A Highly-Evolved Novel AAV Gene Therapy Directly Addresses Fabry Disease Pathology In Vivo by Cell Autonomous Expression in the Heart and Other Target Organs

Abstract 140

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Disclosures

- Full-time employee at 4D Molecular Therapeutics, Inc.
- Co-founder and owner of shares in 4D Molecular Therapeutics, Inc.
- Inventor on patents and/or pending patent applications related to AAV capsid variants and AAV gene delivery.

Fabry Disease: An LSD with High Unmet Medical Need

DISEASE BIOLOGY

Gene: *GLA* gene loss of function mutations lead to an alpha-galactosidase A (AGA) enzyme deficiency.

Biologic Consequence: Insufficient AGA activity results in accumulation of globotriaosylceramide (Gb3) in endothelial, parenchymal and vascular smooth muscle tissues.

Affected Organs: Heart, Kidney, Blood Vessels, Nervous System

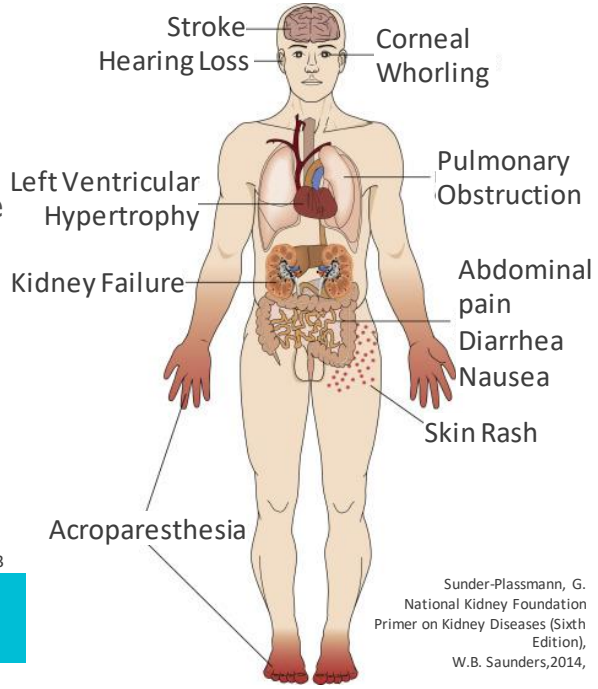
- Cardiovascular disease represents the primary causes of death (75%)

Baig et al Europace. 2018 Sep 1;20(FI2):f153

STANDARD OF CARE

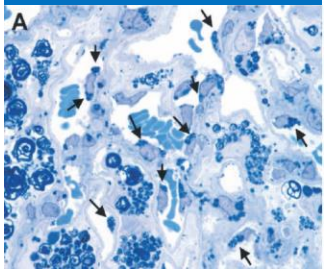
- Existing therapies have accelerated approval only with no therapy obtaining full approval status
- Fabrazyme failed Ph4 composite-endpoint study
- Current therapy fails to clear Gb3 from organ parenchymal cells or vascular smooth muscle cells
- ERT requires biweekly infusion
- Chaperone therapy benefits only 30-40% of patients

A MULTISYSTEM DISEASE

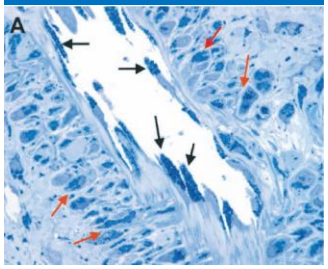


Sunder-Plassmann, G. National Kidney Foundation Primer on Kidney Diseases (Sixth Edition), W.B. Saunders,2014,

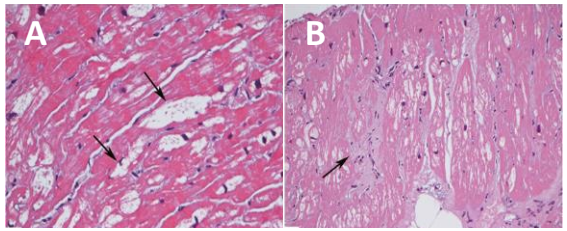
GB3 INCLUSIONS: ENDOTHELIUM



GB3: VASCULAR SMOOTH MUSCLE

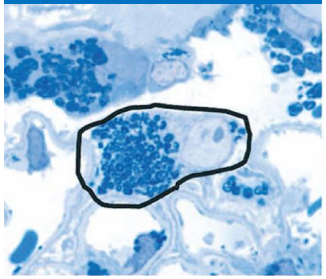


CARDIOMYOCYTE HYPERTROPHY & VACUOLES (A), FIBROSIS (B)



Hsu et al. Orphanet Journal of Rare Diseases 2014, 9:96

PARENCHYMAL GB3 INCLUSIONS

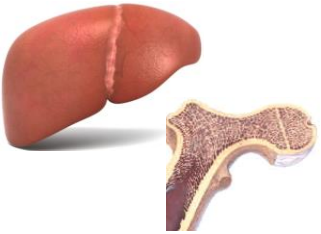


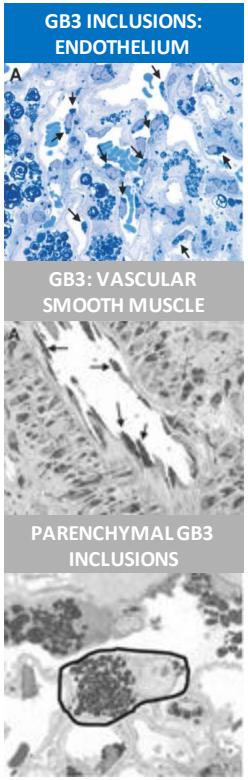
Thurberg et al, Kidney Int. 2002 Dec;62(6):1933

4D-310: Next Generation Gene Therapy Address Target Organs in Fabry Disease

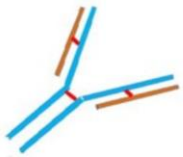
Endothelial Compartment Correction

Strategy	Target	Limitations
Enzyme Replacement	GB3 INCLUSIONS: ENDOTHELIUM	**Persistent Clinical Need**
Bioreactor Approaches	GB3: VASCULAR SMOOTH MUSCLE	**Anti-AGA Antibodies: Presence are Associated with Poor Prognosis**
	PARENCHYMAL GB3 INCLUSIONS	

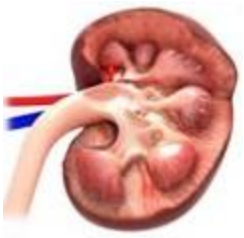


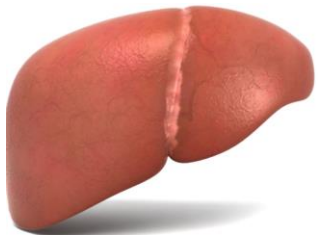


- Cardiomyopathy
- Renal Dysfunction
- Cerebrovascular Disease
- Peripheral Neuropathy
- Gastrointestinal Complaints



Whole Organ Correction





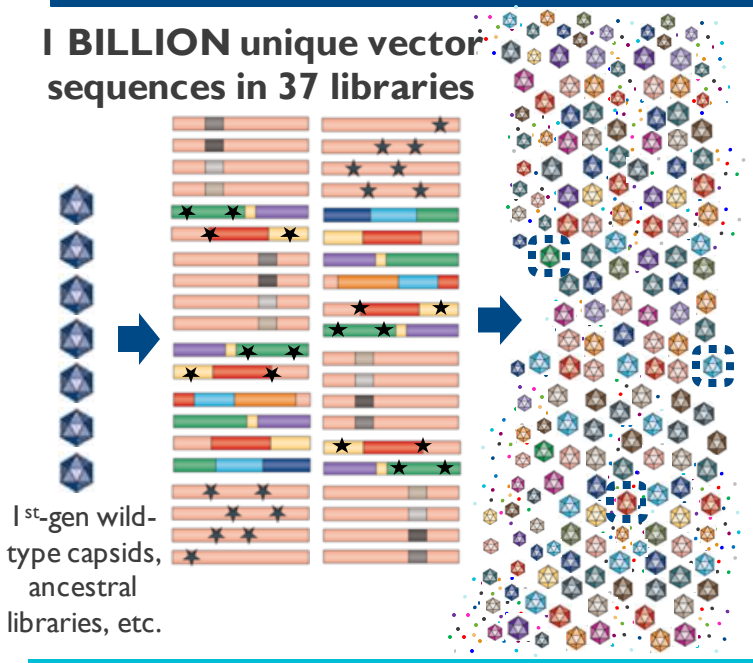
- Circumvents Challenges of Poor Uptake of Circulating AGA
- Potentially Avoids Circulating Anti-AGA Antibodies
- May More Fully Address Fabry Disease Pathophysiology in Classic/Non Classic as well as Female Fabry Disease Patients

Thurberg et al, Kidney Int. 2002 Dec;62(6):1933

Disease-First Approach to Vector Discovery: Therapeutic Vector Evolution

GENERATE MASSIVE AAV VECTOR DIVERSITY (NUMEROUS METHODS)

1 BILLION unique vector sequences in 37 libraries



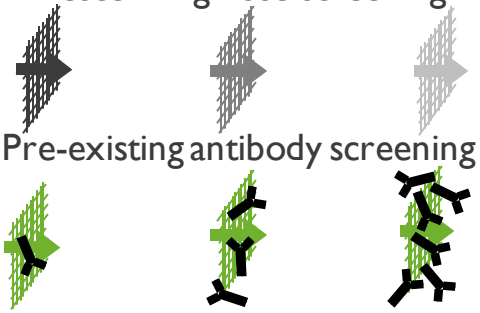
1st-gen wild-type capsids, ancestral libraries, etc.

DISCOVERY: TARGET VECTOR PROFILE

Tissue target, route, dose

Primate screening

Descending dose screening



Pre-existing antibody screening

LEAD CANDIDATES

15 discovery programs completed

IP filed: >300 capsid variants

CHARACTERIZATION & DEVELOPMENT

Lead Vector

In Vivo (NHP)

Human Cell & Disease Models

Clinical Candidate

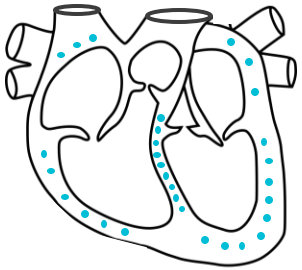
IND Filing

See poster #140

4D-C102: Targets Cardiomyocytes in NHPs

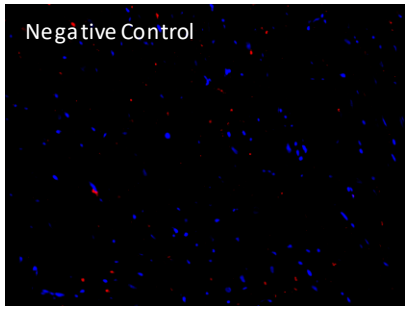
IV 1x1013 vg/kg after 8 weeks

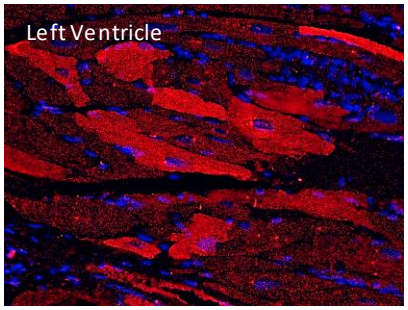
100% (+) GENOME DELIVERY
97% (+) PROTEIN EXPRESSION (N=30)



Primate Heart (N=30)
Left atrium (3)
Right atrium (3)
Left ventricle (9)
Right ventricle (6)
Septum (9)

ROBUST CARDIOMYOCYTE PROTEIN EXPRESSION WITH 4D-C102



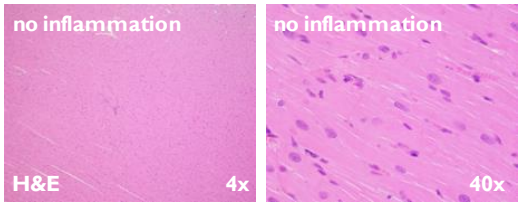


IMPROVED DELIVERY VS AAV8 & AAV9

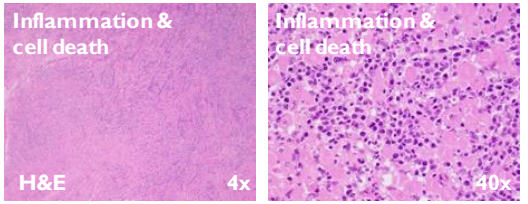
Genomes per ug DNA in heart: 4D-C102 vs AAV8	**13X**
Heart:Liver genome ratio 4D-C102 vs AAV8	**17X**
Heart:Liver genome ratio 4D-C102 vs AAV9	**9X**
4D-C102 **Heart:Liver protein ratio**	**420X**

No Inflammation Seen with 4D-C102

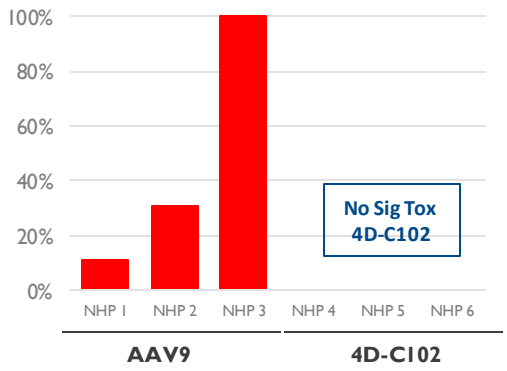
4D-C102



AAV9



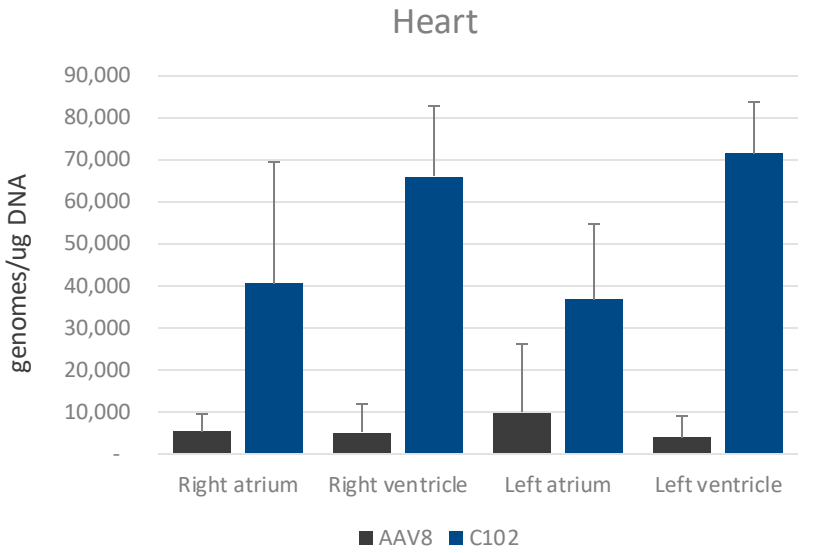
Identical transgene payloads, manufacturing, dose, route

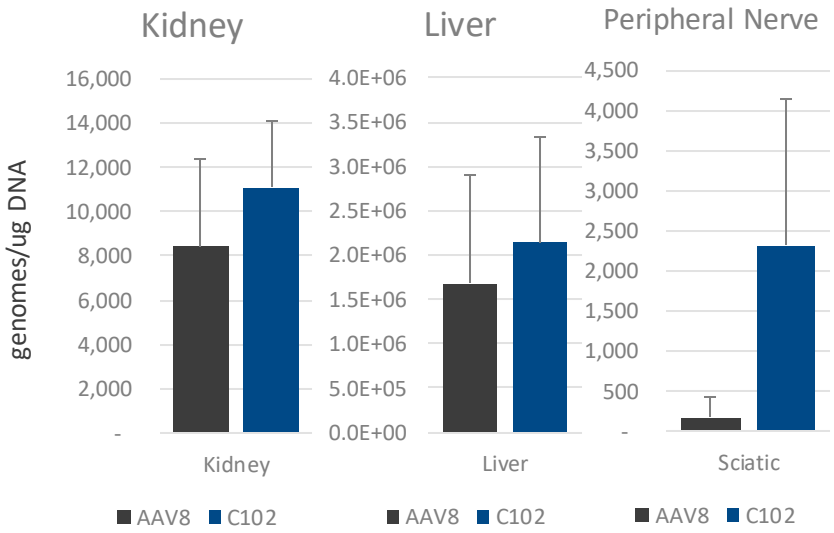


% Tissue Sections with Inflammation Score >1 (4-Point Scale)

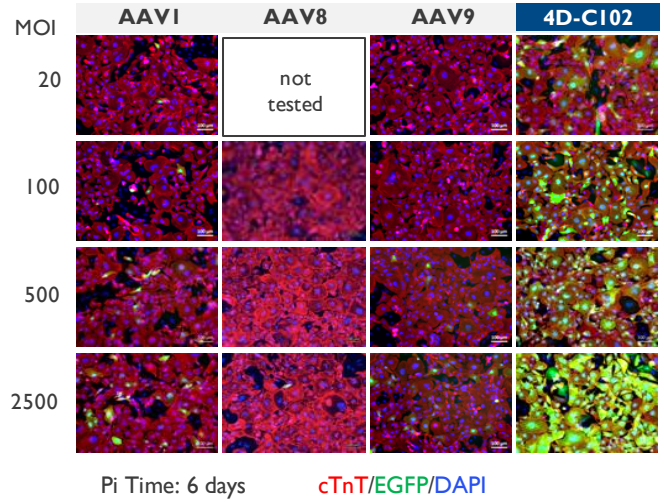
See poster #140

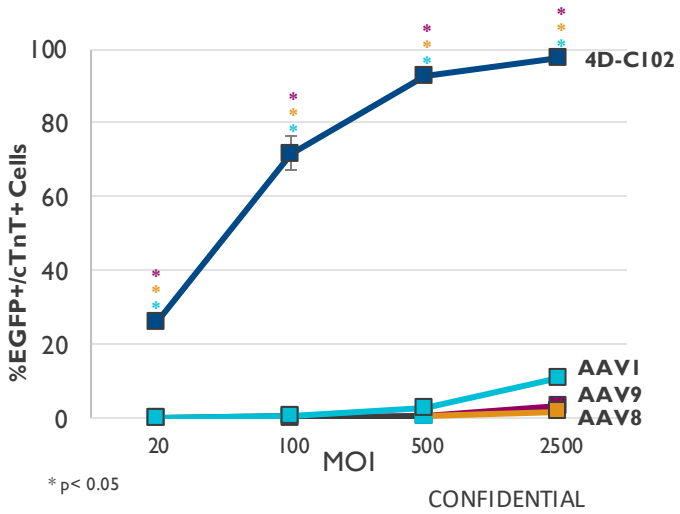
4D-C102: Targets Key Fabry Tissues





4D-C102: SIG. IMPROVED HUMAN CARDIOMYOCYTE TRANSDUCTION

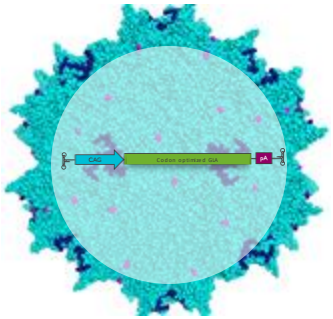




4D-310: Target Tissue Directed Gene Therapy for Fabry Disease

Capsid

4D-C102: Robust delivery to and transduction of heart, kidney, and liver

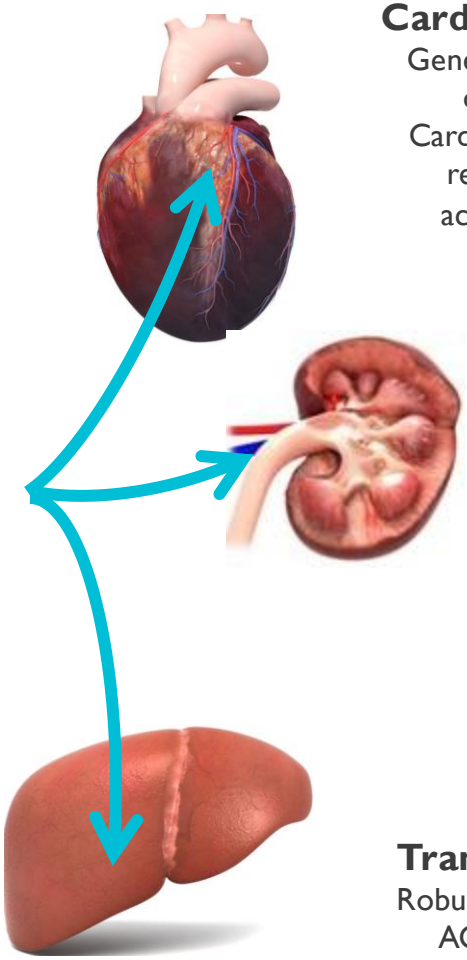


Payload

Ubiquitous promoter (CAG):
Allows expression from multiple cell types

GLA transgene:
Codon optimization

pA



Cardiomyopathy
Gene replacement directly in Cardiomyocyte to reverse Gb3 accumulation

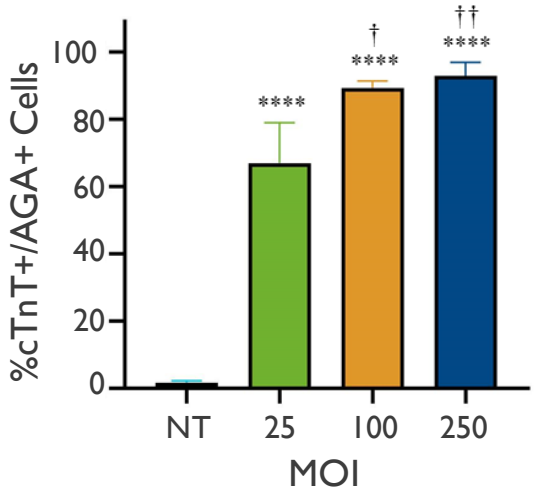
Nephropathy
Gene replacement in kidney parenchymal cells

Liver Transduction
Robust circulating AGA treats endothelial cells systemically

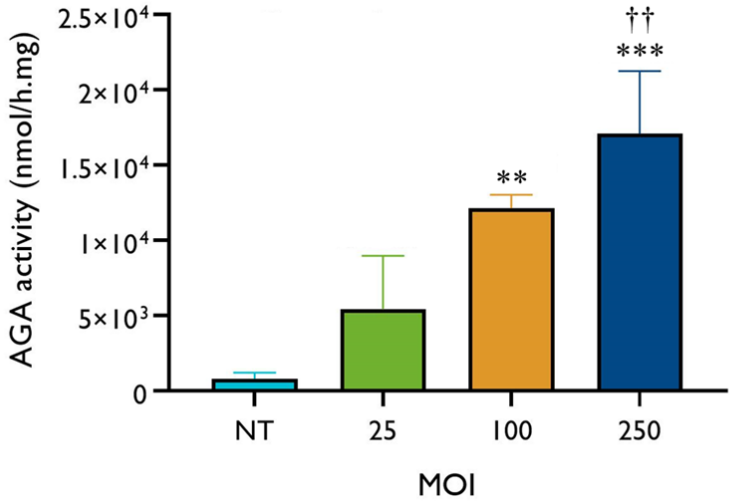
See poster #594

4D-310 Increases AGA Expression & Activity

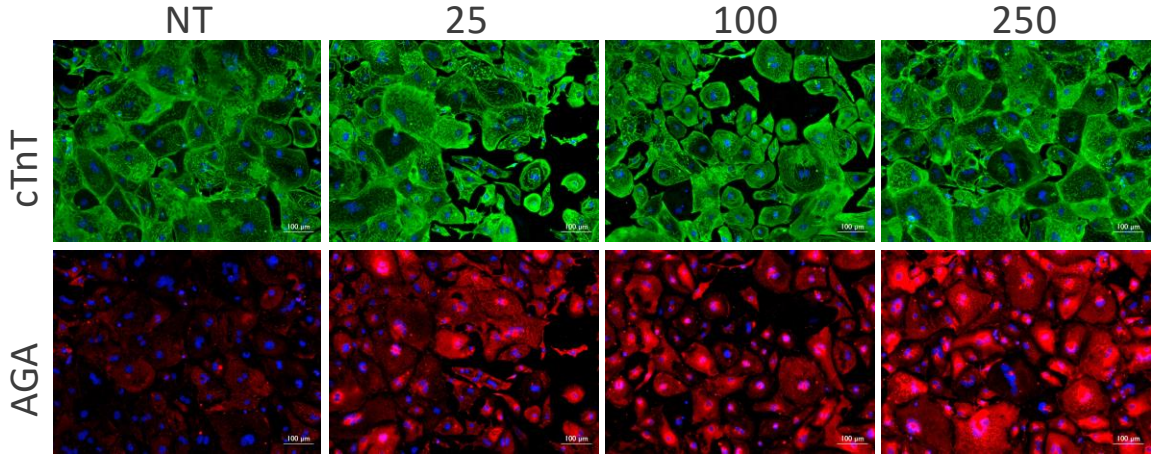
HUMAN FABRY PATIENT IPSC-DERIVED VENTRICULAR CARDIOMYOCYTES



****p<0.0001 compared to NT, ††p<0.001, †p<0.01 compared to MOI 25

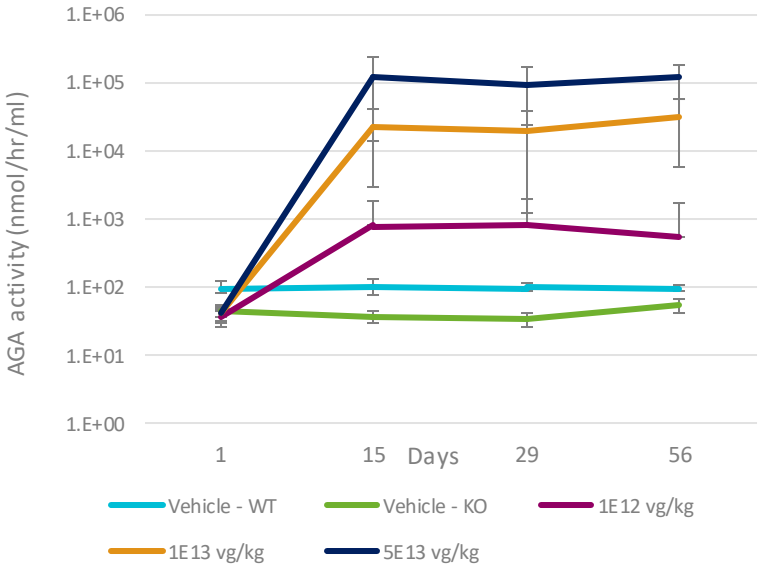


***p<0.004, **p=0.005 compared to NT, †† p<0.004; compared to MOI 25.

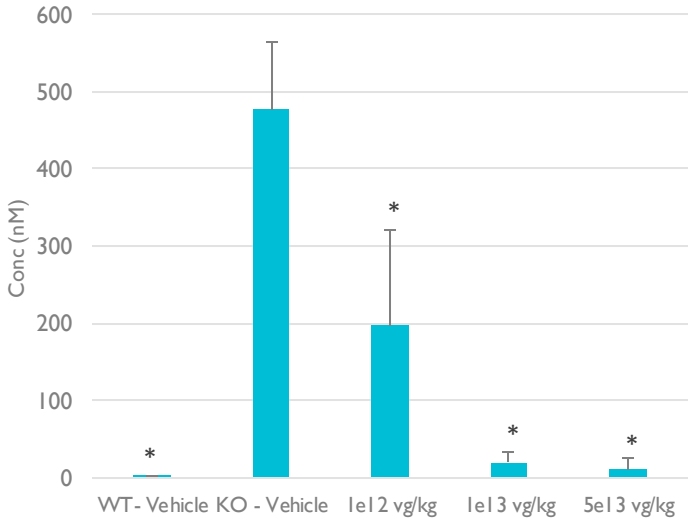


4D-310: High Plasma AGA Activity

PLASMA AGA ACTIVITY IN 4D-310-TREATED FABRY MICE



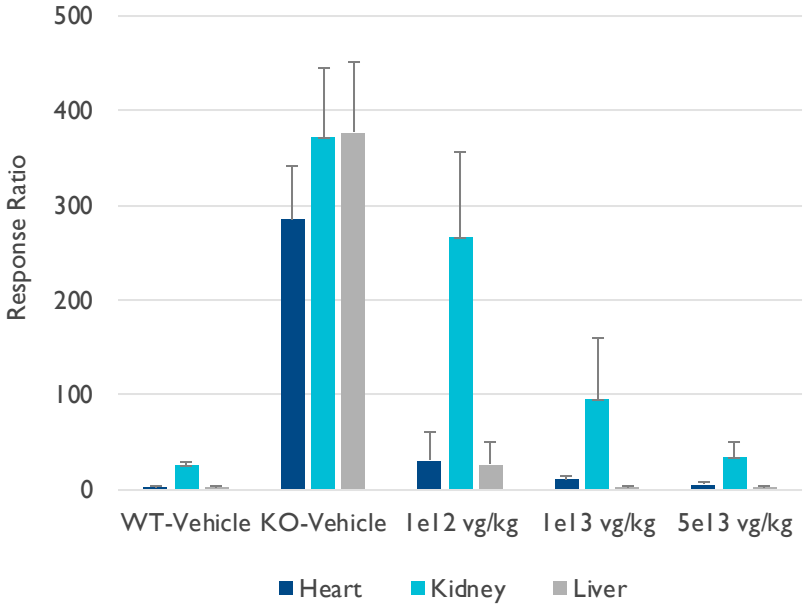
PLASMA LYSO-Gb3 IN 4D-310-TREATED FABRY MICE (WK 8)



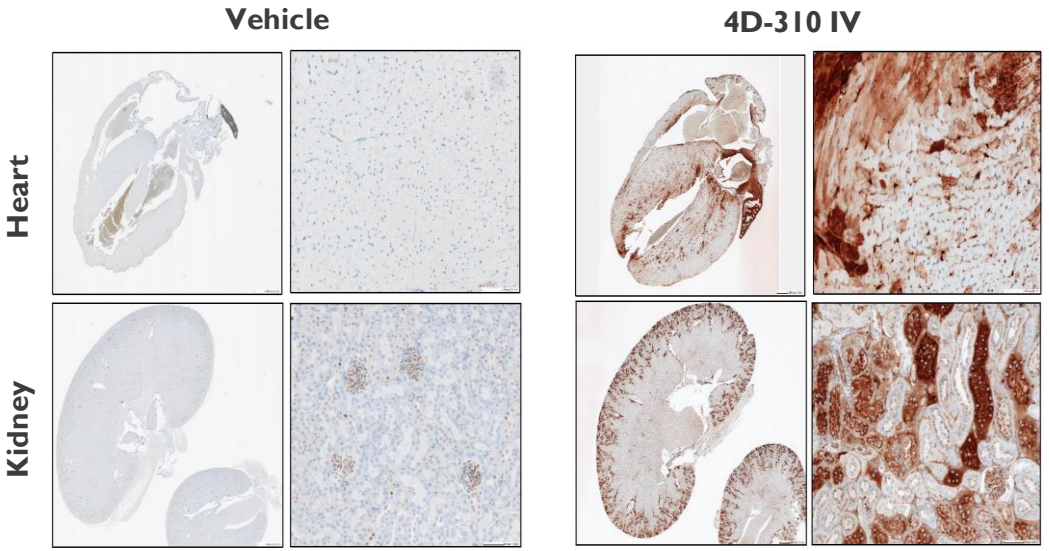
* P<0.01 compared with vehicle-treated Fabry Mice

4D-310: High AGA Activity in Target Organs

TISSUE Gb3 IN 4D-310-TREATED FABRY MICE (WK 8)



WIDESPREAD AGA EXPRESSION IN FABRY MOUSE HEART & KIDNEYS WITH IV 4D-310



4D-310: Mouse GLP Tox Study – No Significant Tox

NO SIG TOXICITY WITH 4D-310 IN NORMAL MICE IN VIVO (GLP STUDY)

GLP Tox Study Results

- No sig toxicities observed
- 3 month in-life
- 132 mice treated w/ 4D-310 (up to 1.5×10^{14} vg/kg)

4D-310: Next Generation Gene Therapy For Fabry Disease

Target Tissue Directed Gene Therapy Approach

- 4D-310 represents a novel approach to the treatment of Lysosomal Storage Disease
- Therapeutic Vector Evolution was used to generate a tissue targeted capsid 4D-C102 that demonstrates:
  - tropism to key target tissues in Fabry (including heart, kidney and liver)
  - minimal/no inflammation
  - highly efficient transduction at low doses
- 4D-310 (derived from C102) drives high AGA activity in the heart and kidney as well as in the systemic circulation
- 4D-310 allows for whole organ correction in addition to treatment of the endothelial compartment
- Cell autonomous AGA expression is expected to circumvent reduced activity due to Anti-AGA antibody binding
- No evidence of toxicity in preclinical studies: 5 Mouse studies, including GLP Tox n=132
- 4DMT is poised to start a clinical trial for Fabry disease with 4D-310 in 2020

Acknowledgments

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- 4DMT HCDM
- 4DMT CMC
- 4DMT Project Management
- Christiane Auray-Blais, Université de Sherbrooke