



# 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)

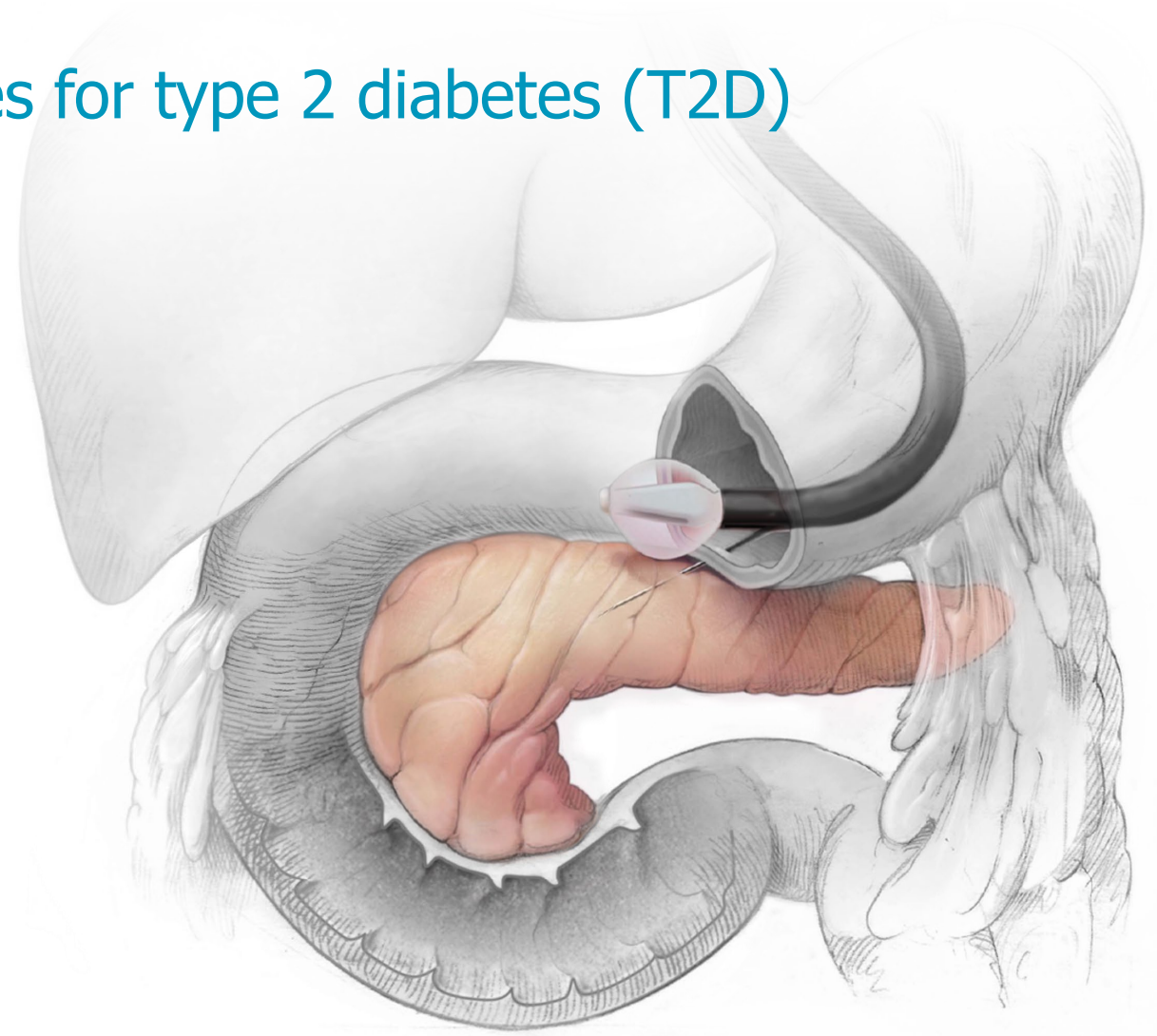
**Complementary development programs targeting key organs in T2D**

**Revita®** (targeting the duodenum)

Endoscopic procedure using hydrothermal ablation in the duodenum

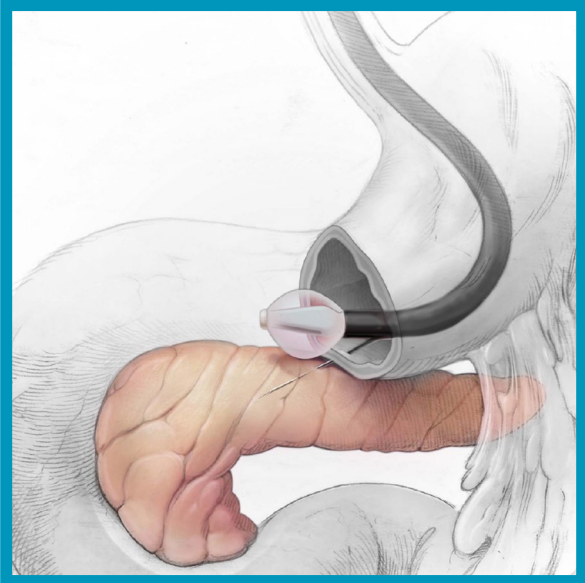
**Rejuva®** (targeting the pancreas)

Adeno-associated virus (AAV)–based pancreatic gene therapy platform

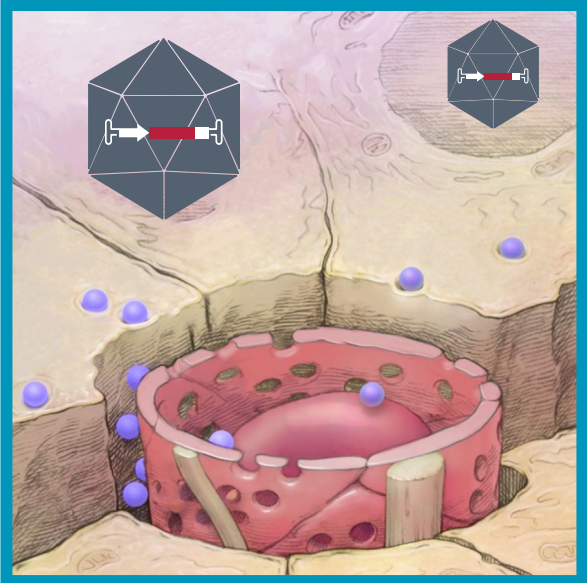


# Rejuva Directly Targets the Pancreas with Gene Therapy

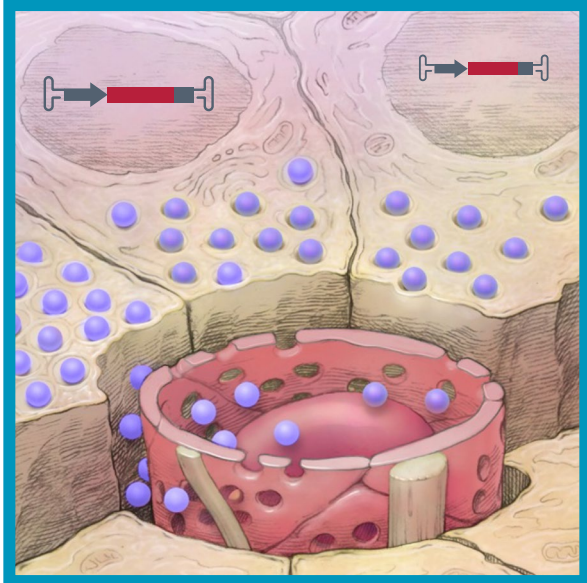
## Key therapeutic elements



**Endoscopic device  
and procedure**



**AAV Gene Therapy  
Delivery Vehicle**



**Tissue-Restricted  
Transgene Expression**



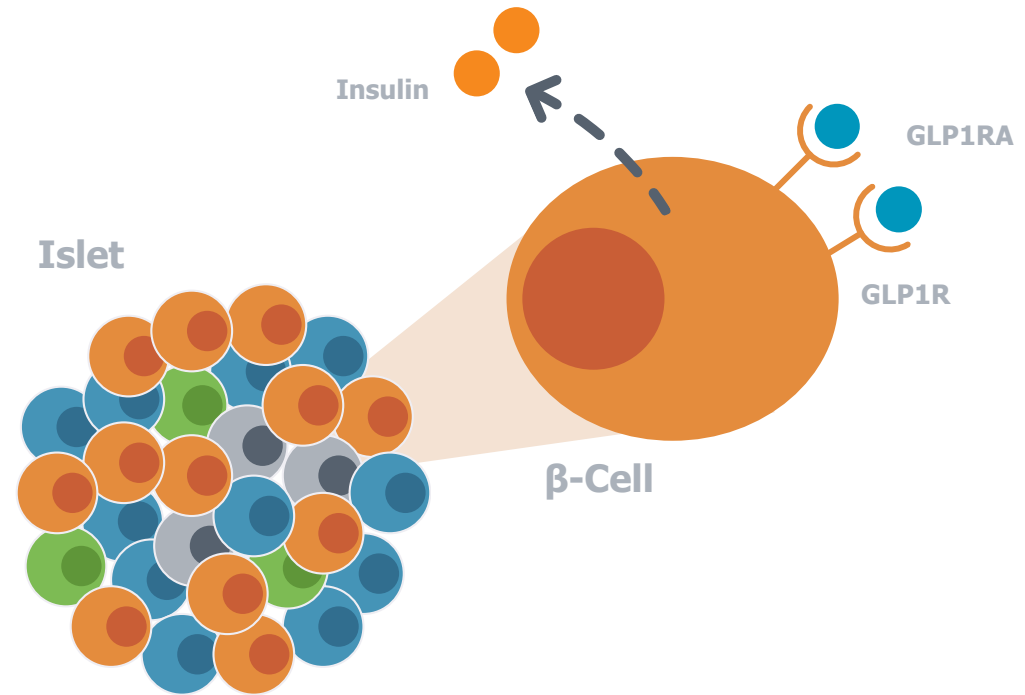
# Rationale for AAV-GLP1RA Gene Therapy to Improve Islet Function

## Declining islet health is an early driver of T2D progression

T2D islet:

- β-cell loss of insulin
- α-cell excess glucagon

GLP1RAs reverse both and improve islet cell health<sup>1</sup>



# Rationale for AAV-GLP1RA Gene Therapy to Improve Islet Function

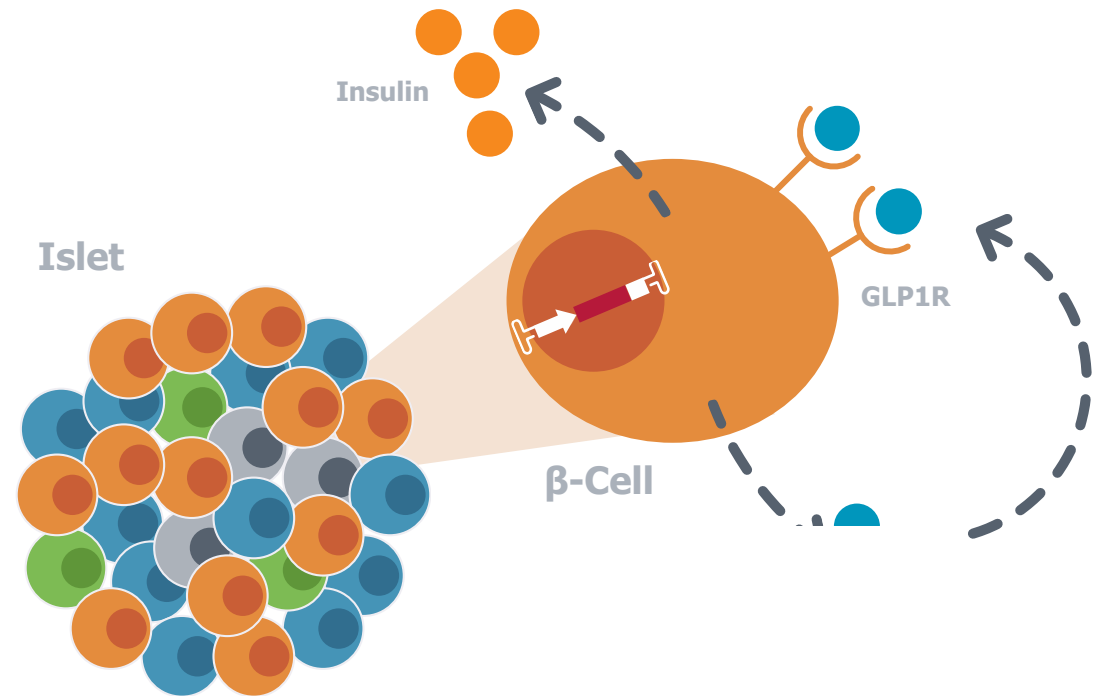
## Declining islet health is an early driver of T2D progression

T2D islet:

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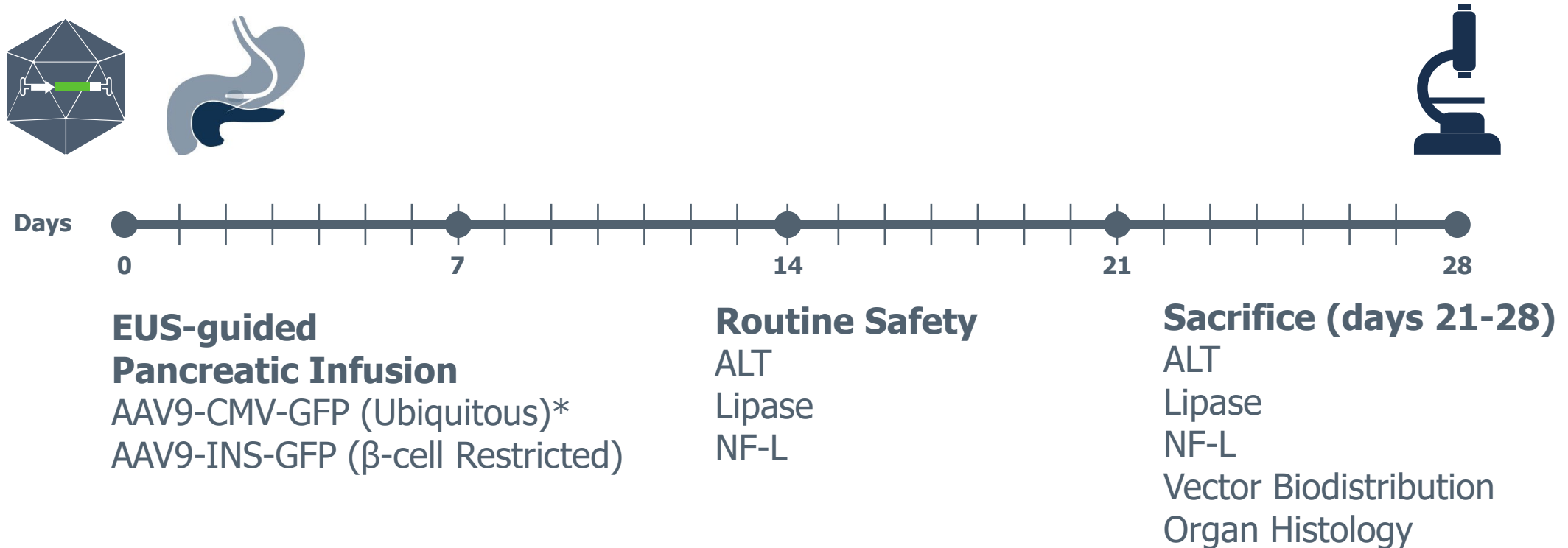
GLP1RAs reverse both and improve islet cell health<sup>1</sup>

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



# EUS-Guided AAV ROA Feasibility in Yucatan Pig

Porcine model approximates human GI tract and pancreas anatomy



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



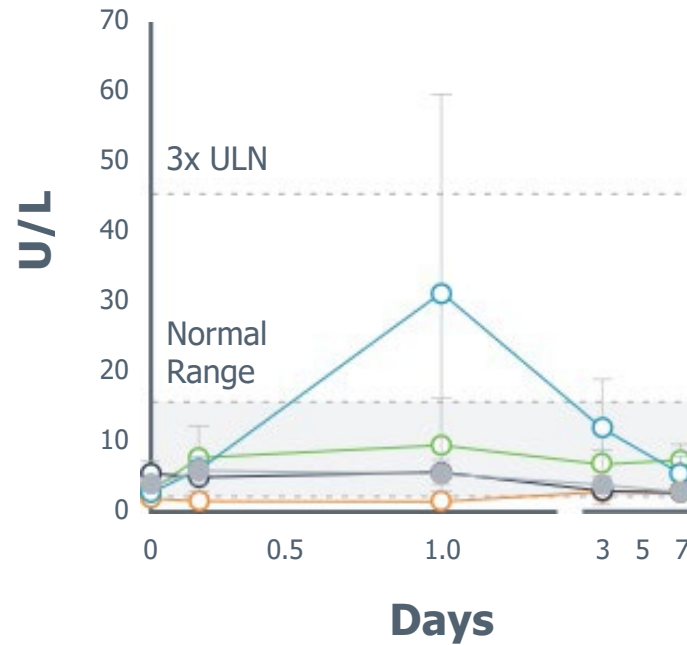
# EUS-Guided AAV ROA in Yucatan Pig

Serum ALT and lipase remained in the normal range across most timepoints

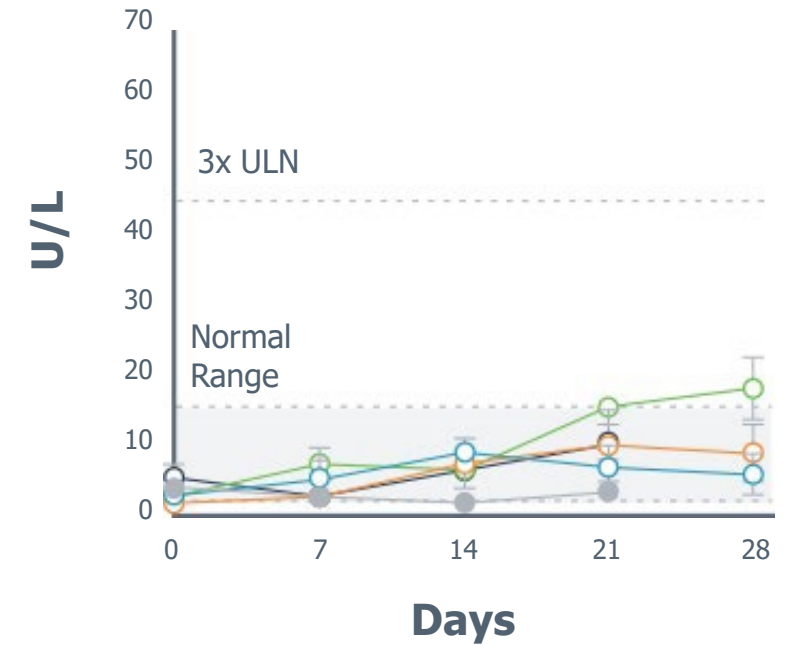
**A) 28-day ALT**



**B) 7-day Lipase**



**C) 28-day Lipase**



● Vehicle    ○ 5.0e12    ○ 1.0e13    ○ 5.0e13    ○ 1.5e14

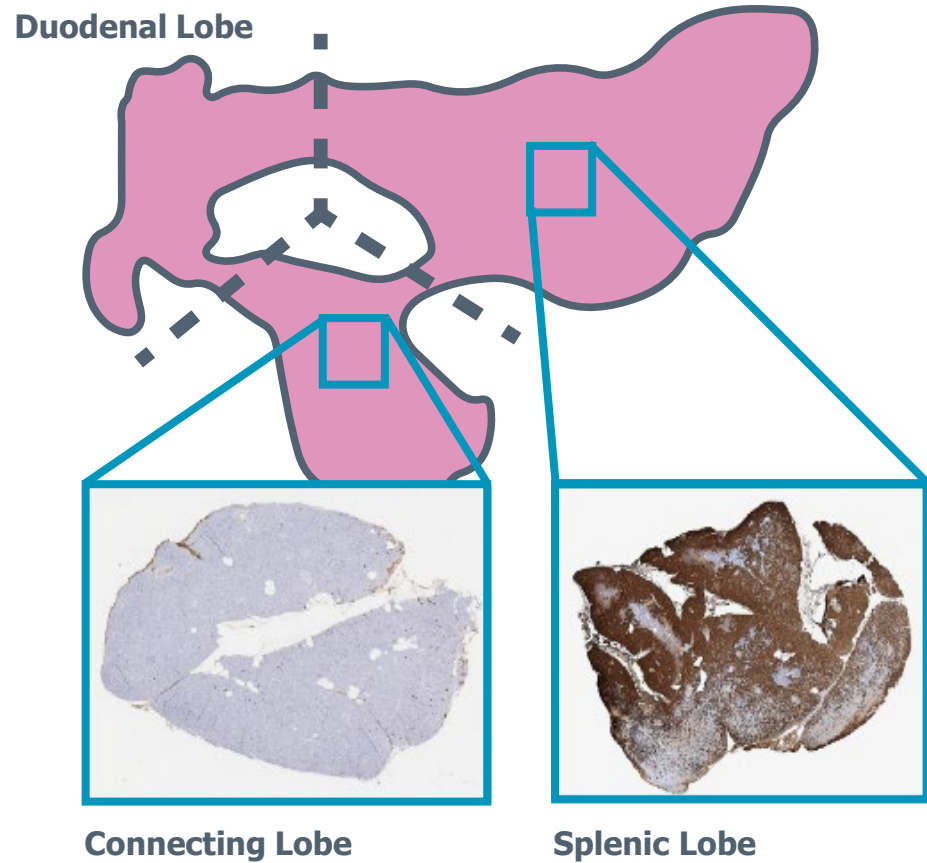




# EUS-Guided AAV ROA in Yucatan Pig

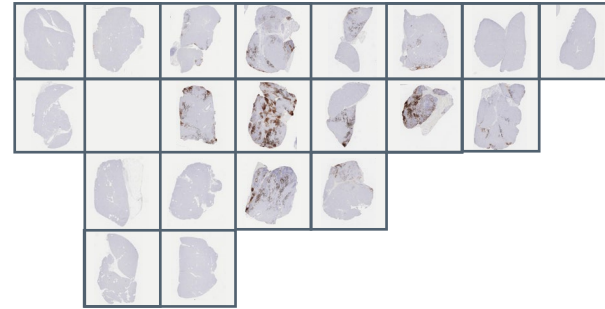
## Dose-dependent expression of GFP throughout targeted splenic lobe

### A) Extensive GFP in Splenic Lobe

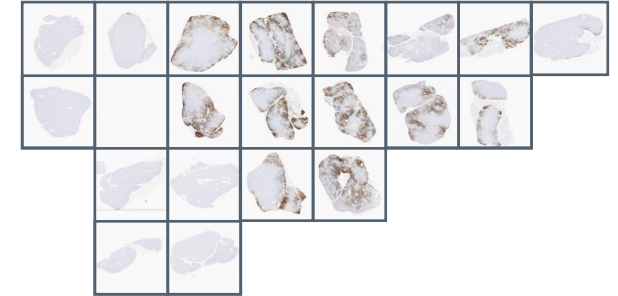


### B) VG Dose-Dependent GFP in Pancreas

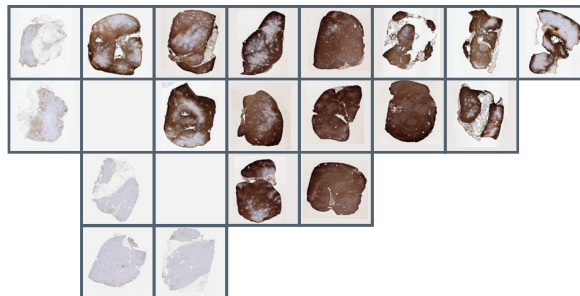
5e12



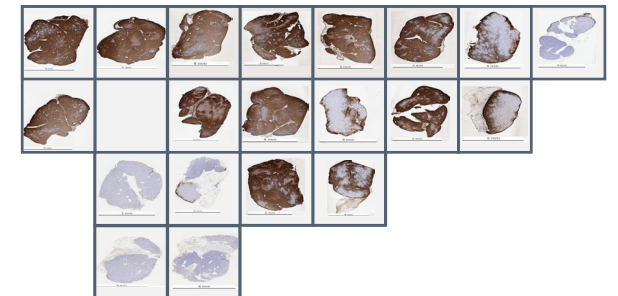
1e13



5e13



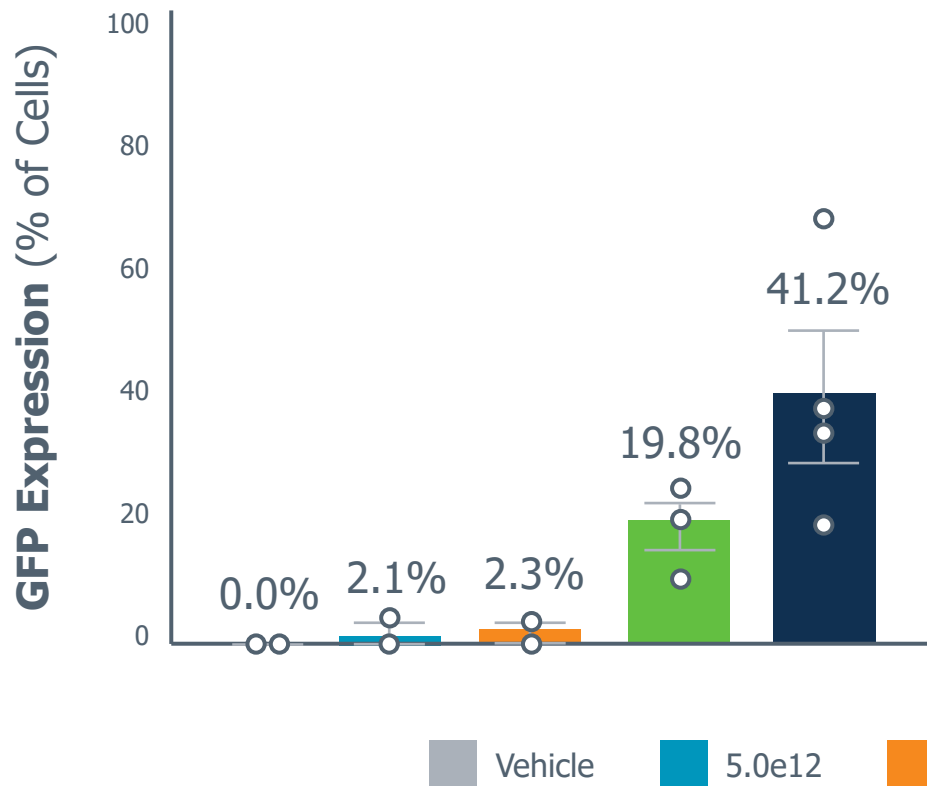
1.5e14



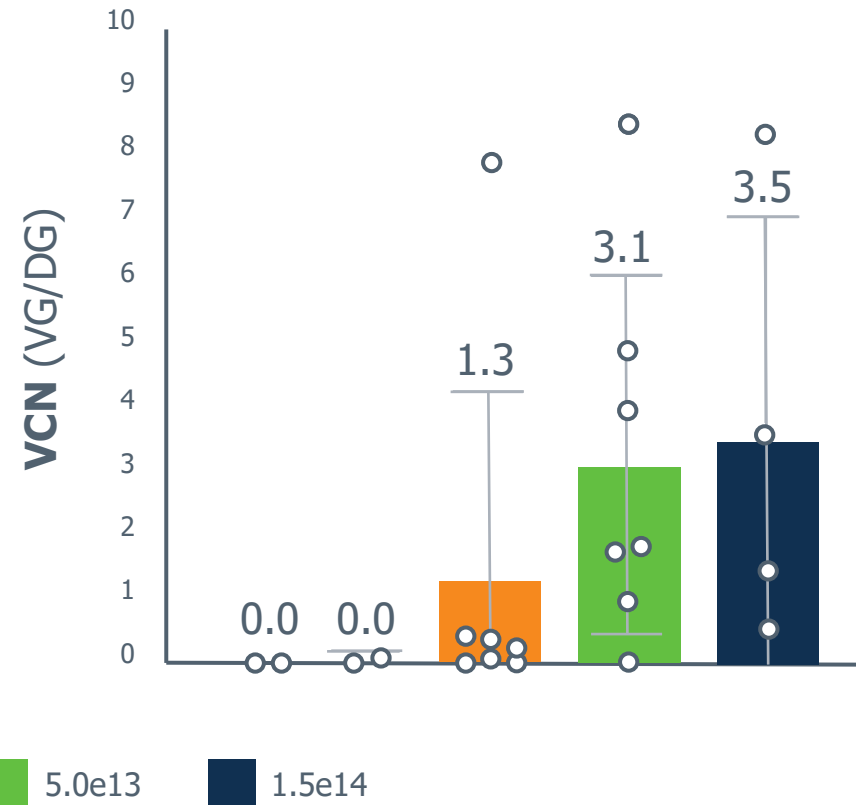
# EUS-Guided AAV ROA in Yucatan Pig

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

### A) Endocrine GFP Expression



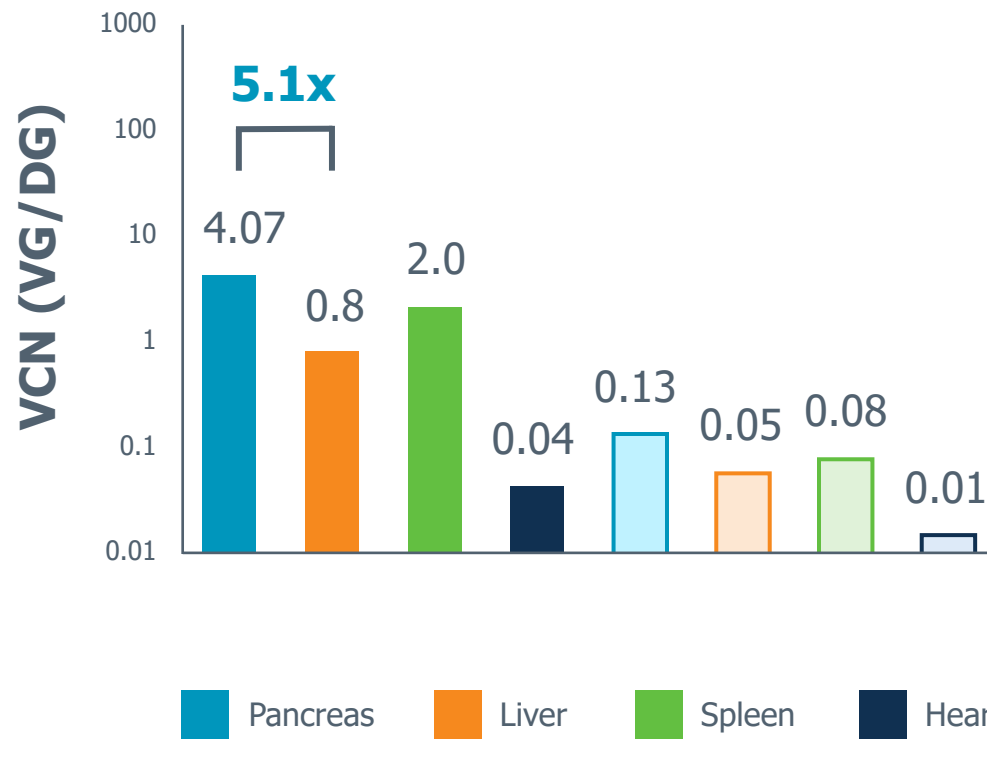
### B) On-target VCN



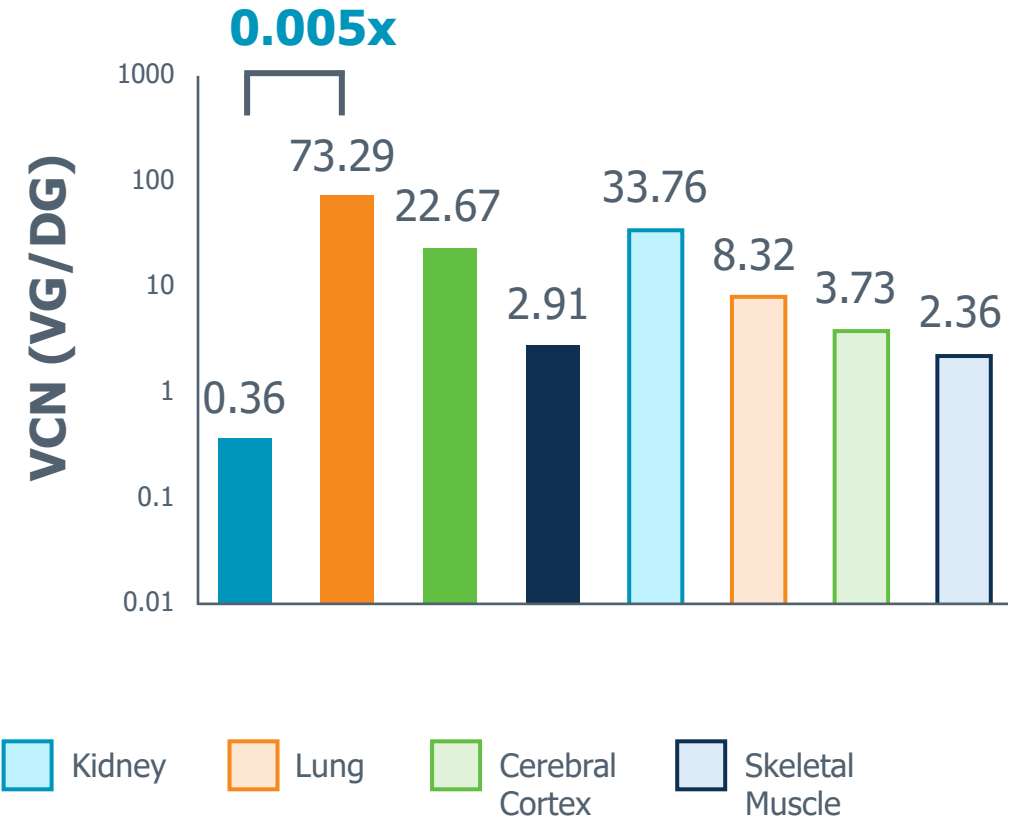
# EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

## Local vs. systemic delivery AAV biodistribution comparison

**A) EUS** (4.2e12 VG/kg)

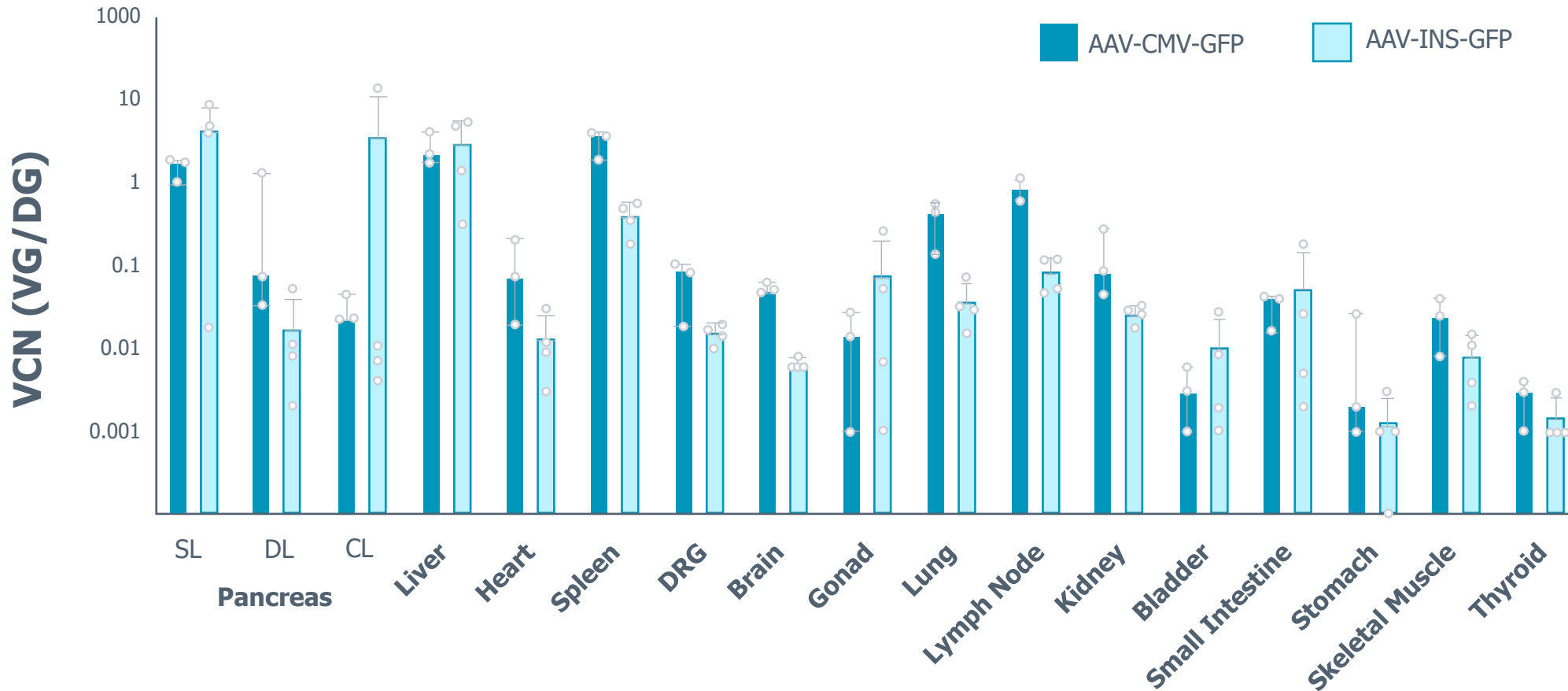


**B) I.V.** (8.3e12 VG/kg, Li et al. 2022<sup>1</sup>)



# EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

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



# EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

## Preliminary AAV-GFP toxicology findings segregated by promotor

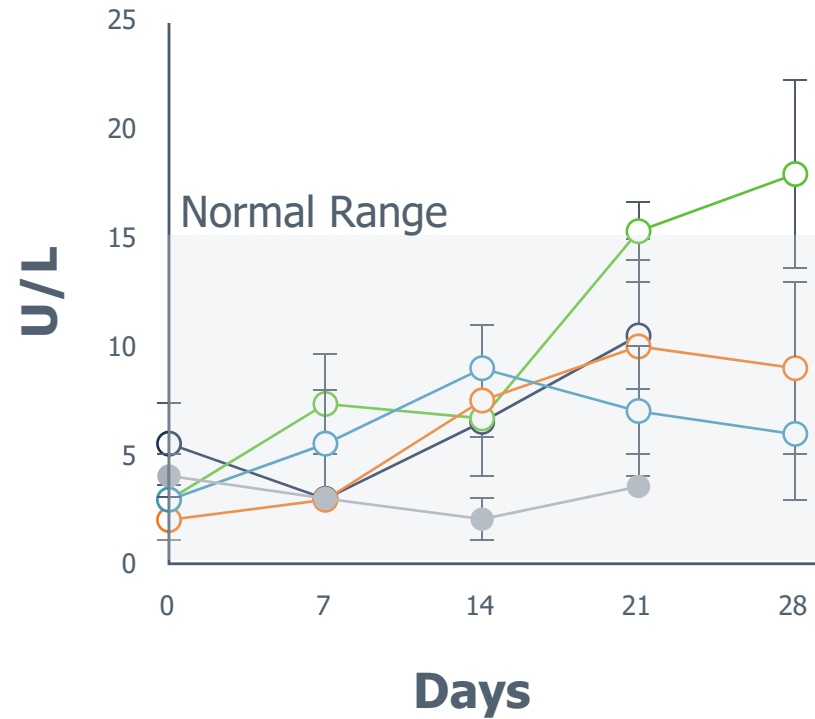
Assessment	AAV-INS-GFP ( $\beta$ -cell Restricted, N=7)	AAV-CMV-GFP (Ubiquitous, N=11)
Clinical findings	None	(n=1) ataxia, mild hindlimb paresis, forelimb knuckling (day 24, 5e13 VG)
Clinical blood chemistries	No relevant changes	(n=1) transient elevation in lipase (< 3x ULN) on day 1; normalized by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



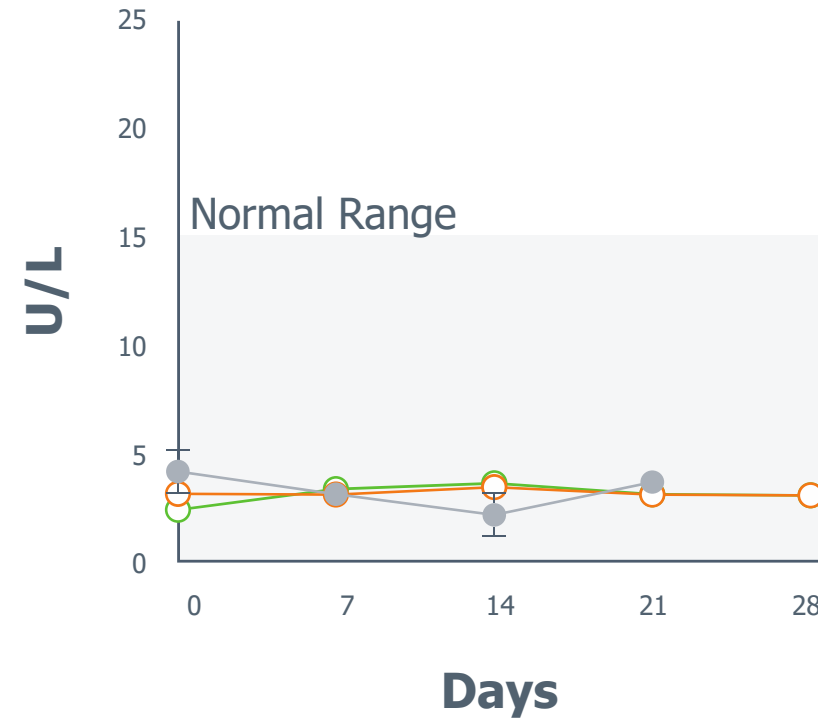
# EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

## Lipase elevation associated with GFP & abolished by promotor restriction

**A) AAV-CMV-GFP 28 Day Lipase**

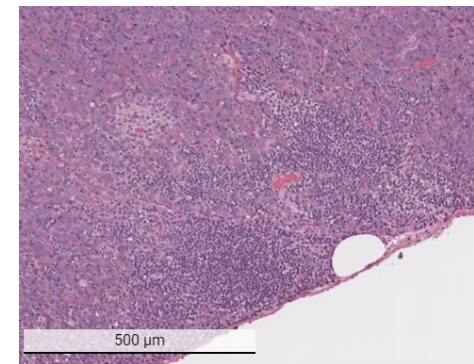
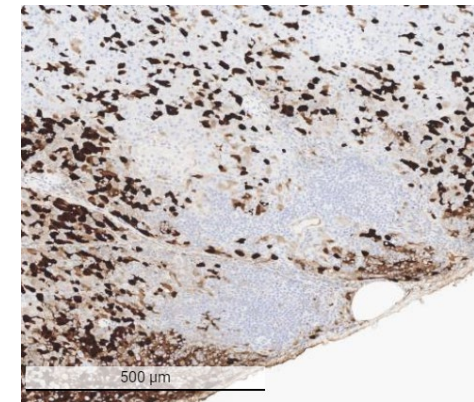


**B) AAV-INS-GFP 28 Day Lipase**



**Elevated lipase likely related to pancreatic GFP expression**

AAV-CMV-GFP



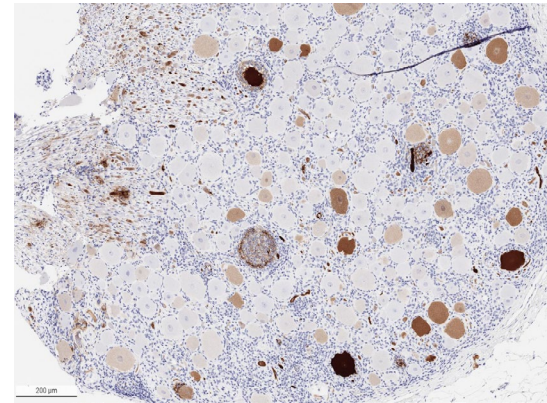
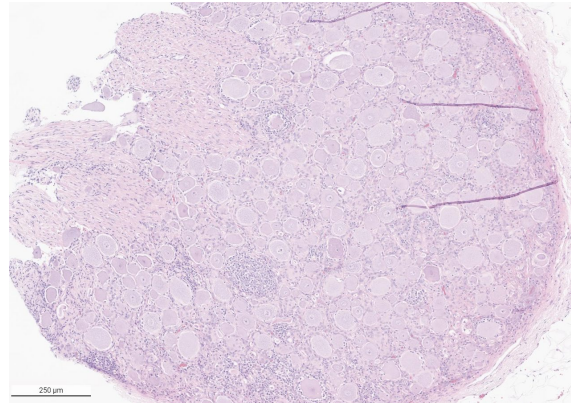
● Vehicle    ○ 5.0e12    ○ 1.0e13    ○ 5.0e13    ○ 1.5e14



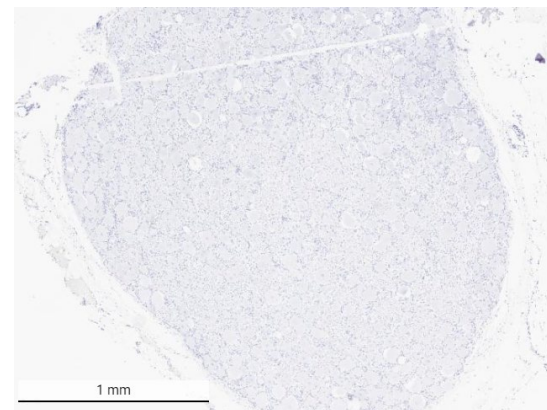
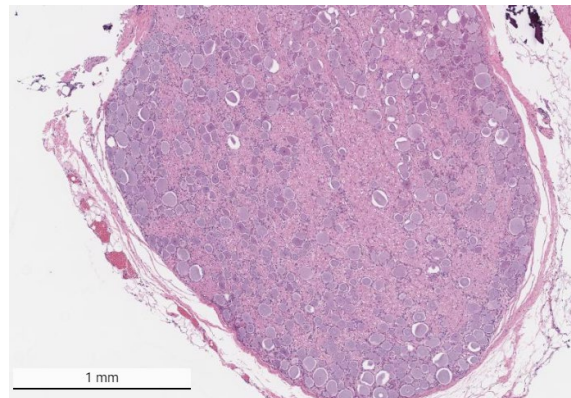
# EUS-Guided AAV Route of Administration Feasibility in Yucatan Pig

AAV-GFP DRG toxicity is mitigated by promotor restriction

## AAV9-CMV-GFP Inflammation, GFP expression



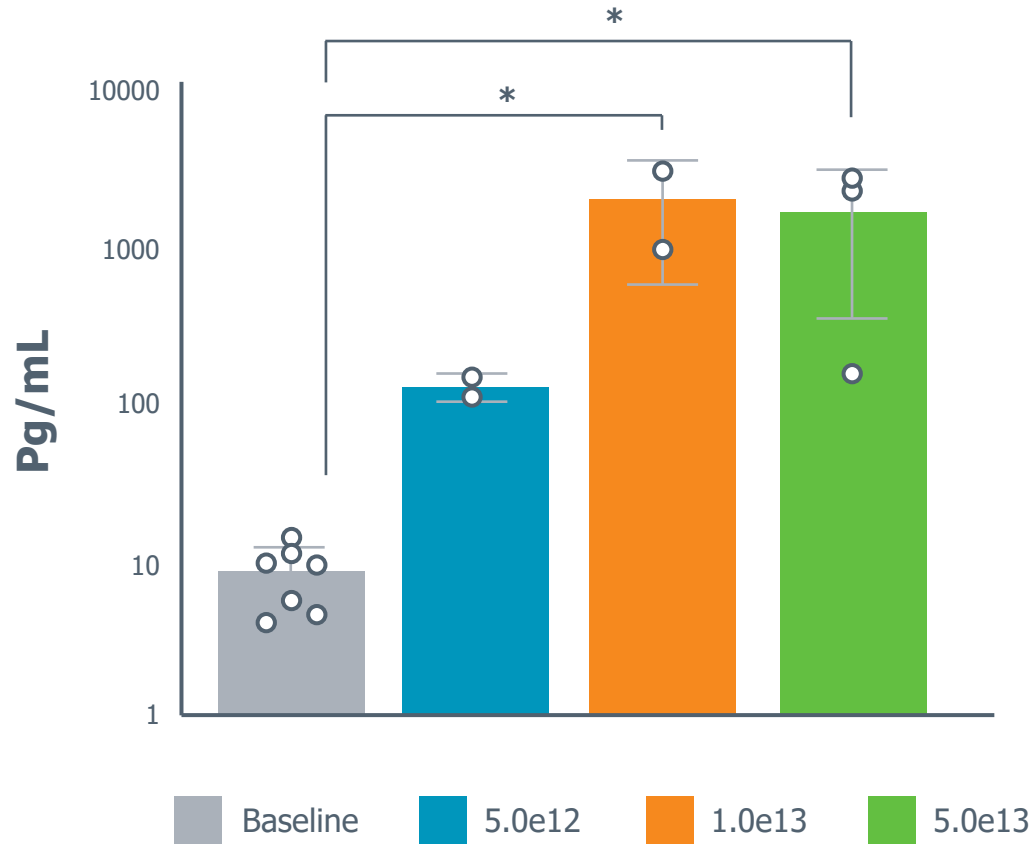
## AAV9-INS-GFP No inflammation, no GFP expression



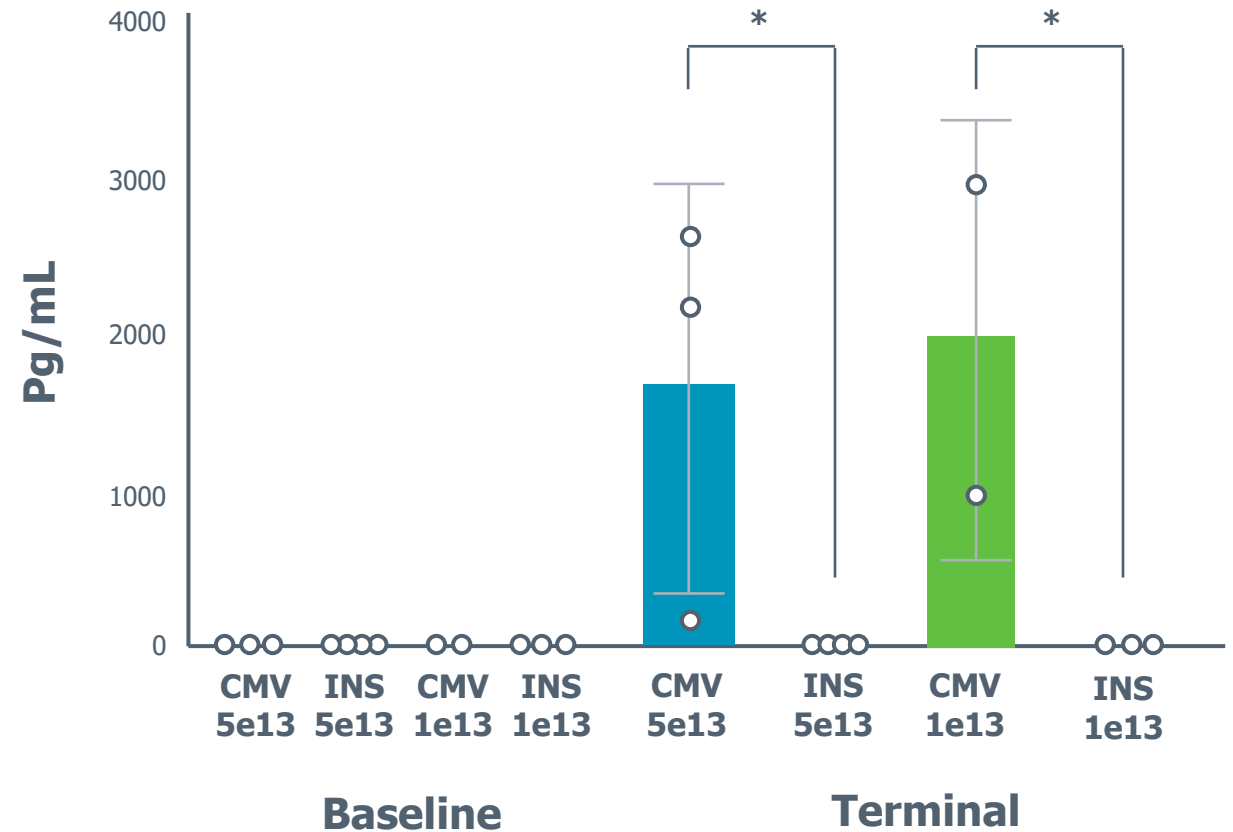
# NF-L Appears to be a Good Biomarker for DRG Toxicity

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**





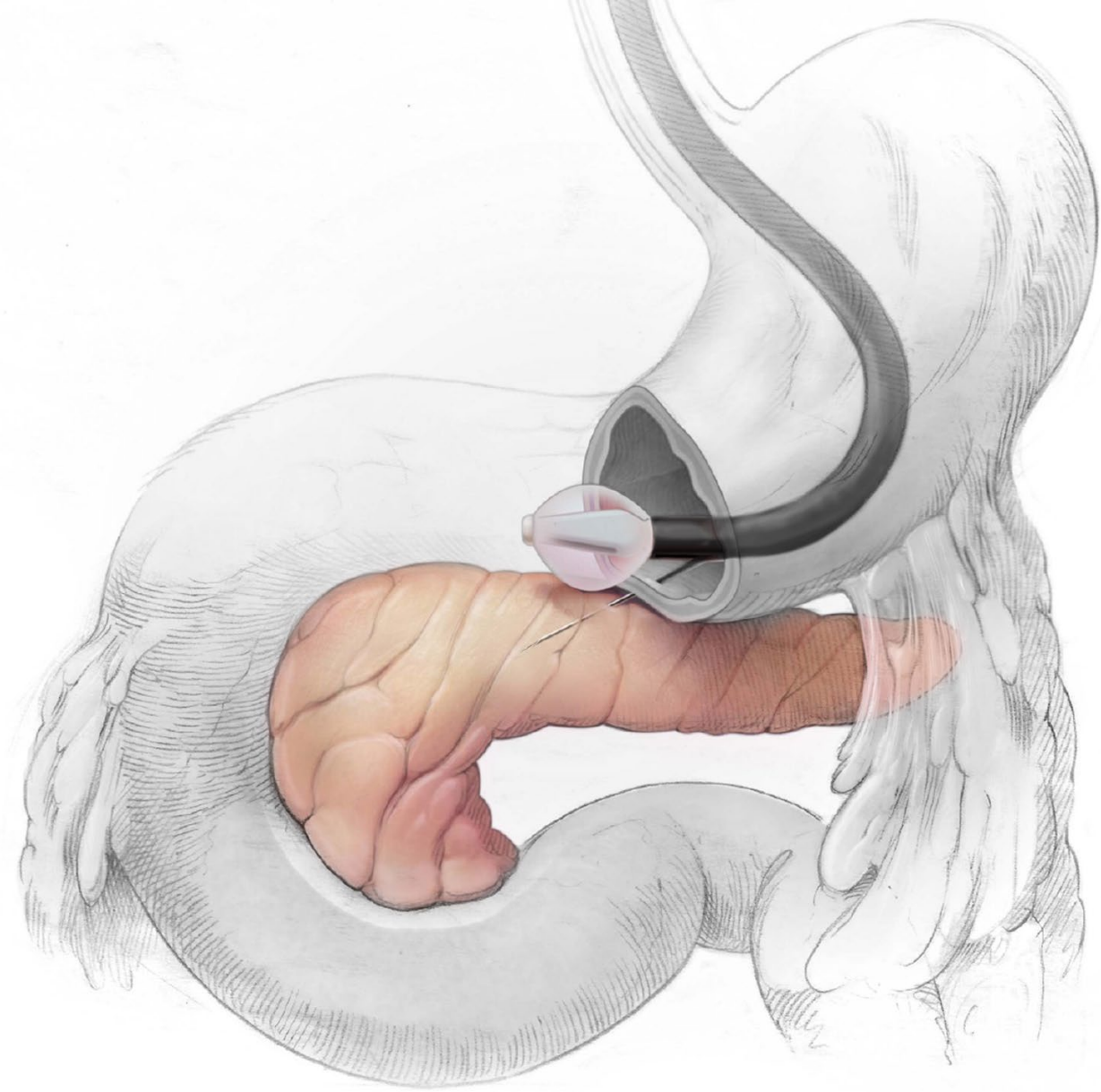
# Conclusions

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

## 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

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