Local Delivery and Tissue Restricted Expression to Optimize Therapeutic Profile for Pancreatic Gene Therapy

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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
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\(^1\)

Figure adapted from: Saikia et al. JCI Insight 2021;6(3):e1418511. 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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T2D islet:
- β-cell loss of insulin
- α-cell excess glucagon

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

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

Figure adapted from: Saikia et al. JCI Insight 2021;6(3):e1418511. 1. Campbell and Drucker. Cell Metabolism 17, June 4, 2013.

AAV=adeno-associated virus, GLP1RA=GLP-1 receptor agonist
EUS-Guided AAV ROA Feasibility in Yucatan Pig
Porcine model approximates human GI tract and pancreas anatomy

**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

B) 7-day Lipase

C) 28-day Lipase

Mean ± SEM shown; N=13, 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 endocrine cells express GFP transgene at highest dose

**A) Endocrine 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
EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig
Local vs. systemic delivery AAV biodistribution comparison

A) EUS (4.2e12 VG/kg)

B) I.V. (8.3e12 VG/kg, Li et al. 2022)

Figure adapted from 1. Li et al. Physiol Genomics 54: 261–272, 2022. EUS, N=4; I.V., N=2; EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, VCN=vector copy number, VG=vector genomes, DG=diploid genomes, I.V.=intravenous.
EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

AAV-GFP biodistribution unaffected by promotor restriction with highest VCN in pancreas

Dose: 5e13 VG, N=2-4 per group; EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, GFP=green fluorescent protein, CMV=cytomegalovirus, INS=insulin, VG=vector genome, DG=diploid genome, VCN=vector copy number, SL=splenic lobe, DL=duodenal lobe, CL=connecting lobe
## EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

Preliminary AAV-GFP toxicology findings segregated by promoter

<table>
<thead>
<tr>
<th>Assessment</th>
<th>AAV-INS-GFP (β-cell Restricted, N=7)</th>
<th>AAV-CMV-GFP (Ubiquitous, N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical findings</td>
<td>None</td>
<td>(n=1) ataxia, mild hindlimb paresis, forelimb knuckling (day 24, 5e13 VG)</td>
</tr>
<tr>
<td>Clinical blood chemistries</td>
<td>No relevant changes</td>
<td>(n=1) transient elevation in lipase (&lt; 3x ULN) on day 1; normalized by day 4</td>
</tr>
<tr>
<td>Hematology</td>
<td>No relevant changes</td>
<td>No relevant changes</td>
</tr>
<tr>
<td>Organ weight</td>
<td>No adverse organ weight changes</td>
<td>No adverse organ weight changes</td>
</tr>
<tr>
<td>Histopathology</td>
<td>No relevant findings</td>
<td>Minimal to moderate DRG inflammation (C2, T7, and L2 vertebrae)</td>
</tr>
<tr>
<td>Immune response</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, GFP=green fluorescent protein, CMV=cytomegalovirus, INS=insulin, VG=vector genome, DRG=dorsal root ganglia
EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig
Lipase elevation associated with GFP & abolished by promoter restriction

A) AAV-CMV-GFP 28 Day Lipase

B) AAV-INS-GFP 28 Day Lipase

Elevated lipase likely related to pancreatic GFP expression

n=2-4 per group; EUS=endoscopic ultrasound, ROA=route of administration, AAV=adeno-associated virus, GFP=green fluorescent protein, CMV=cytomegalovirus, INS=insulin, VG=vector genome
EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig
AAV-GFP DRG toxicity is mitigated by promotor restriction

**AAV9-CMV-GFP** *Inflammation, GFP expression*

**AAV9-INS-GFP** *No inflammation, no GFP expression*
NF-L Appears to be a Good Biomarker for DRG Toxicity
Dose-dependent increases with AAV-CMV but no signal with AAV-INS

A) AAV-CMV-GFP NF-L

B) AAV-CMV-GFP vs AAV-INS-GFP NF-L

N=7, n=2-4 per group; AAV=adeno-associated virus, GFP=green fluorescent protein, CMV=cytomegalovirus, INS=insulin, NF-L=neurofilament light chain
Conclusions

Local AAV9 via EUS shows on target gene expression with low viral dose.

Favorable biodistribution profile to the pancreas compared to other tissues.

The pig model is a very useful and sensitive model for tox assessment.

Mechanical and molecular confinement of transgene expression thus far appear to optimize therapeutic index.
Thank You For Your Attention

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