AAV-Mediated Pancreatic Gene Therapy for Type 2 Diabetes

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Disclosure Statement

Author(s): Alice Liou Fitzpatrick, Nidhi Khanna, Jacob Wainer, Rebecca Reese, Jason West, Jay Caplan, and Harith Rajagopalan are employees and shareholders of Fractyl Health, Inc.
Fractyl Health’s Mission

Target organ-level root causes of metabolic disease

Revita System™ for Duodenal Mucosal Resurfacing (DMR)

Restore morphology and metabolic function to the duodenum

Rejuva® Platform for Pancreatic Gene Therapy

Restore metabolic regulation of the pancreas with locally delivered gene therapy

The Revita™ system 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.
GLP-1 therapies have proven benefits for pancreatic health in T2D but are limited by adherence and tolerability of systemic delivery

- GLP-1 stimulates glucose-dependent insulin secretion and improves overall beta-cell health.¹

- In the US, 50% of patients discontinue therapy within 330 days after initiating weekly GLP-1 therapy.²

- Side effects are primary reason for discontinuation.³

- Discontinuation of GLP-1RA therapy is associated with total loss of metabolic benefit. Ongoing exposure is needed for lasting patient benefit.⁴

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¹ Müller 2019 Mol Metab 30:72-130
² Weiss 2020 Patient Pref Adherence 14:2337-2345
³ Polonsky 2021 Diabetes spectra 34(2):175-183
⁴ RISE Consortium Diabetes Care. 2019;42(9):1742-1751
Gene therapy approach for improvements in beta-cell health

Beta-cell specific GLP-1RA transgene

pA = Polyadenylation
AAV = Adeno-associated virus
ER = Endoplasmic reticulum
Screening a DNA construct library to identify top functional GLP-1RA producers in a beta-cell line

DNA construct template

Beta-cell specific GLP-1RA transgene

Plasmid Library

Transfection

MIN6 Cells

GLP-1RA secretion

25 mM Glucose stimulation

Relative to Tris-EDTA Buffer

Means ± Std Dev. One sample t-test *P<0.05, **P<0.01

MIN6 = Mouse insulinoma cell line 6
eGFP = enhanced green fluorescent protein
GCG = Preproglucagon gene
Screening a DNA construct library to identify top functional GLP-1RA producers in a beta-cell line

**DNA construct template**

**Beta-cell specific GLP-1RA transgene**

![Diagram showing transfection process]

**Plasmid Library**

**Transfection**

**MIN6 Cells**

**cAMP signaling**

CHO-K1 hGLP-1R Gs cell line

**Relative to eGFP Control**

Means ± Std Dev. One sample t-test *P<0.05, **P<0.01, ***P<0.001

**MIN6 = Mouse insulinoma cell line 6**

**eGFP = enhanced green fluorescent protein**

**GCG = Preproglucagon gene**
Confirmed dose-dependent islet-restricted expression of GLP-1RA via AAV-mediated delivery in the BKS db/db mouse

**Single IP injection**
5 weeks old

**Week 10 post-injection**
GLP-1RA transgene expression in mouse islets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pancreatic GLP-1RA protein expression</th>
<th>Whole pancreas GLP-1RA protein expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>% islet expression by IHC</td>
<td>ng/g</td>
</tr>
<tr>
<td>AAV-GLP-1RA Low</td>
<td><strong>4.5</strong> ± 0.5</td>
<td><strong>15.5</strong> ± 1.2</td>
</tr>
<tr>
<td>AAV-GLP-1RA High</td>
<td><strong>6.0</strong> ± 0.8</td>
<td><strong>20.0</strong> ± 2.0</td>
</tr>
</tbody>
</table>

Means ± SEM. One-Way ANOVA, post-hoc Tukey Test

**P<0.01, ****P<0.0001**

*IP = Intraperitoneal
AAV = Adeno-associated virus
IHC = Immunohistochemistry*
Dose-dependent reduced glycemia with elevated insulinemia with AAV-GLP-1RA gene delivery in the BKS db/db mouse

Single IP injection
5 weeks old

Weekly FBGs
4–6 hour fasted

Biweekly insulin
4–6 hour fasted

Means ± SEM. Two-way ANOVA, post-hoc Tukey Test
**P<0.01, ****P<0.0001

IP = Intraperitoneal
AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein
Improved glucose tolerance and 1st phase insulin secretion with AAV-GLP-1RA gene delivery in the BKS db/db mouse

Single IP injection
5 weeks old

Glucose tolerance test

Week 6 post-injection

Glucose-stimulated insulin secretion

- AAV-GLP-1RA Low
- AAV-GLP-1RA High
- Vehicle Control
- AAV-eGFP Control

Means ± SEM. One-Way ANOVA, post-hoc Tukey Test

**P<0.01, ****P<0.0001

IP = Intraperitoneal
AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein
GLP-1RA transgene expression and improved insulin secretion from primary BKS db/db islets ex vivo

- AAV-eGFP Control
- AAV-GLP-1RA

Day 4 eGFP expression

Total GLP-1RA content

Glucose-stimulated insulin secretion

AAV = Adeno-associated virus
eGFP = Enhanced green fluorescent protein
Glc = Glucose
IBMX = 3-isobutyl-1-methylxanthine

Means ± Std Dev. Unpaired t-test, *P<0.05
AAV-mediated delivery of GLP-1RA enhances insulin secretion in a GLP-1R dependent manner in the human beta-cell line EndoC-BH5

AAV = Adeno-associated virus
EX9 = Exendin-9, GLP-1R antagonist
Glc = Glucose

Means ± Std Dev. Two-Way ANOVA, post-hoc Tukey Test ****P<0.0001
Summary

1. Fractyl Health is developing an AAV-mediated gene therapy approach to locally deliver a GLP-1RA to the pancreas to improve beta-cell health and function for T2D.

2. We identified top plasmid constructs yielding functional GLP-1RA production via in vitro screening in a beta-cell line.

3. We tested the metabolic effect of an AAV-delivered GLP-1RA candidate in the db/db mouse model, demonstrating delayed disease progression, improved glycemia and glucose tolerance, and sustained insulin secretion.

4. AAV-GLP-1RA directly improves glucose-stimulated insulin secretion in primary db/db mouse islets and in a human beta-cell line.
Conclusion

• These studies provide proof of concept that a single dose islet-targeted gene therapy durably improves beta-cell function in a diabetic mouse model.

• Targeted gene therapy has the potential to improve glycemic control, modify or reverse disease progression, and reduce therapeutic burden in patients with T2D.

• This approach may offer a durable way to address patient need that is still unresolved with current therapeutics.