Pancreatic Gene Therapy Durably Improves Glycemia and Delays Disease Progression in a Murine Model of Type 2 Diabetes

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June 24th, 2023
Disclosure Statement

Authors
Harith Rajagopalan, Alice Liou, Emily Cozzi, Jacob Wainer, Rebecca Reese, and Jay Caplan are employees and shareholders of Fractyl Health, Inc. Nidhi Khanna and Jason A. West are former employees of Fractyl Health, Inc.

The Pancreatic Gene Therapy (PGTx) is in early development and not approved by any regulatory body for investigational or commercial use.
T2D Progression is Driven by Declining Islet Health

Loss of β-cell function is the sine qua non of T2D

Pancreatic Islets

- Functional
- β-cell hyperplasia & hypersecretion
- Secretory dysfunction
- β-cell loss

Pancreatic Gene Therapy (PGTx) to Improve Islet Function
Potential for durable improvement in β-cell function

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

Intra-islet GLP1 signaling is essential for β-cell function, health, and survival

GLP1-based pancreatic gene therapy (GLP1 PGTx driven by the insulin promoter) may restore islet health in T2D via durable local production of GLP1RA

GLP1=glucagon-like peptide 1, GLP1R=GLP1 receptor, GLP1RA=GLP1R agonist, PGTx=pancreatic gene therapy
GLP1 PGTx Improves Insulin Production and GSIS in *db/db* Islets

Metabolic improvements in isolated islets 10 weeks after PGTx

**A) Islet Transduction**

- **Vehicle**
- **AAV-GLP1RA**

**B) GLP1RA Protein Content**

- GLP1RA (pg/mL)
  - 400
  - 300
  - 200
  - 100
  - 0
  - n.d.

- **AAV-GLP1RA**

**C) Insulin Content**

- Insulin (μg/L)
  - 2000
  - 1500
  - 1000
  - 500
  - 0

- **Vehicle**
- **AAV-GLP1RA**

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

- Glucose (Glu)
  - Stimulation Index
  - 250
  - 200
  - 150
  - 100
  - 50
  - 0

Mean ± SD shown; *p<0.05, ***p<0.001, ****p<0.0001; n=8 per group. D) Glucose stimulation of 16.7 mM +/- IBMX from 2.8 mM baseline. Rajagopalan et al. ASGCT 2023 oral presentation. Abstract no. 191. AAV=adeno-associated virus, GLP1=glucagon-like peptide 1, GLP1RA=GLP1 receptor agonist, Glu=glucose, GSIS=glucose-stimulated insulin secretion, n.d.=not detectable, PGTx=pancreatic gene therapy

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**Islet Transduction** ➔ **GLP1RA Expression** ➔ **Insulin Production** ➔ **Glucose Stimulation**
GLP1 PGTx Improves GSIS in Human Islets and Human β-cell Line
Improved GSIS mediated by GLP1R activation in human cells

A) Human Islet Transduction

B) Human Islet GSIS

C) Human β-cell Line GSIS ± Ex9 (GLP1R Antagonist)

Mean ± SEM shown; *p<0.05, **p<0.01; 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. ASGCT 2023 oral presentation. Abstract no. 191. AAV=adenovirus, Ex9=Exendin-9, GFP=green fluorescent protein, GLP1=glucagon-like peptide 1, GLP1R=GLP1 receptor, GLP1RA=GLP1R agonist, Glu=glucose, GSIS=glucose-stimulated insulin secretion, PGTx=pancreatic gene therapy.
Local Delivery of PGTx
Proprietary endoscopic ultrasound-guided infusion device

Yucatan pig model anatomy similar to humans

Proprietary device and endoscopic procedure previously described\(^1,2\)

>50 animals treated with 100% technical success; no adverse safety signals to date

Dose-dependent AAV-GFP expression in targeted pancreatic lobe\(^1,2\)

**Low viral genome dose with limited systemic virus exposure** – due to local delivery\(^2\)

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Compared to Chronic Semaglutide, Can One-Time GLP1 PGTx:
Improve Glycemia
Delay T2D Progression
and Prevent Weight Gain?
GLP1 PGTx Efficacy Proof of Concept

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

### Single I.P. Injection

(AAV-INS-GLP1RA or Vehicle)

35-day-old mice

Days

0 14 28 42 56 70

### Daily S.C. Injection

Semaglutide (10 nmol/kg*) or Vehicle

*Semaglutide dose selected for glucose-lowering optimization¹

### Efficacy/MOA (day 0-70)

- Weekly Fasting Blood Glucose
- Biweekly Insulin
- Weight

### Sacrifice (days 58-70)

- Organ Histology
- Pancreatic GLP1RA Protein
- Serum GLP1RA Protein

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¹. CDER (2017) Semaglutide NDA Application (209637Orig1s000), Section 4.4 Nonclinical Pharmacology/Toxicology. AAV=adeno-associated virus, GLP1=glucagon-like peptide 1, GLP1RA=GLP1 receptor agonist, INS=insulin promoter, I.P.=intraperitoneal, MOA=mechanism of action, PGTx=pancreatic gene therapy, S.C.=subcutaneous
GLP1 PGTx Expression Restricted to Pancreatic Islets
Safety and feasibility in \textit{db/db} are reassuring thus far

**High specificity for pancreas**
Insulin promoter effectively restricts transgene expression to pancreatic islets
No detectable expression in off-target tissues (e.g., exocrine pancreas)

**Favorable toxicity profile**
No abnormal findings thus far in animal behavior or clinical chemistries
Histopathologic analysis showed no evidence of pancreatitis or pancreatic cancer
Glucose Lowering Efficacy in \textit{db/db} Mouse

GLP1 PGTx improves fasting glucose vs. daily semaglutide

**A)** Fasting Blood Glucose
(Week 8, 4–6 hour fasted)

**B)** Fasting Insulin
(Week 8, 4–6 hours fasted)

Mean ± SEM shown; *p<0.05, **p<0.01, ****p<0.0001; n=8 per group. AAV=adeno-associated virus, Gen=generation, GLP1=glucagon-like peptide 1, GLP1RA=GLP1 receptor agonist, INS=insulin promoter, PGTx=pancreatic gene therapy
Disease Progression and Durability

GLP1 PGTx shifts progression of disease vs. daily semaglutide

AAV=adeno-associated virus, FBG=fasting blood glucose, Gen=generation, GLP1=glucagon-like peptide 1, GLP1RA=GLP1 receptor agonist, INS=insulin promoter, PGTx=pancreatic gene therapy
Body Weight Change

GLP1 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; ****p<0.0001; n=8 per group. AAV=adeno-associated virus, Gen=generation, GLP1= glucagon-like peptide 1, GLP1RA=GLP1 receptor agonist, INS=insulin promoter, PGTx=pancreatic gene therapy
GLP1 PGTx Safety and Pharmacology Studies in Model Systems

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

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

PGTx lead optimization demonstrates potential for even greater efficacy in T2D and obesity with low pancreatic dose.

Data raise important questions about role of pancreatic islet in regulation of metabolic setpoint.

AAV=adeno-associated virus, GLP1=glucagon-like peptide 1, GLP1R=GLP1 receptor, GLP1RA=GLP1R agonist, PGTx=pancreatic gene therapy.
Thank You

Acknowledgements

Fractyl Health

Cell and Animal Models

Alice Liou Fitzpatrick
Camila Lubaczeuski
Becky Reese
Nicole Picard

Virus and Gene Delivery

Lin Quek
Gary White
Suya Wang
Keiko Ishida

Device Engineering

Jake Wainer
Mike Biasella

Advisor Support

ERASE Task Force and Advisors

Randy Seeley, PhD (Michigan School of Medicine)

Alan Cherrington, PhD (Vanderbilt University School of Medicine)

Dave D’Alessio, MD (Duke University School of Medicine)

Geltrude Mingrone, MD PhD (Kings College London and Gemelli Hospital Rome)

Jon Campbell, PhD (Duke University School of Medicine)

John Amatruda, MD (Yale University School of Medicine)