American Diabetes Association.

84TH SCIENTIFIC SESSIONS

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Single-Dose GLP-1-Based Pancreatic Gene Therapy Durably Maintains Body Composition and Glycemia after Semaglutide Withdrawal in a Murine Model of Obesity

Harith Rajagopalan, Alice Liou Fitzpatrick, Suya Wang, Rebecca Reese, Nicole Picard, Emily Cozzi, Timothy Kieffer, Jay Caplan

June 23rd, 2024

Disclosure Statement

Authors

Harith Rajagopalan, Alice Liou Fitzpatrick, Suya Wang, Rebecca Reese, Nicole Picard, Emily Cozzi, Timothy Kieffer, and Jay Caplan are employees and shareholders of Fractyl Health, Inc. Harith Rajagopalan is a board member of Fractyl Health, Inc.

The Pancreatic Gene Therapy (PGTx) platform is in early development and has not been assessed by any regulatory body for investigational or commercial use.

GLP-1 Drugs: Weight Rebound is a Significant Problem

Current GLP-1 drugs do not durably alter metabolic setpoint

Discontinuation of therapy leads to **near total loss of metabolic benefit**[1]

GLP-1 therapies support weight loss and glucose control, **but weight maintenance has become a critical challenge**

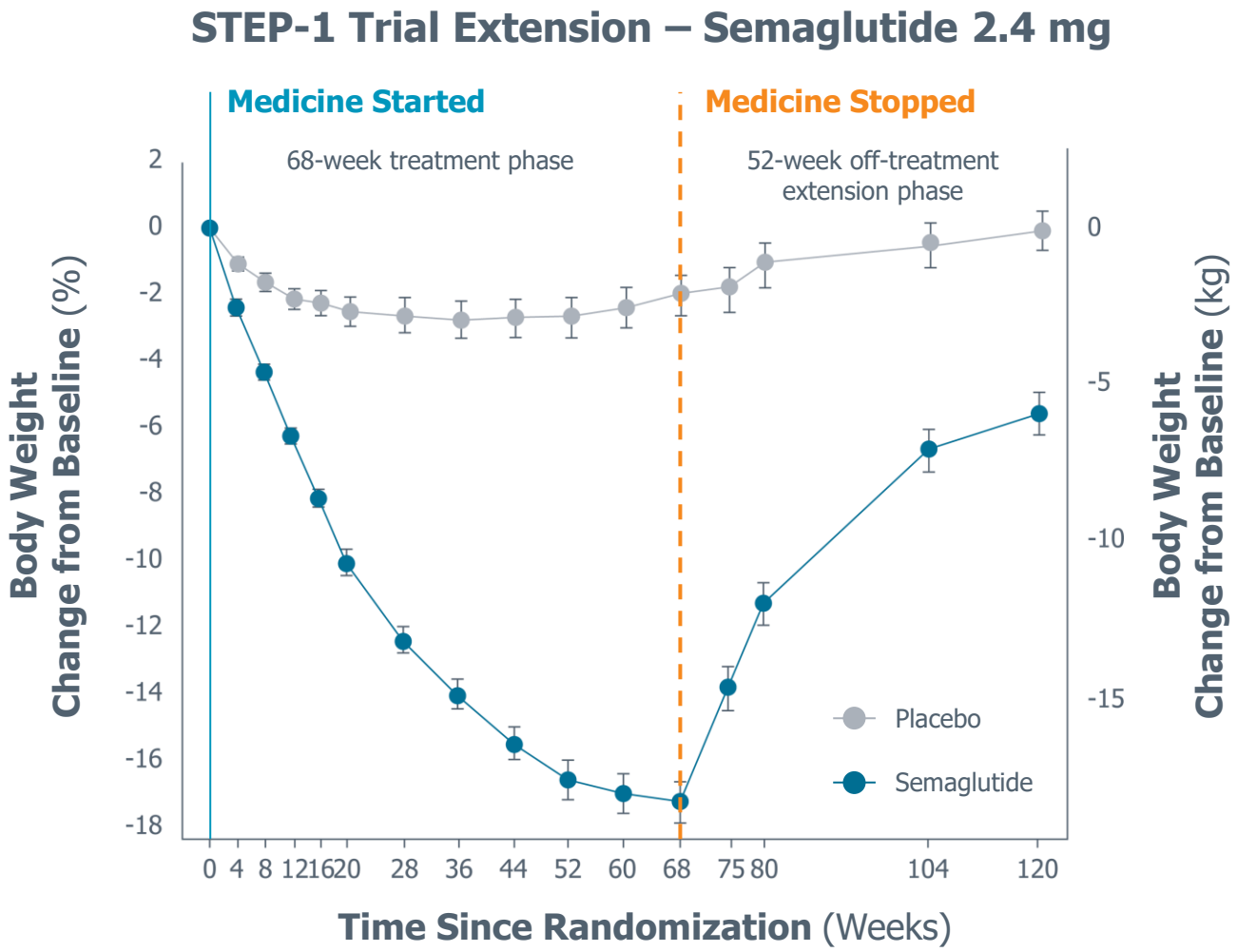


Figure adapted from Wilding et al. Diabetes Obes Metab. 2022 24:1553-1564. 1. Wilding et al. Diabetes Obes Metab. 2022 24:1553-1564. GLP-1=glucagon-like peptide 1

Real-World Evidence Shows High Discontinuation Rates

BCBS data show 50% GLP-1 drug discontinuation within 3 months

Private insurer **survey of ~170K unique GLP-1 drug users** for weight loss from January 2014 to December 2023[1]

Early discontinuation is often due to tolerability, but medium-term discontinuation rates still remain high across a range of tested variables:

- Cost (out of pocket expense)
- Type of prescriber (PCP vs endocrinologist)
- Age of patient
- Comorbidity index

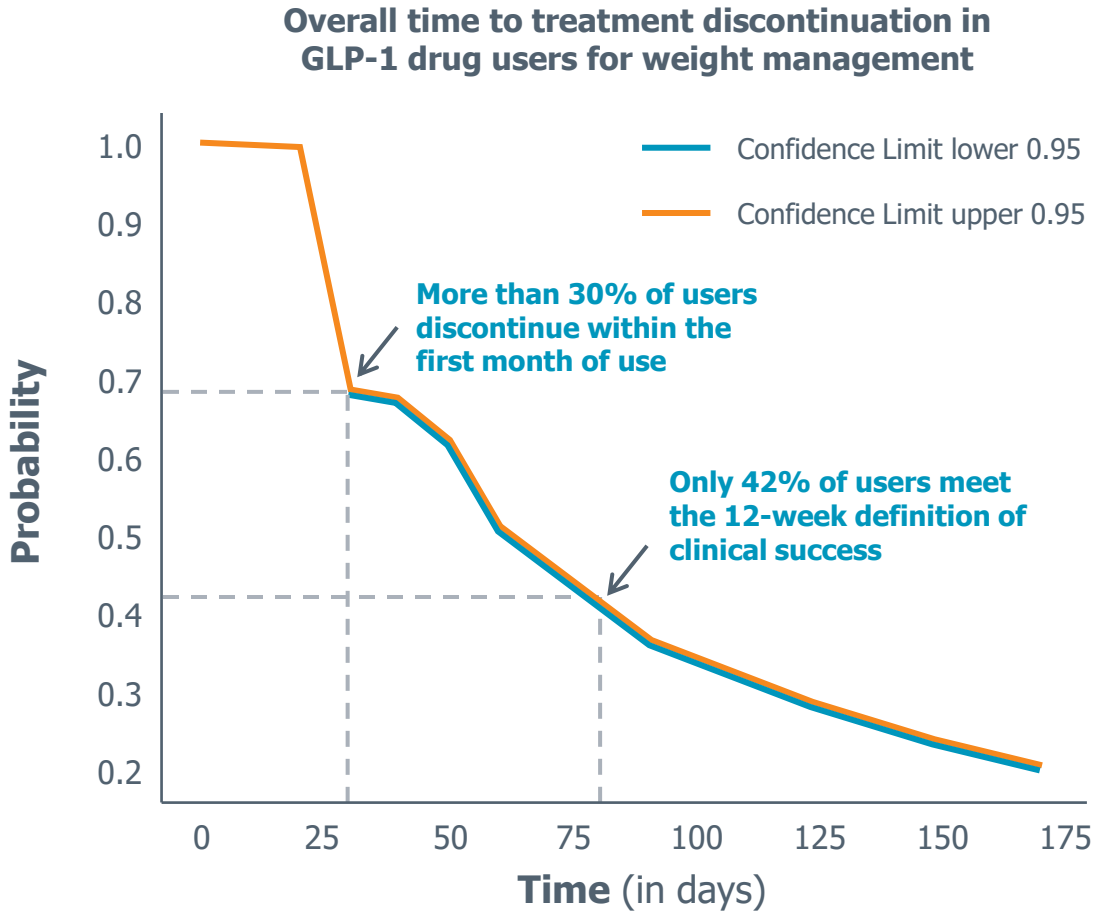


Figure adapted from Blue Health Intelligence, Issue Brief May 2024. 1. Blue Health Intelligence, Issue Brief May 2024. BCBS=Blue Cross Blue Shield, GLP-1=glucagon-like peptide 1, PCP=primary care physician

Pancreatic Gene Therapy (PGTx) to Modify Islet Function

Potential for durable improvement in metabolic health

GLP-1 gene therapy, targeted to pancreatic islets, may offer differentiated benefit

β-cell machinery can be leveraged to produce nutrient-stimulated hormones[1,2]

Islet cells are terminally differentiated,[3] making adeno-associated virus (AAV) suitable for durable effect

Opportunity to amplify islet GLP-1 signaling to improve β-cell health

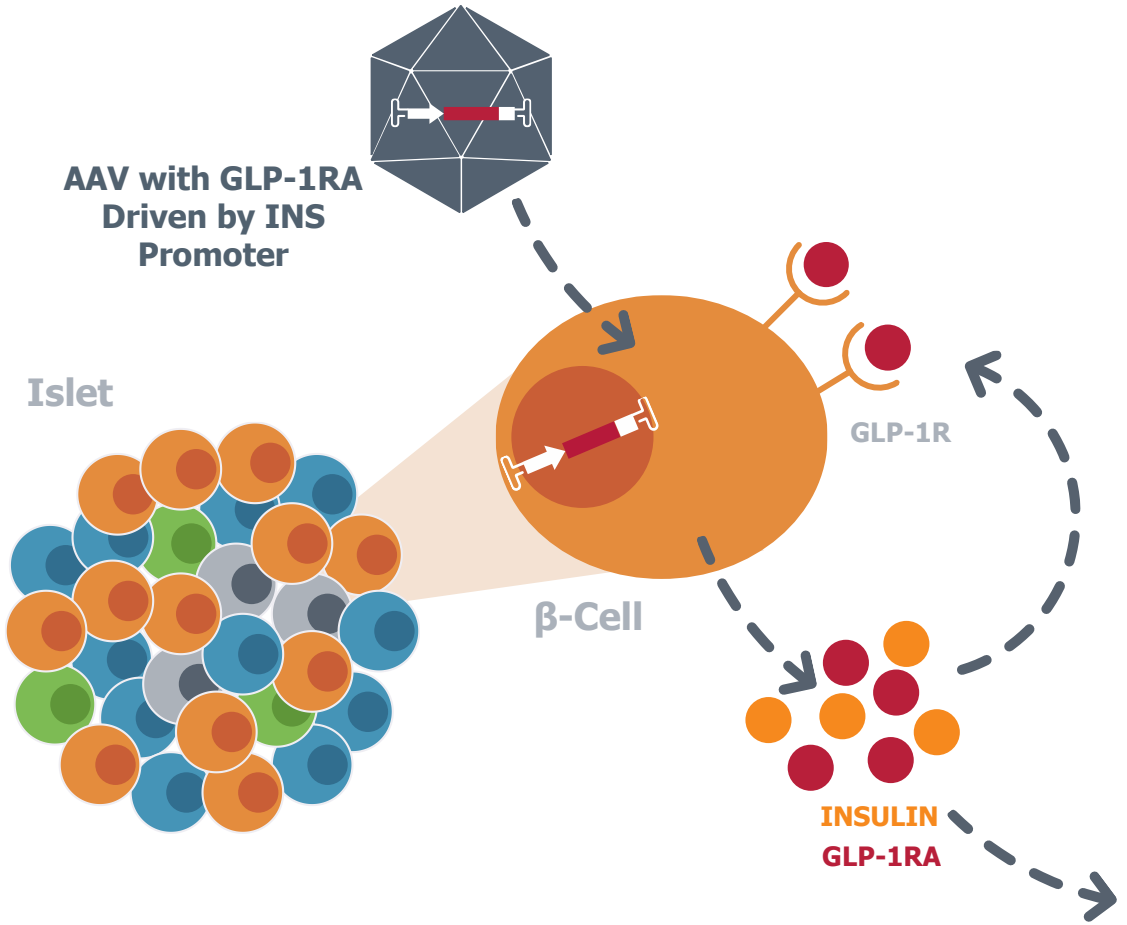
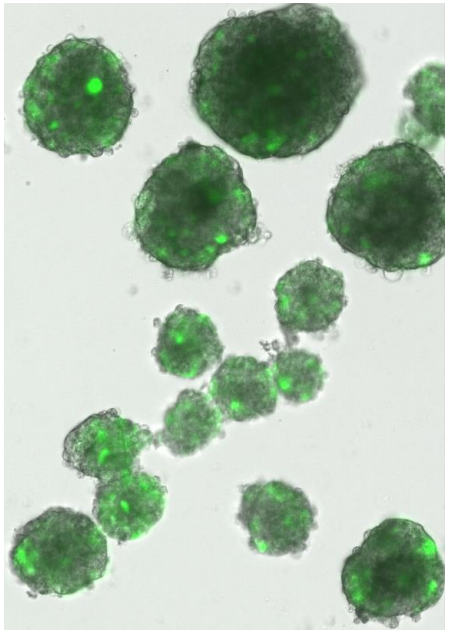


Figure adapted from Saikia et al. JCI Insight. 2021 6:e1418511. 1. Lubaczeuski et al. Keystone 2023 oral presentation. Poster no. 1025. 2. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. 3. Perl et al. J Clin Endocrinol Metab. 2010 95: E234–E239. AAV=adeno-associated virus, GLP-1=glucagon-like peptide 1, GLP-1R=GLP-1 receptor, GLP-1RA=GLP-1R agonist, INS=insulin, PGTx=pancreatic gene therapy

GLP-1RA PGTx in Human Islets and Human β-Cell Line

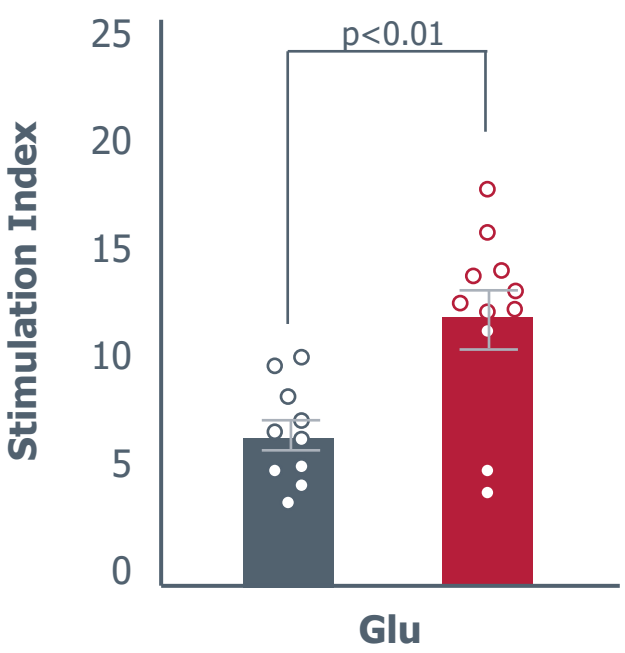
Improves glucose-stimulated insulin secretion via GLP-1R

A) Human Islet Transduction

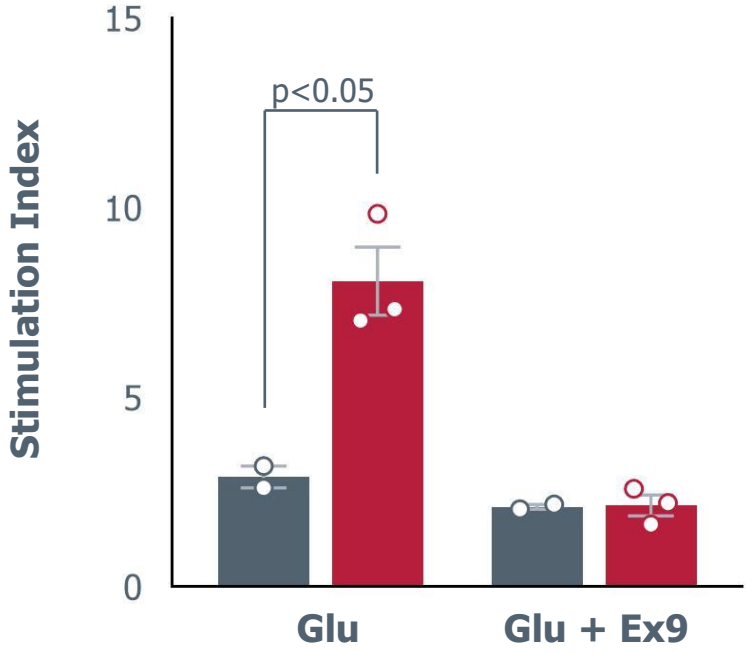


GFP Expression

B) Human Islet GSIS



C) Human β-cell Line GSIS ± Ex9 (GLP-1R Antagonist)



Mean ± SEM shown; n=2-11 per group. B) Glucose stimulation of 16.7 mM from 2.8 mM baseline, C) Glucose stimulation of 11 mM from 0 mM baseline. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. Ex9=Exendin-9, GFP=green fluorescent protein, GLP-1=glucagon-like peptide 1, GLP-1R=GLP-1 receptor, GLP-1RA=GLP-1R agonist, Glu=glucose, GSIS=glucose-stimulated insulin secretion, PGTx=pancreatic gene therapy

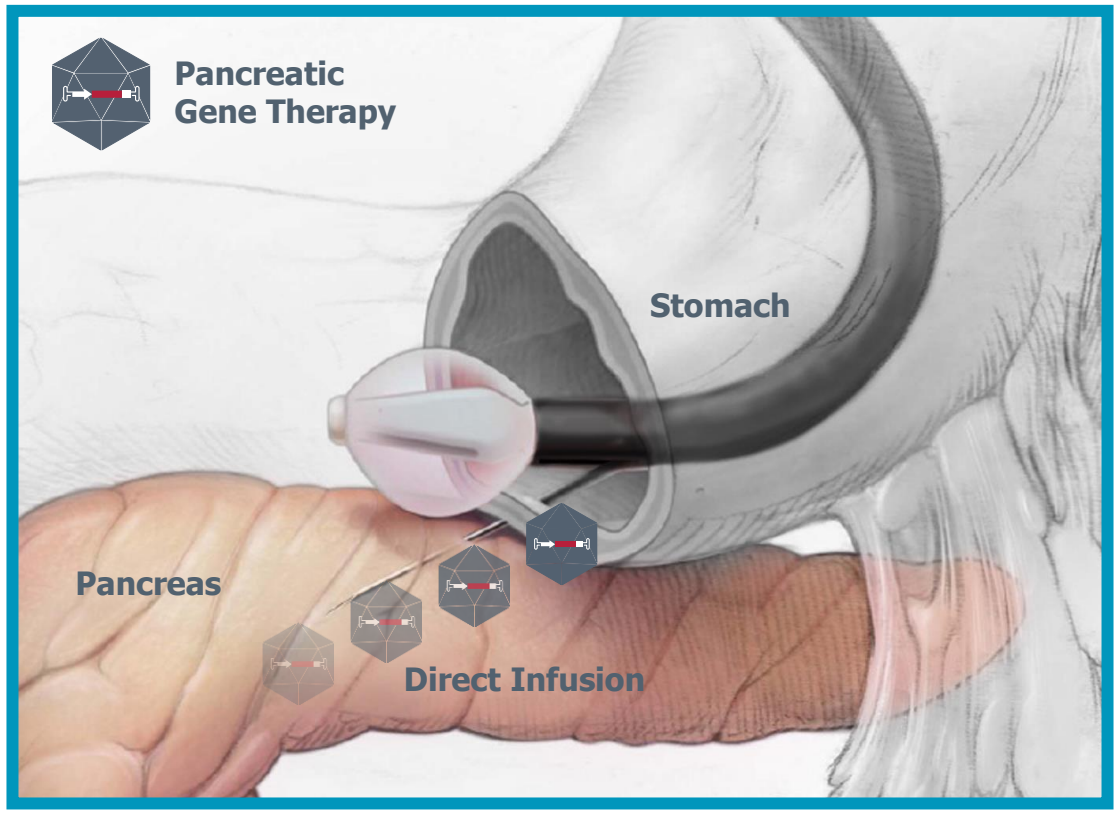
Gene Therapy Route of Administration to Pancreas

Proprietary, automated, endoscopic delivery device

Local delivery enables low viral genome dosing with limited systemic virus exposure[1]

Islets are readily accessible[2,3] via already established, routine, upper endoscopic ultrasound procedures,[4] performed in ~300K patients per year in US[5]

Procedural risk is further mitigated with device design (e.g., needle size, volume, controlled infusion rate)



Endoscopic Procedure & PGTx Delivery

1. Rajagopalan et al. ASGCT 2023 oral presentation. Abstract no. 191. 2. Docherty & Russ. Encyclopedia of Tissue Engineering and Regenerative Medicine 2019, pg. 367-374. 3. Ravi et al. Medicine (Baltimore). 2021 Apr 30;100(17):e25642. 4. Hasan & Hawes. Gastrointest Endosc Clin N Am. 2012 Apr;22(2):155-67. 5. Peery et al. Gastroenterology 2022 Feb: 162(2):621-644. PGTx=pancreatic gene therapy

Dose-Dependent Viral Transduction in Yucatan Pig Model

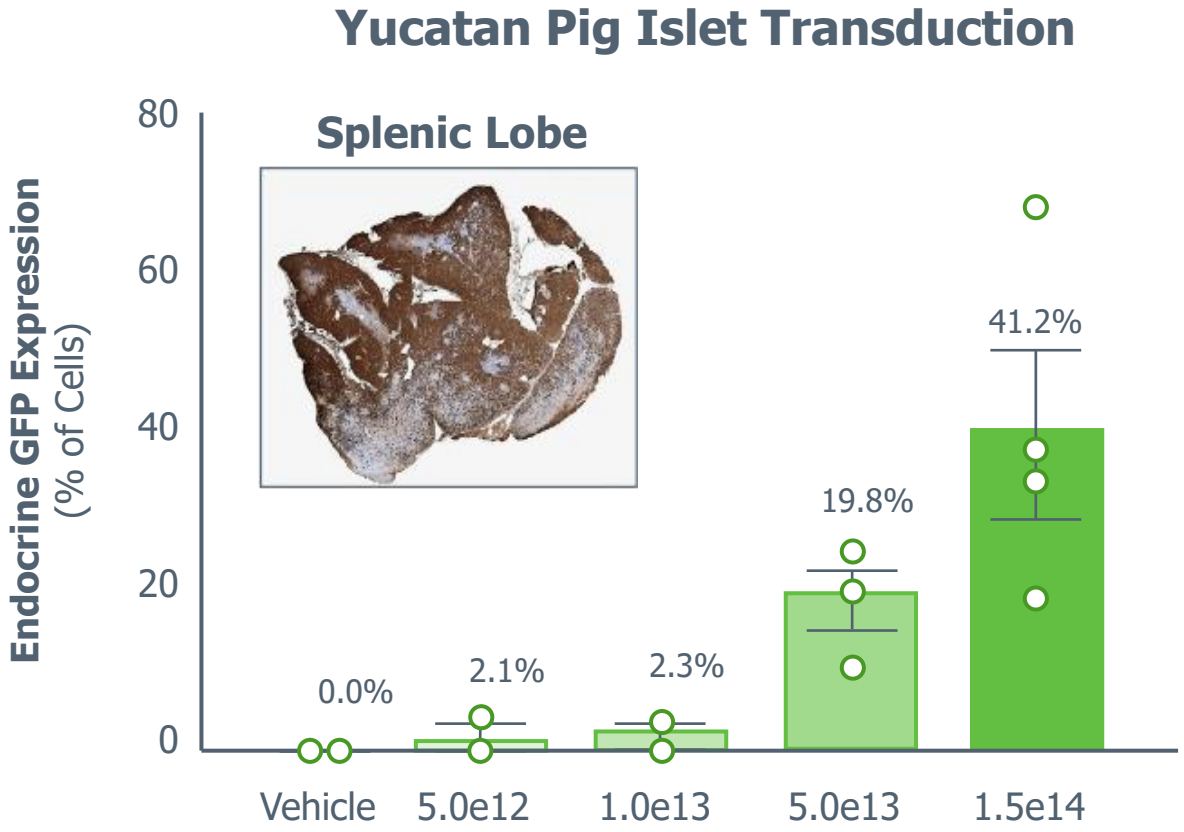
Local delivery effectively and reliably targets islets

Yucatan pig model **anatomy similar to humans**[1]

Dose-dependent AAV-GFP expression in targeted pancreatic lobe[2,3]

>60 animals treated with 100% technical success

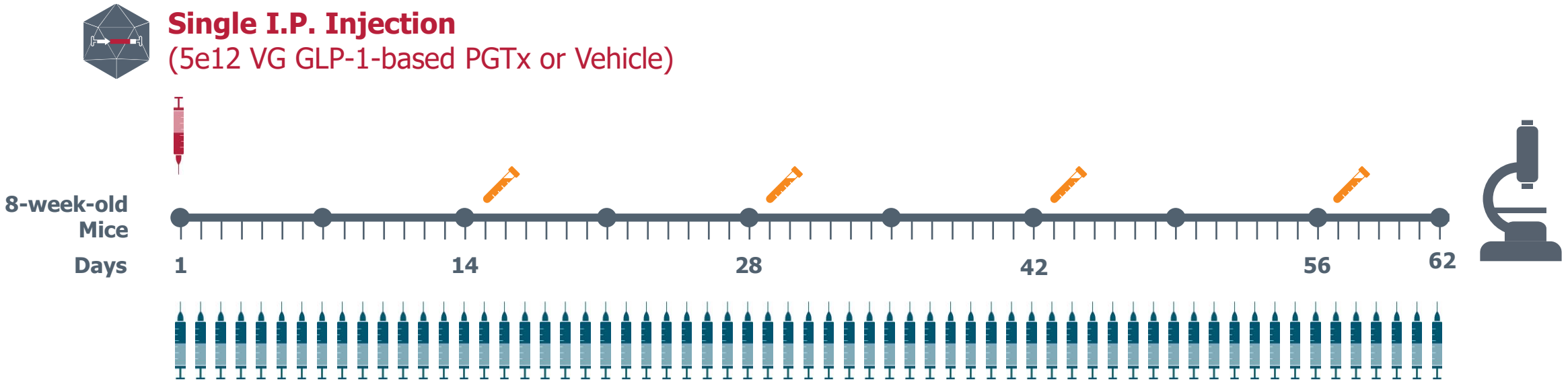
No adverse safety signals to date (e.g., pancreatitis)



Mean ± SD shown; n=2-4 per group. 1. Walters and Prather. Mo Med. 2013 May-Jun;110(3):212-5. 2. Thompson et al. DDW 2023 poster presentation. Control no. 3862948. 3. Rajagopalan et al. ASGCT 2023 oral presentation. Abstract no. 191. AAV=adeno-associated virus, GFP=green fluorescent protein

GLP-1RA PGTx T2D Efficacy Study: Head-to-Head vs. Semaglutide

db/db murine model is *de facto* standard for T2D development

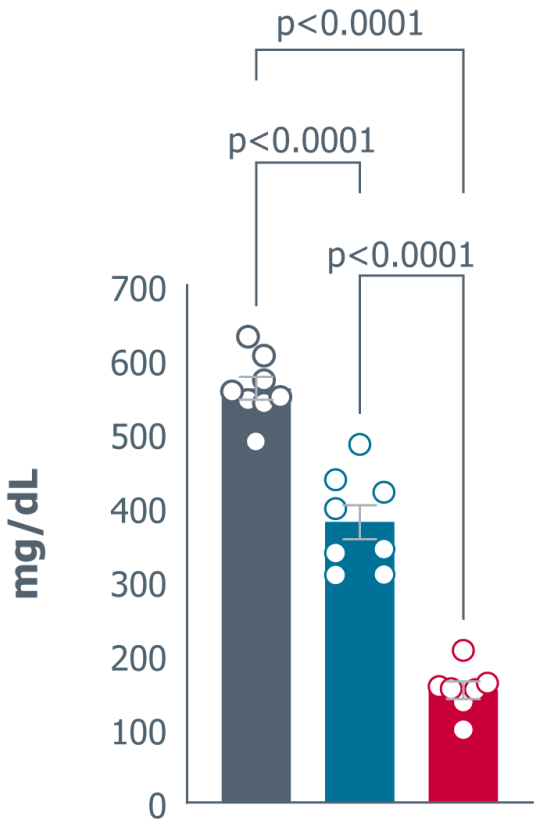


GLP-1=glucagon-like peptide 1, GLP-1RA=glucagon-like peptide 1 receptor agonist, I.P.=intraperitoneal, PGTx=pancreatic gene therapy, S.C.=subcutaneous, T2D=type 2 diabetes, VG=vector genomes

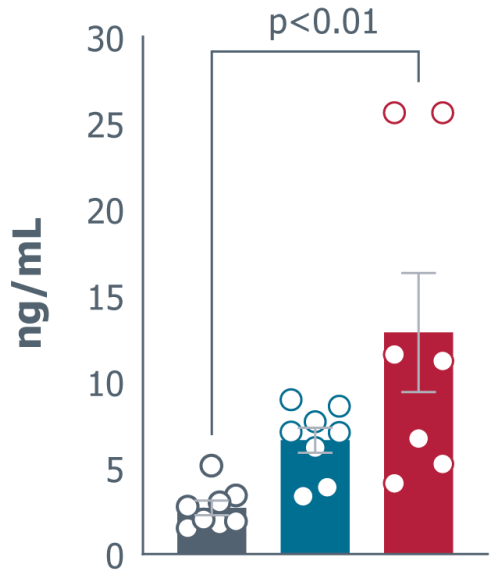
Glucose-Lowering Efficacy in *db/db* Murine Model

GLP-1RA PGTx improves glucose, insulin, and weight vs. daily semaglutide

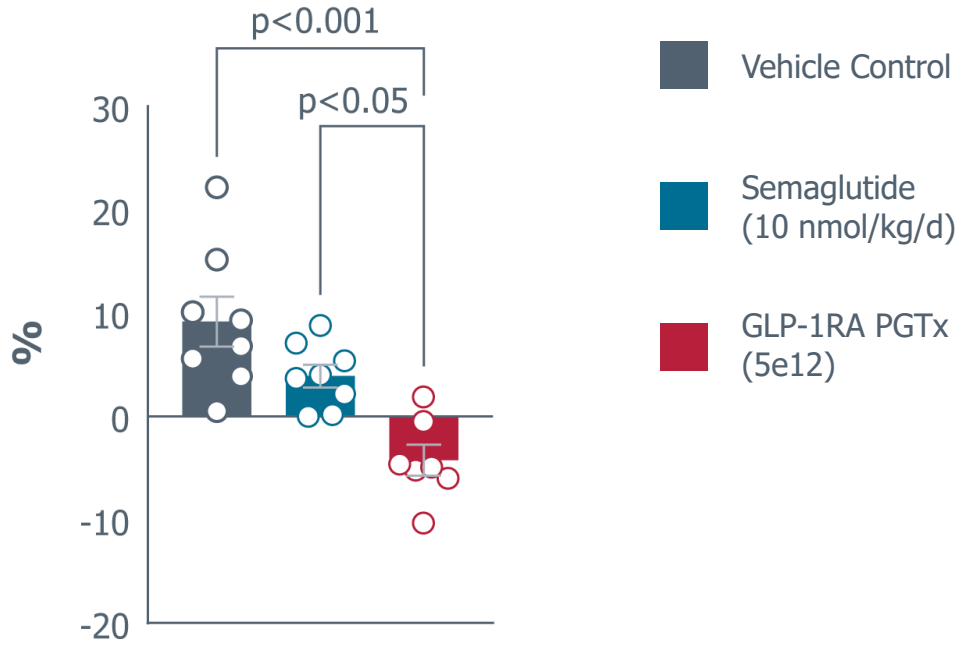
A) Fasting Blood Glucose



B) Fasting Plasma Insulin



C) Body Weight Change from Baseline

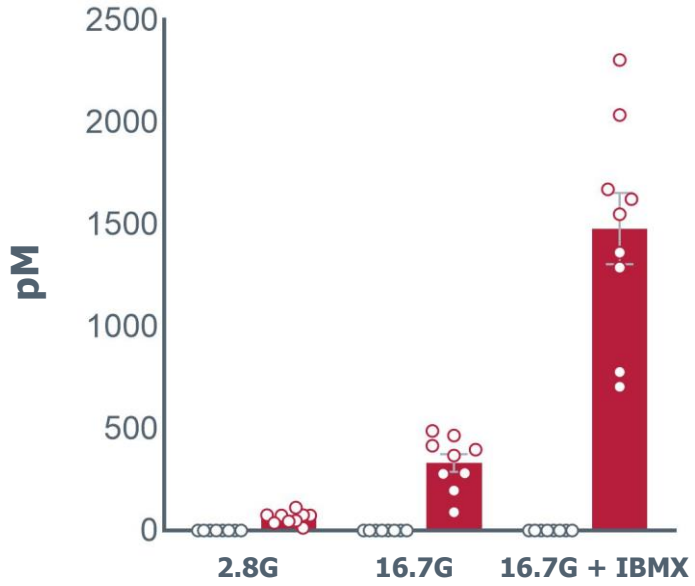


Mean ± SEM shown; n=7-8 per group, day 29 shown, Rajagopalan et al. DDW 2024 oral presentation. Abstract no. 4029196. GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy

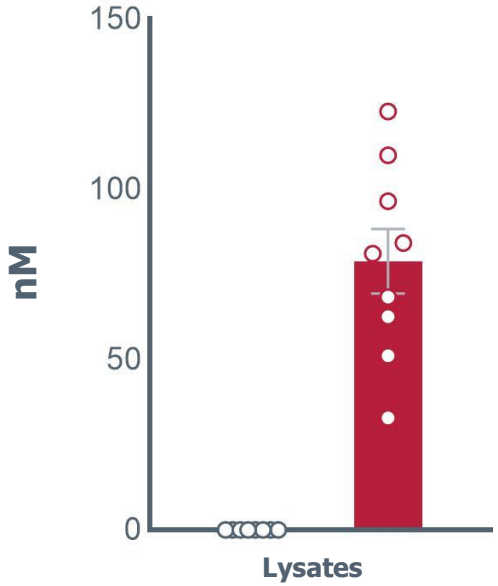
GLP-1RA PGTx Secretion in Isolated *db/db* Islets

Glucose-dependent GLP-1RA release with ample secretory reserve

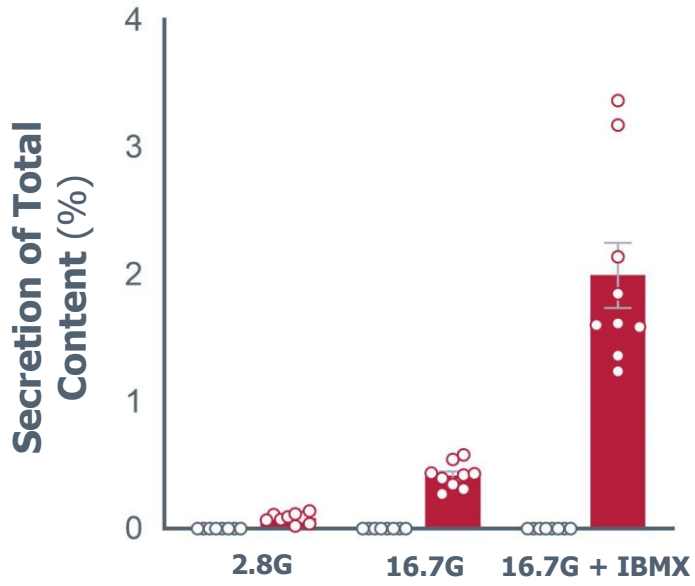
A) GLP-1RA Released



B) GLP-1RA Total Content



C) GLP-1RA Secretion (% of Total Content)



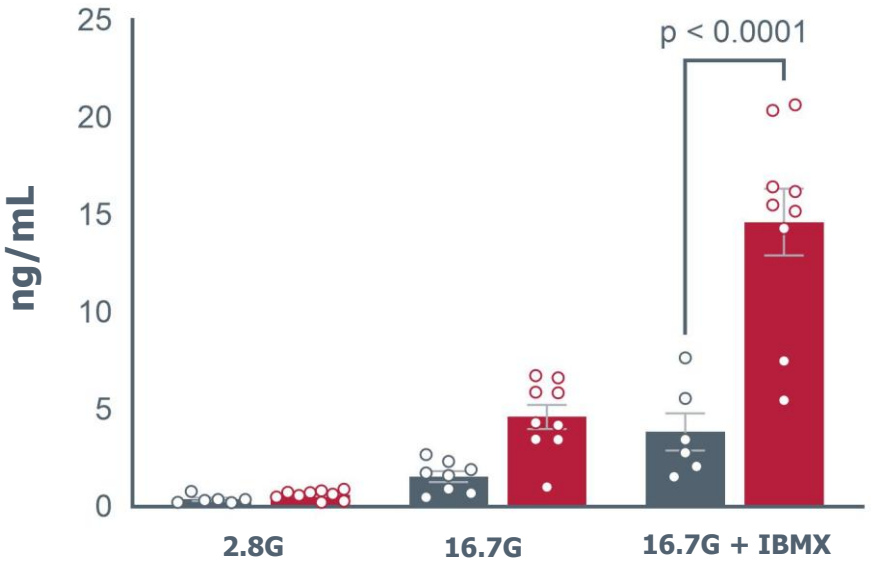
Vehicle Control GLP-1RA PGTx

Islets were isolated 9 weeks post-GLP-1RA PGTx treatment, N=3 mice/group, 15 islets/well, performed in triplicate. 2.8G=2.8mM glucose, 16.7G=16.7mM glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, IBMX=3-isobutyl-1-methylxanthine, PGTx=pancreatic gene therapy

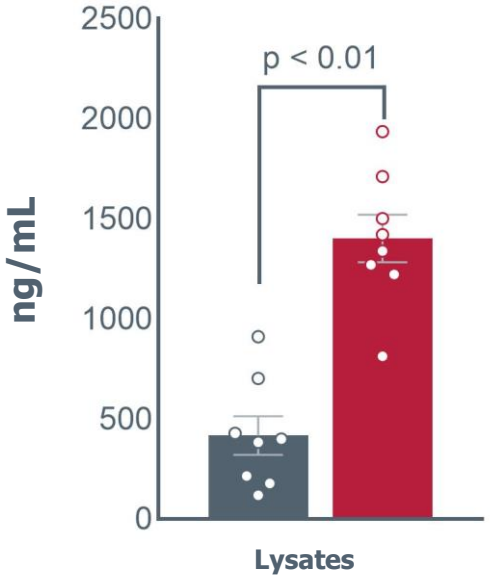
GLP-1RA PGTx-Induced Insulin Secretion in Isolated *db/db* Islets

Increases insulin content and secretion with ample secretory reserve

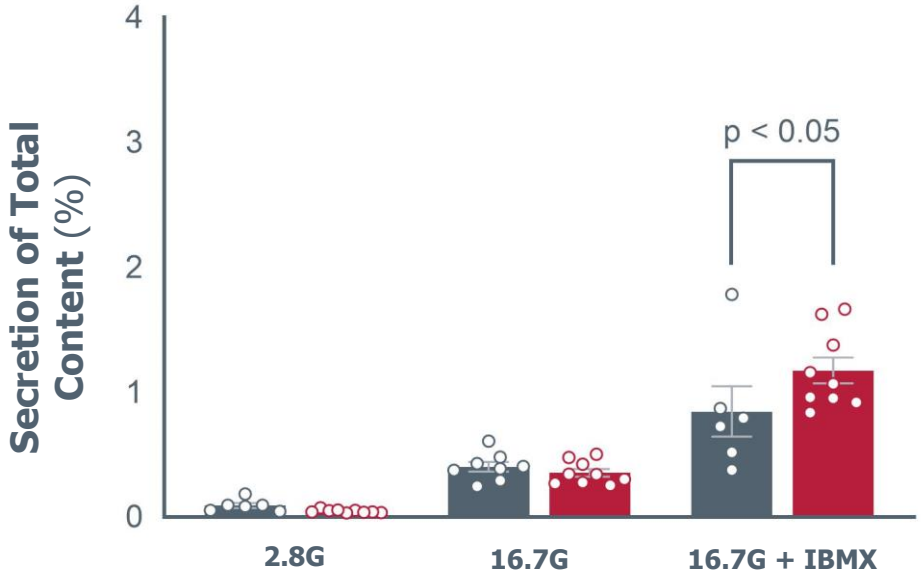
A) Insulin Released



B) Insulin Total Content



C) Insulin Secretion
(% of Total Content)



Vehicle Control GLP-1RA PGTx

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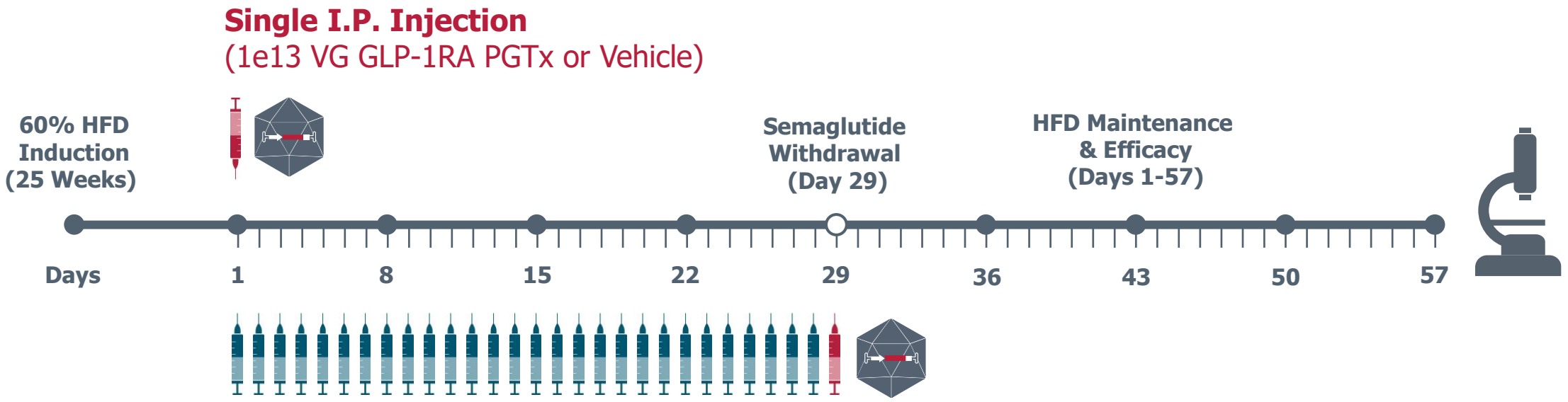
GLP-1RA PGTx Obesity Efficacy Study: Head-to-Head vs. Semaglutide

DIO murine model is *de facto* standard for obesity development

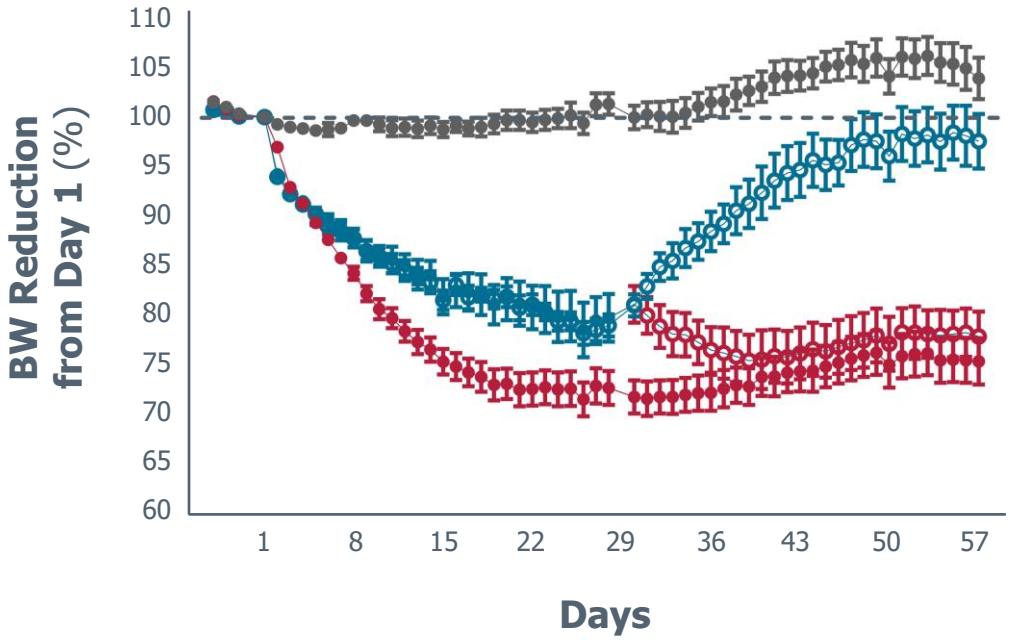


DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, HFD=high fat diet, I.P.=intraperitoneal, PGTx=pancreatic gene therapy, S.C.=subcutaneous, VG=vector genomes

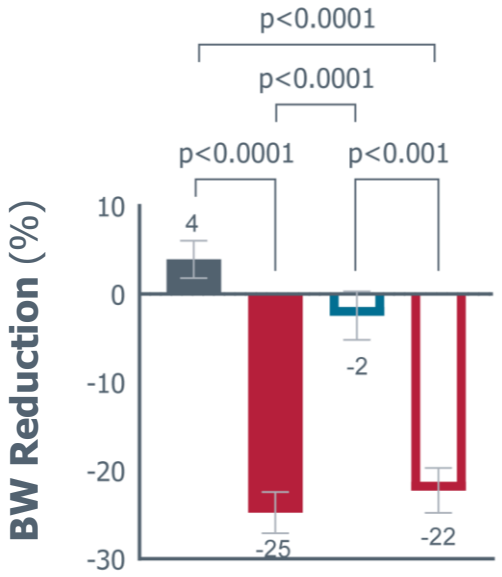
Body Weight Change in DIO Murine Model

Single-dose GLP-1RA PGTx sustains weight loss after semaglutide withdrawal

A) Change in BW Over Time



B) End of Study BW Change



Vehicle Control

Sema (10 nmol/kg/d)

GLP-1RA PGTx (1e13 VG)

Sema Withdrawal + Vehicle

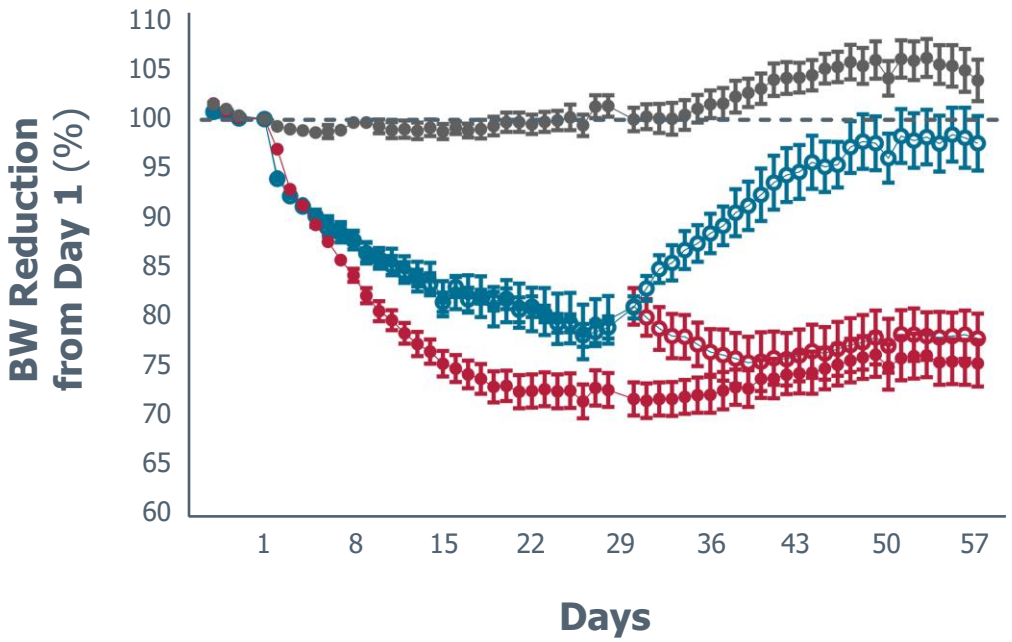
Sema Withdrawal + GLP-1RA PGTx (5e12 VG)

Mean ± SEM shown; n=5-10 per group. Fitzpatrick et al. WCIRDC 2023 oral presentation. Abstract no. 0077. BW=body weight, DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

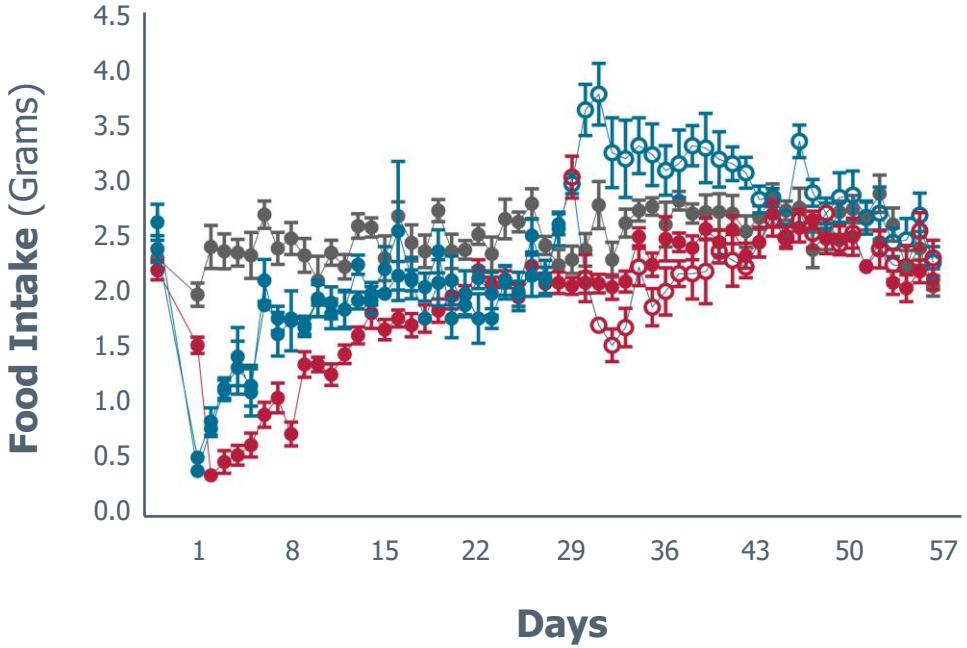
Food Intake Change in DIO Murine Model

Body weight changes are reflected by alterations in food intake

A) Change in BW Over Time



B) Food Intake Over Time



Vehicle Control

Sema (10 nmol/kg/d)

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Sema Withdrawal + Vehicle

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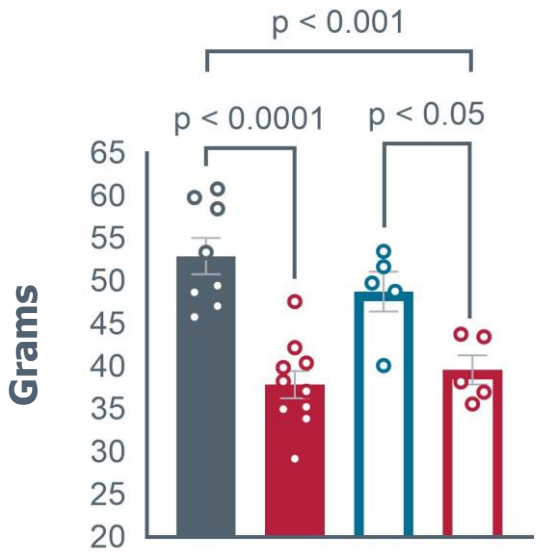
Mean ± SEM shown; n=5-10 per group. Fitzpatrick et al. WCIRDC 2023 oral presentation. Abstract no. 0077. BW=body weight, DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

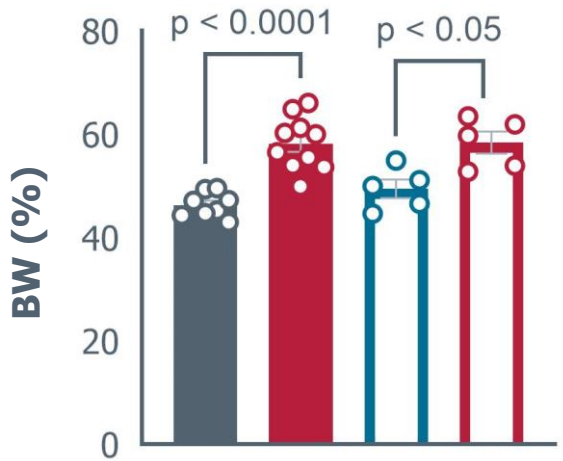
Body Composition Change in DIO Murine Model

Preservation of lean mass: body weight loss primarily from fat mass

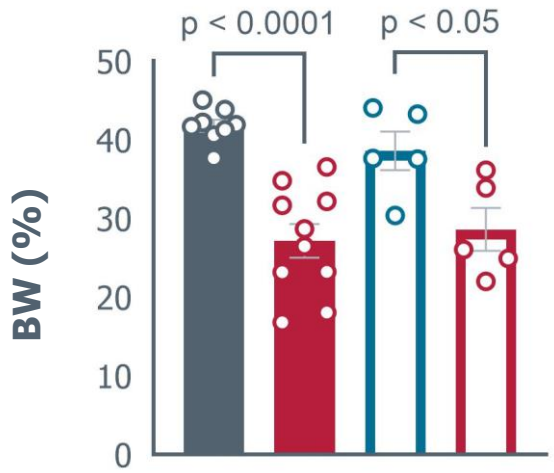
A) Body Weight



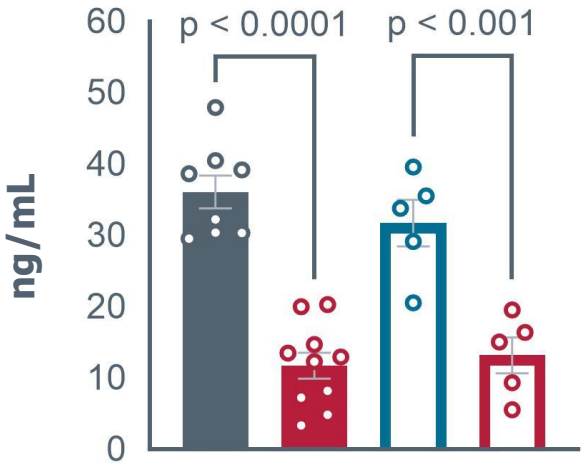
B) Lean Mass

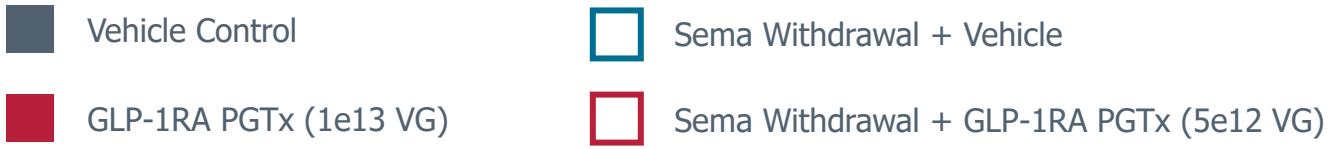


C) Fat Mass



D) Plasma Leptin



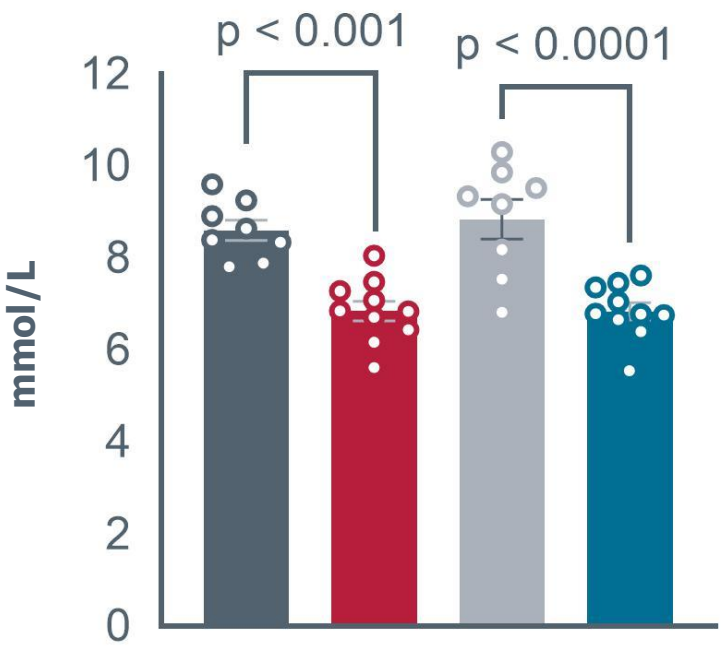


Mean ± SEM shown; n=5-10 per group, 8 weeks post-treatment. BW=body weight, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

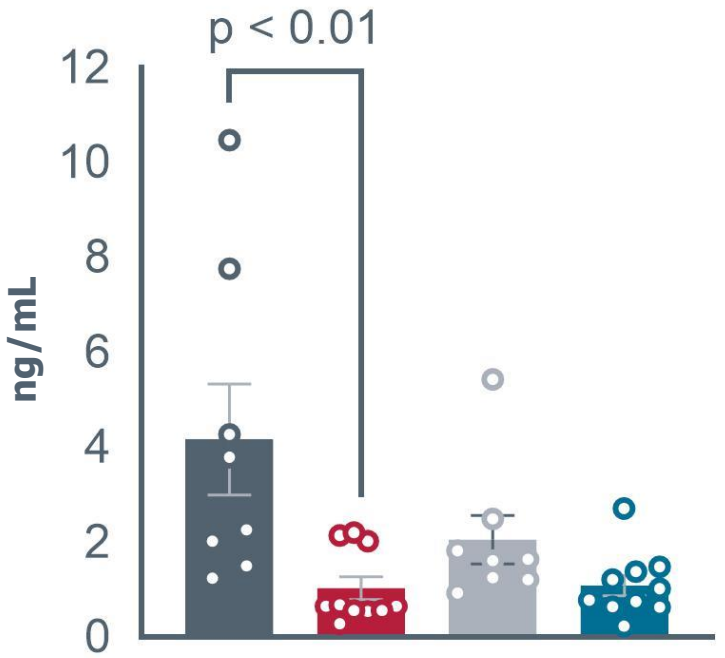
Fasting Blood Glucose and Insulin Changes in DIO Murine Model

Single-dose GLP-1RA PGTx improves FBG and insulin at 4 weeks

A) Fasting Blood Glucose



B) Fasting Plasma Insulin



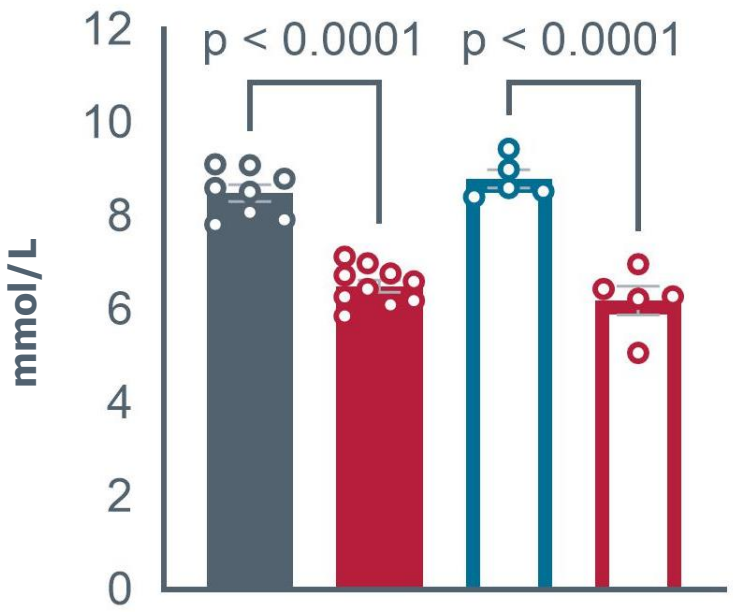
AAV Vehicle Control | GLP-1RA PGTx (1e13 VG) | Sema Vehicle | Sema (10 nmol/kg/d)

Mean ± SEM shown; n=5-10 per group. AAV=adeno-associated virus, DIO=diet-induced obesity, FBG=fasting blood glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

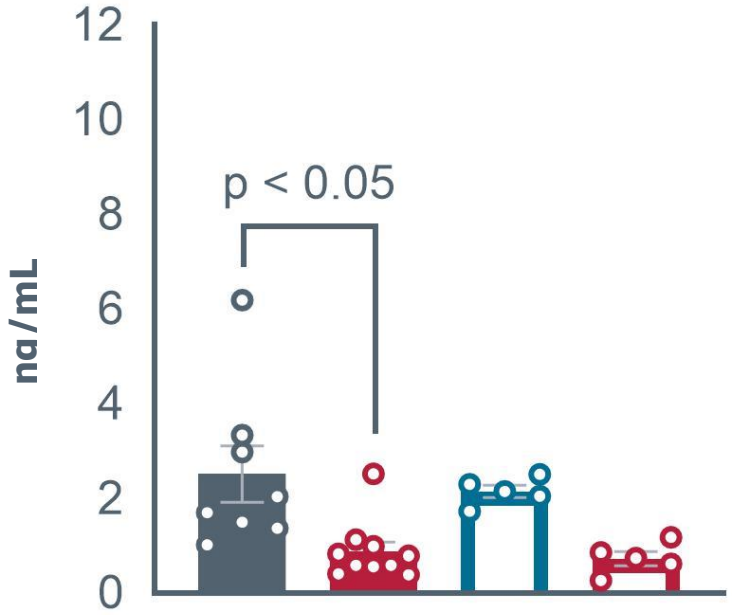
Fasting Blood Glucose and Insulin Changes in DIO Murine Model

Single-dose GLP-1RA PGTx improves FBG and insulin at 8 weeks

A) Fasting Blood Glucose



B) Fasting Plasma Insulin

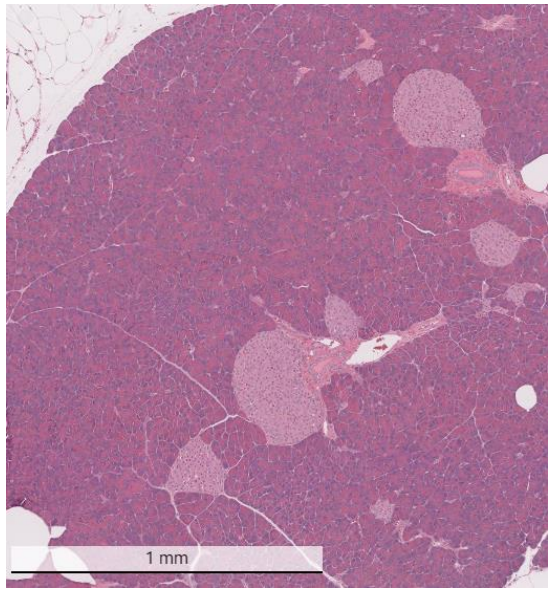
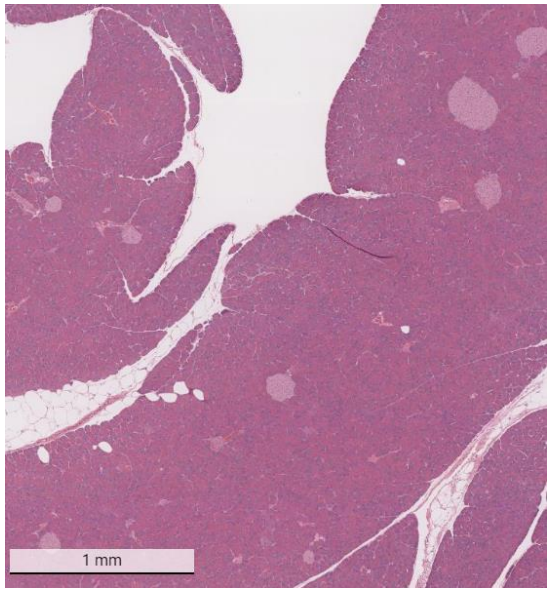
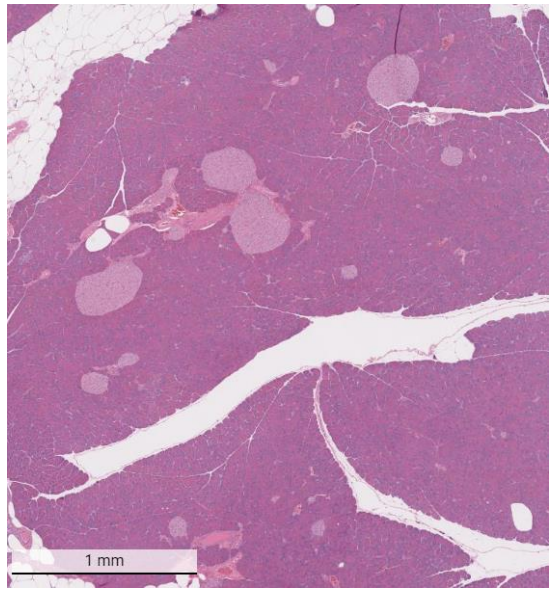
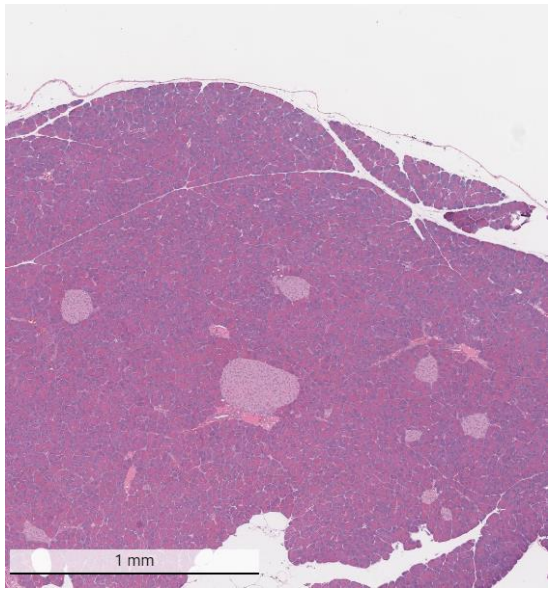


AAV Vehicle Control | GLP-1RA PGTx (1e13 VG) | Sema Withdrawal + Vehicle | Sema Withdrawal + GLP-1RA PGTx (5e12 VG)

Mean ± SEM shown; n=5-10 per group. AAV=adeno-associated virus, DIO=diet-induced obesity, FBG=fasting blood glucose, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide, VG=vector genomes

Pancreatic Islet Histopathology in DIO Murine Model

GLP-1RA PGTx was not associated with inflammation

Vehicle Control	GLP-1RA PGTx	Sema Withdrawal	Sema Withdrawal + PGTx
			
Score: 0.25 ± 0.16	0.30 ± 0.15	0.20 ± 0.20	0.20 ± 0.20

Histopathology Scorecard[1]

0 – No cellular infiltrates
1 – Infiltrates in small foci at islet periphery
2 – Infiltrates surround islets [peri-insulitis]
3 – Intra-islet infiltration <50% islet
4 – Extensive infiltrations = 50% islet, cellular, destruction, prominent cytoarchitectural derangement
5 – Islet atrophy because of beta cell loss

There were no significant differences between groups

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Mean ± SEM shown; n=5-10 per group. 1. Papaccio et al. Endocrinology. 2000 Apr;141(4):1500-5. DIO=diet induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, PGTx=pancreatic gene therapy, Sema=semaglutide

GLP-1RA PGTx Safety and Feasibility Studies in Model Systems

Conclusions to date and next steps

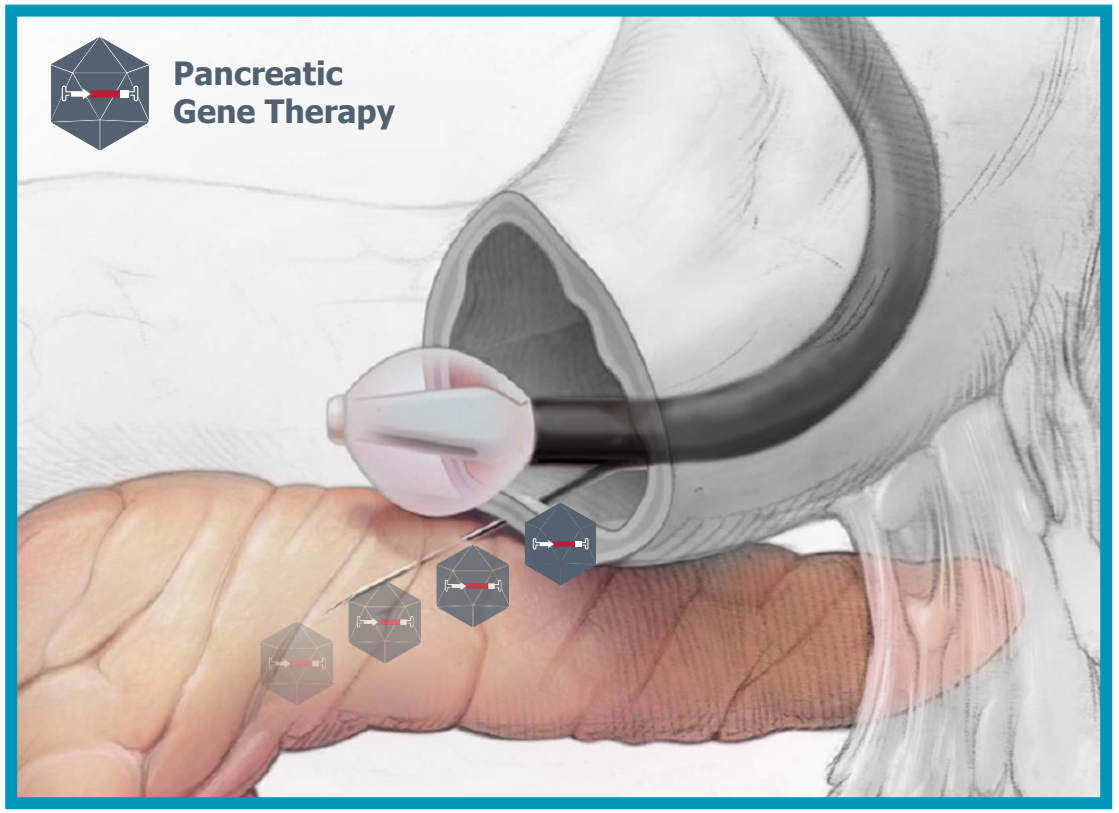
Early safety and feasibility observations in *db/db* and DIO mice and Yucatan pigs are encouraging

First demonstration of **nutrient-responsive GLP-1RA secretion in islets**

PGTx **improves fasting glucose and insulin** in *db/db* and DIO models of T2D and obesity

PGTx leads to **durable weight loss** and **weight and body composition maintenance after semaglutide withdrawal** in DIO mice

RJVA-001 IND-enabling studies are underway



DIO=diet-induced obesity, GLP-1RA=glucagon-like peptide 1 receptor agonist, IND=investigational new drug, PGTx=pancreatic gene therapy, T2D=type 2 diabetes

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