Creating A New Class of Centyrin - Oligonucleotide Therapeutics

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Aro BIOTHERAPEUTICS

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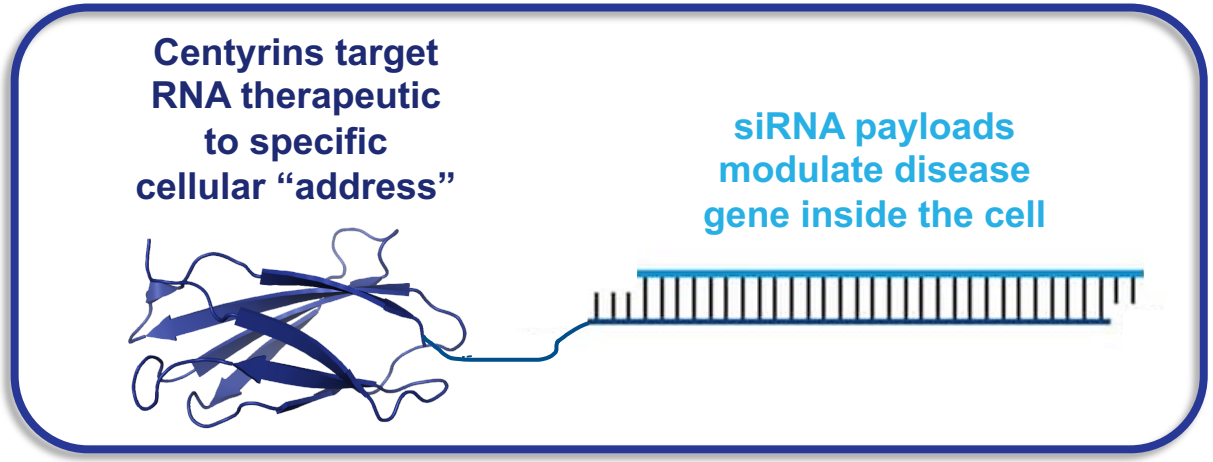
Outline

- Centyrin platform introduction
- CD71 Centyrin – siRNA conjugates demonstrate excellent *in vivo* efficacy
  - Identifying highly specific CD71 Centyrin
  - siRNA screening
  - Conjugate efficacy in murine and NHP models
- CD71 Centyrin – ASO conjugates demonstrate excellent *in vivo* efficacy
  - Conjugate efficacy in human transgenic mouse model
- Summary

Aro's Centyrin platform enables tissue-targeted delivery of RNA medicines, unlocking vast therapeutic potential



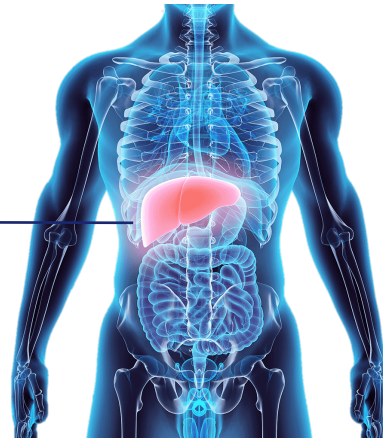
Current FDA approved RNA medicines target genes in the liver

Tegsedi® (inotersen) injection 284 mg/1.5 mL

onpattro® (patisiran) lipid complex injection 10 mg/5 mL

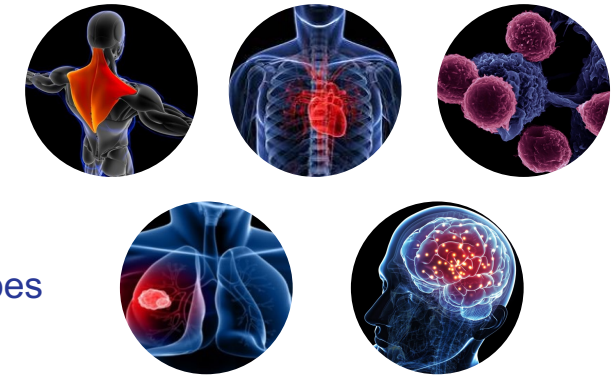
GIVLAARI® (givosiran) injection for subcutaneous use 189 mg/mL

OXLUMO® (lumasiran) for injection 94.5 mg/0.5 mL



Receptor mediated targeting enables tissue specific delivery of RNA medicines to many tissues/cell types:

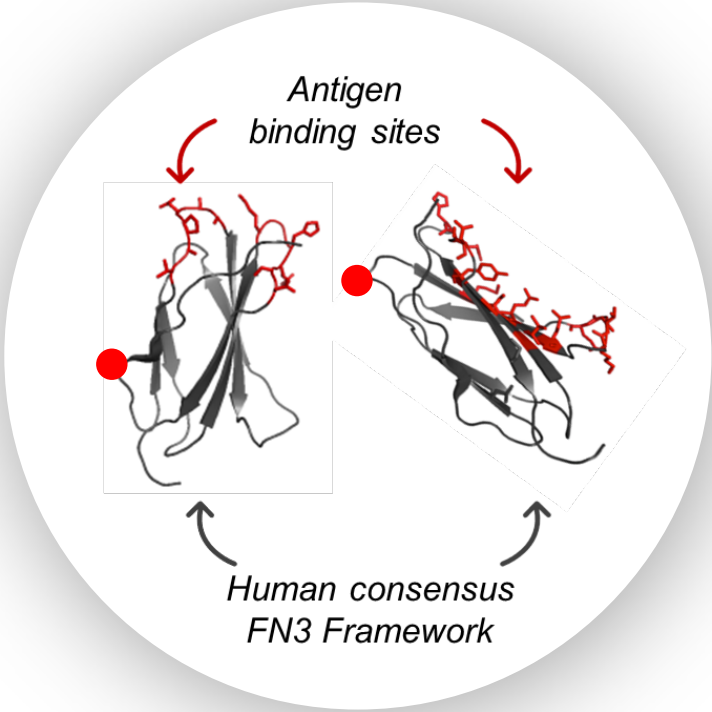
- Skeletal muscle
- Cardiac muscle
- Immune cells
- Tumor tissue
- Additional tissues/cell types



Centyrin overview

Rapid, iterative, flexible and chemically tractable platform for RNA drug targeting

- Proprietary monovalent antigen binding platform
- Built on a consensus human Tenascin C FN3 framework
- Exceptional stability and solubility
- Low immunogenicity risk; no T cell epitopes
- ~1/15 size of standard monoclonal antibodies
- Readily expressed in E. Coli as multi-specific proteins
- Site specific covalent conjugation to drug payloads
- Extensive patent portfolio; strong IP position



Ideal properties for targeted delivery of oligonucleotide therapies

● = Drug Conjugate Site

Centyrins are engineered to exhibit low immunogenicity

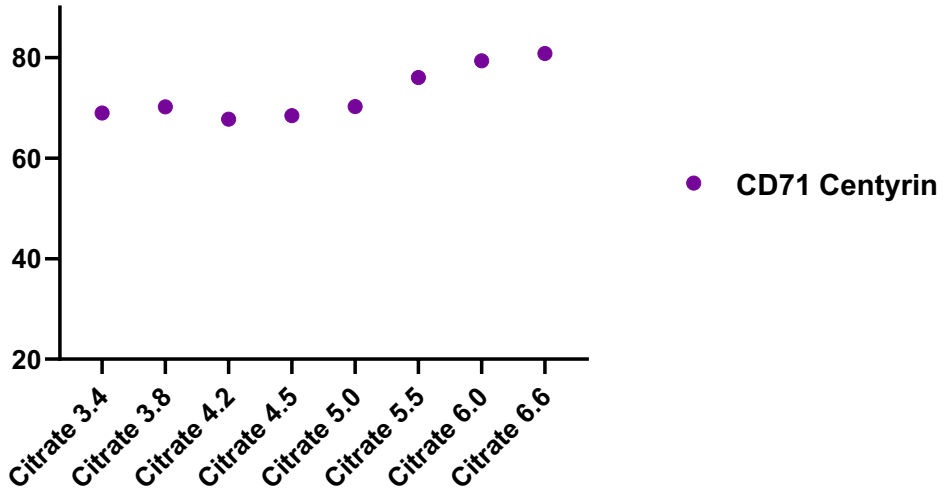
Low immune response index

Protein	Immune Response Index (RI)
PPD (Positive Control 1)	61.39
KLH (Positive Control 2)	32.05
CD71 Centyrin	**0.19**

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

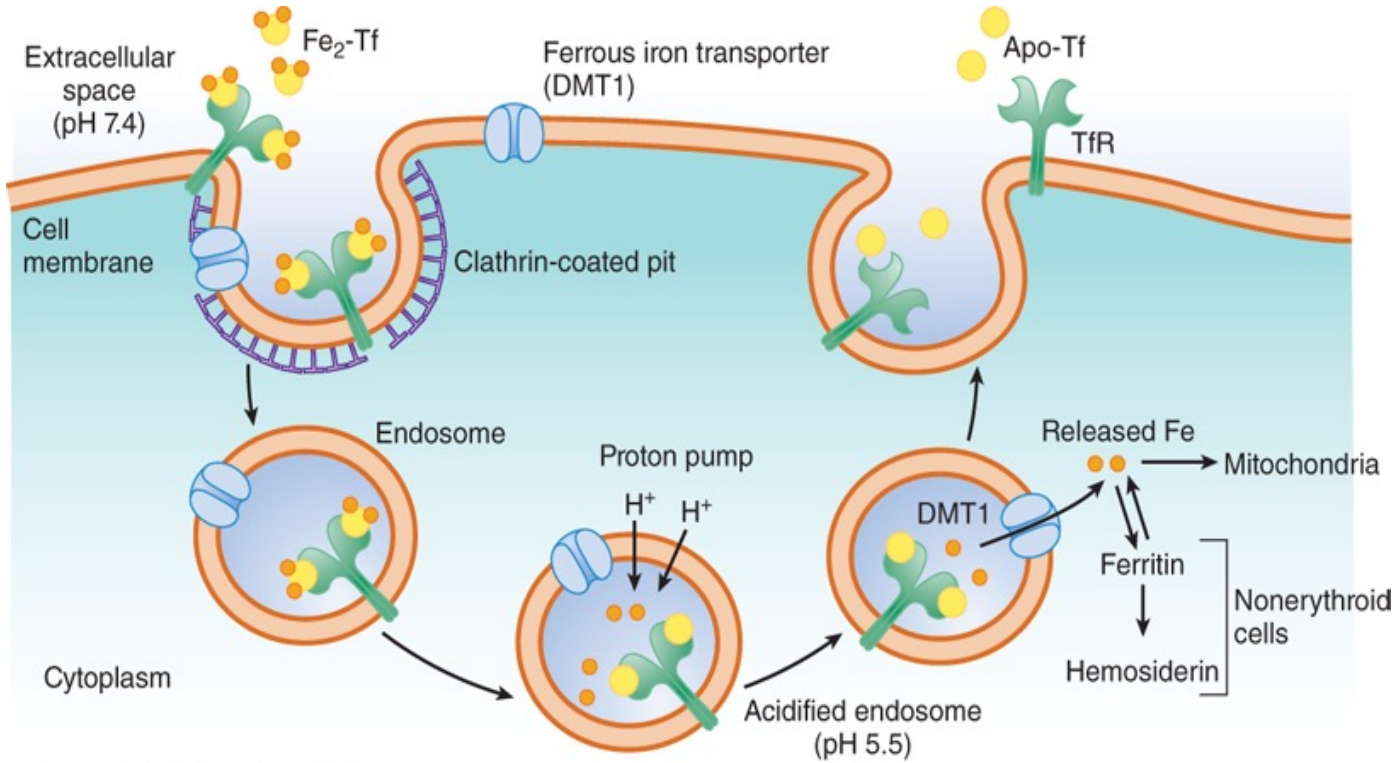
High stability (Tm)



- 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

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
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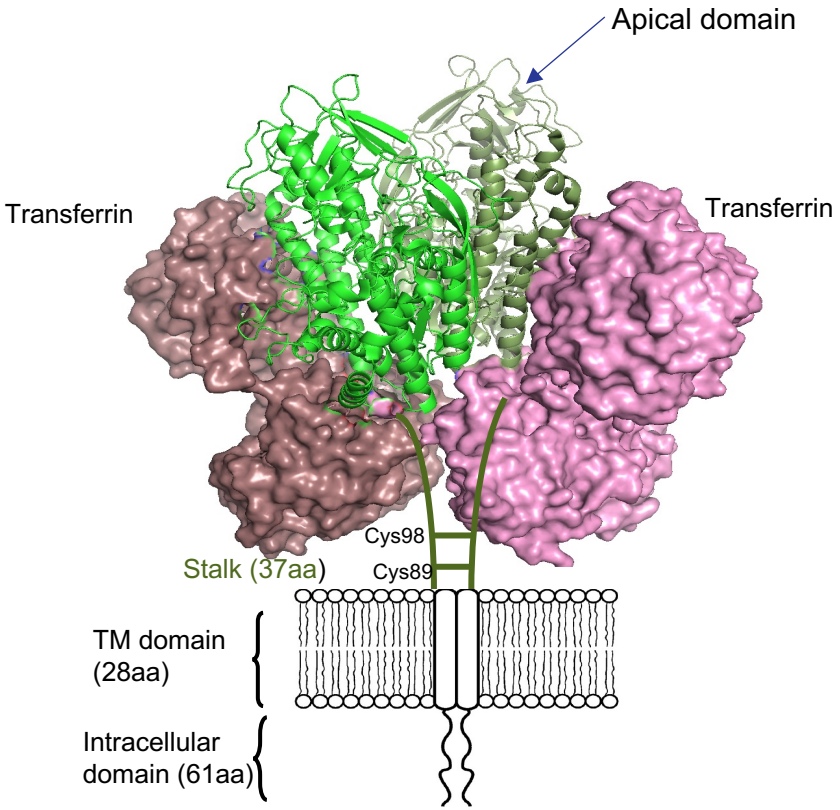
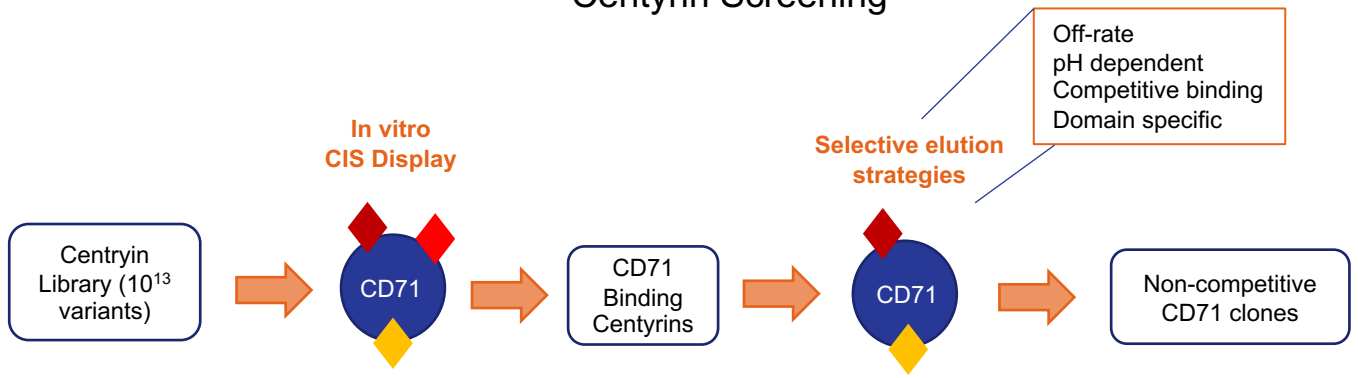
- Essential and ubiquitously expressed receptor responsible for iron transport into cells
- We have generated a large diversity of CD71 Centyrins to enable efficient and customized targeting of various CD71+ cell types
- Demonstrated efficient targeting of CD71 Centyrin-oligo conjugates
  - Monovalent receptor binding does not block transferrin binding or CD71 surface expression
  - No evidence of agonist effect

Transferrin Receptor (CD71): Centyrins bind to apical domain and do not compete with Transferrin

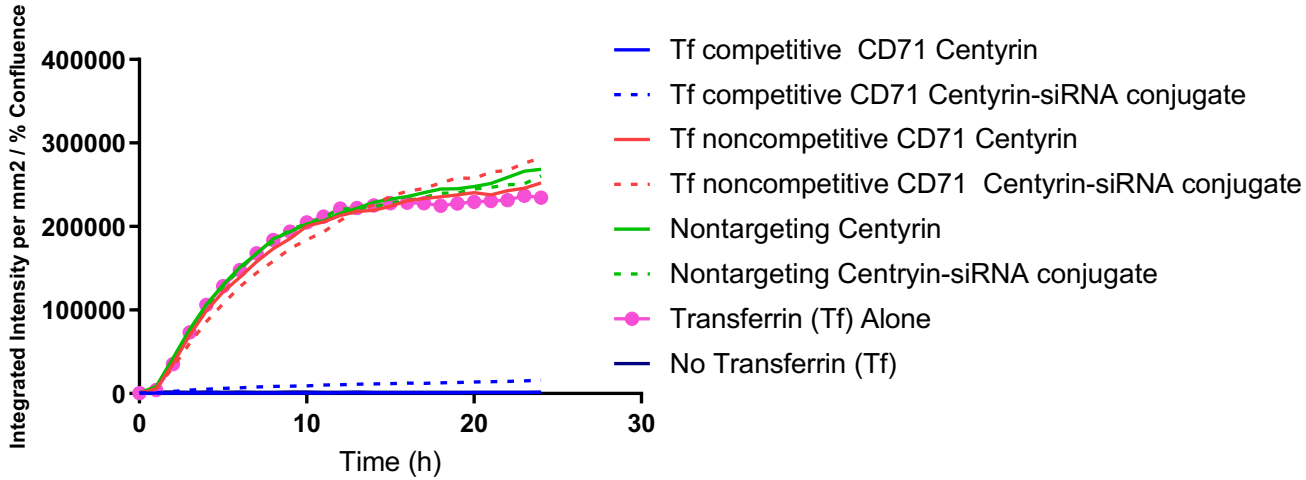
CD71 extracellular domain homodimer



Centyrin Screening



phRodo™ Labeled Transferrin (Tf) Binding Assay



Parallel optimization activities to select development candidate(s)

Progression toward development candidate(s)

Centyrin Libraries (10^{13})

Hu CD71 Binders (~500)

Good Internalization (~350)

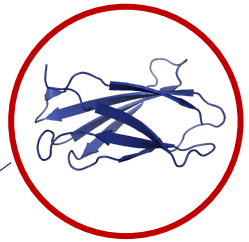
Non-competitive (~70)

Good expression (~40)

Cyno/hu cross reactive (~10)

Cyno in vivo study (6)

Lead Centyrins



Development candidates

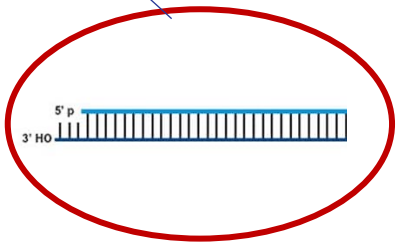
Bioinformatics Screen for hu/cyno sequences (~1,000)

In vitro potency (~100)

In vitro optimization (10)

Rat tolerability (4)

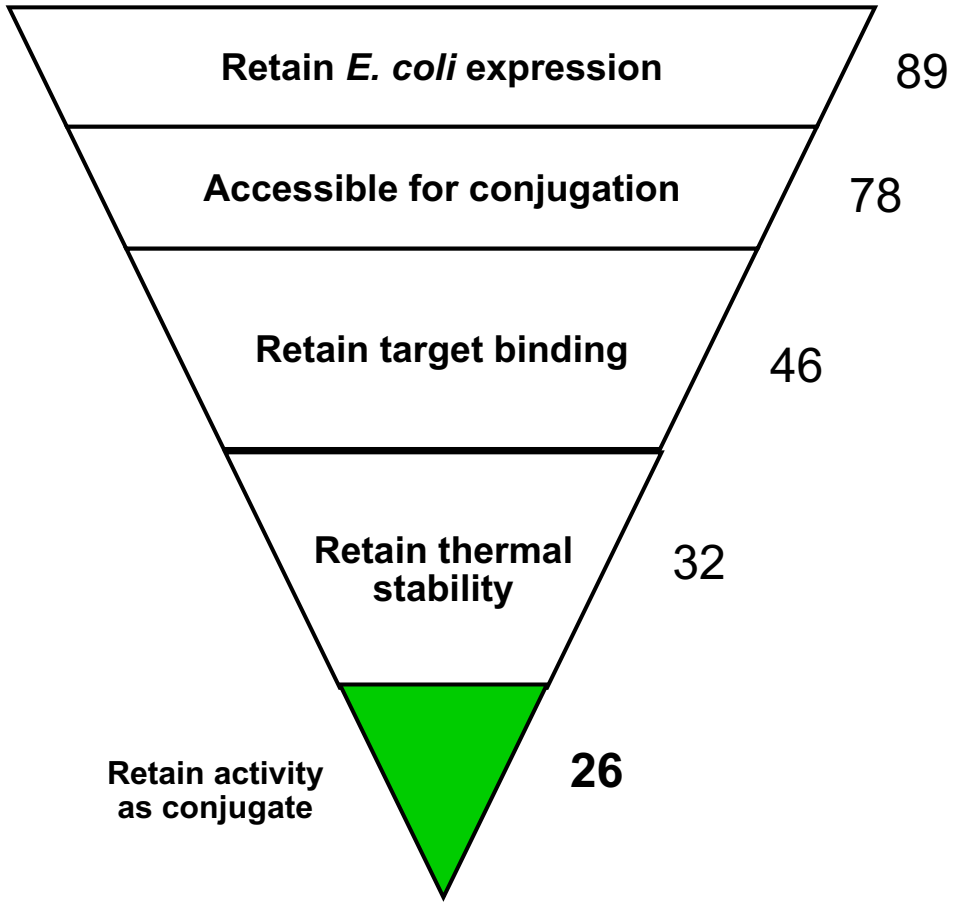
Lead siRNAs



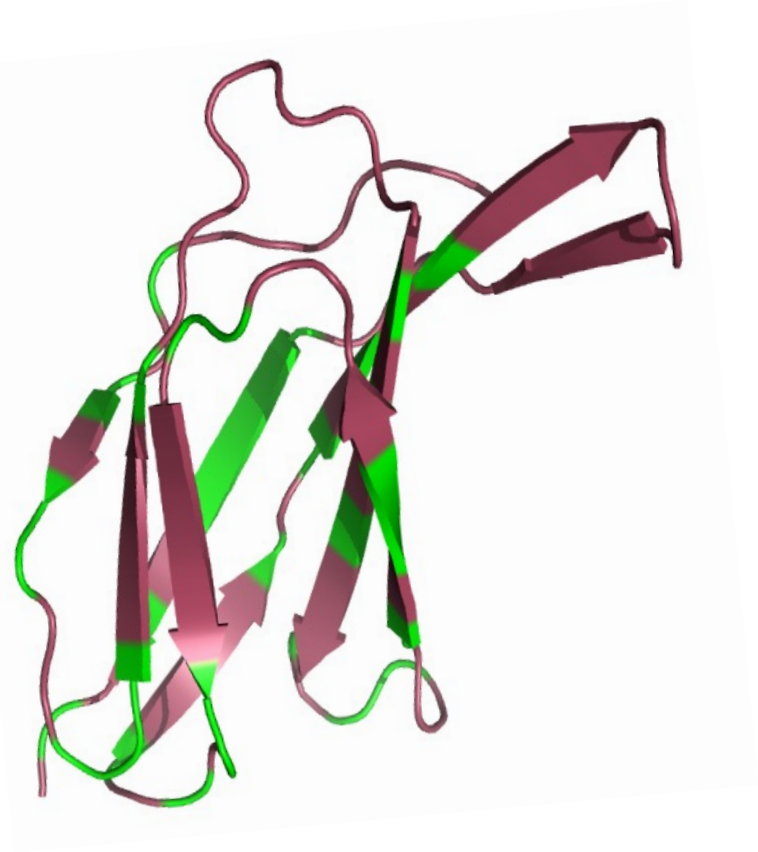
Optimized Sites for Cysteine Conjugation Identified

Adaptable to orthogonal conjugation chemistries

All 96 positions on the Centyrin scaffold were individually mutated to cysteine

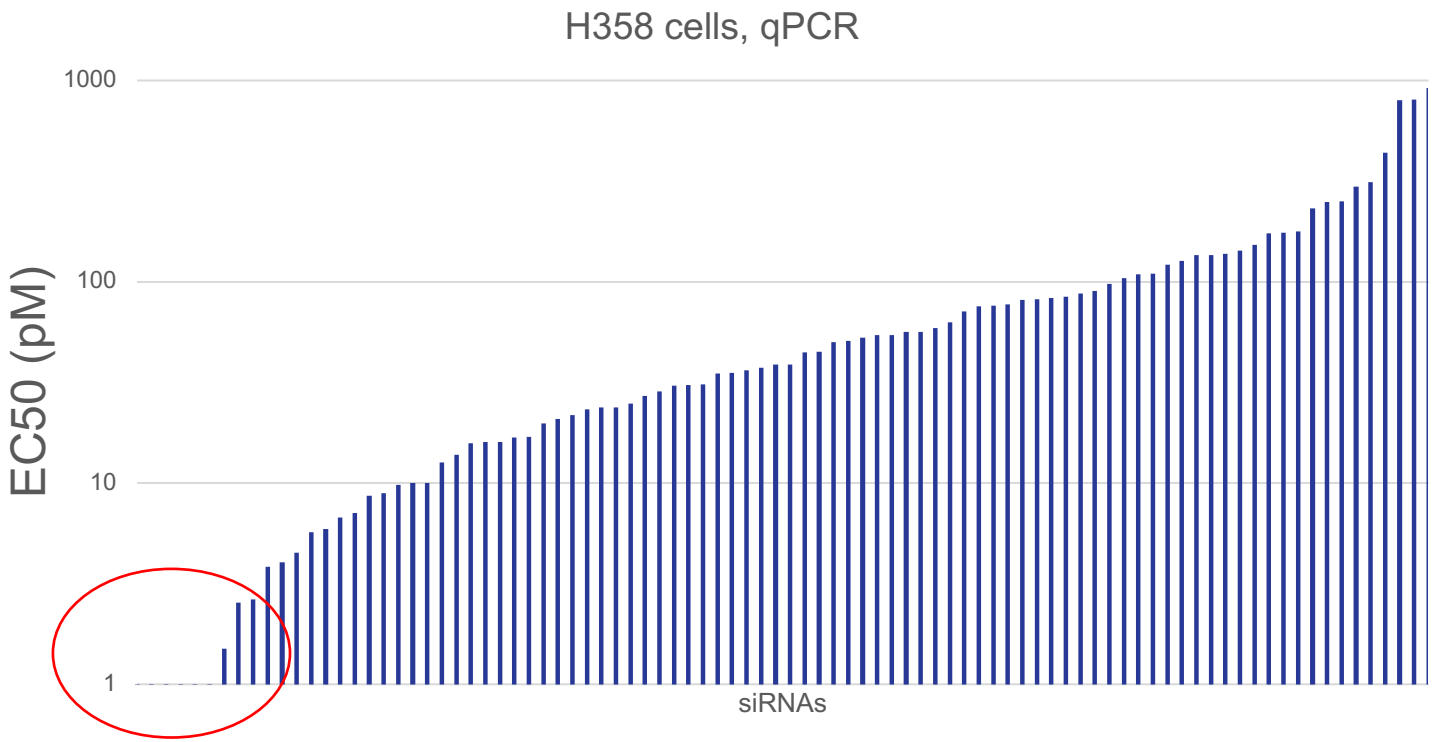


Green are tolerated positions for cysteine conjugation



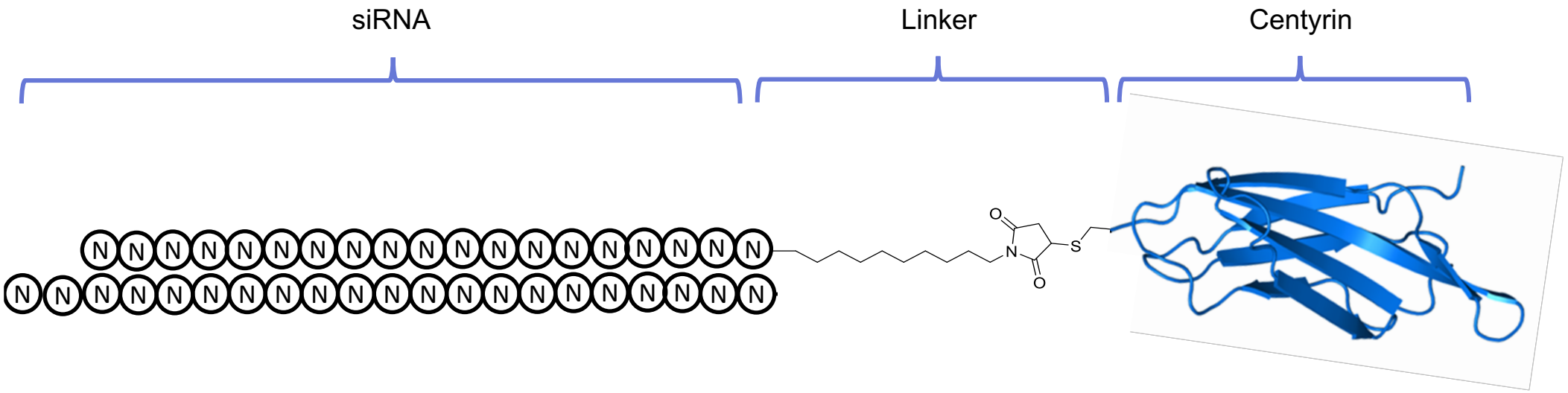
Goldberg et al., PEDS 29 (12) 2016, 563-572

siRNA selection – *In vitro* screening



- Compounds initially transfected using a 4- and 8-point dose curves
- Compounds with low EC50 and high Emax selected for further profiling and conjugation

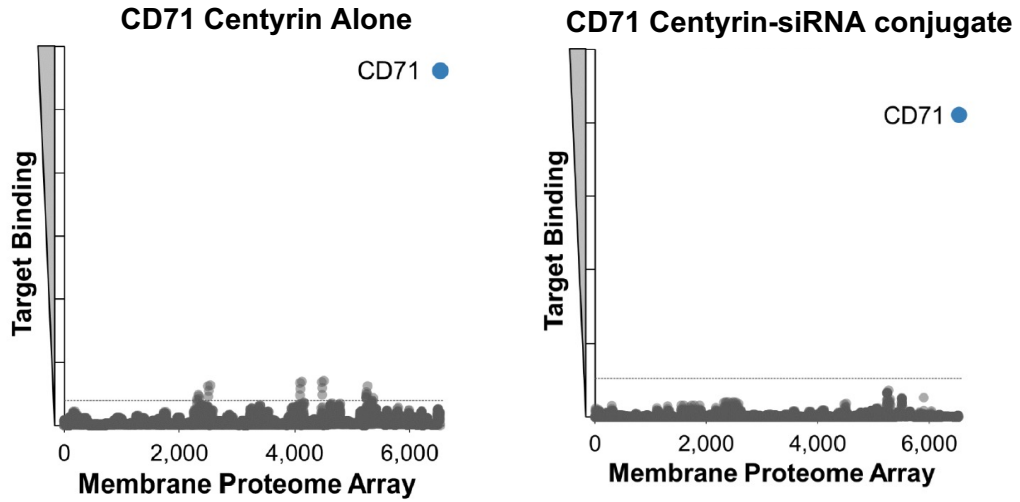
Aro's Centyrin-siRNA conjugate employs clinically proven design principles



- 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 is non-competitive with transferrin, and demonstrates nanomolar affinity and high specificity for CD71 receptor

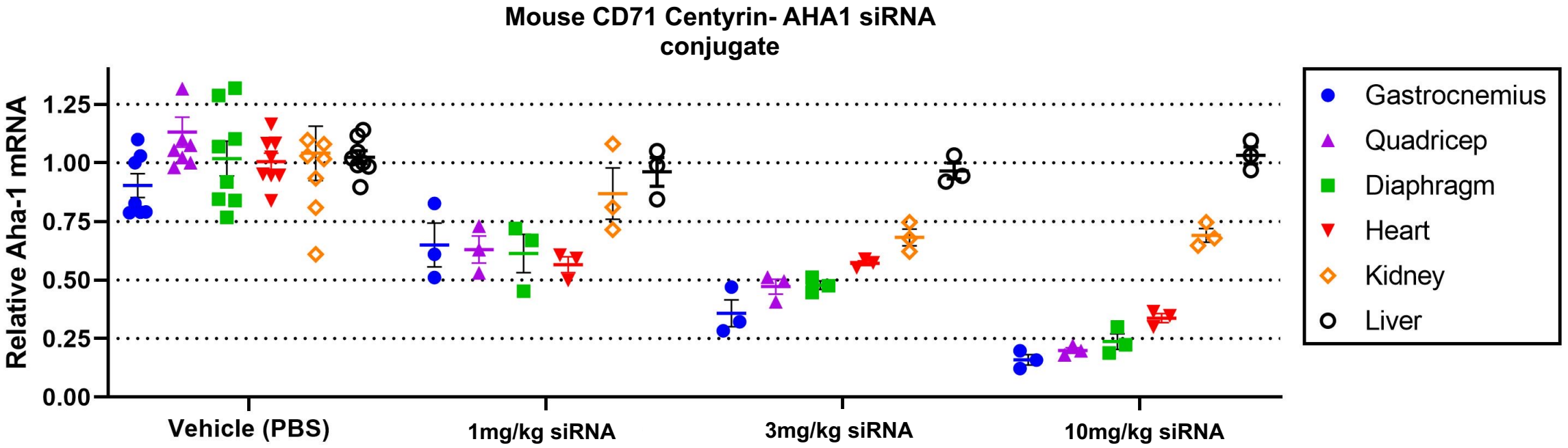
Centyrin	Kd (nM)	Tf competition Centyrin	Tf competition AHA1 conjugate	Human Apical domain MSD	Tm, ^{0}C
CD71 Centyrin	7.3	No	No	Yes	84



- Membrane protein array (MPA) profiles the specificity of ligands that target human membrane proteins and identifies off-target effects
- CD71 is the only confirmed target for the CD71 Centyrin as well as the a CD71 Centyrin-siRNA conjugate

Robust, dose-dependent and selective gene KD in mouse skeletal and cardiac muscle

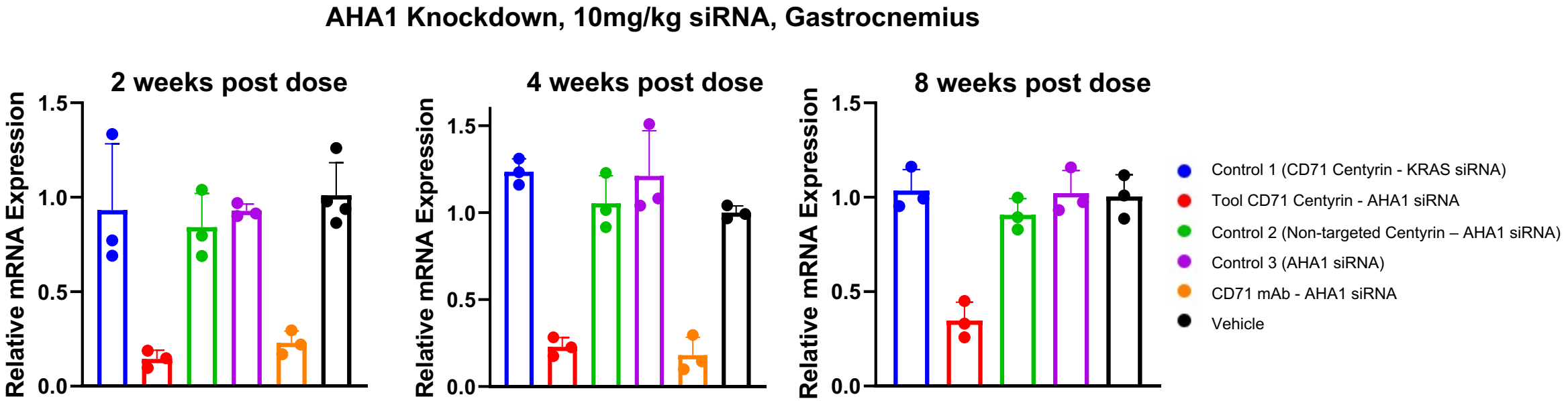
Tool mouse specific CD71 Centyrin-AHA1 siRNA conjugate



- AHA-1 is a ubiquitously expressed housekeeping gene
- Up to 80% gene knockdown observed 2 weeks after single dose
- No / minimal gene knockdown observed in liver / kidney
- Strong dose-response relationship observed

Mice dosed IV with PBS or 1, 3 or 10 mpk (siRNA) of ABX1005 (CD71-AHA1 conjugates)
Tissues collected 2 weeks post single dose

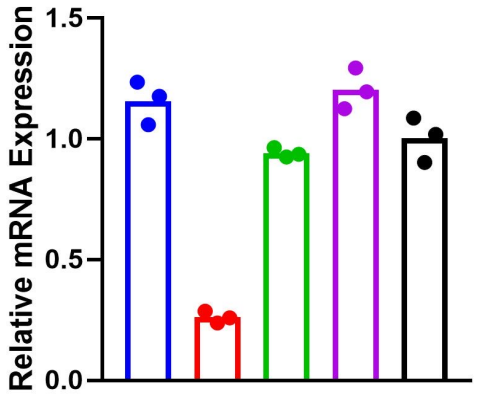
CD71 Centyrin-siRNA conjugate drives sustained gene knockdown at fraction of mAb conjugate dose in mice

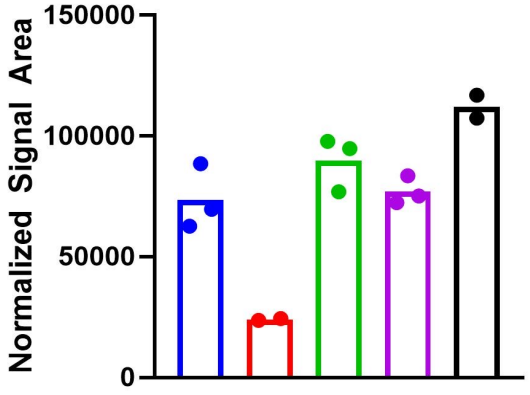


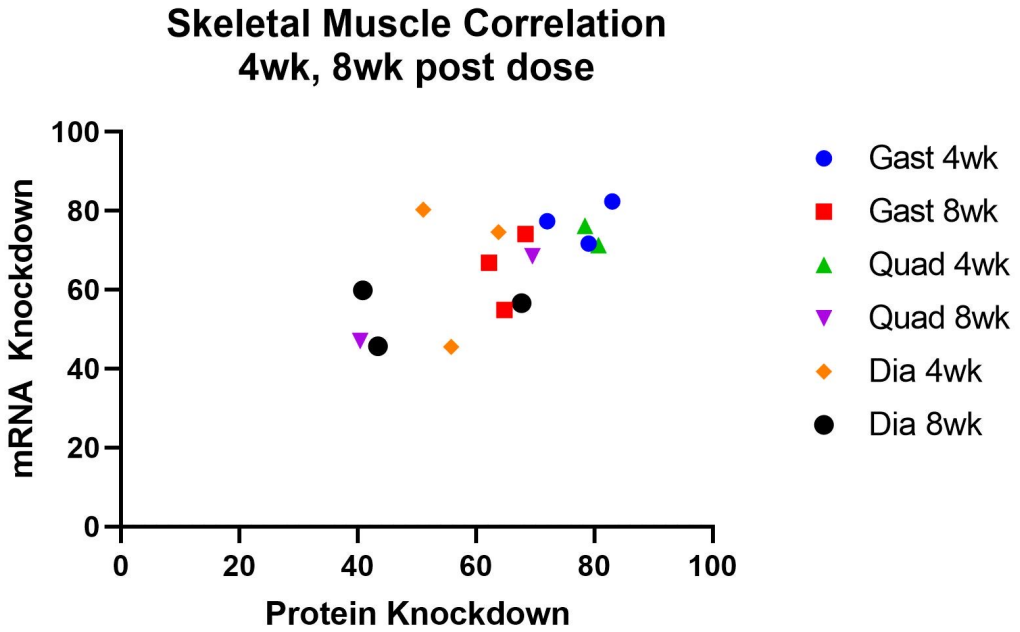
POC study with AHA1 housekeeping gene
C57/B6 mice received single dose of conjugates

	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

mRNA and protein knockdown are well correlated in vivo



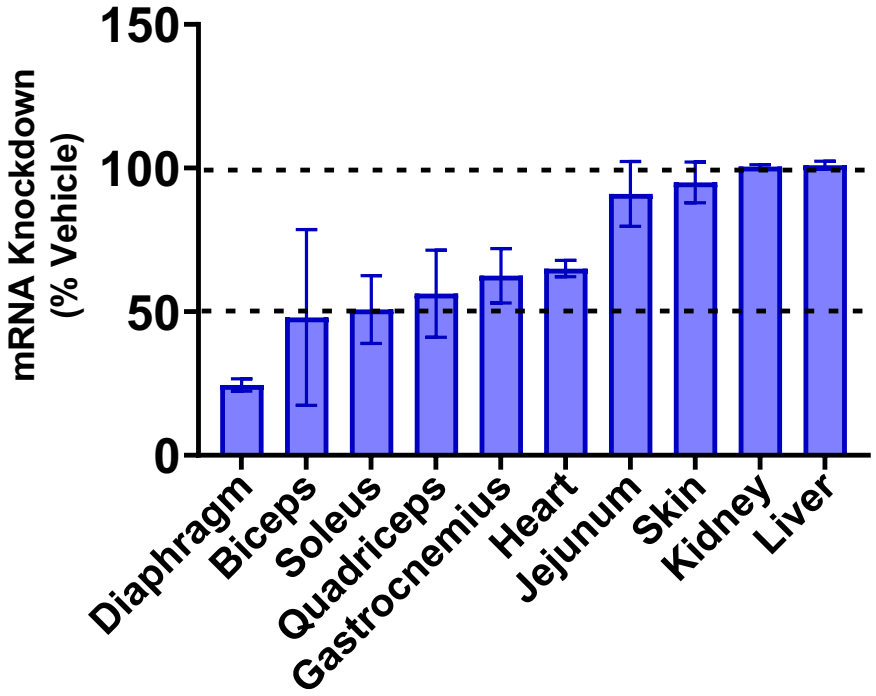




- Control 1 (CD71 Centyrin - KRAS siRNA)
- Tool CD71 Centyrin - AHA1 siRNA
- Control 2 (Non-targeted Centyrin – AHA1 siRNA)
- Control 3 (AHA1 siRNA)
- Vehicle

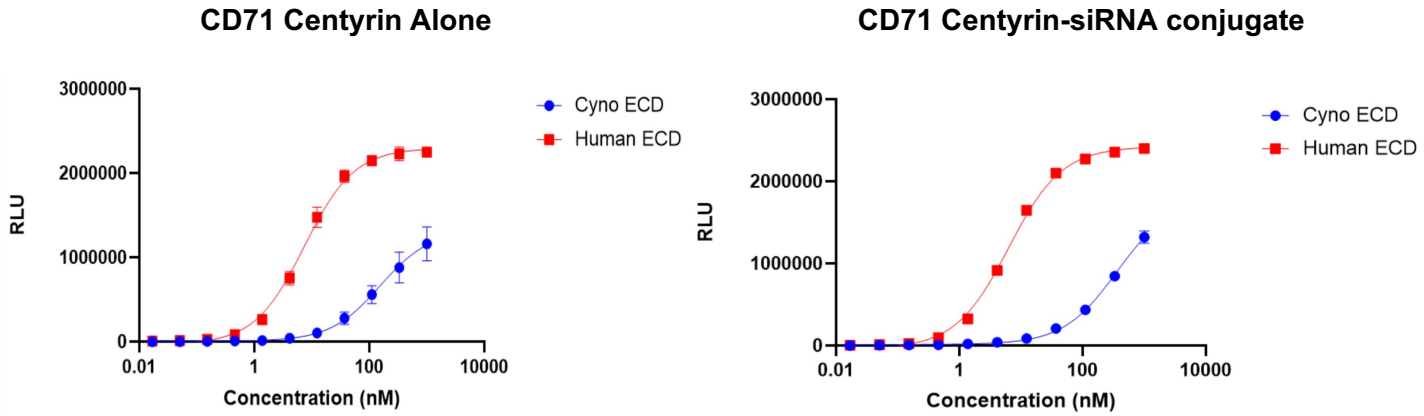
Centyrin-siRNA conjugates are highly active in Cyno skeletal muscles and heart

Centyrin-AHA1 Conjugate mRNA KD



dosed at 10 mg/kg siRNA x3 weekly doses, mRNA KD assessed 28d post three doses

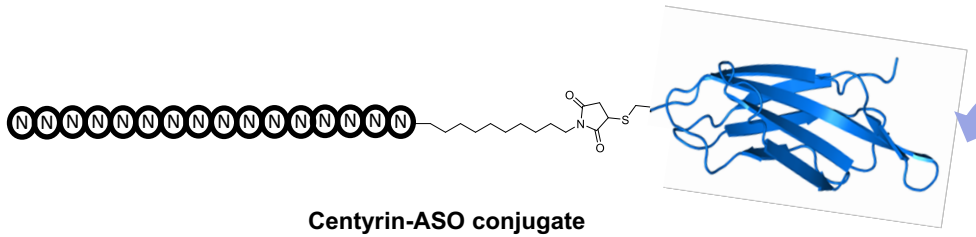
Centyrin binding to Cyno and human CD71



Domain	Centyrin EC50 (nM)	Conjugate EC50 (nM)
Hu CD71 ECD	7.4	6.3
Cyno CD71 ECD	> 150	> 150

- >20x lower binding to cyno vs hu CD71
- siRNA conjugate does not interfere with CD71 Centyrin binding

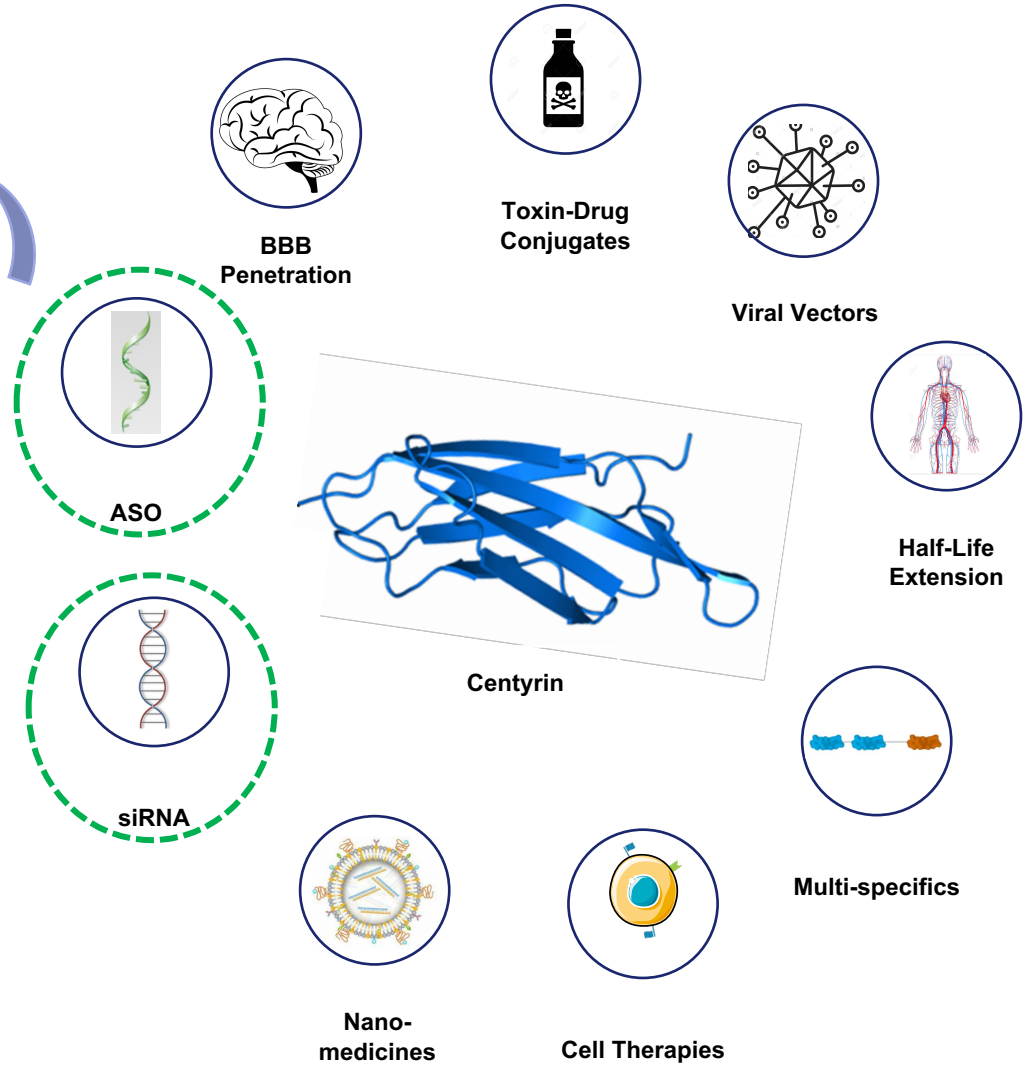
Diverse applications for Aro's Centyrin platform



Centyrin-ASO conjugate

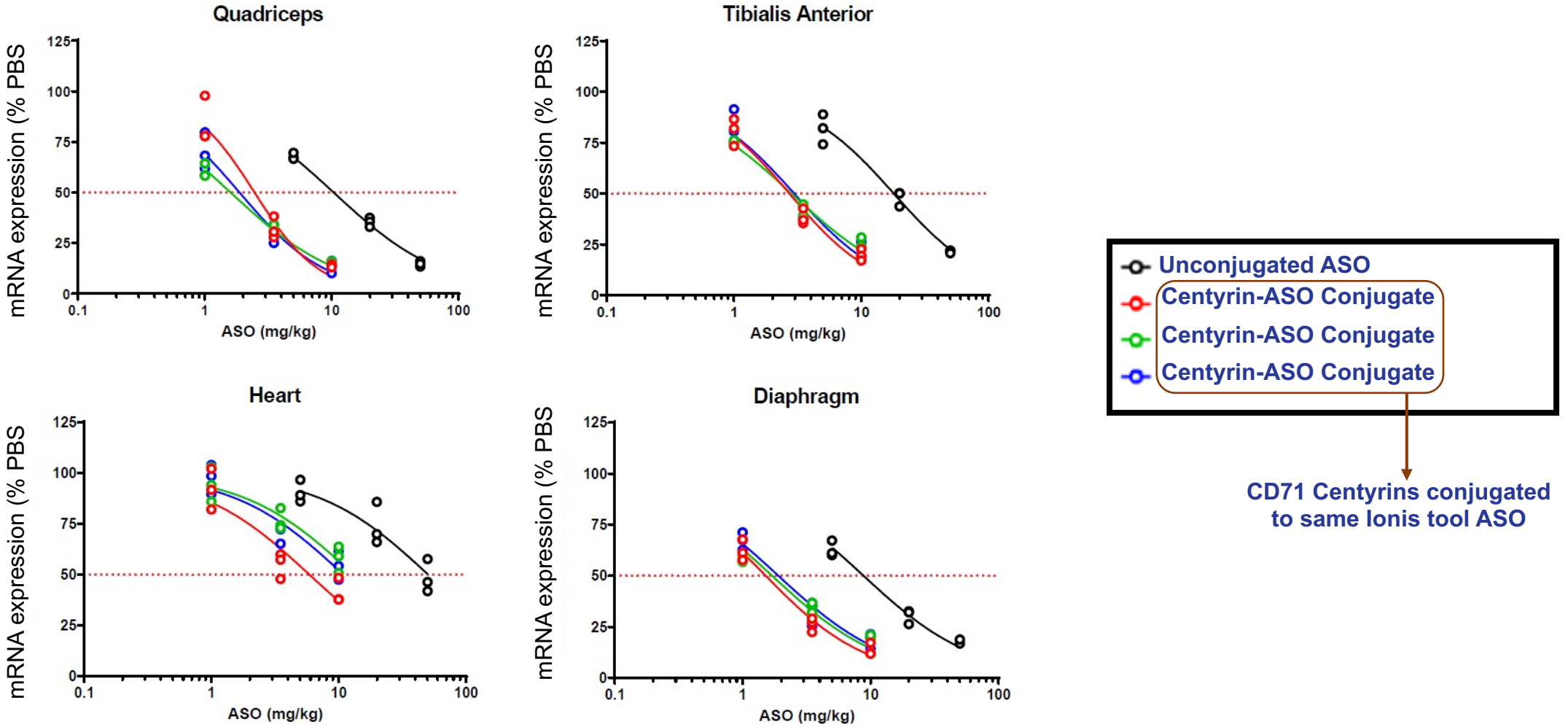
- Centyrin-ASO conjugates generated using site-specific cysteine conjugation
- Standard synthesis, purification and analytical methods employed to generated conjugates in excellent yield
- CD71-ASO conjugates show excellent in vivo efficacy in human CD71 TGx mouse model

Molecule	Mol. Weight. (Da)	Fold Increase
ASO	~5,000	-
Centyrin	~10,000	2
Fab'	~50,000	10
Ab	~150,000	30



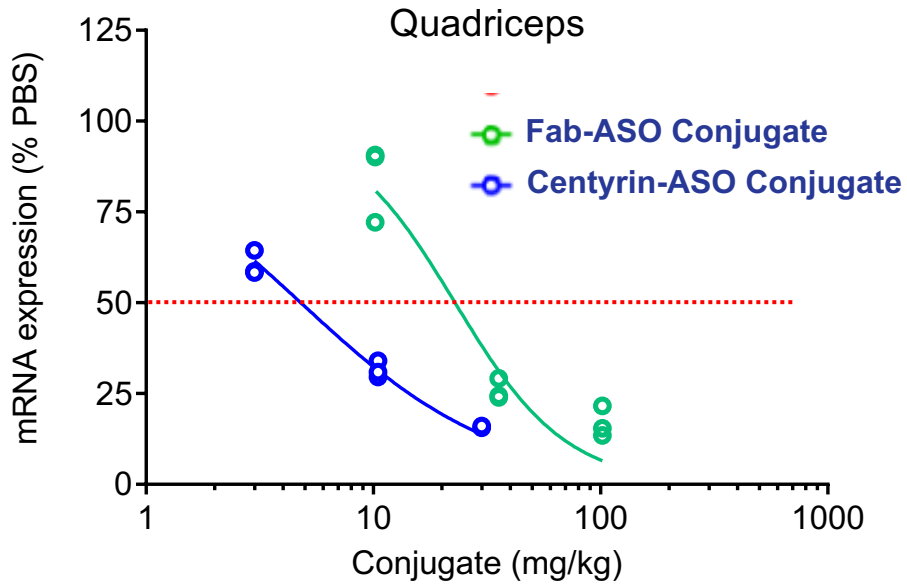
CD71 Centyrin-ASO conjugates demonstrate improved potency versus unconjugated ASO in muscle and heart

hTfRKI/+ mouse model



hTfRKI/+mice dosed IV on days 1, 8 and 15 with unconjugated ASO at 5, 20, 50 mg/kg and Centyrin-ASO conjugate equivalents at 1, 3.5, 10 mg/kg, animals sacked 1-week post final dose

Aro CD71 Centyrin–ASO conjugate demonstrates equivalent activity to Fab–ASO conjugate at a fraction of the dose across mouse skeletal muscle and heart

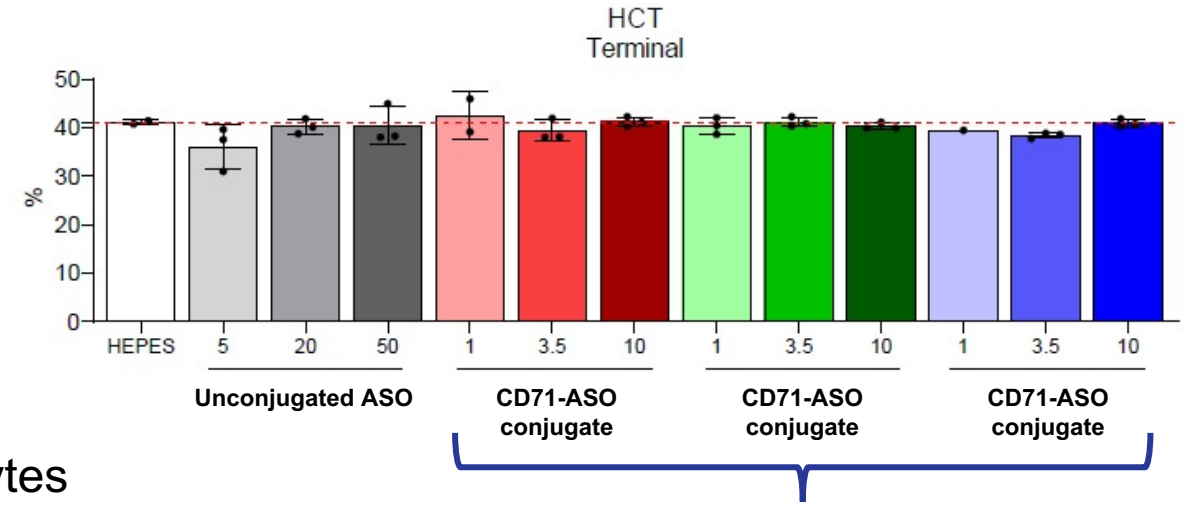


Tissue		CD71 Centyrin-ASO Conjugate ED_{50} (mg/kg)	CD71 Fab-ASO Conjugate* ED_{50} (mg/kg)
Quadriceps	ASO ED_{50} (mg/kg)	2.5	2.2
Quadriceps	Conjugate ED_{50} (mg/kg)	~7.5	~24.5

*CD71 Fab: OKT9

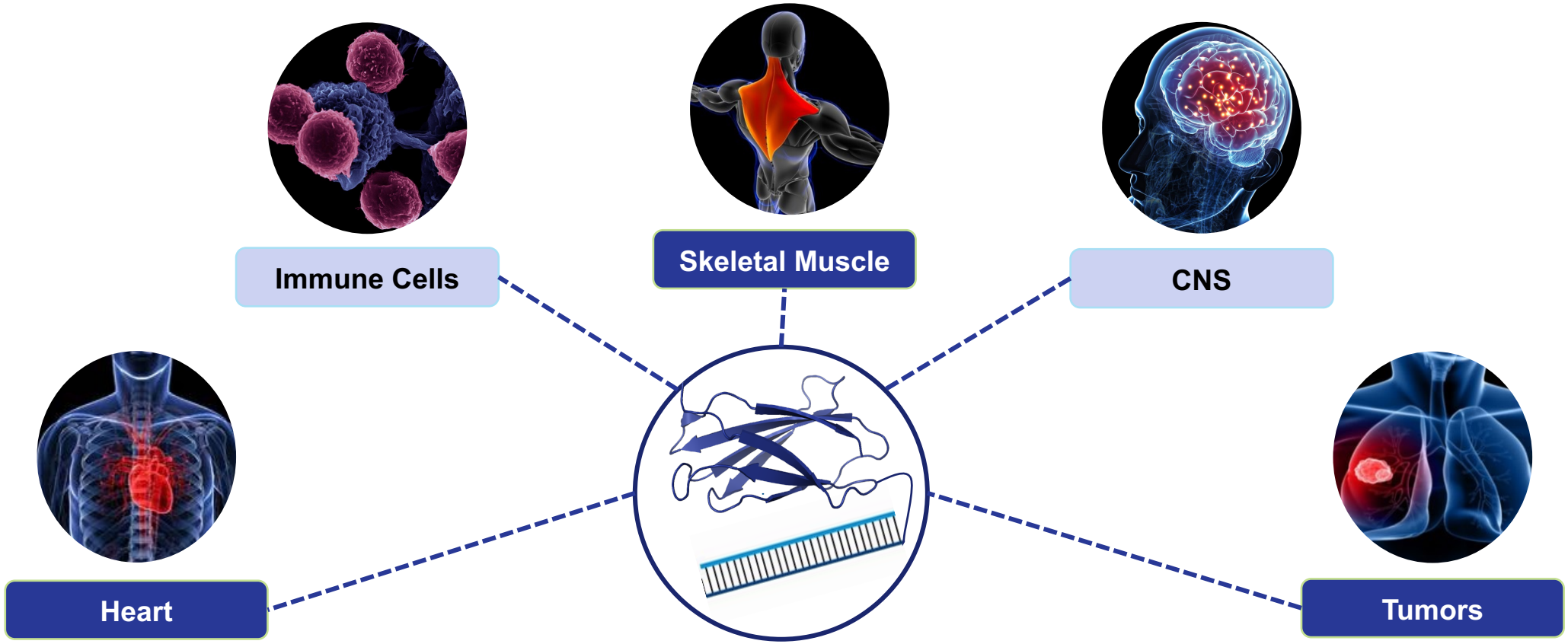
CD71 Centyrin – ASO conjugates are well tolerated in human CD71 transgenic mice with no evidence of anemia

- No abnormalities in body weight changes
- No anemia
- No increase in organ weights
- No concerning increase in liver enzymes
- No concerning changes in platelet numbers and reticulocytes
- No concerning increase in circulatory inflammatory cells



All three conjugates were comprised of different CD71 Centyrins with the same Ionis tool ASO

Aro is exploring Centyrin – siRNA conjugates across a broad range of disease areas



- Rare muscle disease program data to be presented at TIDES 2022

Centyrin-Oligonucleotide Conjugate Summary

- ✓ Panel of CD71 binding Centyrins demonstrate single digit nanomolar affinities and are highly specific for CD71
- ✓ CD71 Centyrin-siRNA conjugates demonstrate potent mRNA knockdown in mouse and cyno animal models
- ✓ CD71 Centyrin-ASO conjugates demonstrate potent mRNA knockdown and are well tolerated in mouse animal models
- ✓ Centyrin-Oligonucleotide conjugates are efficacious at a dose that is a fraction of the total drug dose of antibody (mAb or Fab) oligonucleotide conjugates

Acknowledgements

- Frank Bennett
- Brooke Anderson
- Frank Rigo
- Payman Jafar-nejad
- Michele Carrer
- Punit Seth

Back Up

siRNA Candidate Selection – Iterative and highly reproducible

In silico siRNA screening and "Hit" selection