Single-dose GLP-1-based Pancreatic Gene Therapy Maintains Weight Loss After Semaglutide Withdrawal in a Murine Model of Obesity

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Disclosure Statement

Authors
Harith Rajagopalan, Alice Liou Fitzpatrick, Suya Wang, Emily Cozzi, Timothy Kieffer and Jay Caplan are employees and shareholders of Fractyl Health, Inc. Randy Seeley is a paid consultant for and received research support from Novo Nordisk, Fractyl Health, Congruence, and Eli Lilly; is a paid consultant for CinRx and Crinetics; and received research support from Amgen, Astra Zeneca, and Bullfrog AI.

Pancreatic Gene Therapy (PGTx) is in early development and has not been assessed by any regulatory body for investigational or commercial use.
Incretin Therapies: T2D, Obesity, and Beyond

Broad-based benefit on surrogate biomarkers and hard endpoints

**GLP-1RAs can reduce mortality**
in individuals with obesity and a history of cardiovascular disease

Compounding effect from cumulative exposure in up to 4 years of follow up

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**A) Primary Cardiovascular Composite End Point**

- Hazard ratio, 0.80 (95% CI, 0.72–0.90)
- P<0.001 for superiority

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**B) Death from Cardiovascular Causes**

- Hazard ratio, 0.85 (95% CI, 0.71–1.01)
- P=0.07

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**C) Heart Failure Composite End Point**

- Hazard ratio, 0.82 (95% CI, 0.71–0.96)

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**D) Death from Any Cause**

- Hazard ratio, 0.81 (95% CI, 0.71–0.93)

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Incretin Therapies: Real-world Discontinuation Rates are High

Majority of patients discontinue therapy within first year

Despite proven clinical efficacy in obesity and T2D, up to 2/3rds of patients discontinue weekly GLP-1RA therapy within 1 year

Probability of Treatment Continuation

**Incretin Therapies: Metabolic Rebound Now Well Described**

Current GLP-1RAs do not durably alter metabolic setpoint

Discontinuation of therapy leads to **near total loss of metabolic benefit**\(^1\)

GLP-1RA therapies support weight loss and glucose control, **but how do we maintain these effects?**

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**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-1RA=glucagon-like peptide 1 receptor agonist**
Pancreatic Gene Therapy (PGTx) to Modify Islet Function
Potential for durable improvement in metabolic health

Islet cells terminally differentiated, making adeno-associated virus (AAV) a suitable means of durable genetic modification.

β-cell machinery can be leveraged to produce nutrient-stimulated hormones that modify systemic metabolic function.

GLP-1-based PGTx, driven by the insulin promoter, may offer differentiated benefit via durable local production of GLP-1RA.

GLP-1-based PGTx Improves Insulin Production and GSIS in Islets
Metabolic improvements in isolated *db/db* islets 10 weeks after PGTx

**A)** Islet Transduction

**B)** GLP-1RA Protein Content

**C)** Insulin Content

**D)** Glucose-Stimulated Insulin Secretion (GSIS)

**Mean ± SD shown; n=8 per group. D) Glucose stimulation of 16.7 mM +/- IBMX from 2.8 mM baseline. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. GLP-1=glucagon-like peptide 1, GLP-1RA=GLP-1 receptor agonist, Glu=glucose, GSIS=glucose-stimulated insulin secretion, n.d.=not detectable, PGTx=pancreatic gene therapy.**
GLP-1-based PGTx Improves GSIS in Human Islets and β-cell Line

Improved GSIS mediated by GLP-1R activation

A) Human Islet Transduction

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, Glu=glucose, GSIS=glucose-stimulated insulin secretion, PGTx=pancreatic gene therapy
Proprietary Local Endoscopic Delivery System Extensively Tested
Dose-dependent transduction throughout porcine splenic lobe

A) Extensive GFP in Splenic Lobe

B) VG Dose-dependent GFP in Pancreas

Rajagopalan et al. ASGCT 2023 oral presentation. Abstract no. 191. GFP=green fluorescent protein, VG=vector genomes
Local Endoscopic Delivery Allows High Expression
Dramatically limits systemic exposure to AAV in porcine model

A) Local Delivery (4.2e12 VG/kg)

B) I.V. (8.3e12 VG/kg)

Proof-of-principle Safety with Local Endoscopic Delivery System
ALT and lipase levels remained within normal range across all timepoints

Mean ± SEM shown; n=28. 1. Thompson et al. UEGW 2023 poster presentation. Abstract no. AS-UEG-2023-02238. ALT=alanine transaminase, ULN=upper limit of normal
GLP-1-based PGTx T2D Efficacy Study: Head-to-head vs. Semaglutide

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

**Single I.P. Injection**
(1e13 or 5e12 VG GLP-1-based PGTx or Vehicle)

35-day-old Mice
Days
0 14 28 42 56 70

**Daily S.C. Injections**
Semaglutide (10 nmol/kg/d) or Vehicle

**Efficacy/MOA (Day 0-70)**
Weekly Fasting Blood Glucose
Biweekly Insulin
Weight

**Sacrifice (Days 58-70)**
Organ Histology
Pancreatic GLP-1RA Protein
Serum GLP-1RA Protein

GLP-1=glucagon-like peptide 1, GLP-1RA= GLP-1 receptor agonist, I.P.=intraperitoneal, MOA=mechanism of action, PGTx=pancreatic gene therapy, S.C.=subcutaneous, VG=vector genomes
Glucose-lowering Efficacy in \textit{db/db} Murine Model

GLP-1-based PGTx improves fasting glucose vs. daily semaglutide

\begin{itemize}
  \item \textbf{A)} Fasting Blood Glucose (Week 8, 4-6 Hours Fasted)
  \item \textbf{B)} Fasting Insulin (Week 8, 4-6 Hours Fasted)
\end{itemize}

\begin{itemize}
  \item Mean ± SEM shown; n=8 per group. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. Gen=generation, GLP-1=glucagon-like peptide 1, PGTx=pancreatic gene therapy
\end{itemize}
Body Weight Change in \(db/db\) Murine Model

GLP-1-based PGTx prevents weight gain vs. daily semaglutide

23% lower total body weight with PGTx compared to vehicle

20% lower total body weight with PGTx compared to semaglutide

Mean ± SEM shown; \(n=8\) per group. Rajagopalan et al. ADA 2023 oral presentation. Abstract no. 181-OR. AAV=adeno-associated virus, Gen=generation, GLP-1=glucagon-like peptide 1, PGTx=pancreatic gene therapy.
GLP-1-based PGTx T2D Efficacy Study: Head-to-head vs. Semaglutide

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

**Single I.P. Injection**
(1e13 VG GLP-1-based PGTx or Vehicle)

**Daily S.C. Injection**
Semaglutide (10 nmol/kg/d or Vehicle)

**Single I.P. Injection (½ Dose)**
(5e12 VG GLP-1-based PGTx or Vehicle)

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GLP-1=glucagon-like peptide 1, HFD=high fat diet, I.P.=intraperitoneal, PGTx=pancreatic gene therapy, S.C.=subcutaneous, VG=vector genomes
Body Weight and Food Intake Change in DIO Murine Model
Single-dose GLP-1-based PGTx sustains weight loss after semaglutide withdrawal

A) Change in BW Over Time

B) End of Study

BW Reduction (%)

C) Food Intake Over Time

Days

BW Reduction from Day 1 (%)

Food Intake (Grams)

Mean ± SEM shown; n=8-10 per group. BW=body weight, DIO=diet-induced obesity, GLP-1=glucagon-like peptide 1, PGTx=pancreatic gene therapy, VG=vector genomes
GLP-1-based PGTx Safety and Feasibility Studies in Model Systems

Conclusions to date

Early safety and feasibility observations in \( db/db \) and DIO mice and Yucatan pigs are encouraging.

Compared to chronic semaglutide, single-dose PGTx improves fasting glucose and prevents weight gain in \( db/db \) model of T2D.

Single-dose PGTx can lead to durable weight loss and maintenance of weight loss after semaglutide withdrawal in DIO mice.

PGTx lead optimization demonstrates potential for superior potency in T2D and obesity with low pancreatic dose.

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AAV=adeno-associated virus, DIO=diet-induced obesity, GLP-1=glucagon-like peptide 1, GLP-1R=GLP-1 receptor, GLP-1RA=GLP-1R agonist, PGTx=pancreatic gene therapy
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