Centyrin-targeted siRNA conjugates demonstrate potential new therapeutic approach for reduction of skeletal muscle glycogen in Pompe disease

May 2022

Aro BIOTHERAPEUTICS

ARO DRUG DISCOVERY PLATFORM

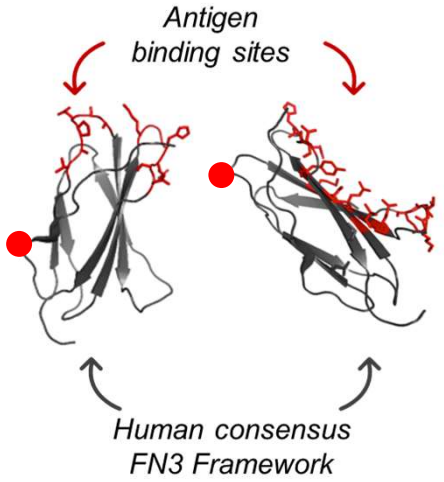
OVERVIEW OF POMPE DISEASE

LEAD SELECTION & CHARACTERIZATION

SUMMARY OF PRECLINICAL DATA

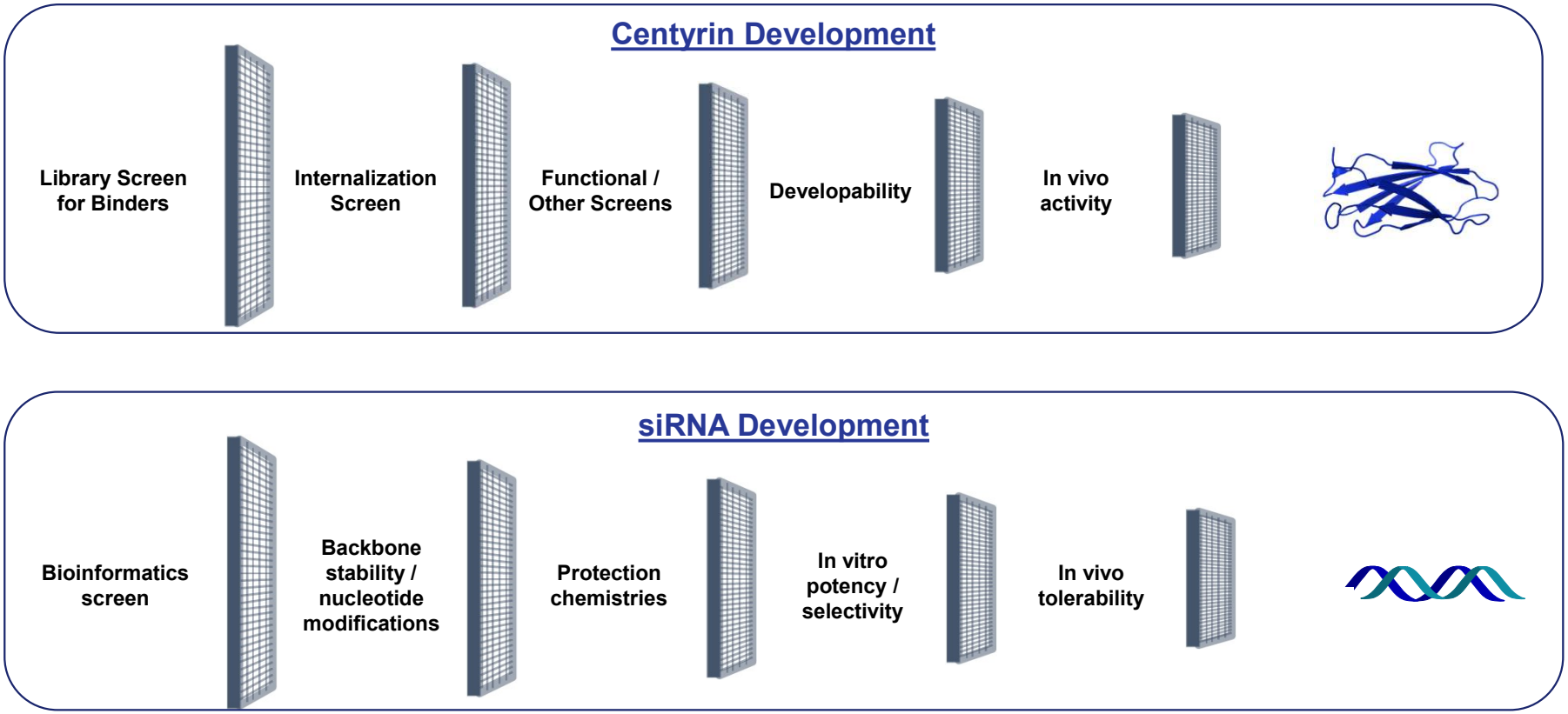
Centyrins are small proteins customized for tissue specific delivery of conjugated drug payloads

- Antigen specific proteins selected for high affinity receptor binding and internalization
- Can be formatted as mono or bi/tri specific binders
- Exceptional stability and solubility
- Low immunogenicity risk; no T cell epitopes
- ~1/15 size of standard monoclonal antibodies
- Readily expressed in E. Coli
- Site specific covalent conjugation to drug payloads
- Extensive patent portfolio; strong IP position

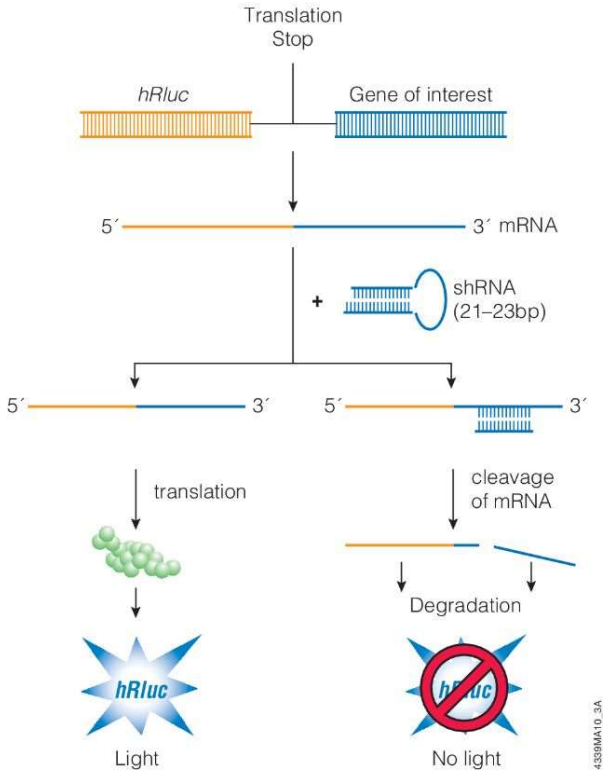


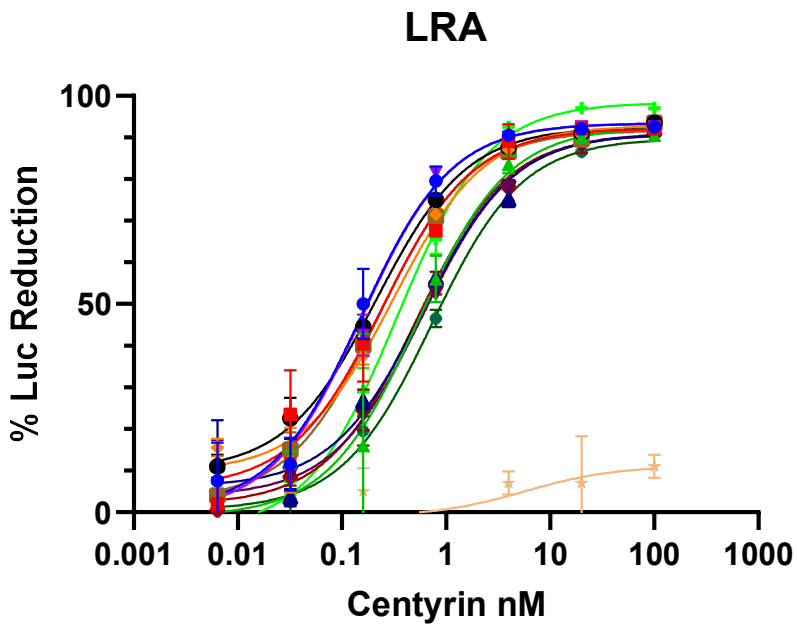
● = Drug Conjugate Site

Parallel discovery efforts enable rapid and modular development of therapeutic candidates



Centyrin – siRNA conjugates with sub-nM potency identified in Luciferase Reporter Assay

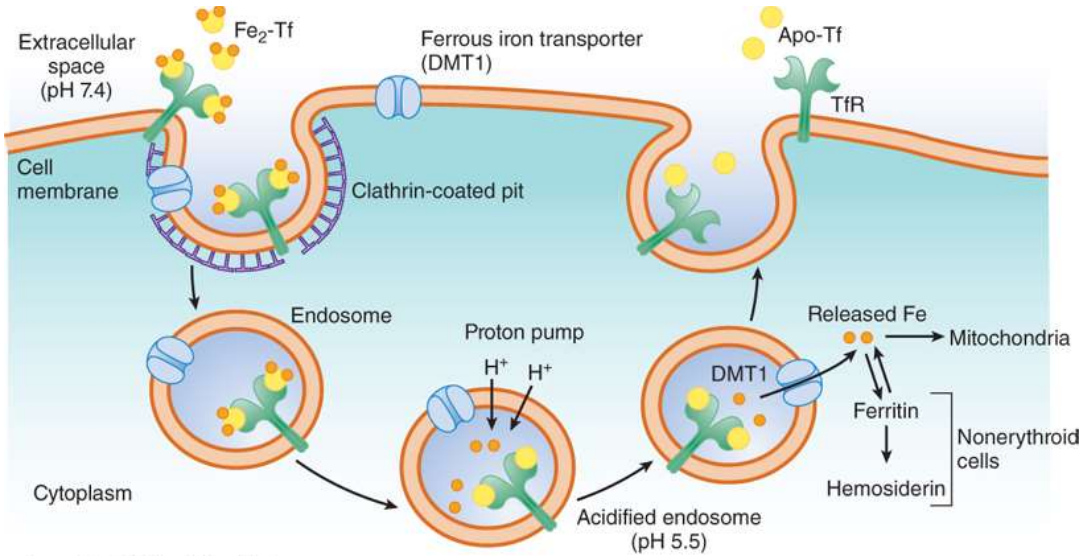




Luciferase Reporter Assay to screen for potent Centyrin-siRNA conjugates in gene knockdown

Aro is developing an industry-leading position in targeting CD71

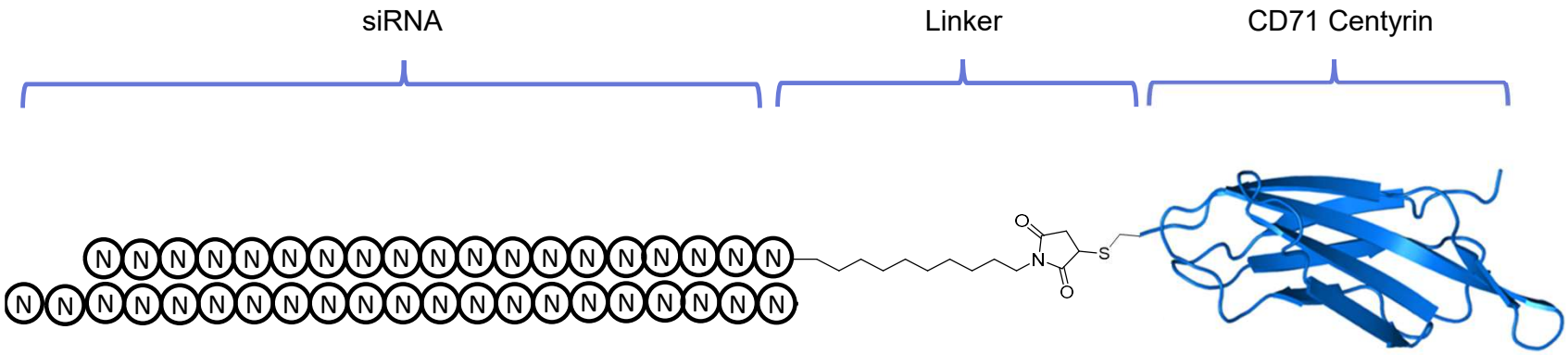
Customized CD71 Centyrins for different tissues to address a broad set of diseases



Source: Jon C. Aster, H. Franklin Bunn:
Pathophysiology of Blood Disorders, Second Edition
www.hemonc.mhmedical.com

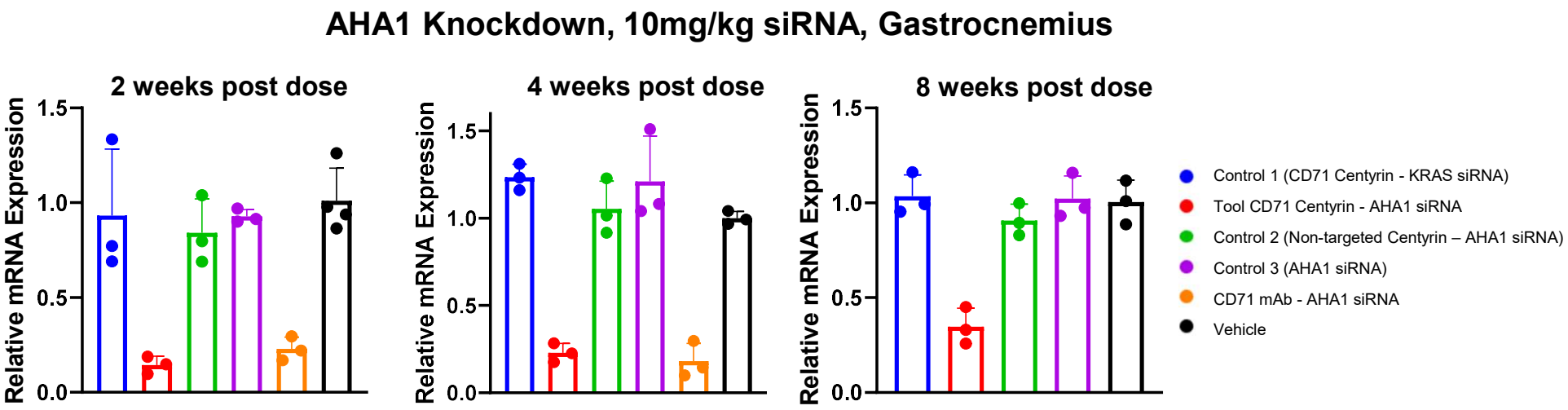
- Essential and ubiquitously expressed receptor responsible for iron transport into cells
- Efficient internalization on muscle
- Non- competitive with respect to transferrin
- We have generated a large diversity of CD71 Centyrins to enable efficient and customized targeting of various CD71+ cell types

Aro's Centyrin-siRNA conjugate employs clinically proven design principles



- Aro's siRNA-linker employs proprietary and clinically tested designs
- Centyrin-siRNA conjugates uses site-specific cysteine conjugation
- Centyrin conjugation onto siRNA does not interfere with loading of the antisense to Ago2
  - Sense-strand 3'- and 5'-linking provide similar activity
- For Centyrin-siRNA conjugates, cleavable linkers have *not* provided clear potency/efficacy advantages *in vivo* or *in vitro*, preclinically

CD71 Centyrin-siRNA conjugate drives sustained gene knockdown at fraction of mAb conjugate dose in mice



POC study with AHA1 housekeeping gene
C57/B6 mice received single dose of conjugates

	Centyrin – siRNA conjugate	mAb – siRNA conjugate
AHA1 knockdown wk2	86%	77%
AHA1 knockdown wk4	77%	82%
AHA1 knockdown wk8	65%	N/A
siRNA dose (mg/kg)	10 mg/kg	10 mg/kg
Conjugate dose (mg/kg)	~18 mg/kg	~120 mg/kg

Centryin and ABX1100 have a low in vitro immune response index and Centyrin has high stability across a wide range of pHs

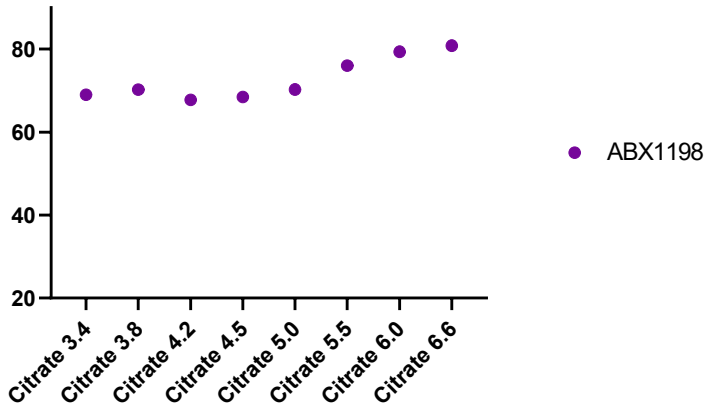
T Cell Activation Assay

Protein	Immune Response Index (RI)
PPD (Positive Control 1)	61.39
KLH (Positive Control 2)	32.05
CD71 Centyrin	**0.11**
ABX1100	**0.12**

Most approved biologic drugs have RI from 0-1

- T cell activation assay (ProImmune)
- 20 donor PBMC samples were HLA typed
- Allele distribution frequency of HLA class II resembled the global population
- T cell activation assessed after 7 days
- siRNA conjugation does not affect immunogenicity

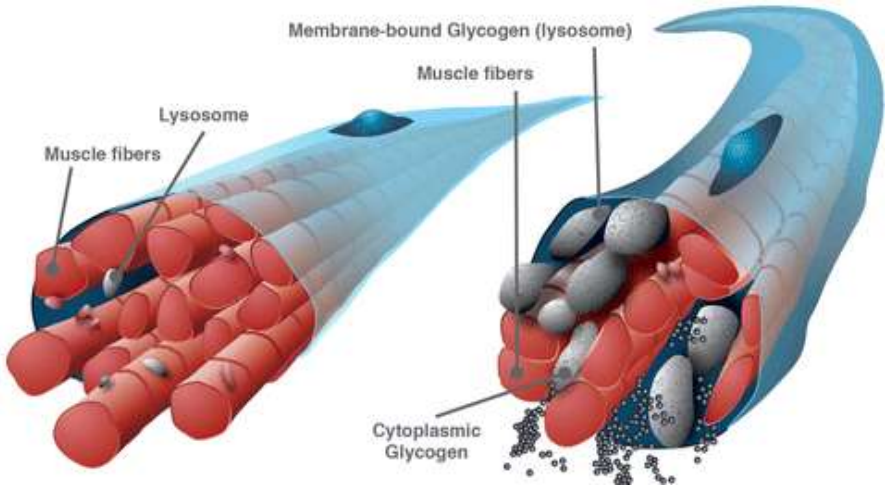
Centyrin Stability (Tm)



- Centyrins have high Tm's indicating extraordinary protein stability
- Stability is retained at low pH environments, such as the endosome
- Inverse relationship between stability and immunogenicity*

*Scheiblhofer S, et.al., Expert Rev Vaccines. 2017

Pompe disease is a rare lysosomal storage disorder caused by deficiency in glycogen metabolizing enzyme acid alpha-glucosidase (GAA)



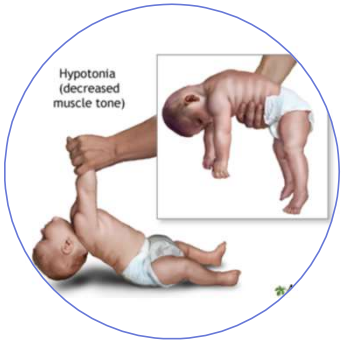
Normal, Healthy Muscle

Diseased muscle with glycogen buildup in lysosomes

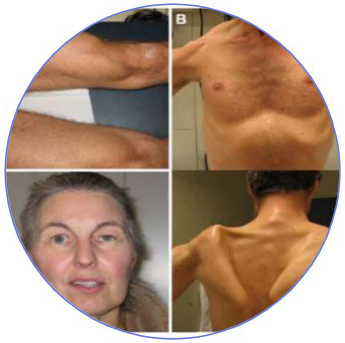
	US	EU5
Annual Incidence	220	206
Calculated True Prevalence	**~10K**	**~8K**
Calculated Dx Prevalence	**~4K**	**~2.8K**

Patient Breakdown

IOPD 25%	LOPD 75%



IOPD life expectancy < 1 year if untreated; can extend to second decade with ERT



LOPD life expectancy depends on age of onset but median age 55 yo

Glycogen buildup has toxic effect on muscle cells and leads to symptoms that include muscle weakness, respiratory distress, cardiomyopathy and loss of independent ventilation

Targeting GYS1 is a novel approach to treatment of Pompe Disease

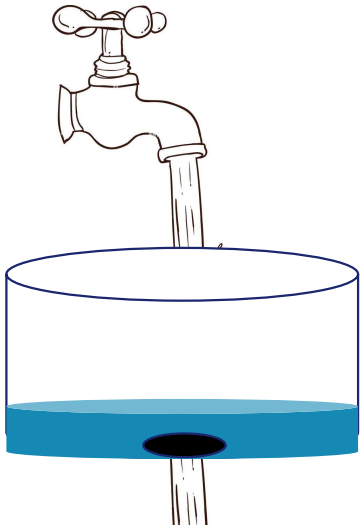
Normal

Pompe Disease

Muscle Glycogen Synthesis (GYS1)

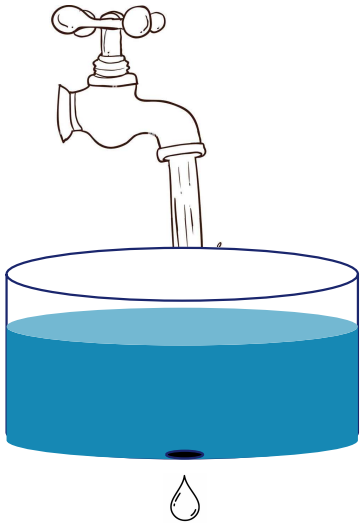
Muscle Glycogen Metabolism (GAA)

Normal GAA



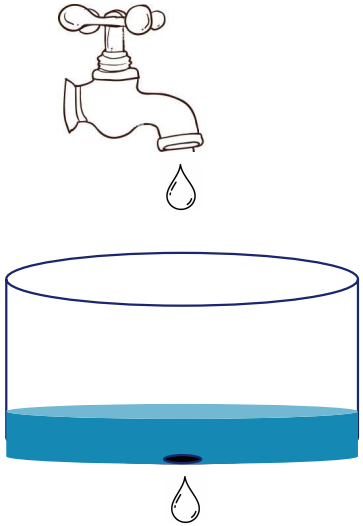
Normal enzyme function; normal glycogen levels

Genetic GAA Mutation



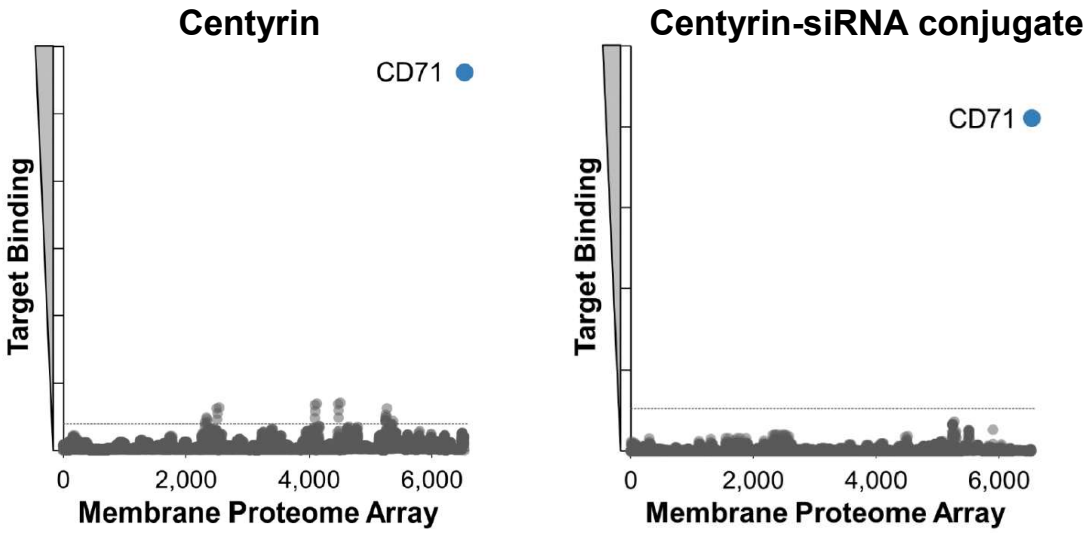
Reduced GAA function; toxic build of glycogen levels

Aro GYS1 siRNA



Reduced GYS1 levels result in normalization of glycogen levels

Binding of lead CD71 Centyrin and siRNA conjugate is highly specific for CD71



- Membrane Proteome Array (MPA) profiles the binding of ligands vs 6,000 arrayed human protein targets
- Determines ligand target specificity and identifies ‘off-target’ binding
- Target receptors are expressed in native conformations on unfixed cells
- Secondary screens confirm ligand binding to specific targets identified in initial screen

MPA confirms specific binding of Centyrin and ABX1100 to CD71; no ‘off target’ binding

siRNA is GYS1 specific and demonstrates pM potency *in vitro*

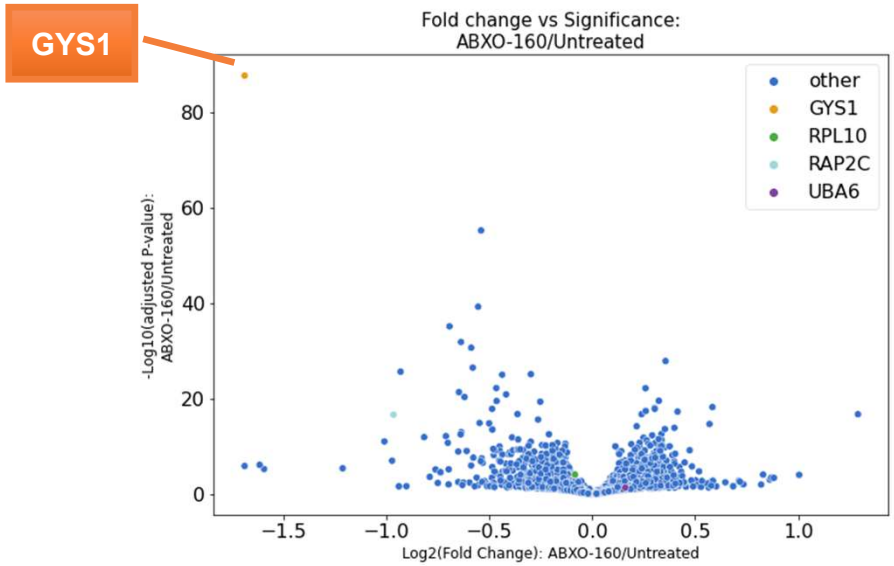
In vitro transfection assay

In vitro screen

siRNA	EC50 (pM)	Emax (%)
ABXO-	**0.8**	**80**
ABXO-	2.6	71
ABXO-	4.0	76
ABXO-	5.7	75
ABXO-	6.7	76
ABXO-	10	80
ABXO-	10	70

RNAseq

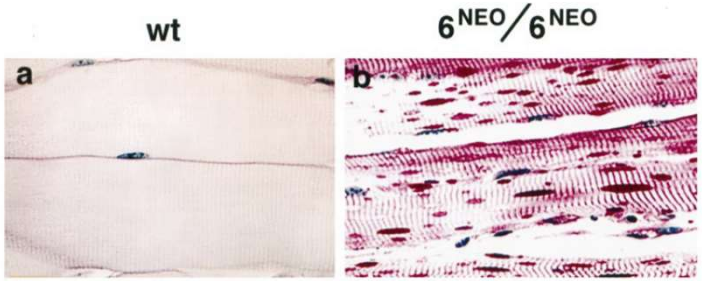
In vitro RNA seq study



- **Highly specific Gys1 RNA knockdown**
- **No in vitro/in vivo activity vs Gys2 mRNA**

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 273, No. 30, Issue of July 24, pp. 19086–19092, 1998
Printed in U.S.A.

Targeted Disruption of the Acid α-Glucosidase Gene in Mice Causes an Illness with Critical Features of Both Infantile and Adult Human Glycogen Storage Disease Type II*



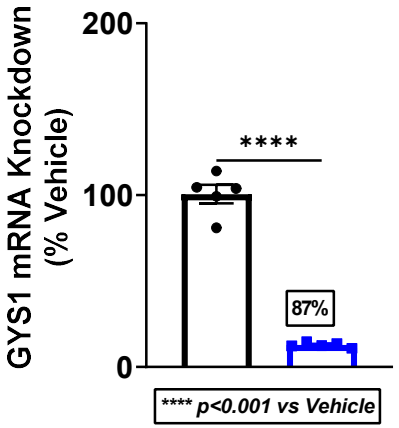
- Complete knockout of alpha acid glucosidase ($GAA^{-/-}$)
- Glycogen accumulation in skeletal and heart muscles
- Does not fully mimic human disease
  - Residual GAA activity generally correlates with disease severity; IOPD patients have the lowest levels of residual GAA activity (most severely affected infants have no detectable residual GAA activity).
  - Demonstrations of glycogen effect are variable across literature (e.g. skeletal muscle)
  - Glycogen measurements
  - Tissue collection
  - Heart vs. skeletal muscle
  - Diet – high vs. low carb
- Gold standard in the field
  - Enzyme replacement therapies (ERT)
  - Translational studies
- Aro is relying on model for dose justification for FIH

CD71 Centyrin-GYS1 siRNA conjugate achieves robust Gys1 mRNA and protein knockdown in Pompe mouse model (GAA$^{-/-}$)

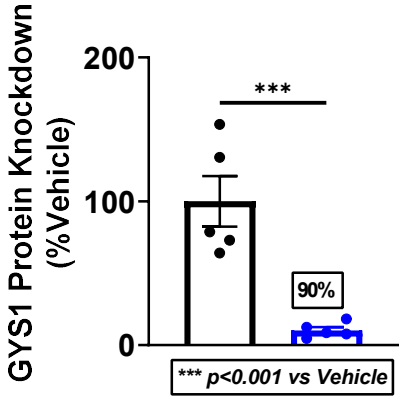
4 weeks post single dose of 3mg/kg siRNA

Gastrocnemius

GYS1 mRNA

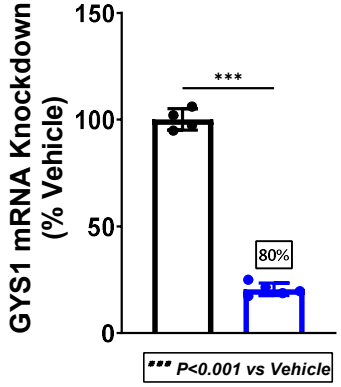


GYS1 protein

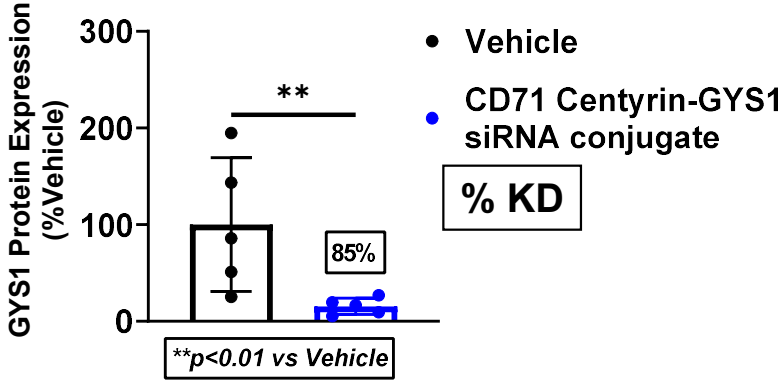


Heart

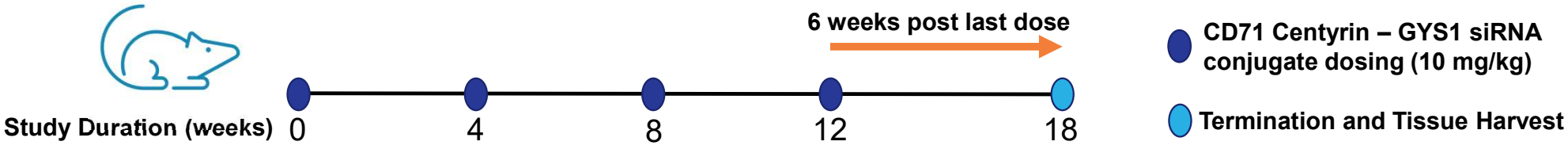
GYS1 mRNA



GYS1 protein



GYS1 mouse glycogen pharmacodynamics study at 18 weeks after initiating monthly dosing

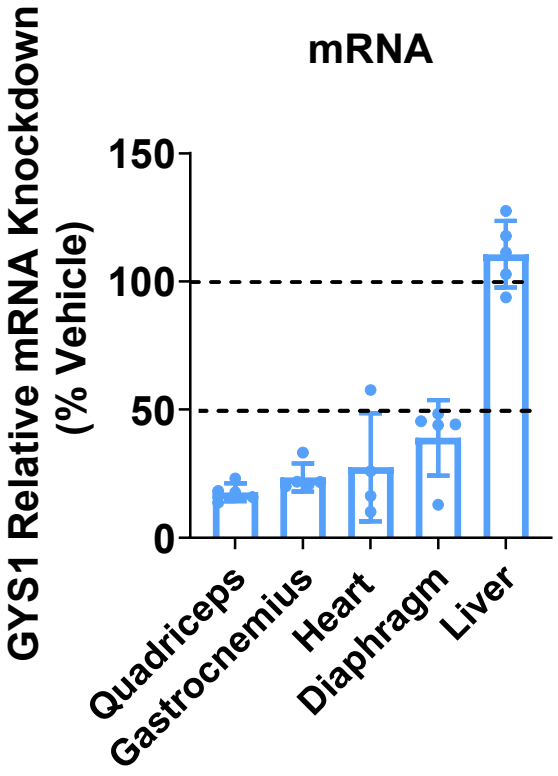


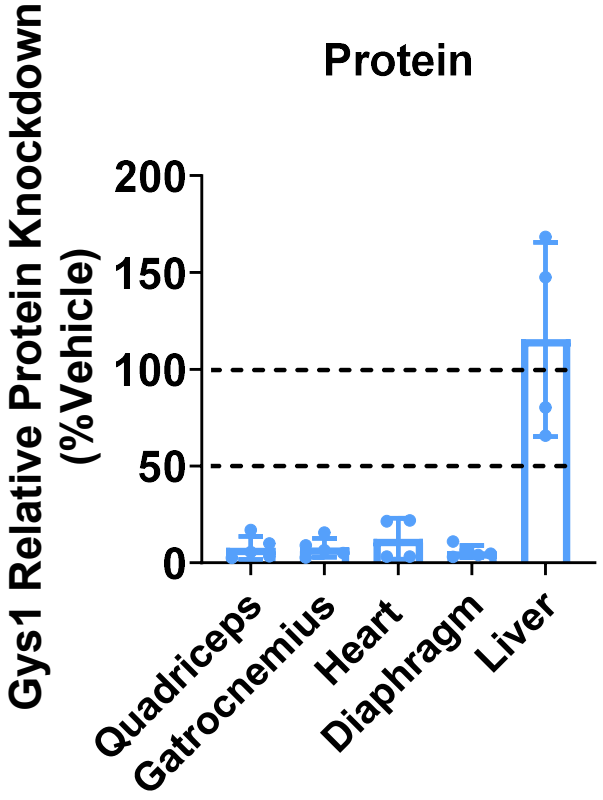
Objectives

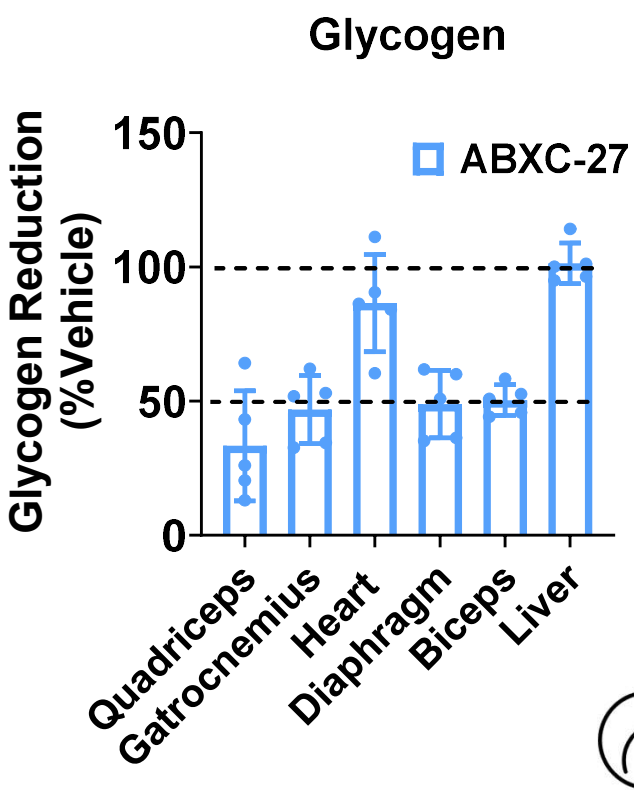
- Assess reduction of **glycogen levels** after long-term dosing
- Determining the correlation between Gys1 protein knockdown and reduction of glycogen levels

Pharmacodynamic effects of CD71-GYS1 conjugate (ABXC-27)

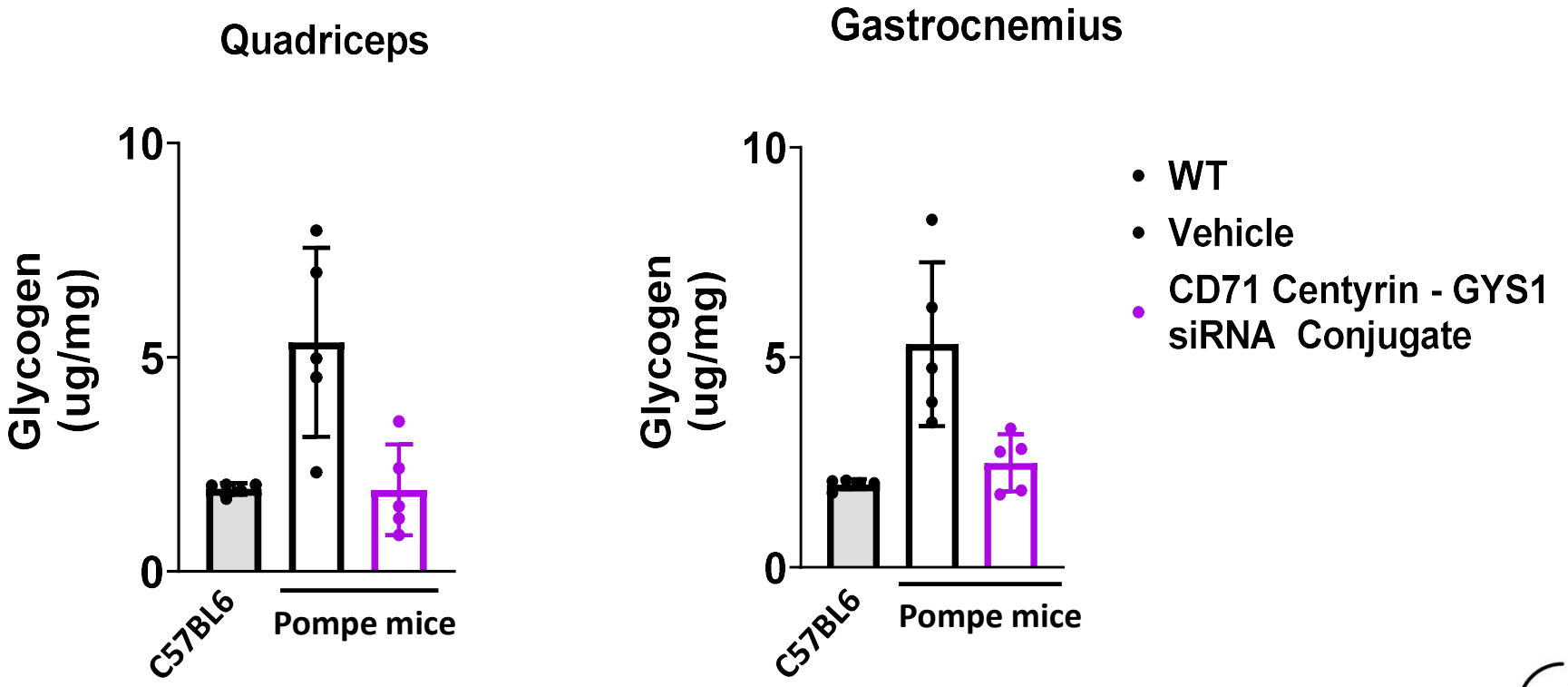
mRNA, protein, and glycogen levels assessed 6 weeks post 4x dosing at 10mg/kg every 4 weeks







Glycogen levels in the treated Pompe mice were reduced to the levels observed in age matched wild type mice

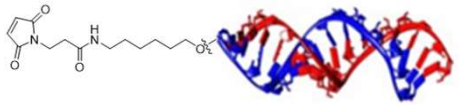


Highly site-specific bioconjugation routinely generates quality Centyrin-siRNA conjugates in gram quantity

CMC process scale-up for IND enabling studies

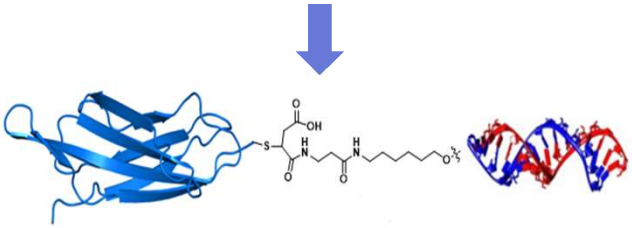
Centyrin

- 20 L fermentation scale-up completed
- Soluble expression of Centyrin in cytoplasm (no refolding required)



siRNA

- 40 gram scale-up completed to support non-GLP Tox



Centyrin-siRNA Conjugate

- 35 g conjugation scale-up completed for non-GLP tox studies

Supports pharmacology and toxicology studies

Aro's Pompe Disease program

On track to be first to the clinic in Pompe Disease with innovative GYS-1 siRNA conjugate

- Durable and tissue-specific pharmacodynamic effects in muscle with no / limited effect in liver and kidney
- Highly selective and potent CD71 Centyrin and GYS-1 siRNA; no off-target effects
- Potent mRNA and protein reduction - leading to robust glycogen reduction equivalent or better than ERT - in skeletal muscle in Pompe mouse model
- Activity established in NHPs; no evidence of toxicity across multiple mouse and NHP in vivo studies
- Centyrin siRNA conjugates demonstrate lack of immunogenicity in human T-cell assays and are well tolerated in preclinical in vivo models
- CMC scale-up commenced with high soluble expression of CD71 Centyrin in microbial system and established conjugation chemistry
- Non-GLP tox studies initiated

Aro BIOTHERAPEUTICS

Thank You!

Contact:

Scott Greenberg (Chief Operating Officer) sgreenberg@arobiotx.com

www.arobiotx.com