A Pancreatic Gene Therapy Delivery Platform for the Treatment of Type 2 Diabetes

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Disclosure Statement

Authors: Harith Rajagopalan, Jacob Wainer, Alice Liou, Rebecca Reese, Suya Wang, Keiko Ishida, Nicole Picard, Camila Lubaczeuski, Emily Cozzi, and Jay Caplan are employees and shareholders of Fractyl Health, Inc. Jason A. West and Nidhi Khanna are former employees of Fractyl Health, Inc. Christopher C. Thompson, Linda S. Lee, and Rob P. Trasolini are employees of Brigham and Women's Hospital and Harvard Medical School.

Revita® is for investigational use only in the United States. The Rejuva® platform is in early development and not approved by any regulatory body for investigational or commercial use.
Fractyl Health, Inc.
Pioneering new treatment approaches for type 2 diabetes (T2D)

Complementary development programs targeting key organs in T2D

**Revita®** (targeting the duodenum)
Endoscopic procedure using hydrothermal ablation in the duodenum

**Rejuva®** (targeting the pancreas)
Adeno-associated virus (AAV)–based pancreatic gene therapy platform
Rejuva Directly Targets the Pancreas with Gene Therapy

Key therapeutic elements

- Endoscopic device and procedure
- AAV Gene Therapy Delivery Vehicle
- Tissue-Restricted Transgene Expression
GLP1RAs for T2D Have Proven Clinical Benefit But Are Limited
Opportunity to advance GLP1RA field with local gene therapy approach

Glucagon-like peptide 1 receptor agonists (GLP1RAs) have proven clinical efficacy

Limited by adherence and tolerability of systemic delivery\(^2,3,4\)

In the US, 50% of patients discontinue therapy within 330 days\(^1,3,4\)

Discontinuation of GLP1RA therapy is associated with total loss of metabolic benefit\(^5\)

Rationale for AAV-GLP1RA Gene Therapy to Improve Islet Function

Declining islet health is an early driver of T2D progression

T2D islet:
- \(\beta\)-cell loss of insulin
- \(\alpha\)-cell excess glucagon

GLP1RAs reverse both and improve islet cell health\(^1\)

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Fig. 1. Campbell and Drucker. Cell Metabolism 17, June 4, 2013.

AAV = adeno-associated virus, GLP1RA = GLP-1 receptor agonist
Rationale for AAV-GLP1RA Gene Therapy to Improve Islet Function

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AAV-GLP1RA gene therapy may address limitations by driving local, durable production of GLP1RA to improve islet function

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AAV=adeno-associated virus, GLP1RA=GLP-1 receptor agonist
Rationale for AAV-GLP1RA Route of Administration & Islet Targeting

Islets are most easily accessed via endoscopic ultrasound

Endoscopic ultrasound (EUS) is routine (~300K cases per year)\(^1\)

EUS via stomach provides direct access to pancreatic body and tail

Islets are 1-2% of total pancreas mass, predominantly in tail, and mostly terminally differentiated\(^2,3,4\)

Risk of procedural pancreatitis can be mitigated with device and procedure steps

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EUS-Guided AAV ROA Feasibility in Yucatan Pig

Porcine model approximates human GI tract and pancreas anatomy

Days

EUS-guided
Pancreatic Infusion
AAV9-CMV-GFP (Ubiquitous)*
AAV9-INS-GFP (β-cell Restricted)

Routine Safety
ALT
Lipase
NF-L

Sacrifice (days 21-28)
ALT
Lipase
NF-L
Vector Biodistribution
Organ Histology

*AAV9-CMV-GFP used to assess on target efficacy and acute safety

EUS=endoscopic ultrasound, ROA=route of administration, GI=gastrointestinal, ALT=alanine aminotransferase, NF-L=neurofilament light chain, AAV=adeno-associated virus, GFP=green fluorescent protein, CMV=cytomegalovirus, INS=insulin
EUS-Guided AAV ROA in Yucatan Pig
Serum ALT and lipase remained in the normal range across most timepoints

A) 28-day ALT

Mean ± SEM shown; n=2-4 per group. ALT=alanine transaminase, EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus
EUS-Guided AAV ROA in Yucatan Pig
Dose-dependent expression of GFP throughout targeted splenic lobe

**A) Extensive GFP in Splenic Lobe**

**B) VG Dose-Dependent GFP in Pancreas**

EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, GFP=green fluorescent protein, VG=vector genomes
EUS-Guided AAV ROA in Yucatan Pig

~ 40% of splenic lobe islet cells express GFP transgene at highest dose

A) Islet GFP Expression

B) On-target VCN

Mean ± SD shown; n=2-7 per group; EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, GFP=green fluorescent protein, VCN=vector copy number, VG=vector genomes, DG=diploid genomes
**In Vivo and In Vitro AAV-GLP1RA Efficacy Proof of Concept**

Murine model of T2D progression and islet analyses

- **I.P. Injection** (35-day-old db/db mice)
  - AAV9-INS-GLP1RA
  - Vehicle

- **Efficacy/MOA**
  - Weekly Fasting Blood Glucose
  - Biweekly Insulin
  - IPGTT

- **Sacrifice (days 42-100+)**
  - Organ Histology
  - Pancreatic GLP1RA Protein
  - Serum GLP1RA
  - Islet Isolation

**AAV**= adeno-associated virus, **INS**= insulin, **GLP1RA**= GLP-1 receptor agonist, **I.P.**= intraperitoneal, **IPGTT**= intraperitoneal glucose tolerance test
**In Vivo** AAV-GLP1RA Efficacy Proof of Concept in Db/db Mouse

AAV-GLP1RA targets pancreas with ~4% of islet cells transduced

**A)** Pancreatic Islets 10 Weeks Post I.P. Injection

**B)** Islet GLP1RA Protein Expression

**C)** Whole Pancreas GLP1RA Protein Expression

Note: GLP1RA expression restricted to islet and below limit of quantification in serum

Mean ± SEM shown; **p<0.01, ****p<0.0001; n=5-8 per group; AAV=adeno-associated virus, GLP1RA=GLP-1 receptor agonist, I.P.=intraperitoneal, VG=vector genomes
**In Vivo** AAV-GLP1RA Efficacy Proof of Concept in Db/db Mouse

AAV-GLP1RA increases fasting insulin and reduces fasting blood glucose

**A)** Biweekly Insulin (4–6 hour fasted)

**B)** Weekly FBG (4–6 hour fasted)

Mean ± SEM shown; *p<0.05, **p<0.01, ***p<0.001; n=8 per group; AAV=adeno-associated virus, GLP1RA=GLP-1 receptor agonist, GFP=green fluorescent protein, FBG=fasting blood glucose
**In Vitro AAV-GLP1RA Efficacy – Isolated Islets from Treated Mice**

AAV-GLP1RA increases db/db islet GLP1RA, insulin, and subsequent GSIS

**A) VCN within Islets**

**B) Transgene Content**

**C) Insulin Content**

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

Mean ± SD shown; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; n=2-8 per group; AAV=adeno-associated virus, GLP1RA=GLP-1 receptor agonist, VCN=vector copy number, VG=vector genome, DG=diploid genome

Islet Transduction → GLP1RA Expression → Insulin Production → Glucose Stimulation

Fractyl Health 2023
In Vitro AAV-GLP1RA Efficacy Proof of Concept in Human β-cell Line

AAV-GLP1RA induces GLP1RA protein secretion and improves β-cell function

Mean ± SEM shown; *p<0.05, ****p<0.0001; n=2-3 per group. AAV=adeno-associated virus, Ex9=Exendin-9, GLP1RA=GLP-1 receptor agonist, Glu=glucose

A) GLP1RA Secretion

B) GSIS ± GLP1R Blockade with Ex9
**In Vitro** AAV-GLP1RA POC Efficacy in Human Islet

AAV-GLP1RA targets up to 30% of human islet β-cells and improves function.

**A) β-cell Transduction**

- **Untransduced**
- **AAV-GFP Low Dose**
- **AAV-GFP High Dose**
- **AAV-GLP1RA High Dose**

**B) Transduction Efficiency**

- **GFP**
- **GFP+ NKX6.1**

**C) GSIS**

- **Stimulation Index**

Mean ± SEM shown; **p<0.01, ***p<0.001, ****p<0.0001; n=9-11 per group. AAV=adeno-associated virus, GFP=green fluorescent protein, GLP1RA=glucagon-like peptide 1 receptor agonist, Glu=glucose, NKX6.1=NK6 Homeobox 1, POC=proof of concept**
Conclusions

EUS-guided ROA is a feasible and novel platform for pancreatic gene therapy

AAV9 can dose-dependently target the pancreatic islet and drive transgene expression

POC efficacy for GLP1RA-based gene therapy to restore β-cell function and improve glycemic control in rodents and human cells

No unexpected safety signals observed in models studied to date

Expanded biodistribution and safety analyses will be presented on May 20th, 8:00 AM, Petree Hall C (Abstract 312)
Thank You For Your Attention

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