more intact and insulin positive-staining NICC grafts compared with non-selected Xn Treg group at day 60. Only very few insulin positive-staining cells whereas a large number of human CD3 and CD8 infiltrated the islet graft in non-CD27+HLADR+ Treg group. However in CD27+HLADR+ Treg group there were few CD3 and CD8 human leukocytes surrounded but not infiltrated the islet graft. The porcine C-peptide results also confirmed the finding from immunohistochemistry. After glucose stimulation, the porcine C-peptide was 551.8 ± 140.2 pmol/L in CD27+HLADR+ Treg group, with only 232.5 ± 60.4 pmol/L and 130.9 ± 40.7 pmol/L in Xn Treg group and non-CD27+HLADR+ Treg group.

Conclusion: Compared with the previously reported Treg number (2×10^6), only 1/5 number of the CD27+HLADR+ Treg can prevent the islet xenograft rejection which suggested this memory like Treg may be more specific to the xenograft and have potential as an effective cell therapy in immunomodulation in xenotransplantation.

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301.3 | Tolerance in xenotransplantation by use of regulatory T cells with SLA-specific chimeric antigen receptor

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Introduction: Replacement of terminally diseased organs is restricted by limited availability of donor organs and tissues. This represents the biggest hurdle in the medical care of patients with end-stage organ dysfunction. In contrast, xenotransplantation might represent an alternative. However, this development is still accompanied by an immunological challenge, which is constituted by a strong immune response against the xenogeneic graft and which hinders currently the clinical translation. Regulatory T cells (Tregs) are involved in graft-specific tolerance after allogeneic solid organ transplantation and in xenotransplantation. However, adoptive transfer of polyspecific Tregs alone is insufficient to prevent graft rejection even in rodent models, indicating that graft-specific Tregs are strongly required.

Methods: SLA-specific scFvs were identified by an antibody-phage display panning using a swine leucocyte monomer and were used to generate a xenospecific CAR (Xeno-CAR). Specificity studies of generated Xeno-CAR were performed in NFAT-GFP hybridoma assay. Transduction of nTregs with Xeno-CAR enabled the possibility to change their specificity. In vitro experiments regarding nTreg phenotype were performed by flow cytometry, TSDR- and transcriptome analysis and by suppression assays. In vivo studies were performed in the humanized mouse model including the regulation of strong immune responses against xenogeneic cells, porcine skin as well as pig islet cells transplanted under the kidney capsule.

Results: NFAT-GFP hybridoma assay revealed an exclusive specificity of Xeno-CAR against porcine target structures. Any cross-reactivity with human cells or self-activation could be ruled out. Generated Xeno-CAR Tregs changed their specificity without alteration of the regulative phenotype with no significant difference in the expression of Treg-specific surface molecules. Furthermore, both the TSDR analysis and Xeno-CAR transcriptome showed no difference to nTregs. In contrast, the use of Xeno-CAR Tregs in a xenogeneic driven MLR showed a highly significant impact on regulation of Teff cell proliferation. Even in the highest dilution (Treg:Teff), CAR-Tregs showed a suppressive effect compared to nTregs. Most importantly, Xeno-CAR Tregs completely prevented rejection of allogeneic target cells, porcine skin and islets in reconstituted humanized mice in the absence of any immunosuppression leading to graft-specific tolerance.

Conclusion: The generation of xenospecific CAR Tregs describes an essential advance in the application of a cell therapeutic intervention in transplantation medicine. The ability to induce xeno-specific tolerance without any perturbation of the general immune competence opens up entirely new ways to successfully treat patients with end-stage organ dysfunction in times of organ shortage.

ORAL ABSTRACTS SESSION 325 ON ISLET XENOTRANSPLANTATION

325.1 | Improved secretory function of pancreatic islets from InsGLP1M3R piglets

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Background & aims: Clinical pig islet transplantation for the treatment of type I diabetes is still hindered by obvious immunological considerations and less investigated physiological incompatibilities. Cellular encapsulation, donor genetic engineering and host immunomodulation can help improve islet survival in a xenotransplantation context but adequate insulin output from transplanted islets remains the ultimate goal to be achieved for islet transplantation to be clinically efficient. We have previously shown that adenovirus-driven expression of a cassette carrying a dipeptidyl peptidase-resistant form of glucagon-like-peptide-1 (GLP1) and a constitutively activated form of type 3 muscarinic receptor (M3R) in isolated neonatal and adult pig islets significantly increases their secretory response to in vitro glucose stimulation that is otherwise 4–10 times

lower than human islets. Our aim in the current study was to replicate our previous results in a transgenic pig model with beta-cell specific expression of our GLP1M3R cassette, to characterize in vivo and in vitro islet function of these pigs and to verify whether their offspring exhibit the same improved insulin secretion as founder animals.

Material & methods: Cloned transgenic pigs were produced using Talens technology and validated by TLA sequencing analyses. Pancreata were collected from 14-day old piglets. Insulin secretion in response to different stimuli was evaluated during dynamic islet perifusion experiments. After reaching adult age, selected animals were subjected to IVGTT to study in vivo islet function. Transgenic animals were then bred and islet function of their offspring was studied as for founder animals.

Results: Transgenic cloned pig founders (male and female) expressing the GLP1M3R cassette at the beta cell level were successfully obtained. Islets isolated from these piglets showed a 5.5 to 7.5-fold increase of their insulin output upon stimulation with 15 mM glucose. They maintained regulated insulin secretion as basal unstimulated secretion was not significantly increased: stimulation indices of 3.7–7.5 compared to 2.6 for control wild type islets. In vivo islet function during IVGTT was evaluated in two animals that both showed a 2 to 3-fold increase of insulin secretion compared to controls. Finally, we examined secretory function of islets isolated from piglets born from transgenic cloned parents. In vitro perifusion experiments showed increased stimulated insulin secretion from transgenic piglets compared to their wild type siblings.

Conclusion & perspectives: We obtained the first lines of genetically modified pigs exhibiting enhanced function of their pancreatic islets. Specific expression of modified GLP1 and activated M3R at the beta-cell level enhanced insulin secretion both in vivo and in vitro without affecting viability or fertility and was transmitted to the descendants of cloned animals while maintaining its positive effect on beta-cell function.

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325.2 | Enhancing islet quality during pre-transplant culture using a novel necroptosis inhibitor

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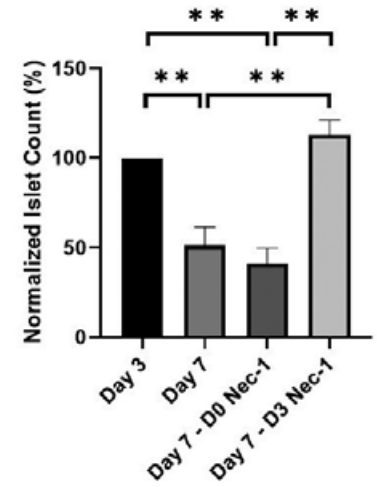
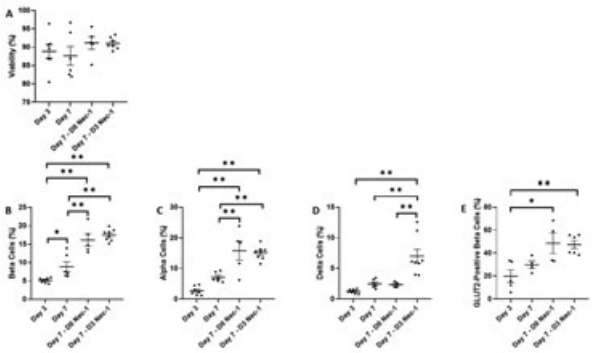
Introduction: The current pre-transplant islet culture protocol is associated with marked islet loss. Necrostatin-1 (Nec-1), a necroptosis inhibitor, has been shown to promote human islet survival. This study assessed the effects of Nec-1 supplementation on day 0 or day 3 of culture on islet recovery, maturation, and function of pre-weaned porcine islets (PPIs).





Methods: PPIs were isolated from pancreata of pre-weaned Yorkshire piglets (4–11 days old) and cultured in optimized islet culture media (37°C, 5% CO2). Nec-1 (100 μM, Sigma) was added to the culture media on day 0 (D0 Nec-1, n = 6) or day 3 (D3 Nec-1, n = 8) of culture, and cultured to day 7 for evaluation. Islet recovery was calculated based on the IEQ/g of pancreas and normalized to day 3 of the untreated group. Flow cytometry (NovoCyte 3000VYB) was used to assess the islet viability, composition, GLUT2 expression, and differentiation. Islet function was determined by glucose-stimulated insulin release assay. Data was expressed as mean ± SEM and evaluated using ANOVA.

Results and Discussion: On day 7 of culture, islets treated with D3 Nec-1 had a significantly higher islet recovery compared to untreated islets or D0 Nec-1 treated islet (untreated = 51.3 ± 10.2% vs D0 Nec-1 = 41.2 ± 8.6% vs D3 Nec-1 = 112.8 ± 8.4%, $P < 0.01$). Nec-1 treatment on day 0 or day 3 of culture increased the beta-cell and alpha-cell composition by twofold (β-cells: untreated = 8.9 ± 1.3% vs D0 Nec-1 = 16.1 ± 1.7% vs D3 Nec-1 = 17.5 ± 0.6%, $P < 0.01$; α-cells: untreated = 7.1 ± 0.6% vs D0 Nec-1 = 15.8 ± 3.1% vs D3 Nec-1 = 15.1 ± 0.8%, $P < 0.01$). However, only D3 Nec-1 treated islets had a threefold increase in the delta-cell composition (untreated = 2.5 ± 0.3% vs D0 Nec-1 = 2.4 ± 0.2% vs D3 Nec-1 = 7.0 ± 1.1%, $P < 0.01$). Additionally, D0 Nec-1 and D3 Nec-1 treated islets, but not day 7 untreated islets, had a twofold increase in GLUT2-positive beta cells compared to day 3 untreated islets (day 3 untreated = 19.9 ± 5.6% vs day 7 untreated = 29.9 ± 3.2%, P = NS;