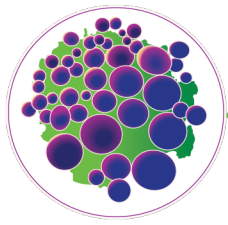


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BEST ABSTRACTS SESSION III



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Abstract 1:

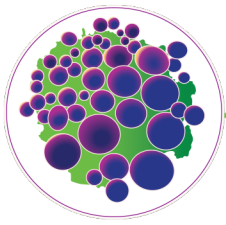
HYPOIMMUNE ISLET CELLS MEDIATE INSULIN INDEPENDENCE AFTER ALLOGENEIC TRANSPLANTATION WITHOUT IMMUNOSUPPRESSION

Xiaomeng Hu¹, Kathy White¹, Chi Young¹, Ari Olroyd¹, Paul Kievit⁴, Andrew Connolly³, Tobias Deuse², Sonja Schrepfer¹.

¹Hypoimmune Platform, Sana Biotechnology, San Francisco, CA, United States; ²Surgery, UCSF, San Francisco, CA, United States; ³Pathology, UCSF, San Francisco, CA, United States; ⁴OHSU, Portland, OR, United States

Treatment of type 1 diabetes mellitus (T1DM) via allogeneic donor transplant has limited success due to morbidities from immunosuppression (IS) and a gradual loss of engrafted pancreatic islet function. We report that allogeneic transplantation of engineered, primary, hypoimmune, pseudo-islets (HIP p-islets) engraft into a fully immunocompetent, diabetic non-human primate (NHP), provide stable endocrine function, and enable insulin independence without inducing any detectable immune response in the absence of IS. NHP cadaveric islet cells were engineered to disrupt function of MHC class I and II and overexpress CD47 thus rendering them hypoimmune (HIP). Diabetes was induced in the NHP with streptozotocin and daily insulin injections started to re-establish glucose control. After 78 days, NHP underwent transplantation of HIP p-islets by intramuscular injection resulting in insulin independence. As early as one week after the transplantation, the NHP's serum c-peptide level had normalized and remained stable throughout the follow-up period of 6 months. The NHP showed tightly controlled blood glucose levels for 6 months, was completely insulin-independent, and continuously healthy. Up to 6 months after HIP p-islet transplantation, PBMCs and serum were obtained for immune analyses. HIP PI showed no T cell recognition, no graft-specific antibodies, and were protected from NK cell and macrophage killing. To prove that the monkey's insulin-independence was fully dependent on the HIP p-islets graft and there was no regeneration of his endogenous islet cell population, we triggered the destruction of the HIP p-islet transplant using a CD47-targeting strategy resulting in loss of glycemic control and return to exogenous insulin dependence. These data demonstrate evidence for immune evasion of HIP p-islets, graft mediated insulin-independence of the diabetic NHP, and a potential safety strategy.

In summary, this successful NHP study provides proof of concept for an upcoming clinical trial using allogeneic, HIP-edited primary islets in patients with type 1 diabetes.



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Abstract 2: IsletRxPlus, CD26⁻ and CD49A⁺ enriched hESC-derived Islet cells, improves safety and identity profile in diabetic mice at multiple implantation sites.

Kfir Molakandov¹, Judith Chebath¹, Michal Izrael¹, Daniel Lazar¹, Itzik Toledo¹, Ariel Revel¹, Arik Hasson¹, Michel Revel¹.

¹Diabetes Cell Therapy, Kadimastem Ltd, Ness Ziona, Israel

Introduction: Cell therapy of diabetes aims at restoring the physiological control of blood glucose by transplantation of functional pancreatic islet cells. A potentially unlimited source of cells for such transplantations would be islet cells derived from an in vitro differentiation of human pluripotent stem cells (hESC/hiPSC), that often contains undesired off-target cells, as byproduct obtained along the differentiation stages. Here, we demonstrate that following the removal of these off-target cells from islets preparations, newly formed **IsletRx^{Plus}** cells significantly improves the efficacy and safety profiles in vivo.

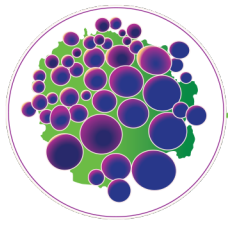
Methods: Islet cells were enriched by MACS for CD26⁻ and CD49A⁺ cells. Following re-aggregation, IsletRx^{Plus} cells were analyzed for identity (flow cytometry) and potency (GSIS). Both enriched and non-selected Islet cells were implanted either encapsulated in alginate hydrogel intraperitoneally or implanted 'naked' in the epidural fat pad (EFP). Glycemia and Human c-peptide and glucose concentrations were followed in mouse blood in function of time in vivo and grafts were analyzed upon study termination by histology and immunostaining for graft size and cellular identity¹.

Results: Non-selected islet cells implantation either microencapsulated in alginate or in the EFP sites, resulted in the development of islet-off-target structures (cystic, tubular or fibrillary) already at 2-3 months post implantation besides the endocrine insulin-producing cells (Figure 1). IsletRx^{Plus} cells, enriched for CD26⁻ and CD49A⁺ cells, resulted in stable and long term (8 months) pancreatic endocrine phenotype, without evidence for islet-off-target structures. Interestingly, the same safety and identity profile was achieved when IsletRx^{Plus} cells were implanted in confined settings (microencapsulated in alginate) and when implanted as 'naked' islets in EFP highly vascularized and ECM enriched tissue, implying of stability of identity regardless of direct contact with mouse parenchyma.

Discussion: IsletRx^{Plus} cells demonstrated an improved potency in correction of hyperglycaemia¹. Concomitantly, IsletRx^{Plus} cells improved stem-cells derived islets safety: in-vivo, IsletRx^{Plus} cells enabled the complete reduction of unwanted cells. These results have significant implications for current diabetes stem cells-derived therapy approaches explored in preclinical development studies and future clinical settings.

References:

[1] Molakandov K. Selection for CD26⁻ and CD49A⁺ Cells From Pluripotent Stem Cells-Derived Islet-Like Clusters Improves Therapeutic Activity in Diabetic Mice. *Front Endocrinology*, 2021 12:635405)



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Abstract 3:

The beta cell “invisibility cloak” – Developing stem cell-derived pancreatic islets that are protected from the immune system

Caterra Leavens¹, Dana Baiu², Connie Chamberlain¹, Ellen Abad Santos¹, Ayesha Palwasha Khan¹, Sara Sackett¹, Peter Chlebeck¹, Daniel Tremmel¹, Sierra Raglin⁴, Xianming Wang³, Matthew Brown¹, Jenny Gumperz², Danwei Huangfu³, Jon Odorico¹.

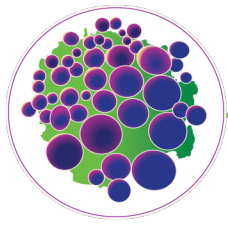
¹Transplantation and Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States; ²Medical Microbiology, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States; ³Developmental Biology, Memorial Sloan Kettering Cancer Center, Sloan Kettering Institute, New York, NY, United States; ⁴Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States

Introduction: Type 1 diabetes is an autoimmune disorder that destroys pancreatic beta cells leading to insulin deficiency and hyperglycemia. While pancreas and islet cell transplantation are effective in controlling glycemia, challenges exist, including the need for life-long immunosuppression to prevent allograft rejection and autoimmune reoccurrence. We have genetically engineered a human embryonic stem cell (hESC) line that is devoid of immunostimulatory HLA Class I and II expression (double knockout, DKO) and expresses doxycycline-inducible T cell inhibitors, CTLA4Ig (iC) and PD-L1 (iP). We hypothesize these DKO;iCP hESCs can be differentiated to phenotypic stem cell-derived islet-like clusters (SCILCs) and have the ability to avoid immune system detection and destruction.

Methods: DKO;iCP hESCs were differentiated to SCILCs based on an established protocol. Mature SCILCs were exposed to doxycycline for 48h to induce CTLA4Ig and PD-L1 expression. Flow cytometry was subsequently performed to show upregulation of doxycycline-induced CTLA4Ig and PD-L1. To examine the ability of DKO;iCP SCILCs to avoid immune cell detection from NK, $\gamma\delta$ T cells, CD8, and CD4 T cells, IncuCyte based co-culture experiments were performed. Results were compared to HUES8 SCILCs and cadaver islets.

Results: Here we report that DKO;iCP hESCs can be successfully differentiated into SCILCs with similar morphology to human cadaver islets. Flow cytometry results indicated that doxycycline-induced DKO;iCP SCILCs are upregulated for PD-L1 and CTLA4Ig compared to untreated DKO;iCP SCILCs. IncuCyte assessment of cell cytotoxicity reveals that human islets and HUES8 SCILCs are targeted by NK cells, $\gamma\delta$ T cells, and less so, by naïve CD8+ T cells.

Discussion: Our results demonstrate the ability to differentiate genetically modified hESCs into SC-islets. Moreover, SCILC DKO;iCPs are resistant to destruction by the effector immune cells examined in the assay, suggesting the ability of SCILCs to evade the immune system, in vitro. Ongoing studies are examining the in vivo ability of DKO;iCP SCILCs to evade the immune system using a humanized mouse model. Successful results may indicate these genetically modified SCILCs provide a scalable cell source for therapeutics that can reverse diabetes while preventing immune rejection.



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Abstract 4: Study of interactions between human immune systems and SC-islets from T1D individuals in a novel NSG MHC-DKO Tg(hIL15) mouse

MICHAEL BREHM¹, Mina Seedhom¹, Ryan Tarpey¹, Jamie Kady¹, Lindsay Ferreira¹, Catherine Kooyomijan¹, Sean O'Brien¹, Leonard Shultz², Dale Greiner¹.

¹Diabetes Center of Excellence, UMass Chan Medical School, Worcester, MA, United States; ²The Jackson Laboratory, Bar Harbor, ME, United States

Introduction: Our current understanding of the pathogenesis of type 1 diabetes (T1D) has been shaped significantly by traditional animal models and by studies with blood and islet specimens from individuals with T1D. These experimental paradigms have provided important insights into the disease process for human T1D, but there is a paucity of robust in vivo models to directly study interactions between autoreactive T cells and autologous beta cells. We have developed induced pluripotent stem cells (iPSC) from individuals with T1D and from individuals without T1D and have demonstrated that these iPSC lines can be differentiated into glucose sensing, insulin-secreting beta cells. Islet-like structures derived from the iPSC lines can be transplanted into immunodeficient mice, which then become functional, glucose sensing, insulin-secreting islets. These humanized mouse models are now being used to study immune cell interactions with stem cell (SC)-islets and human T cells.

Methods: The NOD-scid IL2rgnull (NSG) mouse is a severely immunodeficient mouse model that enables engraftment of human peripheral blood mononuclear cells (PBMCs) and SC-islets. However, survival of human innate immune cell populations, such as NK cells, in NSG mice is lacking and survival of PBMC-engrafted NSG mice is limited by development of xenogeneic graft versus host disease (xGVHD). Human PBMC and human SC-islets were implanted into novel NSG strains that do not develop acute xeno-GVHD to study the immune response in vivo.

Results: We have developed a novel NSG mouse that lacks expression of murine MHC class I and II and expresses human IL15 (NSG MHC-DKO Tg(hIL15)) that enables efficient engraftment of human T cell and NK cells without the development of acute xenogeneic GVHD. Moreover NSG MHC-DKO Tg(hIL15) support implant and survival of SC-islets, and this platform can be used to study the rejection of SC-islets by allogeneic human immune systems. NSG MHC-DKO Tg(hIL15) mice implanted with autologous SC-islets and immune cells from T1D individuals are being used to examine donor-specific T cell responses, define antigen specificities, and evaluate T cell phenotype and function.

Conclusion: The novel NSG MHC-DKO Tg(hIL15) mouse has a long-life span after engraftment of human PBMCs and supports studies of the interactions between human immune systems and SC-islets. This model can be used to study rejection of SC-islets by both human alloreactive T cells and autologous autoreactive T cells in the absence of xGVHD.

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