A Nutrient-Regulated GIP/GLP-1 AAV Gene Therapy to Durably Treat Obesity

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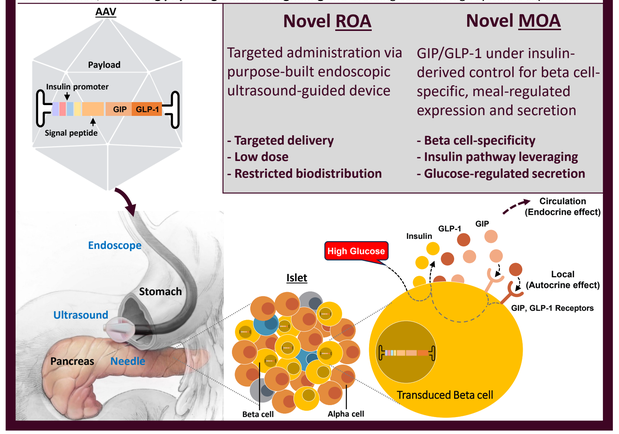
Rejuva Gene Therapy Platform

PANCREAS-TARGETED GIP/GLP-1 GENE THERAPY TO TREAT OBESITY

Glucagon-like peptide-1 (GLP-1) analogues have revolutionized the treatment of obesity, and dual GLP-1/glucose-dependent insulinotropic polypeptide (GIP) agonism has further improved metabolic efficacy. However, these pharmacotherapies require chronic administration and are limited by gastrointestinal side effects, low patient adherence, and rapid weight regain and metabolic rebound on discontinuation - highlighting the need for a durable, physiologically tuned therapeutic alternative. To address this need, we leveraged our *Rejuva* Platform to develop a beta cell-targeted AAV gene therapy enabling co-production and secretion of GIP and GLP-1 that is triggered by metabolic demand. Expressed as a monocistronic polyprotein designed for post-translational cleavage, trafficking, and release, the dual incretin cassette is modulated by an engineered chimeric insulin-derived promoter. After investigating its specificity and mechanism of action in vitro, we evaluated its metabolic efficacy with an obese murine model in a proof-of-concept study.

Figure 1. Endoscopic ultrasound-guided pancreatic delivery and targeted expression

AAV-sINSp-GIP/GLP1 targets pancreatic beta cells to drive nutrient-responsive GIP and GLP-1 expression and secretion, **mimicking physiologic incretin signaling** and avoiding constant high systemic exposure.

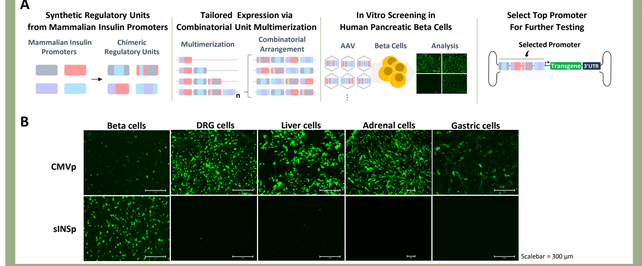


Novel ROA	Novel MOA
Targeted administration via purpose-built endoscopic ultrasound-guided device	GIP/GLP-1 under insulin-derived control for beta cell-specific, meal-regulated expression and secretion
- Targeted delivery - Low dose - Restricted biodistribution	- Beta cell-specificity - Insulin pathway leveraging - Glucose-regulated secretion

BETA CELL SPECIFICITY VIA A SYNTHETIC INSULIN PROMOTER

Figure 2. Beta cell-restricted transgene expression via rational promoter design

(A) Differentiated insulin-based synthetic regulatory units assemble into a robust, beta cell-restricted tandem array promoter. (B) The synthetic insulin promoter (sINSp) shows strong beta cell GFP expression with minimal activity in relevant off-target human cell lines vs a ubiquitous control (CMVp).

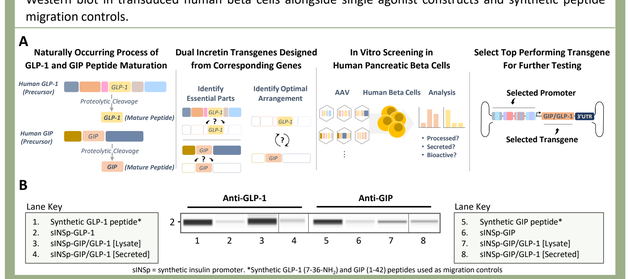


Design and function

DUAL INCRETIN OUTPUT FROM SINGLE TRANSGENE ORF

Figure 3. Monocistronic transgene design to produce dual incretins from a single ORF

(A) Transgene design enables delivery and co-production of dual incretins from a single vector as a polyprotein releasing the individual peptides. (B) Mature GIP and GLP-1 peptides confirmed by capillary Western blot in transduced human beta cells alongside single agonist constructs and synthetic peptide migration controls.

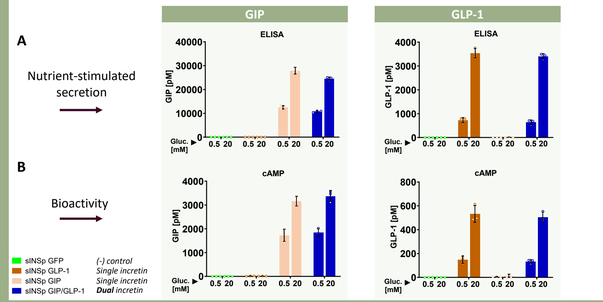


sINSp = synthetic insulin promoter. *Synthetic GLP-1 (7-36-NH_2) and GIP (1-42) peptides used as migration controls

GLUCOSE-REGULATED SECRETION IN HUMAN BETA CELLS

Figure 4. Dual incretin secretion is stimulated by high glucose

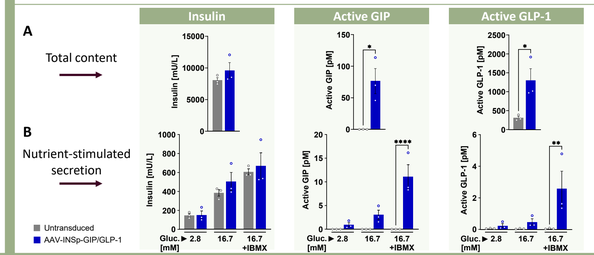
Nutrient-stimulated secretion of GIP and GLP-1 is observed in transduced human beta cells (ELISA), with both peptides demonstrating bioactivity via corresponding receptor binding and signal induction (cAMP).



FUNCTIONAL ACTIVITY IN HUMAN ISLETS

Figure 5. Dual incretin vector demonstrates function in human primary islets

Increases in (A) incretin content and (B) nutrient-stimulated incretin secretion in human primary islets.

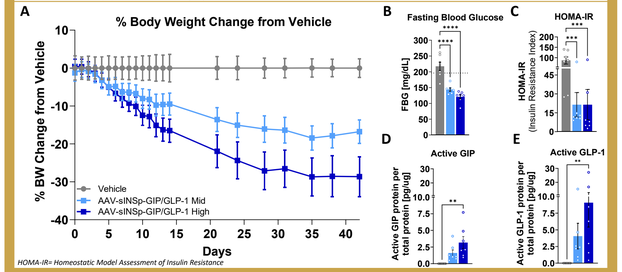


Metabolic Efficacy

WEIGHT AND METABOLIC IMPROVEMENTS IN OBESE MICE

Figure 6. Strong and durable efficacy in treated diet-induced obese (DIO) mice

A single dose of the AAV9 dual agonist vector led to significant, dose-responsive reductions in (A) body weight and (B) fasting blood glucose (FBG) and (C) improvements in insulin sensitivity. Detection of (D) active GIP and (E) active GLP-1 peptide forms well above vehicle levels was observed in the pancreas.

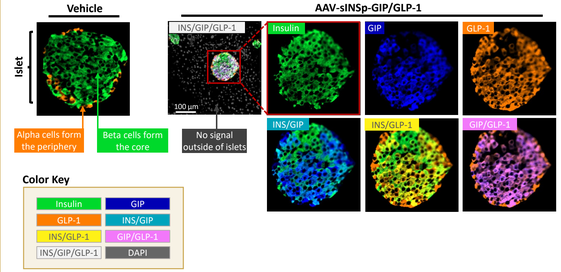


HOMA-IR= Homeostatic Model Assessment of Insulin Resistance

BETA CELL-RESTRICTED EXPRESSION CONFIRMED IN PANCREAS

Figure 7. Confirmed robust and restricted beta cell transgene expression

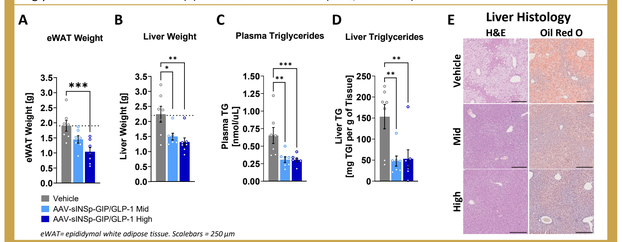
Immunofluorescence staining for insulin, GIP, and GLP-1 in the pancreas demonstrated GIP and GLP-1 expression in insulin-positive cells (beta cells) within the islets with no signal detected in exocrine cells.



REDUCED FAT/LIVER WEIGHT AND SYSTEMIC/HEPATIC LIPIDOSIS

Figure 8. Improved fat/liver weight and systemic/liver triglycerides and steatosis

Marked reduction in (A) adipose and (B) liver weight and in (C) systemic triglyceride and (D) liver triglyceride levels mirrored in (E) reduced liver steatosis (H&E, Oil Red O).



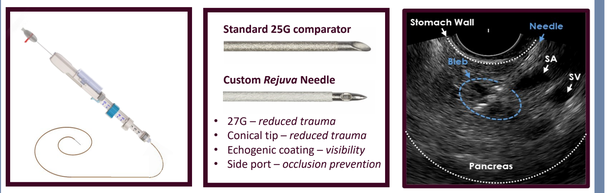
eWAT= epididymal white adipose tissue. Scalebars = 250 µm

Targeted Delivery

PURPOSE-BUILT DEVICE FOR TARGETED AAV DELIVERY

Figure 9. The *Rejuva System* for targeted intrapancreatic gene therapy delivery

The *Rejuva System* for targeted intrapancreatic delivery utilizes a purpose-built device, catheter and custom needle designed to administer gene therapy under endoscopic ultrasound (EUS) guidance in a ~20 minute out-patient procedure.

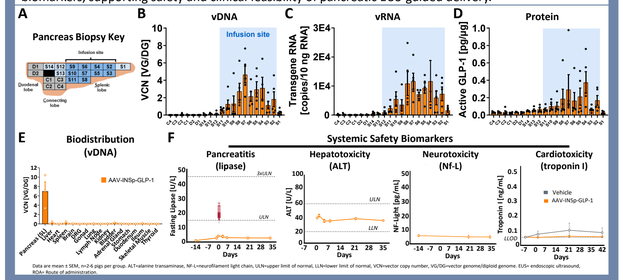


Catheter not to scale. SA = splenic artery, SV = splenic vein

SAFE AND EFFECTIVE DELIVERY AND EXPRESSION VIA EUS ROA

Figure 10. EUS-guided pancreatic delivery of a GLP-1 AAV vector is effective and safe

EUS-guided infusion of a GLP-1-producing AAV vector in Yucatan pigs resulted in enrichment within the (A) targeted pancreatic splenic lobe of (B) viral DNA (vDNA), (C) vRNA, and (D) GLP-1 protein at 6-weeks post-dose, with (E) minimal biodistribution in off-target tissues and (F) no elevations in systemic safety biomarkers, supporting safety and clinical feasibility of pancreatic EUS-guided delivery.



Data are mean ± SEM, n=2-6 pigs per group. ALT=alanine transaminase; Nf-L=neurofilament light chain, ULN=upper limit of normal, LLN=lower limit of normal, VCN=vector copy number, VG/DG=vector genome/diploid genome. EUS= endoscopic ultrasound, ROA= Route of administration.

CONCLUSIONS

Unmet Need

- GLP-1 pharmacotherapies have demonstrated their potential to treat obesity but chronic dosing, tolerability concerns, and frequent discontinuation and rebound highlight the need for a durable, single-intervention alternative - **A Nutrient-Regulated Dual GIP/GLP-1 Gene Therapy.**

Design and Function

- The synthetic insulin-derived regulatory features showed robust, specific expression in human beta cells with nutrient-regulated secretion of bioactive peptides

Metabolic Efficacy

- A single treatment in DIO mice showed substantial and durable improvements in body weight, adipose weight, liver weight, hepatic steatosis, and glycemic control.

Targeted Delivery

- EUS-guided intrapancreatic infusion is a safe and effective means to deliver AAV. Combined with a dual incretin gene therapy, this platform has the potential to - **Shift treatment paradigm from chronic pharmacotherapy to one-time metabolic reprogramming.**

Rejuva is an investigational gene therapy program in preclinical development. RJVA-001 is the subject of planned first-in-human clinical studies, pending regulatory and ethics approvals in the Netherlands and Australia. RJVA-002 remains in preclinical development and has not been submitted to any regulatory authority.