The Harmine and Exendin-4 Combination Markedly Expands Human Beta Cell Mass In Vivo: Quantification and Visualization By iDISCO+ 3D Imaging

Short Title: iDISCO and Human Beta Cell Mass Expansion

*Carolina Rosselot PhD, *Alexandra Alvarsson PhD, *Peng Wang PhD, *Yansui Li PhD, Kunal Kumar PhD, Robert J. DeVita PhD, #Andrew F. Stewart MD, #Sarah A. Stanley MB Chir. PhD, #Adolfo Garcia-Ocaña PhD.

* Indicates these authors contributed equally.
# Indicates these authors contributed equally.

From the Diabetes Obesity Metabolism Institute, and the Drug Discovery Institute, The Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

Correspondence To:

Adolfo García-Ocaña PhD
Diabetes Obesity Metabolism Institute
The Icahn School of Medicine at Mount Sinai
Atran 5, PO box 1152
One Gustave Levy Place.
New York, NY 10029

Phone: 212-241-3993
Email: adolfo.garcia-ocana@mssm.edu
Abstract

Since all diabetes results from reductions in numbers of functional pancreatic beta cells, beta cell regenerative drugs are required for optimal and scalable future diabetes treatment. While many diabetes drugs are in clinical use, none increases human beta cell numbers. We have shown that a combination of the DYRK1A inhibitor, harmine, with the GLP1 receptor agonist, exendin-4, markedly increases human beta cell proliferation in vitro. However, technological limitations have prevented assessment of human beta cell mass in vivo. Here, we describe a novel method that combines iDISCO+ tissue clearing, insulin immunolabeling, light sheet microscopy, and volumetric quantification of human beta cells transplanted into immunodeficient mice. We demonstrate a striking seven-fold in vivo increase in human beta cell mass in response to three months of combined harmine-exendin-4 combination infusion, accompanied by lower blood glucose levels, increased plasma human insulin concentrations and enhanced beta cell proliferation. These studies unequivocally demonstrate for the first time that pharmacologic human beta cell expansion is a realistic and achievable path to diabetes therapy, and provide a rigorous, entirely novel and reproducible tool for quantifying human beta cell mass in vivo.

Introduction

All forms of diabetes ultimately result from inadequate numbers of normally functioning pancreatic beta cells. It follows that regeneration of sufficient numbers of fully functioning human beta cells could reverse diabetes. Unfortunately, human beta cells only proliferate during a brief window in early childhood, and are resistant to regeneration in adulthood. The recent discovery of human beta cell regenerative drugs of the DYRK1A inhibitor class, used alone - and particularly in combination with other drugs such as glucagon-like peptide (GLP1) receptor agonists (GLP1RA’s) already in widespread use in people with diabetes - has provided hope that therapeutic human beta cell regeneration is achievable in adults (1-7). To date, these studies have employed surrogate markers of human beta cell proliferation, exemplified by Ki67, BrdU, EdU and phospho-histone-H3 co-immunolabeling with insulin-positive cells, to suggest that authentic beta cell proliferation can be achieved. Unfortunately, since human beta cells cannot survive for more than a week in tissue culture, it remains unproven whether these potential therapies can translate into actual increases in human beta cell mass over the longer term.

Here, we describe a reliable method for precise quantification of long-term changes in human beta cell mass in vivo in response to potential human beta cell regenerative drugs. We have used adult human cadaveric islets transplanted into the kidney capsule of immunodeficient NOD-SCID-gamma (NSG) mice (1-3), followed by chronic treatment with a DYRK1A inhibitor (harmine) alone or in combination with a widely used GLP1RA peptide (exendin-4) over one to three months. We have combined this standard animal model with advanced iDISCO+...
tissue clearing, light-sheet microscopy and 3-D image rendering techniques to quantify human beta cell mass (7,8). We find that harmine and exendin-4 co-administration increases human beta cell mass by a factor of 7-fold over three months, without significant alterations in alpha cell mass. These observations, together with prior reports that the harmine and exendin-4 combination enhances human beta cell differentiation and function in vitro and in vivo (1-3), provide an attractive new path to diabetes treatment, as well as a rigorous, entirely novel and reproducible method to quantify human beta cell mass and response to potential future therapies in vivo.

Results

Procedures employed for human beta cell mass measurement are depicted in Fig. 1 (1-3,7,8). Methods and detailed protocols are provided in Supplemental Material. First, human cadaveric islets derived from human organ donors are obtained from the National Institute Diabetes Digestive and Kidney Disease (NIDDK) Integrated Islet Distribution Program (IIDP) or other distributors. Second, islets are transplanted into the renal subcapsular space of immunodeficient NSG mice as illustrated. Third, mice are treated with vehicle or drugs of interest (in this case the GLP1 receptor agonist, exendin-4, harmine, or a combination of harmine and exendin-4). Treatment is administered by continuous infusion using osmotic minipumps. Fourth, animals are anaesthetized and perfused with 4% paraformaldehyde (PFA) to in vivo fix antigens of interest. Fifth, human islet-bearing kidneys are removed, processed, labeled with insulin or glucagon antisera, and cleared using a modification of the iDISCO+ method (7,8). Sixth, whole kidneys are imaged using light sheet microscopy. Seventh, 3-D images are generated and volumes of cell types of interest are quantified using computational image analysis. Eighth, 3-D video images are rendered for visualization of results.

Quantitative validation of the human beta cell volume assay is illustrated in Fig. 2. Fig 2A is a video (see Suppl. Video 1) illustrating beta cell mass visualization within the renal subcapsular space. Beta cells are immunolabeled in green, and a “housekeeping” protein, smooth muscle actin, is shown in red, to highlight the entire kidney and illustrate human islet graft placement under the kidney capsule. Fig. 2B provides representative images (see Suppl. Videos 2-100, 2-300, 2-500 and 2-1000) of human islet grafts transplanted in increasing doses from 100 to 1000 human islet equivalents (IEQ, one IEQ = 125 μm diameter islet) from three different organ donors into three sets of mice. Mice were perfused with PFA, kidneys harvested immediately (5 min.) after transplantation to quantify beta cell mass, 3-D videos generated and beta cell volume calculated. Fig. 2C shows the theoretical volume of human islets transplanted (assuming that one human islet is a sphere of 125 μm diameter), and compares this to actual measured beta cell volume immediately after islet transplant (64±11% of the islet volume). Fig. 2C also illustrates that beta cell mass had declined rapidly by two weeks post-transplant (49±7% of the initial beta cell mass), likely reflecting the rapid or immediate cell death in human islets transplanted into the renal subcapsule, devoid of a blood supply for the first several days post-transplant (9,10). These “standard curves” and the changes at the two-week time point provide reassurance that beta cell mass measured using these methods and progressively larger numbers of islets is accurate.
To examine potential human beta cell mass changes in response to treatment with vehicle (saline), exendin-4, harmine, or the harmine and exendin-4 combination, we employed osmotic minipumps that deliver their active contents by continuous infusion over one month. For studies lasting three months, we replaced the original minipumps with new minipumps containing fresh drugs at months 1 and 2. Fig. 3A-B provides representative images of human islet grafts obtained from animals treated with vehicle or harmine plus exendin-4 for three months (see Suppl. Videos 3A and 3B). These make it clear that a dramatic increase in human beta cell mass has occurred in the drug-treated group. Indeed, Fig. 3C demonstrates that a three-month treatment of seven different sets of human islets and animals with the harmine-exendin-4 combination led to a significant, progressive 7-fold increase in human beta cell volume. Notably, harmine alone appeared to increase human beta cell volume by a factor of 2- to 3-fold at three months when compared to the vehicle or exendin-4 groups, although this did not achieve statistical significance. Exendin-4 by itself had no effect.

Fig. 3D confirms prior reports (1-3) that harmine and harmine plus exendin-4 increase Ki67 co-immunolabeling with insulin in the human islet grafts in vivo. Figs. 3E-F indicate that blood glucose declined in all three treatment groups, and circulating human insulin concentrations increased in the harmine plus exendin-4 group. Fig. 3G demonstrates that there was no significant difference in body weight gain among the four groups following three months of vehicle or active drug treatment.

Suppl. Fig. 1 shows the results observed in four additional groups of mice treated with vehicle (saline), exendin-4, harmine, or the harmine and exendin-4 combination for a period of one month. Suppl. Fig. 1A demonstrates that the harmine plus exendin-4 combination is able to double actual human beta cell volume in just four weeks. Suppl. Fig. 1B confirms that harmine and harmine plus exendin-4 increase Ki67 co-immunolabeling with insulin in the human islet grafts in vivo after one month of treatment. Suppl. Fig. 1C-D indicate that blood glucose significantly declined, and circulating human insulin concentrations increased, albeit not significantly, in the harmine plus exendin-4 group. As in the mice treated for three months, no significant difference in body weight gain was observed among the four groups following one month of treatment (Suppl. Fig. 1E).

The overall results on human beta cell mass in vivo are illustrated in Fig. 4. The black line, derived from Figs. 2 and 3, summarizes human beta cell volume at baseline, one month and three months in human islets transplanted and treated with vehicle. As reported previously (9,10), beta cell mass declines precipitously following transplant and remains unchanged thereafter. Indeed, although we did not assess beta cell volume in the first week post-transplant, the initial decline in beta cell volume likely occurs within the first day or two post-transplant (9,10), as suggested by the green dotted line. Exendin-4 treatment alone has no effect, whereas harmine alone maintains beta cell mass at a volume similar to baseline. Notably, in the harmine plus exendin-4 group, human beta cell volume is similar to baseline at one-month post-transplant (presumably having recovered from an initial decline as illustrated in the dotted green line). By three months, beta cell volume is 3-fold higher.
than the initially transplanted beta cell mass. When compared to the nadir of beta cell mass at three months in the controls, this represents a 7-fold increase, as shown by the vertical green arrow.

Harmine also increases proliferation in alpha cells \textit{in vitro} (1-4,17). To examine whether the increase in human beta cell mass was accompanied by an increase in alpha cell mass, we analyzed alpha cell volume in the same samples described in \textbf{Fig. 3} and \textbf{Suppl. Fig. 1}. \textbf{Fig. 5A-B} provides representative 3-D images of human islet grafts obtained from animals treated with vehicle or harmine plus exendin-4 for one month and stained for both insulin and glucagon (see Suppl. Videos 4A and 4B). Complete one and three-month studies revealed no difference in human alpha cell mass among the four different treatment groups (\textbf{Fig. 5C&E}). Human alpha cell proliferation was not significantly altered in the grafts from mice treated with any of the active drugs compared with vehicle-treated mice at one month (\textbf{Fig. 5D}). Following three months of treatment, mice treated with harmine plus exendin-4 showed a mild but significant increase in human alpha cell proliferation compared with vehicle-treated mice (\textbf{Fig. 5F}).

\textbf{Discussion}

Collectively, these studies provide two essential and novel advances. First, they provide the first reproducible, rigorous, quantitative tools for assessing total human beta and/or alpha cell mass in any \textit{in vivo} system, and for defining changes over time with drug treatment. This toolset fulfills a critical unmet need in human diabetes research in general and in beta cell regenerative research in particular. We expect that this will become the new benchmark for quantifying human beta cell mass in animal models. Second, the studies provide unequivocal documentation that treatment with the harmine-exendin-4 combination (or indeed, any GLP-1R agonist drug) can lead to actual increases in human beta cell mass. This is an important observation: although there are some 30 different diabetes drugs currently in clinical use globally, none of these leads to expansion of human beta cell mass. Thus, these studies begin to fill a critical therapeutic void in diabetes: restoration of human beta cell mass.

The current study adds to an expanding beneficial profile of the harmine-exendin-4 combination. For example, the combination not only increases human beta cell mass, it also reverses diabetes in a standard \textit{in vivo} NSG STZ-diabetic mouse marginal mass human islet graft model of Type 1 diabetes (3), enhances expression in human beta cells of a spectrum of human beta cell differentiation markers, such as PDX1, NKX6.1, MAFA, MAFB, SLC2A2, GLP1R among others, and enhances glucose-stimulated insulin secretion, both \textit{in vitro} and \textit{in vivo} (1-3). Thus, the harmine-exendin-4 combination is capable not only of restoring human beta cell mass, but also beta cell function \textit{in vitro} as well as \textit{in vivo}, two key goals of Type 1 diabetes research. Equally importantly, the harmine-exendin-4 combination enhances beta cell differentiation, proliferation and function in islets derived from organ donors with Type 2 diabetes (3).
While these are important steps in human beta cell regenerative research, they prompt a series of additional opportunities for research that the field should address over the next few years. First, although three months is a prolonged benchmark for NSG mouse human islet transplant studies, it does not approach the human lifetime duration of Type 1 and Type 2 diabetes. Thus, longer studies (e.g., 6-12 months) will be required to determine how long human beta cell mass will continue to expand, how long beta cell proliferation will persist, and how long glycemic control is maintained. It is also critical to ascertain whether human beta cell proliferation will cease when the harmine-exendin-4 treatment is terminated, and whether the remarkable increase in beta cell mass will be retained following drug withdrawal.

This study raises intriguing mechanistic questions. While the 7-fold increase in human beta cell mass over three months is gratifying, it seems unlikely that the increase is an exclusive result of beta cell proliferation. The human beta cell proliferation rates from harmine (1), harmine plus TGFβ inhibitors (2) and harmine plus exendin-4 (3) are substantially higher in vitro than they are in vivo. For example, harmine alone induces a beta cell Ki67 labeling index in human islets in vitro of 1.5-2.5% (1-5), harmine plus TGFβ inhibitors yields 5-8% (2), and harmine plus exendin-4 yields a similar 5-8% Ki67 labeling index in vitro (3). The corresponding Ki67 labeling indices in in vivo studies grafts are 0.7% for harmine alone, 1.5% for harmine plus TGFβ inhibitors, and 1.5% for harmine plus exendin-4 (1-3). We speculate that the lower in vivo proliferation rates reflect local production of anti-proliferative growth factors in the renal capsular milieu that are absent or diminished in vitro. Whatever the cause, the relatively modest in vivo proliferation rates in the current and prior studies seem unlikely to cause a 7-fold increase in human beta cell mass over three months. It is of course possible that we may have missed transient higher rates of proliferation at times not assessed here, for example 2, 6, 8 or 10 weeks.

We believe it likely that proliferation is one important contributor to beta cell mass expansion from the harmine-exendin-4 combination. We also believe that additional mechanisms contribute to this effect. GLP1 family drugs have repeatedly been shown to enhance beta cell survival (11). Enhanced beta cell survival following islet transplant may be a likely contributor in the early days post-transplant, assuming that drugs can access the poorly vascularized islets in the first few days post-transplant. It is also likely that enhanced expression of beta cell transcription factors, differentiation markers and glucose-stimulated insulin secretion alluded to above (1-3) may enhance human islet graft performance, and also the number of insulin-positive cells detected by immunolabeling, contributing to the increase in beta cell mass. Finally, it is also possible that transdifferentiation of alpha cells or other endocrine cells into beta cells may also play a role. Indeed, there is evidence that this can occur among human islet cells, and can be enhanced by treatment with GLP1 receptor agonists (12-14). In this regard the increase in alpha cell proliferation in the absence of an increase in alpha cell mass is particularly intriguing: is it possible that newly replicating alpha cells are becoming beta cells?

It remains unknown what precise rate and duration of beta cell regeneration will be required to reverse either Type 1 or Type 2 diabetes in people. The 7-fold increase in human beta cell mass over three months suggests
that therapeutically relevant increases are clearly achievable. Indeed, one wonders if this rate may be too aggressive, and may need to be attenuated by altering dosing and administration schedules.

Harmine and exendin-4 were delivered systemically in this study, without attempts to target them specifically to beta cells. Although, no alterations were observed in alpha cell mass here, nor were they observed in liver, kidney, spleen, heart, or pancreas in our earlier study (3), we cannot be certain that expansion in other cells or organs would not occur in response to systemic harmine and exendin-4 treatment. Some might prefer specific beta cell-targeted drug delivery, yet at present, there is no perfect human beta cell-specific targeting molecule, nor is it unequivocally clear that beta cell-specific targeting of a harmine-exendin-4 conjugate or other molecule is required. Clarifying these questions is a primary goal of the NIDDK and other diabetes funding agencies around the world.

Overall, the diabetes field has progressed from the widespread belief that therapeutic human beta cell regeneration is unachievable, to the current state in which it appears to be achievable. The combination of human islet transplantation models and iDISCO+ 3-D human beta cell mass measurement methodology applied to transplanted human islets as described herein provide essential tools for the next steps in this process.

**Methods**

**Human pancreatic islets.** Human adult pancreatic islets from 22 non-diabetic donors were provided by the Integrated Islet Distribution Network and Prodo Laboratories. The average donor age was 48±3 and 59% were from male donors. Additional details are provided in Supplemental Table 1.

**Chemicals.** Harmine-HCl was synthesized in the Drug Discovery Institute at Mount Sinai (15,16). Exendin-4 was purchased from MedChemExpress (Monmouth Junction, NJ).

**Human islet transplantation into NSG mice.** Human islets were transplanted into the renal subcapsular space as described in detail previously (1-3). Numbers of human islet equivalents are described in the Figure Legends. All protocols were performed with the approval of and in accordance with guidelines established by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

**Minipump Delivery of Harmine and Exendin-4.** Harmine and exendin-4 dissolved in water were loaded into Alzet (Cupertino, CA) model 1004 mini-osmotic pumps at a concentration of 27 mg/ml and 1 mg/ml, respectively, to permit subcutaneous delivery of harmine and exendin-4 for one month at a continuous rate of 3 mg/kg/day and 0.1 mg/kg/day, respectively. For three-month studies, pumps were replaced at 28 days and 56 days with new pumps and fresh harmine and exendin-4.
Renal Islet Graft Fixation and Harvesting. At the time of graft harvesting, animals were anaesthetized with inhalant isoflurane (3%), and in vivo perfused with 4% PFA. Whole kidneys were harvested and placed in 4% PFA overnight.

Modified iDISCO+ Tissue Immunolabeling and Clearing. Kidneys were dehydrated, peroxidase-bleached, rehydrated again and permeabilized before immunolabeling with anti-insulin (A5064, Agilent, Santa Clara, CA), anti-glucagon (ab137817, Abcam, Cambridge, MA) and anti-smooth muscle actin (A5228, Millipore-Sigma, St. Louis, MO) antibodies and secondary antibodies (A-21450 and A-32754, Thermo Scientific, Waltham, MA). After immunolabeling, tissue was dehydrated and clearing was achieved using a modified iDISCO+ clearing method (7,8) as detailed in Supplemental Material.

Light-Sheet Microscopy. Z-stacked optical sections were acquired using an UltraMicroscope II (LaVision BioTec GmbH, Germany) as detailed in Supplemental Material.

Computational Data Analysis, Image Rendering and Beta Cell Mass Quantification. Whole kidneys were imaged at 1.3x with dynamic focus, and at 4x with multiple z-stacks with 20% overlap, and tiled using the plugin TeraStitcher through the ImSpector Pro software (LaVision Biotec, Bielefeld, Germany). Digital visualization and quantification of volume and surface images were created by using Imaris Software (Bitplane, Belfast, United Kingdom), using the surface function with absolute intensity as the threshold for detection of beta cells.

Blood Glucose and Human Insulin Measurement. Blood was obtained by tail vein nicking. Glucose was measured using AlphaTrak2 glucometer (Abbott, Alameda, CA). Plasma human insulin was measured using the human insulin ELISA kit (Mercodia, Winston Salem, NC).

Human Islet Graft Ki67 and Insulin Immunohistochemistry. Tissue immunolabeling for Ki67 (MA5-14520, Thermo Scientific) and insulin or glucagon were performed on neutral-buffered formalin-fixed, paraffin-embedded sections as detailed previously (1-3).

Statistics. The data are presented as means ± SE. Statistical analysis was performed using one-way ANOVA with Tukey or Holm post hoc HSD (http://astatsa.com) for comparison among groups. P < 0.05 was considered statistically significant.

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Declarations of Interest

For A.F.S., K.K., R.J.D. and A.G-O.: The Icahn School of Medicine has submitted patents on the work described herein. S.A.S is a named inventor of the intellectual property, "Compositions and Methods to Modulate Cell Activity", co-founder of, consults for, and has equity in the private company Redpin Therapeutics (preclinical stage gene therapy company developing neuromodulation technologies).

Author Contributions

A.G.-O., S.A.S., R.J.D, and A.F. S. conceived of the studies, oversaw the experiments and wrote the manuscript. C.R., A.A., P.W., Y.L., and K.K. performed the studies.

References.


**Figure Legends.**

**Figure 1. A schematic depiction of the work flow.** Isolated adult human islets are provided are obtained from authorized vendors, and transplanted into the renal subcapsular space. This may be done in one or both kidneys, depending on experimental design. Following islet transplant, depending on experimental design, osmotic minipumps loaded with drugs of interest, in this case harmine and exendin-4 diluted in water, are implanted subcutaneously in the interscapular space. Pumps function for 28 days, at which time the experiment may be completed, or a fresh replacement pump may be inserted. At a preselected time point, animals are in vivo fixed with 4% paraformaldehyde (PFA), and intact kidneys harvested, immunolabeled and clarified using iDISCO+. Clarified kidneys are examined by light-sheet microscopy, Z-stacks converted to 3-D images, and beta cell volumes calculated using commercial software.

**Figure 2. Human Beta Cell Mass Quantification and Validation.** A. An example of a 3-D video displaying a human beta cell graft using insulin immunolabeling. Smooth muscle actin is used to visualize the entire kidney. B. Four video images of grafts harvested immediately following transplantation with 100, 300, 500 and 1000 adult human islet equivalents (IEQ). Representative of four different experiments. C. Comparison of the theoretical beta cell volume in the four groups in B, assuming that human islets are spheres of 125 µm in diameter, with actual beta cell volumetric measurements at time zero and following two weeks after transplant. At time zero, measured beta cell mass is approximately 64% of the theoretical islet volume. At two weeks, as expected, beta cell volume is approximately 49% lower, reflecting beta cell death in the immediate peri-transplant period (9,10).

**Figure 3. Quantitative Effects of Harmine and Exendin-4 on Human Beta Cell Mass and Function.** A.B. Examples of videos of human islet grafts harvested at three months (84 days) following treatment with vehicle (water) or harmine plus exendin-4 (H+E) and immunolabeled for insulin. C. Beta cell mass in human islet grafts in seven to eight sets of human islets in animals receiving vehicle (V), exendin-4 (E), harmine (H), or harmine
plus exendin-4 (H+E) for three months. D. Insulin and Ki67 co-immunolabeling in the four groups in panel B. E. Blood glucose in the four groups in panel B. F. Plasma human insulin in the four groups in panel B. G. Body weight changes in the four groups in B. * indicates p<0.05 vs. V; and ** indicates p<0.05 vs. V and E, both by one-way ANOVA and Tukey or Holm post-hoc tests.

Figure 4. Schematic of the Effects of the Harmine-Exendin-4 Combination on Human Beta Cell Mass. A. Data are derived from Figs. 2 and 3. Human beta cell mass in vehicle-treated NSG mice (black line) declines following transplant and remains stable over the ensuing two months. Exendin-4 alone (grey line) does not affect beta cell volume, whereas harmine alone (blue line) leads to a significantly higher beta cell volume than vehicle or exendin-4. In the harmine-exendin-4-treated mice (red line), beta cell volume remains stable during the first month and increases markedly by the end of month 3. Since beta cell mass declines markedly in the first day or days following transplant (8,9), the actual course of events likely resembles the dotted green line. B. The seven-fold increase in beta cell volume shown in the green arrow reflects the difference between the vehicle-treated and combined harmine-exendin-4-treated groups at three months. The grey box suggests potential mechanisms that may underline the increase in human beta cell mass. See text for additional details.

Figure 5. Quantitative Effects of Harmine and Exendin-4 on Human Alpha Cell Mass and Proliferation. A,B. Examples of videos of human islet grafts harvested at one month following treatment with vehicle (water) or harmine plus exendin-4 (H+E) and immunolabeled for glucagon. C. Alpha cell mass in human islet grafts in five to six different sets of human islets and animals receiving vehicle (V), exendin-4 (E), harmine (H) or harmine plus exendin-4 (H+E) for one month. D. Glucagon and Ki67 co-immunolabeling in the four groups in panel B. E. Alpha cell mass in human islet grafts in seven to eight sets of human islets in animals receiving vehicle (V), exendin-4 (E), harmine (H), or harmine plus exendin-4 (H+E) for three months. F. Glucagon and Ki67 co-immunolabeling in the four groups in panel E. * indicates p<0.05 vs. V by one-way ANOVA and Tukey or Holm post-hoc tests.
1. Isolate Human Islets From Cadaveric Organ Donors

2. Transplant Human Islets into Kidney Capsules of 8-12 week old Male Rag1\(^{-/-}\) Mice

3. Continuous Infusion Via Implantable Minipumps
   - Day 0
   - 28 - 84 days

4. 4%PFA
   - In Vivo Perfusion/Fixation

5. Harvest Kidney, Immunolabel, Tissue Clearing

6. Light Sheet Microscopy

7. Image Analysis

8. 3-D Image Rendering

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Figure 1
Figure 2

(A) Insulin / Smooth Muscle Actin

(B) Insulin

(C) Theoretical

Time 0

2 Weeks

Human Islet Equivalents Transplanted

Beta Cell Volume (mm$^3$)

y = 0.001x

R² = 1

y = 0.0008x

R² = 0.993

y = 0.0005x

R² = 0.9773

0.00
0.20
0.40
0.60
0.80
1.00
1.20
0 500 1000
Figure 3

A 3 Months

Vehicle Insulin

B 3 Months

H+E Insulin

C

Beta Cell Volume (mm$^3$)

V E H H+E

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8

D

Insulin$^+$ Ki67$^+$ Cells (%)

V E H H+E

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

E

Blood Glucose (mg/dl)

V E H H+H

0 40 80 120 160 200 240

F

Plasma Human Insulin (mU/L)

V E H H+H

0 2 4 6 8 10 12 14

G

Body Weight Gain (g)

V E H H+H

0 2 4 6 8 10
Figure 4

A. Graph showing the human beta cell volume (mm^3) over months after islet transplant with different treatments:
- **Harmine + Exenatide**
- **Harmine**
- **Exenatide**
- **Vehicle**

B. Text box listing potential benefits of the treatment:
- Proliferation
- Enhanced Function
- Survival
- Transdifferentiation
- Combinations

**7X Increase**
Figure 5

A. Vehicle vs. Glucagon

B. H+E vs. Glucagon

C. 1 Month

D. 1 Month

E. 3 Months

F. 3 Months

Alpha Cell Volume (mm$^3$)

Glucagon$^+$Ki67$^+$ Cells (%)

* indicates significance.