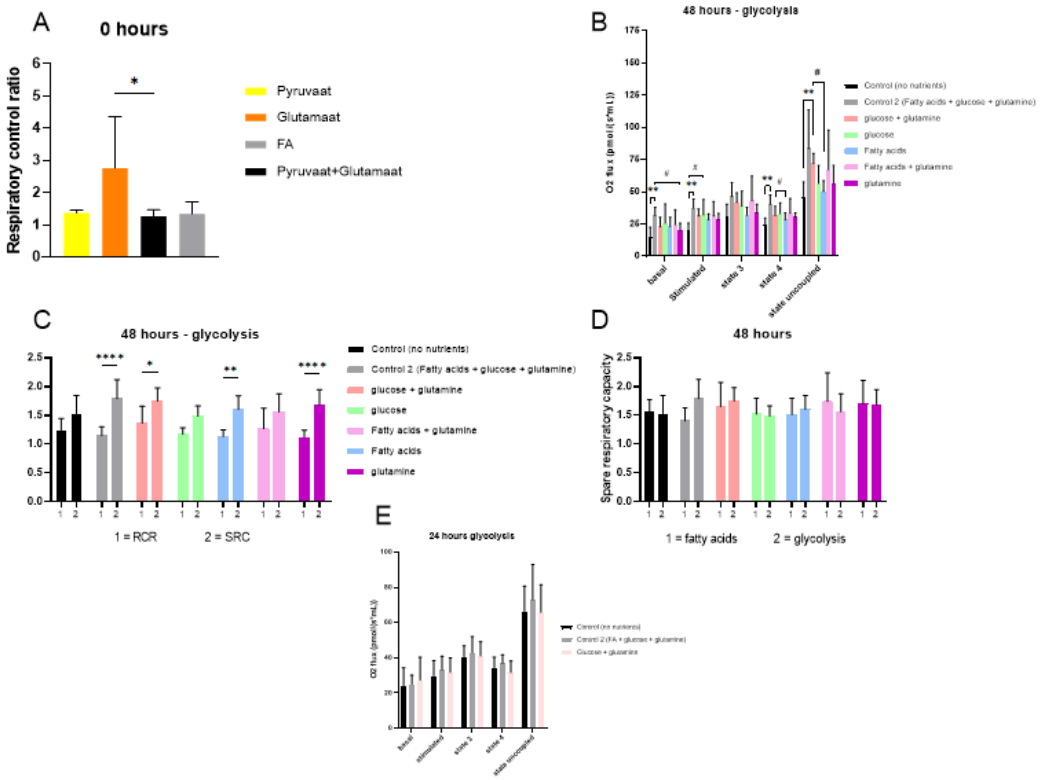

Figure 1. *A*: Respiratory control ratio after slicing. Significant higher RCR with stimulation with glutamate compared to pyruvate and glutamate. * p<0,05. *B:* Oxygen consumption after 48 hours of incubation, stimulated with pyruvate and glutamate. #p<0,05. **p<0,005. *C:* Respiratory control ratio and spare respiratory capacity after 48 hours of incubation, stimulated with pyruvate and glutamate. *p<0,05. **p<0,005. ****p<0,0001. *D:* spare respiratory capacity after 48 hours of incubation, stimulated with fatty acids or glycolysis. *E:* Oxygen consumption after 24 hours of incubation of human PCKS, stimulated with pyruvate and glutamate.

POS61

CHIMERISM AND BIDIRECTIONAL ALLORESPONSES IN PATIENTS RECEIVING LUNG TRANSPLANTATION

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Background: Long-term outcomes after lung transplantation (LuTx) are suboptimal, with 5-year survival < 60%. Graft rejection is a major complication limiting success. Lung grafts carry large numbers of T, B and antigen-presenting cells, leading to two-way alloresponses: graft-vs-host (GvH) and host-vs-graft (HvG). Despite the importance of T cells in driving

alloresponses, their dynamic repopulation, clonal distribution and alloreactivity after LuTx are largely unknown.

Methods: Serial bronchoalveolar lavage (BAL) provides a unique opportunity for temporal analysis of immune cells in lung grafts. We utilized a combination of flow cytometry that includes HLA-specific antibodies to distinguish donor- and recipient-derived cells, with high-throughput sequencing of donor and recipient T cells within pre-Tx lymphoid tissues and identification of alloreactive T cell clones in the GvH and HvG directions in mixed lymphocyte reactions.

Results: Donor T/B/monocytes in BAL post-Tx were gradually replaced by the recipient (Fig.1A-B). The turnover dynamics were faster in Pt4 compared to Pt1, especially for T cells. Donor CD8 T cells showed persistent high expression of TRM markers (CD69, CD103 and CD49a) and low expression of the effector T cell (Teff) marker CD28 (Fig.1C-D). However, recipient graft-infiltrating CD8 T cells gradually downregulated CD28 during the process of acquiring TRM features, indicating a phenotypic transition from circulating Teffs to lung-resident TRMs. An enrichment of HvG- compared to GvH-reactive sequences was observed in Pt4 among both pre-Tx mappable repertoires (Fig.1E) and all sequences in BAL post-Tx (Fig.1F). However, an opposite trend was shown in Pt1. Despite obvious differences in the turnover dynamics and alloreactive repertoire of T cells in BAL of these two patients, histology on the lung transbronchial biopsies taken on the same day reported no signs of rejection for either.

Conclusions: Dynamic replacement of donor T cells by the recipient, and the presence and dominance of GvH and HvG-reactive T cell sequences in lung allograft, may reveal the underlying mechanisms of graft acceptance and rejection and eventually help predict graft outcomes and develop novel strategies to promote tolerance after LuTx.

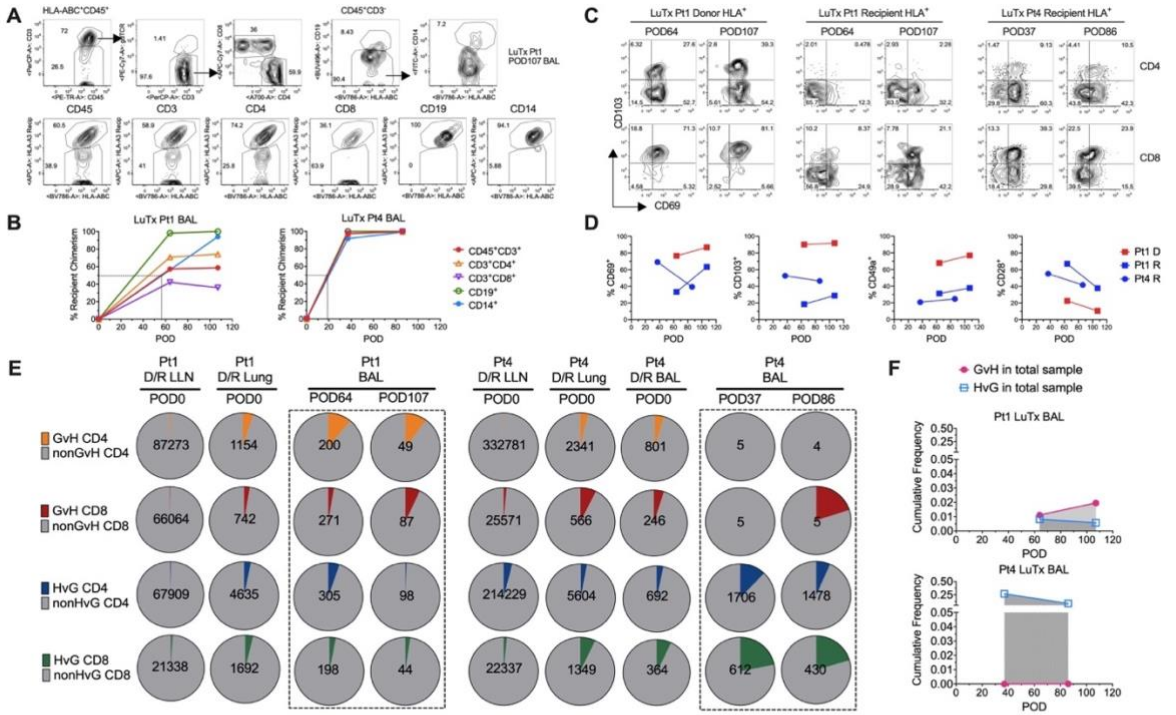

Figure 1. T cell immune profiling and sequencing data generated from Pts 1 and 4 were reported here, where we had a relatively complete tissue collection, including the donor and recipient tissues pre-Tx to perform mixed lymphocyte reactions to define GvH and HvG clones and two time points of BAL specimens post-Tx (POD <120) to track chimerism and clonal distribution. (A-D) Dynamic changes of multilineage chimerism and phenotypes of donor- and recipient-derived T cells in BAL specimens collected from Pts 1 and 4 after LuTx. (A) Representative gating of T/B/monocyte chimerism in post-Tx BAL sample collected from Pt1 POD107. (B) Percentages of total T cell, CD4 and CD8 T cell, B cell and monocytes in BAL collected from Pt1 POD 64 and POD107 and from Pt4 POD37 and POD68. Black dotted lines indicates the estimated PODs when donor T cell replacement by the recipient reached 50%. (C) TRM phenotypes ($CD69^{+}CD103^{+/-}$) of donor- and recipient-derived CD4 and CD8 T cells in post-Tx BAL. (D) Percentages of $CD69^{+}$, $CD103^{+}$ $CD49a^{+}$ and $CD28^{+}$ CD8 T cells in the donor or recipient compartments of BAL collected from Pts 1 and 4. (E-F) Distribution and frequencies of GvH- and HvG-reactive T cell sequences in pre-Tx donor and recipient lung associated tissue sites (LLN, lung, BAL) and in post-Tx BAL of LuTx Pts 1 and 4. LLN: lung associated lymph nodes. (E) Cumulative frequencies of alloreactive sequences detectable in Pt1 and Pt4 are shown in pie charts, including GvH CD4 among donor-mappable CD4 sequences, GvH CD8 among donor-mappable CD8 sequences, HvG CD4 among recipient-mappable CD4 sequences, and HvG CD8 among recipient-mappable CD8 sequences. The counts of mappable unique sequences are annotated in the center of each pie chart. D/R: Donor or Recipient. (F) Cumulative frequencies of GvH (pink) and HvG (blue) clones among total samples at indicated time points are shown in the BAL of Pt1 and Pt4. Cumulative frequency was calculated as a percentage of all sequences weighted by copy numbers in designated populations. Mappable clones refers to clones that were detectable in sequenced pre-Tx unstimulated LLN and/ or $CFSE^{lo}$ T cell populations in mixed lymphocyte reactions (MLRs) from the donor or recipient.

POS63

COMPARISON OF TWO QUANTITATIVE REAL-TIME PCR ASSAYS FOR PLASMA TTV-DNA MONITORING POST KIDNEY TRANSPLANTATION

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Background:
Plasma viral load of the highly prevalent, non-pathogenic torque teno virus (TTV) reflects the strength of immunosuppression after solid organ transplantation. An optimal TTV range has been defined for risk stratification of graft rejection and infection in the first year post kidney transplant applying an in-house PCR at the Medical University of Vienna (MUV). Due to the lack of an international standard the optimal plasma TTV range has to be verified for each distinct TTV PCR assay set-up. Recently, a commercial PCR - the TTV R-GENE® kit - has been CE certified for clinical use. In this analysis, TTV R-GENE® was compared with the in-house TTV PCR.

Methods:
Patients and events were selected from the prospective TTV-POET trial including all 628 consecutive adult recipients of a kidney allograft transplanted at the MUV, between January 2016 and July 2020. Patients were followed for 12 months post-transplant. TTV was quantified longitudinally by the TTV R-GENE® and our in-house PCR. Analyses for both assays were performed in parallel using the same nucleic acid extracts.

Results:
A total of 342 plasma samples from 314 renal transplant recipients were tested. The study cohort included 79 infectious events and 18 biopsy-proven rejections. All but three samples (n=339) were tested TTV positive with both assays. Mean $\log_{10}$ c/mL TTV load was 7.52 (SD 1.98) and 6.16 (SD 1.62) for in-house PCR and TTV R-GENE®, respectively. The results of the two assays were highly associated (intercept: estimate -0.86, 95% CI -1.04 to -0.67; R 0.91). Bland-Altman analysis showed a mean $\log_{10}$ TTV load of -1.38 c/mL detected by TTV R-GENE® kit compared to in-house PCR. Difference in TTV load of >2 $\log_{10}$ to this mean difference was observed in 13 samples, for which 4 and 1 test results showed additional clinical discordance concerning prediction of infectious events and rejections for TTV R-GENE® and in-house PCR, respectively.

Conclusions:
Under consideration of the conversion factor quantitative results of both assays are highly comparable resulting in similar diagnostic accuracy for infections and rejections after kidney transplantation. Further analysis including single strain analysis and sequencing will focus on discrepant results.

POS64

CYTOF INVESTIGATIONS OF CMV IMMUNE RESPONSE PROFILES: THE CICERONE STUDY

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Background:
CMV remains a threat for kidney transplant recipients (KTR). Deciphering the impact of CMV on the immune system of KTR has helped improving CMV outcomes and management. In this study, we combined phenotype and functional response analyses to investigate the early immune response to CMV of KTR.

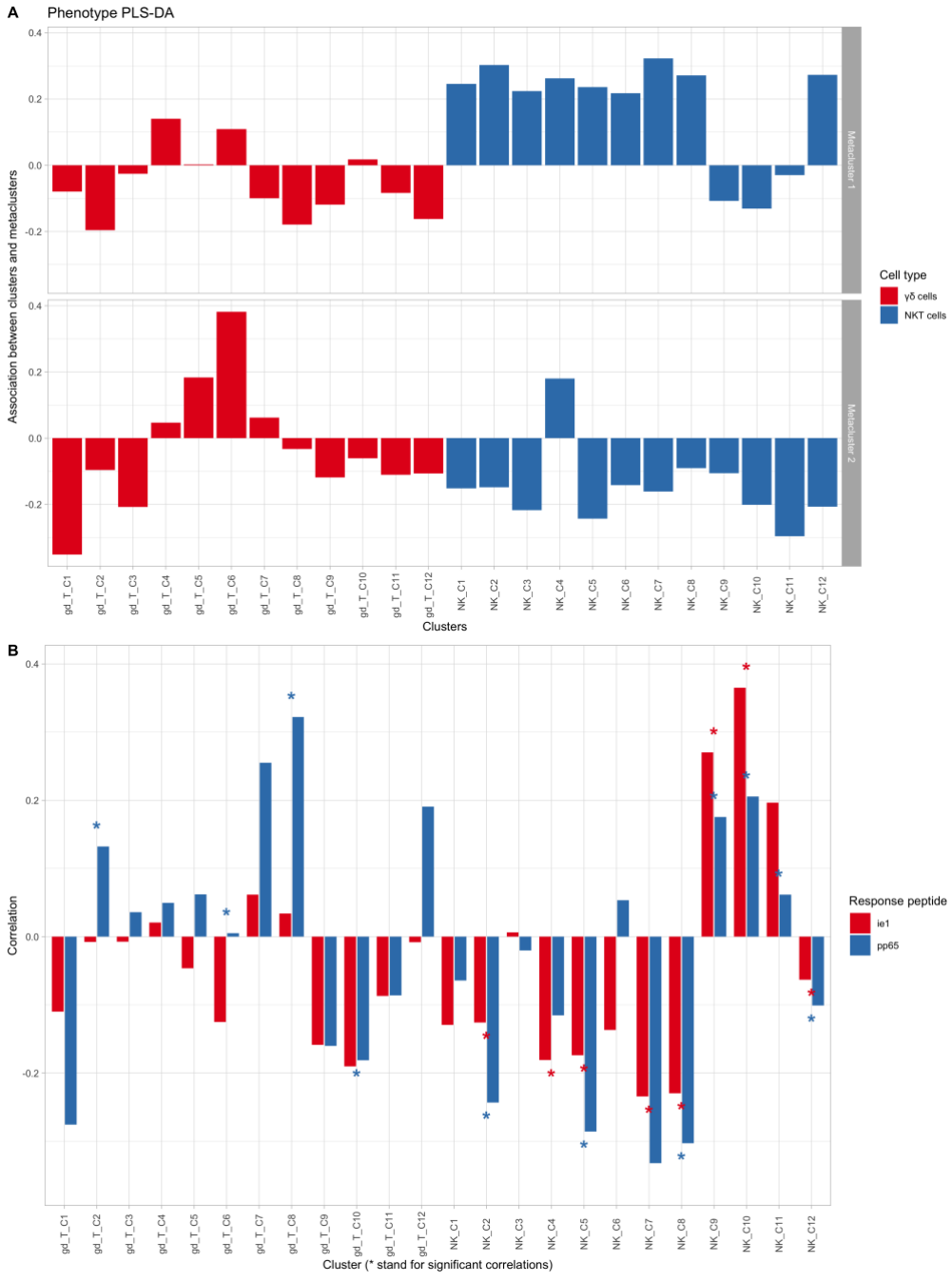
Methods:
In a population of 28 CMV positive KTR, we sampled PBMC on 4 occasions: pre-transplantation, then at months 1, 3, and 12. For each sample, we simultaneously assessed a CyTOF (Cytometry by Time Of Flight) T phenotype using 35 antibodies, the CMV-specific T-ELISpot functional response against IE1 and pp65 antigens, and the CMV replication events. Using semi-supervised clustering of the phenotypes, we identified Natural Killer T cells (NKT) subsets and gamma-delta T cells subsets and evaluated their association with CMV replication events and with both functional responses.

Results:
Among the 28 patients, 12 patients underwent a CMV-replication event, at a median time of 1.8 (IQ 1-3) months. Overall, the whole populations of NKT or gamma-delta cells were not associated with the outcome nor the functional

response. On the other hand, senescent CD4 and exhausted CD8 populations were positively associated with both functional responses.

In the CyTOF analysis, there were 12 NKT and 12 gamma-delta clusters. We show heterogeneity within NKT and gamma-delta cells. In a Projection to Latent Structures – Discriminant Analysis (PLS-DA), we combined clusters in meta-clusters associated with a CMV replication event. A subset of 3 NKT clusters was inversely associated with both CMV replication events and with concomitant functional responses, when compared to the 9 other clusters that had the inverse behavior (figure 1). These 3 NKT clusters were defined by CD11b (ITGAM) expression. Similarly, a subset of gamma-delta T cells was positively correlated with the pp65 response, expressing CD11c. The other cluster expressing CD11c but negatively correlated with the functional response expressed PD1, a known exhaustion marker.

Conclusions:
Using the extended phenotyping allowed by CyTOF analysis, we show the added value of subpopulations delineation among known lymphocytes populations, when compared to traditional flow cytometry. This suggests investigating CD11b on NKT cells and CD11c on gamma-delta cells.



POS65

DIFFERENCES IN SARS-COV-2-SPECIFIC ADAPTIVE IMMUNITY BETWEEN CONVALESCENT AND VACCINATED SOT PATIENTS AND IMMUNOCOMPETENT INDIVIDUALS

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Background: Short-term adaptive immune memory has been reported among immunocompetent (IC) and convalescent Solid Organ Transplant (SOT) individuals following SARS-CoV-2 infection as well as after active vaccination. However, quality and longevity of anti-viral immune memory comparisons between natural and active immunization has not been thoroughly assessed among SOT.

Methods: SARS-CoV-2-specific adaptive immune memory was assessed at different compartments (serological, memory B cells [mBC] and cytokine [Th1: IFN-γ, IL-2, IFN-γ/IL-2 and Th2: IL-21 and IL-5] producing T cells) by ELISA and FluoroSpot-based assays, respectively, in 41 convalescent patients with severe COVID-19 (22 SOT and 19 IC) and 39 vaccinated patients (19 SOT and 20 IC) with a mRNA-based vaccine) at different time-points post immunization (T1=21days after infection/1st dose; T2=3months after infection/2nd dose; T3=6months after infection/2nd dose). Additionally, a group of convalescent mild (19 SOT and 19 IC) and asymptomatic patients (9 SOT and 10 IC) were also evaluated at T3.

Results: Statistically significant higher immune responses in all immune compartments were observed in convalescent patients than among those after vaccination. After vaccination, low seropositivity rates (5,88%) were observed among SOT after 1st dose, whereas seroconversion was fully achieved in IC patients and SOT with severe COVID-19 ($p<0.001$). Similarly, while the presence of mBc after vaccination progressively increased over time, it was less pronounced and significantly delayed among SOT than convalescent patients in all time points ($p<0.001$ T1, T2 and T3). SARS-CoV-2-specific Th1 and Th2 frequencies were significantly higher among vaccinated IC patients than SOT, being these significantly lower than those observed in convalescent among SOT and IC patients ($p<0.001$ T1, T2 and T3). At 6 months after vaccination, IgG titters, mBc frequencies and Th1/Th2 T-cell responses after two-dose vaccination in SOT mimicked those observed in convalescent SOT with an asymptomatic/mild clinical COVID-19 infection.

Conclusions: The type of immunization against SARS-CoV-2, either natural or active after vaccination, clearly differentiates the quality and length of adaptive immune memory, with a clear weaker immune response observed among SOT.

POS67

FK-BINDING PROTEIN 12 AND P-GLYCOPROTEIN AND RELATIONS TO THE DIFFERENCES OF THE INTRACELLULAR TACROLIMUS CONCENTRATION IN T CELLS AND MONOCYTES

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Background:

Little is known about the pharmacokinetics of intracellular tacrolimus, particularly in $CD3^+$ T lymphocytes and $CD14^+$ monocytes. We demonstrated a significantly lower intracellular tacrolimus concentration in $CD3^+$ ($TAC_{[CD3]}$) compared with $CD14^+$ cells ($TAC_{[CD14]}$). Our objective was to investigate the role of 2 important proteins involved in intracellular tacrolimus distribution, namely FK-binding protein 12 (FKBP12) and P-glycoprotein (P-gp) to explain these differences in intra-cellular tacrolimus concentrations.

Methods:

Rhodamine (Rh), a substrate of P-gp, was used to determine the P-gp activity in $CD3^+$ T cells and $CD14^+$ monocytes obtained from healthy volunteers. FKBP12 and P-gp expression was semi-quantified by Western blot and flow cytometry comparing between $CD3^+$ T cells and $CD14^+$ monocytes. To determine the effect of P-gp on intracellular tacrolimus concentration, verapamil, was added to kidney transplant recipient's blood before the cell isolation process. Results were compared with the same blood samples not treated with verapamil.

Results:

$CD3^+$ T cells showed a significantly lower percentage of Rh-positive cells after 2 hours incubation at 37°C (61±14%) and 25°C (80±9%) compared with 4°C (94±4%) ($p<0.001$). Adding verapamil completely negated this temperature effect, suggesting that tacrolimus is pumped out of $CD3^+$ T cells if not processed at 4°C or in the absence of a P-gp inhibitor. Flow cytometric analysis revealed a significantly higher expression of P-gp on $CD3^+$ T cells than $CD14^+$ monocytes and a lower intensity of FKBP12 in $CD3^+$ T cells than $CD14^+$ monocytes. Western blot confirmed that $CD3^+$ T cells had higher P-gp and lower FKBP band density than $CD14^+$ monocytes. By adding verapamil to patient samples, $TAC_{[CD3]}$ was 53-100% higher than samples from the same patients in the absence of verapamil.

Conclusions:

The higher activity of P-gp and the lower concentration of FKBP-12 explain the lower $TAC_{[CD3]}$ compared with $TAC_{[CD14]}$. A substantial amount of tacrolimus is actively pumped out from $CD3^+$ T lymphocytes during the cell isolation process if P-gp is not properly inhibited. Verapamil blocks this process and gives reliable intracellular tacrolimus T lymphocyte concentrations.

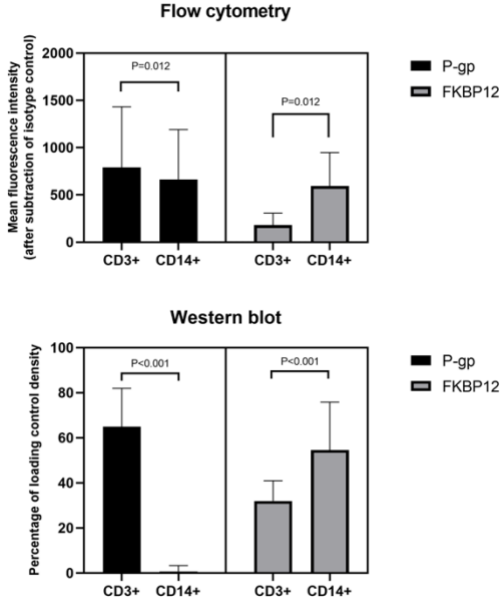

Figure 1: Difference of P-gp and FKBP-12 between $CD3^+$ T lymphocytes and $CD14^+$ monocytes.

POS68

HUMORAL RESPONSE TO 3 AND 4 DOSES OF MRNA COVID-19 VACCINE IN KIDNEY TRANSPLANT RECIPIENTS WITHOUT PRIOR SEROCONVERSION

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Background:

Since growing evidence shows diminished response to mRNA vaccination in organ transplant recipients, a 3rd and 4th dose strategy of mRNA vaccination was recommended for this population.
We aimed to achieve seroconversion after mRNA Covid-19 vaccine in prior seronegative kidney transplant recipients (KTR) by administering a 3rd and 4th dose following national recommendations and described characteristics of KTR unresponsive to mRNA vaccine.

Methods:

We included 323 KTR in this retrospective single-center study and analyzed humoral response to–mRNA vaccine measuring anti-spike protein IgG antibody levels after the 3rd and 4th vaccination dose. For the 1st and 2nd dose both mRNA vaccine types depending on availability were administered (n=74, 22,9% mRNA-1273; n=249, 77,1% BNT162b2). Based on our published data showing improved response after mRNA-1273, this vaccine was preferred for the 3rd (n=222, 91,9% mRNA-1273) and 4th dose (n=41, 90,2% mRNA-1273). Titers were measured 37 days (IQR 30-40) after admission of the 3rd and 26 days (IQR 26-27) after the 4th dose using SARS-CoV-2 IgG II Quant assay, Abbott. A negative response was defined as a titer <7,1 BAU/ml by the manufacturer. GraphPad Prism was used for descriptive analyses. The study was approved by the Ethics Committee of JKU Linz.

Results:

Out of 159 KTR being seronegative after two doses, 129 followed our recommendation to receive a 3rd dose (n=117, 90,7% mRNA-1273; n=12, 9,3% BNT162b2). 32 KTR (24,8%) remained seronegative after 3 doses and were predominately male (n=23, 71,9%), aged median 69 years (IQR 64-73), transplanted median 4,3 years (IQR 2,3-10,7) ago and

almost all were maintained on Calcineurin inhibitors (CNI; n=30, 93,8%) and Mycophenolate (n= 30, 93,8%).

Fourth dose data were available for 21 out of 32 seronegative KTR after 3 doses (n=13, 92,9% mRNA-1273/ n=1, 7,1% BNT162b2). After the 4th dose two thirds (n=14, 66,7%) remained seronegative, were predominately (n=9, 64,3%) male, aged median 71 years (IQR 68- 76), transplanted median 3,4 years ago (IQR 1,2-12,0). Immunosuppression contained CNI (n=14, 100%) and Mycophenolate (n=13, 93,8%).

Conclusions:

We report a seroconversion rate of 75,2% in prior seronegative KTR after 3 doses of mRNA vaccine. Seronegative KTR after 3 doses showed a humoral response in 33,3% after the 4th dose.

POS69

IMMUNE PROFILING OF MIGRATING AND GRAFT-ASSOCIATED ΓΔ T CELLS AFTER HUMAN INTESTINAL TRANSPLANTATION REVEALS UNIQUE INNATE AND ADAPTIVE FEATURES

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Background: Innate- and adaptive-like features of human γδ T cells are associated with different T cell receptor (TCR) repertoires, defined as Vγ9^{+}δ2^{+} and non-Vγ9δ2, respectively. Despite comprising a significant portion of lymphocytes residing in many organs, the role of γδ T cells in transplantation outcomes is unclear.

Methods: We performed phenotypic and clonal tracking of donor- and recipient-derived γδ T cells after human intestinal transplantation (ITx) in blood, intestinal graft and bone marrow (BM).

Results: We previously demonstrated that donor T cell macrochimerism (peak level ≥4% in blood) is associated with less rejection. We now show that high levels of γδ T cell blood chimerism were only observed in patients with macrochimerism. Remarkedly, donor γδ T cells were detected in recipient BM 105-357 days post-Tx. Single-cell profiling of BM-infiltrating donor γδ T cells revealed both Vδ1- and Vδ2-dominant clonotypes with cytotoxic effector phenotypes that might contribute to graft-vs-host responses. In one multivisceral Tx patient, the top dominant donor γδ TCR clone (Vγ8Vδ1) detectable during the peak T cell chimerism in blood (8-20 days post-Tx) was also predominantly present in the recipient BM 126 days post-Tx, with highly persistent cytotoxic profiles (GZMB/PRF1/GNLY) but reduced proliferation (MKI67) and BM-homing (CXCR4) features. BM-infiltrating donor Vδ2 clonotypes tended to be more “public” and were shared by three pediatric patient post-Tx BM specimens and in pre-Tx repertoires across pediatric donors and tissues. Many of these Vδ2 clones are Vγ9δ2 with zero N-additions that likely originate from fetal liver and cord blood. However, these Vδ2-dominant clones were not present in adult lymphoid tissues, gut or BM, suggesting an age-related distribution and migration pattern. In contrast to αβ T cells, the turnover dynamics of γδ T cells in the graft showed a stronger association with donor age than with the status of macrochimerism. Graft-repopulating recipient γδ T cells showed activated effector phenotypes early post-Tx and gradually developed into cytotoxic resident-memory T cells with “private” Vδ1 clonotypes.

Conclusions: γδ T cells have the potential to modulate immune responses locally and systemically. They may participate in host defense and graft rejection after ITx.

POS70

INTEGRATED ADVANCED MODEL-INFORMED PRECISION DOSING OF TACROLIMUS IN DE NOVO RENAL RECIPIENTS.

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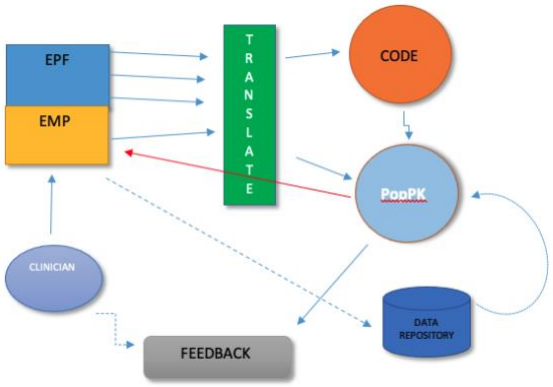
development team, Leuven, Belgium, [4]*University of Leuven, Leuven Biostatistics and Statistical Bioinformatics Center, Leuven, Belgium*, [5]*University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium*

Background: Tacrolimus has a narrow therapeutic index. Pre-dose concentrations are used to guide dose adaptations aimed at predefined target concentrations that vary with time after grafting and according to clinical conditions.
Empirical Bayesian Estimation (EBE) is the key algorithm behind model-informed precision dosing (MIPD). However EBE is sensitive to misspecifications induced by unaccounted trends which can be improved by applying Model-Predictive Control leading to superior predictive performance.

Methods: Based on a retrospective dataset of 315 kidney recipients a 1-compartment PK model with time-dependent clearance was built. MIPD performance was evaluated by calculating error to predict future concentrations, which is directly related to dosing precision and probability of target attainment (PTA). By incorporating correlated within-patient variability when predicting future individual concentrations, PTA improved beyond the theoretical upper limit which was set at 45% based on the initial model residual error. *In silico* testing yielded a Bayesian feedback dosing algorithm accurately predicting future trough concentrations and adapting each dose to reach a target concentration.

Results: The advanced MIPD tool was integrated in the hospital Electronic Patient File (EPF) for *de novo* renal recipients using automatically retrieved individual model covariates to generate a model-informed dosing advice every time a new tacrolimus trough concentration becomes available in the EPF (Fig). The future tacrolimus dose advice is automatically fed into the Electronic Medication Prescription (EMP red arrow) awaiting formal validation (or overruling) by the clinician. A prospective randomized-controlled clinical validation study comparing physician dosing with MIPD is currently recruiting (target n=200). MIPD performance is evaluated by 3 PK endpoints: average PTA, median time to 3 concentrations in target, and mean log-squared distance to target.

Conclusion: Prior *in silico* testing is mandatory to establish (f)utility of integrating advanced MIPD tools into real-life clinical EPF/EMP in order to support clinicians with individualized precision dosing of narrow therapeutic index drugs at the point of care.



POS71

INTRACELLULAR TACROLIMUS CONCENTRATION IN CD3+ T LYMPHOCYTES AND CD14+ MONOCYTES AND THE ASSOCIATION WITH ACUTE REJECTION

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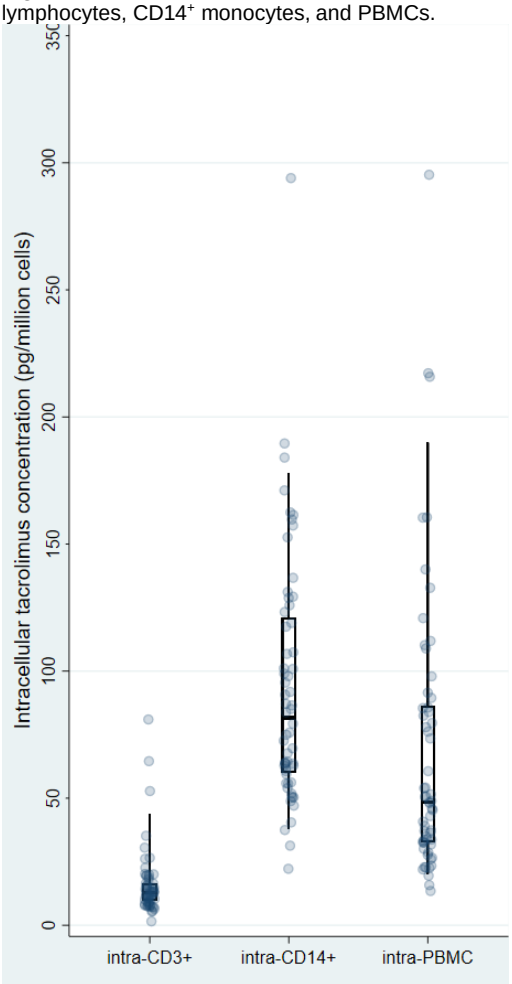
Background:
The intracellular tacrolimus concentration in peripheral blood mononuclear cells (PBMCs) ($TAC_{[PBMC]}$) was proposed to better represent the active concentration than the pre-dose whole blood concentration (C_0). However, in previous studies, the $TAC_{[PBMC]}$ did not correlate well with acute rejection. Since tacrolimus acts within T lymphocytes and other white blood cells such as monocytes, we investigated the association between the tacrolimus concentration in $CD3^+$ T lymphocytes ($TAC_{[CD3]}$) and $CD14^+$ monocytes ($TAC_{[CD14]}$), and acute rejection after kidney transplantation.

Methods:
From total 61 samples in this case-control study, 28 samples were obtained during biopsy-proven acute rejection (rejection group) and 33 samples were obtained in the absence of rejection (control group). PBMCs were collected from both cryopreserved samples (retrospectively) and freshly obtained samples (prospectively). $CD3^+$ T lymphocytes and $CD14^+$ monocytes were isolated from PBMCs and measured for their intracellular tacrolimus concentration.

Results:
The correlations between the tacrolimus whole blood and intracellular concentrations were poor. $TAC_{[CD3]}$ was significantly lower than $TAC_{[CD14]}$ (median 12.8 *vs* 81.6 pg/million cells; p<0.001). No difference was found between the rejection group and control group regarding the $TAC_{[PBMC]}$ (48.5 *vs* 44.4 pg/million cells; p=0.82), $TAC_{[CD3]}$ (13.4 *vs* 12.5 pg/million cells; p=0.28), and $TAC_{[CD14]}$ (90.0 *vs* 72.8 pg/million cells; p=0.27). However, PBMCs that were freshly isolated showed significantly higher $TAC_{[PBMC]}$ compared with PBMCs from cryopreserved samples. Subgroup analysis of the intracellular tacrolimus concentrations from freshly isolated cells again did not show difference between the rejectors and non-rejectors.

Conclusions:
The correlations between tacrolimus whole blood and intracellular concentrations were poor. The difference in $TAC_{[CD3]}$ and $TAC_{[CD14]}$ between patients with and without rejection could not be demonstrated. However, further optimization of the cell isolation process is required because a difference in $TAC_{[PBMC]}$ between fresh and cryopreserved cells was demonstrated.

Figure 1: Intracellular tacrolimus concentration in $CD3^+$ T lymphocytes, $CD14^+$ monocytes, and PBMCs.



POS73

MACHINE LEARNING IN KIDNEY TRANSPLANTATION: A NEW POSSIBILITY FOR THE GRAFT SURVIVAL PREDICTION

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Background: One of the major open questions in the field of kidney transplantation is the graft survival, and therefore life expectancy of the receiver.
In the last decades the information available about the recipient, the donor and the organ transplanted have increased considerably. In addition, the knowledge about transplantation has grown, so it could be possible to make a more accurate prediction about the outcome.
In the current state of the art, these analyses are conducted by applying traditional machine learning techniques, such as multilayer perceptrons, decision trees, etc. However, these algorithms require a large and precise amount of data.
Our aim is to create a new machine learning technique to predict our patients' life expectancy and organ durability without requiring complete information and huge amounts of data to be able to produce reliable results.

Methods: Due to the incomplete nature of the data, in order to trace a patient's life expectancy, similarity metrics among the involved patients are searched and compared with the actual lifespan of a very similar real patient. Therefore, we have applied algorithms, that can be traced back to recommendation systems, that suggest life expectancy by comparing the available data of a patient with those of all other patients who have already undergone a transplant. After that, results are processed again with an unsupervised machine learning technique, obtaining a numeric result that corresponds to the life expectancy of the transplanted patient in years.

Results: In our study, we have considered a cohort of 377 patients, who underwent kidney transplantation in Ospedale di Circolo, Varese, from 2013 to 2021. In order to create the first algorithm, we focused primarily on serum creatinine level during the first year after transplantation and on the comorbidities of both donor and recipient, recipients' age and years in dialysis treatment. Data processing is still ongoing, due to the limited number of the statistical sample.

Conclusions: Our method could be a valid starting point to apply machine learning advanced techniques to life expectancy and organ durability estimates, which could complement other scores already validated. Further studies are needed to make the learning machine more accurate.

POS75

PERFUSATE IL-6 LEVELS DURING LIVER NMP ARE PREDICTIVE FOR HEMODYNAMIC RESPONSE AND CATECHOLAMINE DEMAND AFTER REPERFUSION IN THE RECIPIENT

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Background:
Normothermic liver preservation (NMP) has become a clinical routine at several transplant centres. Reperfusion-syndrome occurs less often in recipients of NMP-livers compared to cold stored livers. We hypothesized that perfusate interleukin (IL)-6 during liver NMP correlate with recipient hemodynamics in the post-reperfusion period.

Methods:
Consecutive NMP-liver transplants at a single-centre were prospectively analysed. Perfusate samples were collected at 1 and 6 hours of NMP and at the end of perfusion and analysed for IL-6 levels. Median arterial pressure (MAP) and catecholamine need during surgery were recorded. The anhepatic phase was defined as baseline for MAP and catecholamine requirements.

Results:
Over a period of 36 months, IL-6 perfusate measurements were assessed in 77 livers undergoing NMP and transplantation; 15/77 (19.5%) were DCD organs. The median donor age was 61 (15-87) years, median recipient age was 60 (19-73) years. Median (IQR) cold ischemia time was 6.2 (2.1) hrs, NMP-time and overall preservation time were 17.6 (10.4) hrs and 23.6 (10.6) hrs. Median (IQR) IL-6 levels (ng/L) after 1, 6 hrs and NMP-end were 52 (175), 278 (674) and 174 (2171). Neither duration of CIT nor NMP correlated with IL-6 levels over time. NMP-livers were stratified for the median of the last IL-6 measurement. Recipients receiving NMP-livers with perfusate IL-6 levels above the median developed significantly lower post-reperfusion MAP (dropping 20% from baseline) and displayed a significant higher demand of catecholamines (increase of 25% from baseline) up to 30 minutes after reperfusion (figure 1A-B). Perfusate IL-6 did not correlate with the occurrence of early allograft dysfunction.

Conclusions:
Perfusate IL-6 levels during liver NMP are clinically relevant as they help to predict the post-reperfusion hemodynamics in recipients.

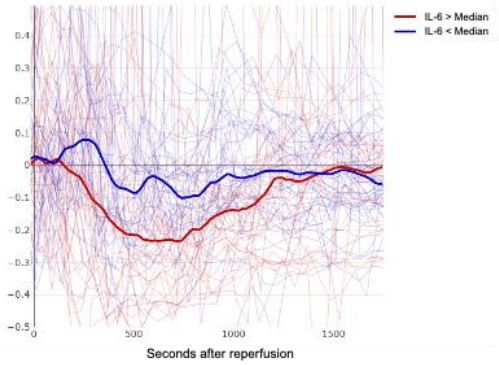

Figure 1A) MAP compared to baseline

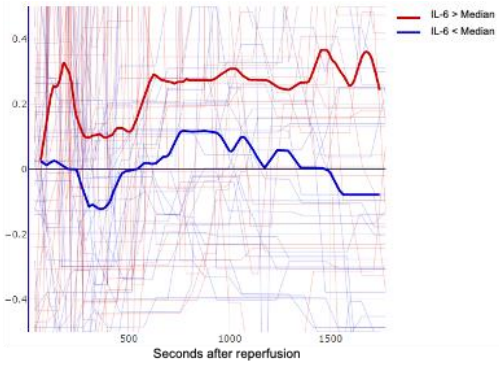

Figure 1B) Catecholamine demand compared to baseline

POS76

QUOD BIOBANK: THE FUTURE OF TRANSPLANT RESEARCH

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Background: The UK QUOD Biobank was launched in 2013 to provide a not-for-profit, nationwide repository of samples from deceased donor organs retrieved for transplant linked to national organ donation and transplant data. Researchers can combine biological sample analysis with clinical donor data with the aim to help clinicians decide on suitable organs and ultimately, impact on patient outcomes. QUOD is a unique partnership between UK academic transplant centres and NHS Blood & Transplant, hosted by the University of Oxford. This report describes QUOD's achievements.

Methods: Donor families are approached by Specialist Nurses for QUOD consent. QUOD samples are collected at set time points during the retrieval process. Longitudinal blood, urine, bronchial lavage, and abdominal and cardiothoracic tissue samples are recovered in ICU and by National Organ Retrieval Service teams, then processed in dedicated QUOD laboratories, before being sent to Oxford hub for final processing and storage. Full sample traceability is maintained via a bespoke database.

Results: Rates vary but consistently >80% of all UK donors also donate to QUOD, resulting in collection of samples from 5,925 donors from 60 hospital trusts; a total of 109,561 samples (>56,000 blood, >13,000 urine and >41,000 tissue biopsies) since June 2013.

Since conception, QUOD has received 71 research applications for access to samples of which 54 have been approved by Steering Committee. 31,807 samples have been issued resulting in 55% of all donors having samples used in research. QUOD samples are credited in more than 30 international publications and presentations.

Conclusions: QUOD is a unique opportunity and tremendous achievement improving scientific knowledge and offering donors and their families a way to maximise the impact of their generous gifts of donation. In addition, QUOD has developed multi-centre scientific platforms facilitating collaborative research focusing on genetic, multi-omics and imaging/histopathological analyses to assess organ quality, predict graft outcomes or increase insight in mechanisms of injury and repair.

Consent rates are high and sample collection is safe ensuring that this large scale, collaborative transplant biobank will continue to provide a unique platform for transplant research and investigation.

POS78

TACROLIMUS LEVELS: DOCUMENT-ATION IN THE OUTPATIENT SETTING

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Background: Tacrolimus (TAC) is a commonly used immunosuppressant in renal transplant patients. The trough level is measured in the outpatient clinic to ensure therapeutic range. The aim of our audit was to review the effectiveness of documentation of tac levels on our IT system and communication of the results to the patient.

Methods: A retrospective audit was performed in the renal transplant department at Manchester Royal Infirmary (MRI) over a five day period. We set the standard of every result being recorded, reviewed and communicated to the patient within 24 hours of the result being available.

Results: Over this 5 day period there were 206 patient encounters in the renal transplant outpatient clinics. 57 patient encounters were excluded (28 did not attend, 16 patients not taking TAC, 6 coded incorrectly, 4 did not have TAC blood test, 2 admitted to hospital from clinic and 1 attended for different reason) leaving 149 patient encounters. 99 of these patients had face to face clinical appointments whilst 50 patients had a virtual appointment. Of 149 patients, only 2 patients had the TAC level and dose documented on our electronic noting system within 24 hours of the TAC level being reported.

Conclusions: Based on this audit we have identified a lack of documentation of TAC levels which could lead to wrong dosage and therefore have detrimental effects for the patient. We have implemented a new policy of acknowledgement of the result on the IT system, discussion of every result in the post clinic MDT and asking our colleagues to write a

note on the IT system if any result is not in range.

POS79

UTILITY OF CONTRAST-ENHANCED ULTRASOUND (CEUS) IN PREDICTING KIDNEYTRANSPLANTATION OUTCOME: A MONOCENTRIC PROSPECTIVE STUDY

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Background: Renal transplantation is the gold standard replacement therapy when irreversible chronic kidney disease occurs, owing its superiority in survival and quality of life compared to hemodialysis.
An acute kidney injury always occurs after kidney transplantation and too frequently progresses to the clinical diagnosis of delayed graft function (DGF), involving 25% of patients undergoing renal transplant and requiring at least one dialysis treatment in the first post-transplantation week. DGF is a major obstacle for allograft survival as it can be responsible for an increasing risk of acute rejection and chronic allograft nephropathy (CAN).
The aim of our prospective study is to conceive a new predictive model for grafts outcome based on kidney CEUS examination, with the purpose to establish a more tailored immunosuppressive therapy.

Methods: The study was performed with EPIQ ultrasonoscope (Philips Healthcare, Andover, MA) using SonoVue (Bracco Company, Milan, Italy) as contrast medium. The Ultrasound Dynamic evaluation was performed for 1 minute. A 10mm side square region of interest (ROI) was defined on the superior polar renal cortex. QLAB Software was used to obtain quantitative analysis of renal perfusion including:

- time-intensity curve,
- slope rate of ascending curve (A),
- time to peak (TTP),
- derived peak intensity (DPI),
- area under the curve (AUC).

Results: 22 patients were included in the study. Among them, 3 patients developed a DGF and 1 developed a Primary Non Function (PNF). The results revealed that TTP in patients with DGF was significantly later on than patients with Early Graft Function (EGF). DPI and AUC were lower in the DGF group than EGF group. The patient who developed a PNF, had TTP, AUC and DPI undetectable. No correlation between A and functional recovery of the graft was found.

Conclusions: CEUS seems to provide a better evaluation of grafts' microcirculation and perfusion, allowing a better prediction of its functionality and outcome.
Further studies are needed to obtain a Perfusion Index, that will be the cornerstone of a new predictive model of functional recovery.

POS80

VALIDATION OF THE OPTIMAL TT VIRUS RANGE FOR RISK STRATIFICATION OF GRAFT REJECTION AND INFECTION IN KIDNEY TRANSPLANT RECIPIENTS BY TTV R-GENE®

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Background: The non-pathogenic and ubiquitous Torque Teno virus (TTV) plasma load is associated with immunosuppression in solid organ transplant recipients. An optimal TTV range has been defined for risk stratification of graft rejection and infection in the first year post kidney transplant applying an in-house PCR. Recently, a commercial PCR - the TTV R-GENE® kit - has been CE certified for clinical use. The present study was designed to validate and refine the optimal TTV range applying the TTV R-GENE® kit.

Methods: Patients and events were selected from the prospective TTV-POET trial including all 628 consecutive adult recipients of a kidney allograft transplanted at the Medical University of Vienna, between January 2016 and July 2020. Patients were followed for 12 months post-transplant or until drop out. TTV was quantified longitudinally by the TTV R-GENE®

kit. The primary outcome was biopsy proven graft rejection and the secondary end-point was infection.

Results: A total of 78 patients with 85 graft biopsies (rejection, n=18) and 274 patients with 80 infectious events following TTV quantification after reaching steady state in month 3 post-transplantation were selected. TTV was quantified at a median of 17 days before rejection was diagnosed and 63 days before onset of infection. The risk for rejection decreased by 25% with every log level increase in TTV load (RR 0.75, 95% CI 0.67-0.85; p<0.001). For TTV loads <5log10 c/mL a high specificity of 90% for rejection was calculated. The risk for an infection increased by 6% with every log level increase of TTV load (RR 1.06, IQR 1.00-0.12; p=0.047). For TTV loads >7log10 c/mL a high specificity of 91% for infection was calculated. Multivariate modelling revealed an independent associated between TTV and rejection and infection, respectively.

Conclusions: These data support the value of TTV quantified by TTV R-GENE® for risk stratification of graft rejection and infection in the first year post kidney transplantation. The optimal TTV range defined within this study will be applied in an interventional randomized controlled trial to assess the safety of TTV-guided immunosuppression: the TTVguideIT trial.