

CREATING A NEW CLASS OF RECEPTOR TARGETED GENETIC MEDICINES

Aro Biotherapeutics

OTS September 2021

Aro
BIOTHERAPEUTICS

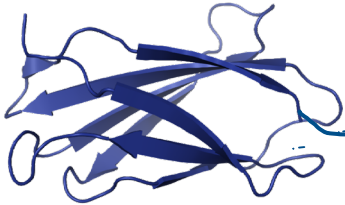
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Our vision

Unlock the potential of RNA medicines by enabling specific targeting to diseased tissues

Initial Focus: Centyrin – siRNA Conjugates

Centyrins target RNA therapeutic to specific cellular “address”



siRNA payloads modulate disease gene inside the cell



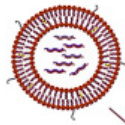
Wholly Owned Pipeline

Additional Pipeline Opportunities



IONIS™

Antisense Oligos



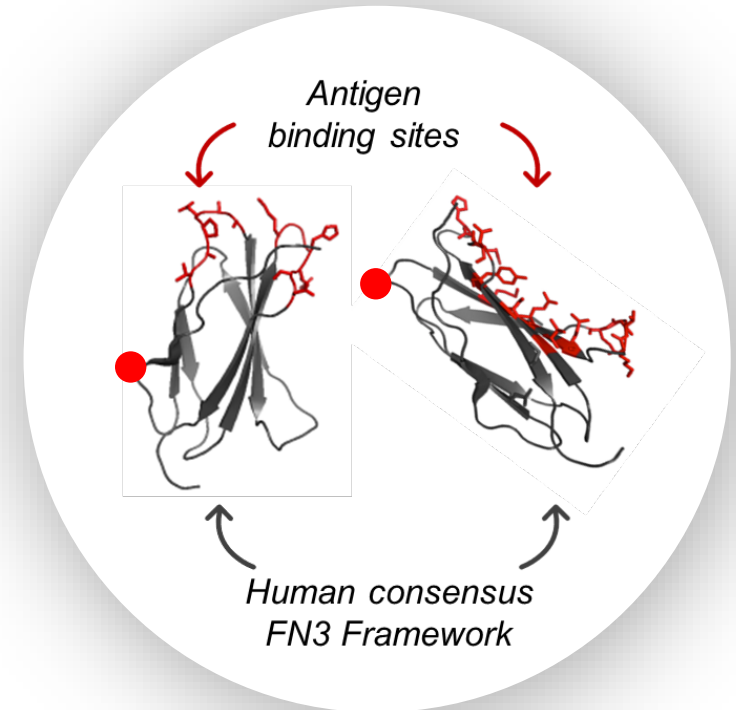
Messenger RNA

Centyrin overview

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

- Proprietary antigen binding platform
- Built on a consensus human Tenascin C FN3 framework
- Exceptional stability and solubility
- ~1/15 size of standard monoclonal antibodies
- Readily expressed in E. Coli as multi-specific proteins
- Facile site specific covalent conjugation to drug payloads

Ideal properties for targeted delivery of oligonucleotide therapies



● = Drug Conjugate Site

Centyrins are exceptionally stable proteins with T_m 's > 80 degrees, and remain folded at early endosomal pH

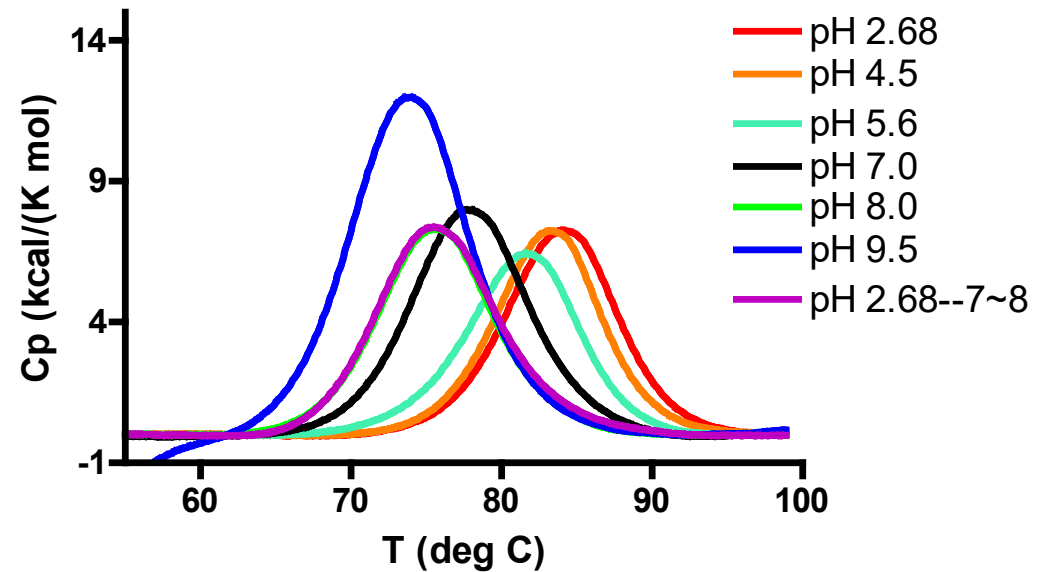
Early Endosome
~pH 6

Late Endosome
~pH 5-6

Lysosome
<pH 5



pH dependent melting temperatures support potential for Centyrin stability from early endosome to lysosome

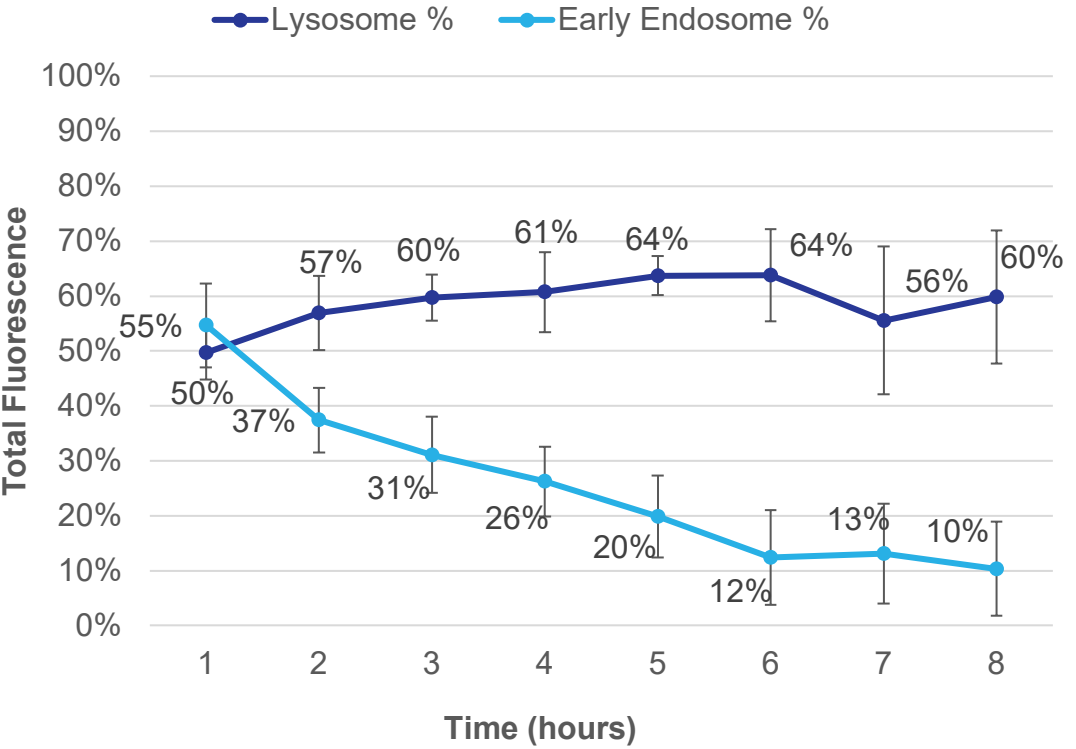


Centyrin Stability =

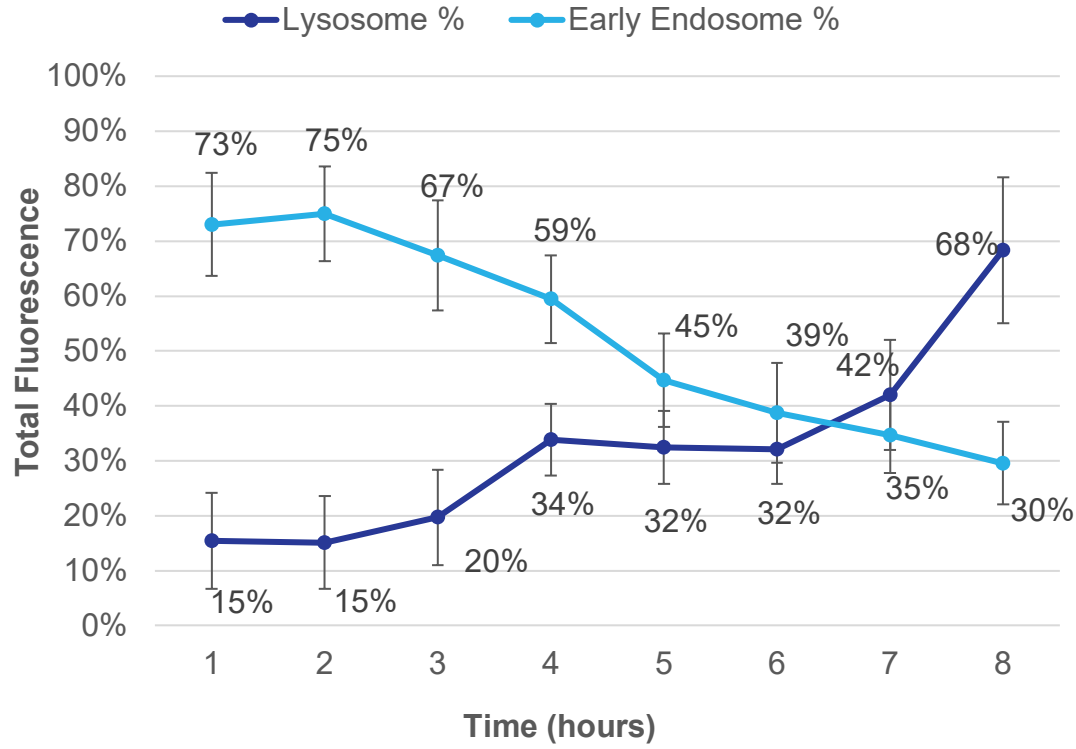
- Prolonged endosomal “depot”
- Reduced immunogenicity risk

Centyryn early endosome depot may be critical for observed intracellular activity of Centyryn-siRNA conjugates

Cetuximab



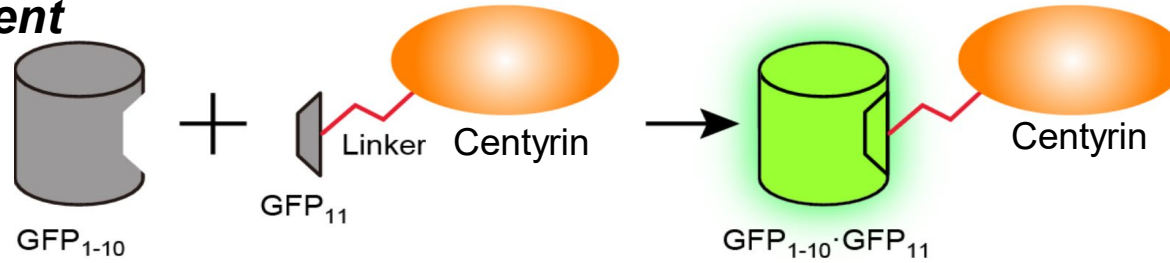
Centyryn



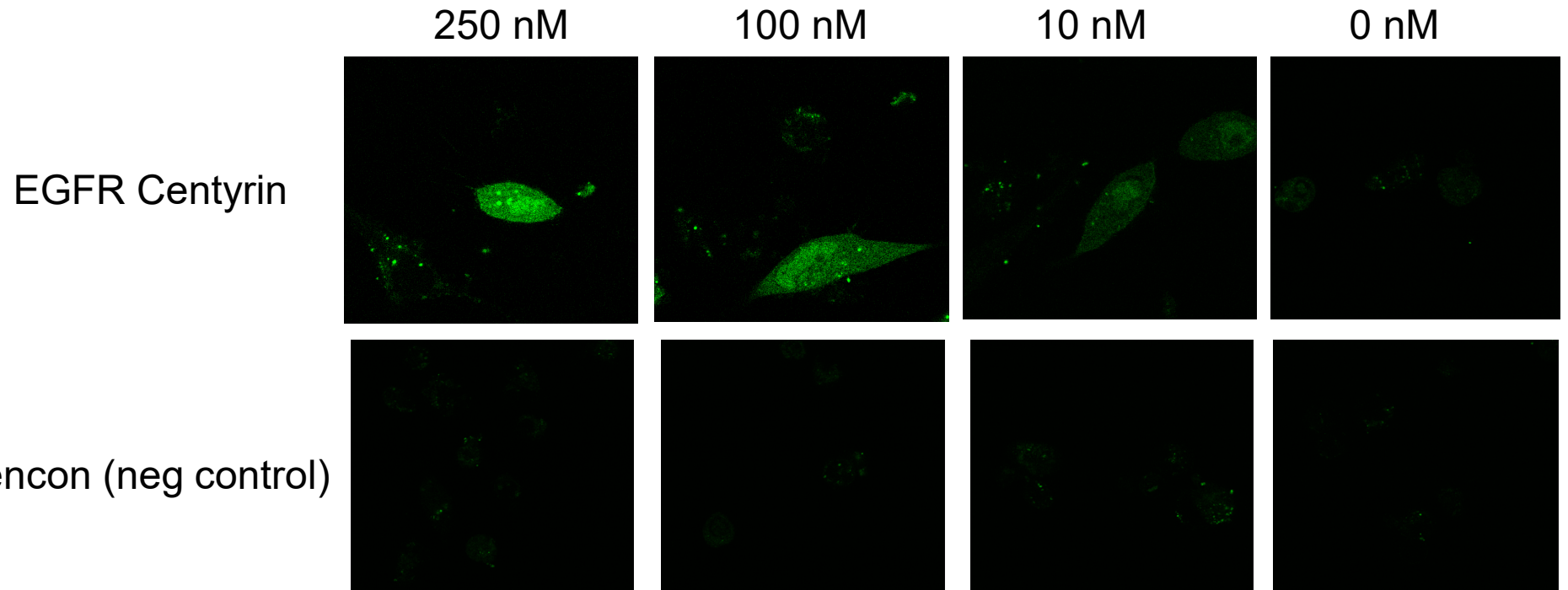
Percentage in Organelles Quantified by FIJI ImageJ in Cal27

Centyrins escape the endosome and traffic to the cytosol

“Split GFP” experiment



- GFP (1-10) expressed in cytoplasm and only fluoresces upon binding of GFP (11) peptide



24 hour treatment of HCC827-GR5 Lung cell line with varying concentrations of EGFR-GFP11 Centyrin or Tencon-GFP11 construct

Centyrin Scaffold lacks immunogenicity in human T cell assays

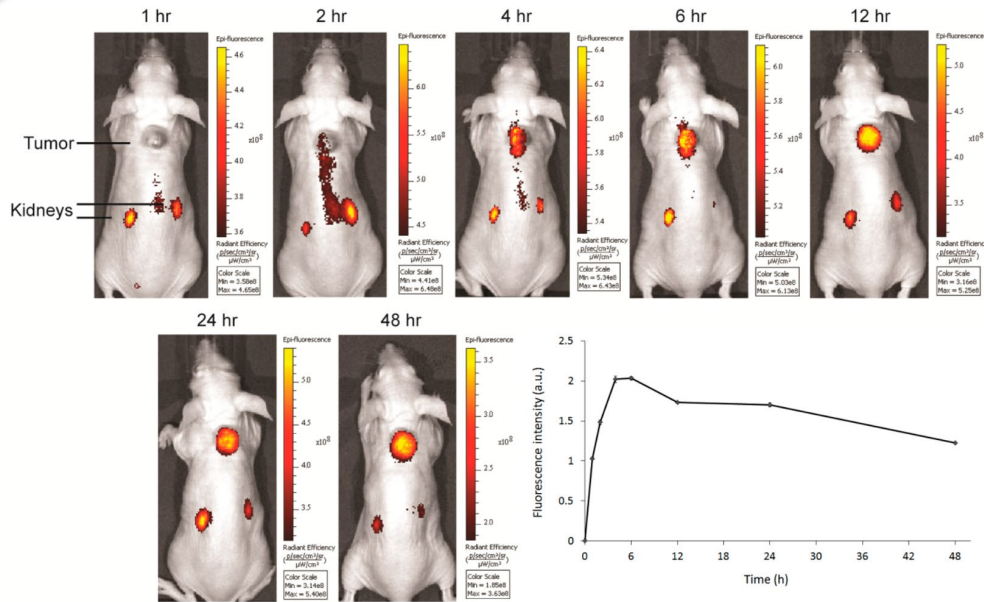
Dendritic cell:T cell assay co-culture was used to assess immunogenicity potential (ProImmune, Inc)

- 20 donor PBMC samples were HLA typed
- Allele distribution frequency of HLA class II across DRB1, DQB1, DPB1 was similar to that across the global population
- T cell activation assessed after 7 days

Table 4. RI Values for each Test Protein as Generated by Percent Antigenicity and Percent Stimulation (Percentage Stimulation above Background $\geq 0.5\%$, SEM=2)

Percentage Stimulation above Background $\geq 0.5\%$, SEM=2			
Protein ID	Percentage Antigenicity	Strength of Response (Mean %Stimulation)	Response Index (RI)
Ctrl 1 PPD	100.00	61.39	61.394
Ctrl 2 KLH	100.00	32.05	32.053
Tencon40	25.00	1.36	0.341

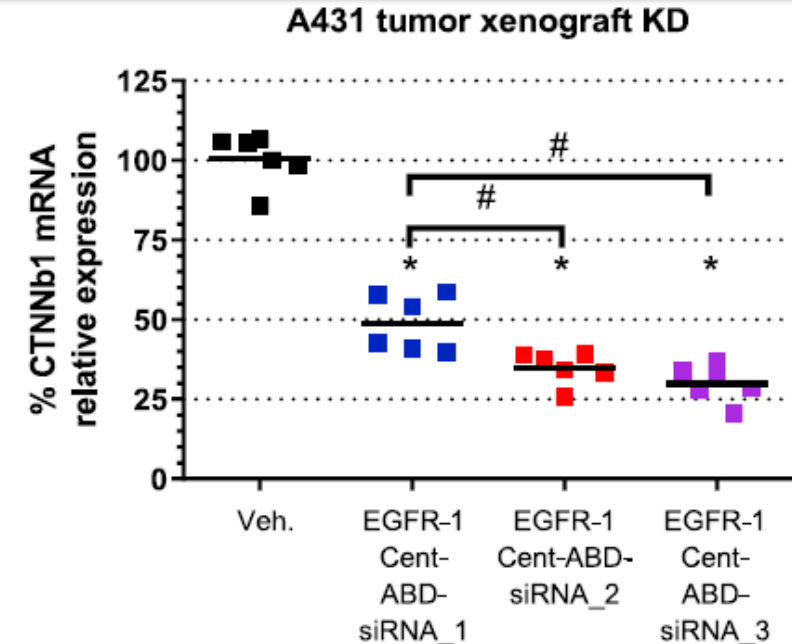
Initial Centyrin in vivo POC for payload delivery established in oncology



- EGFR Centyrin labeled with near infrared dye in mice bearing HCC827 tumor cells*
- Centyrin remains localized in tumor up to 48 hours

*Mahalingham, SM, et al. Bioconjugate Chemistry, 2017

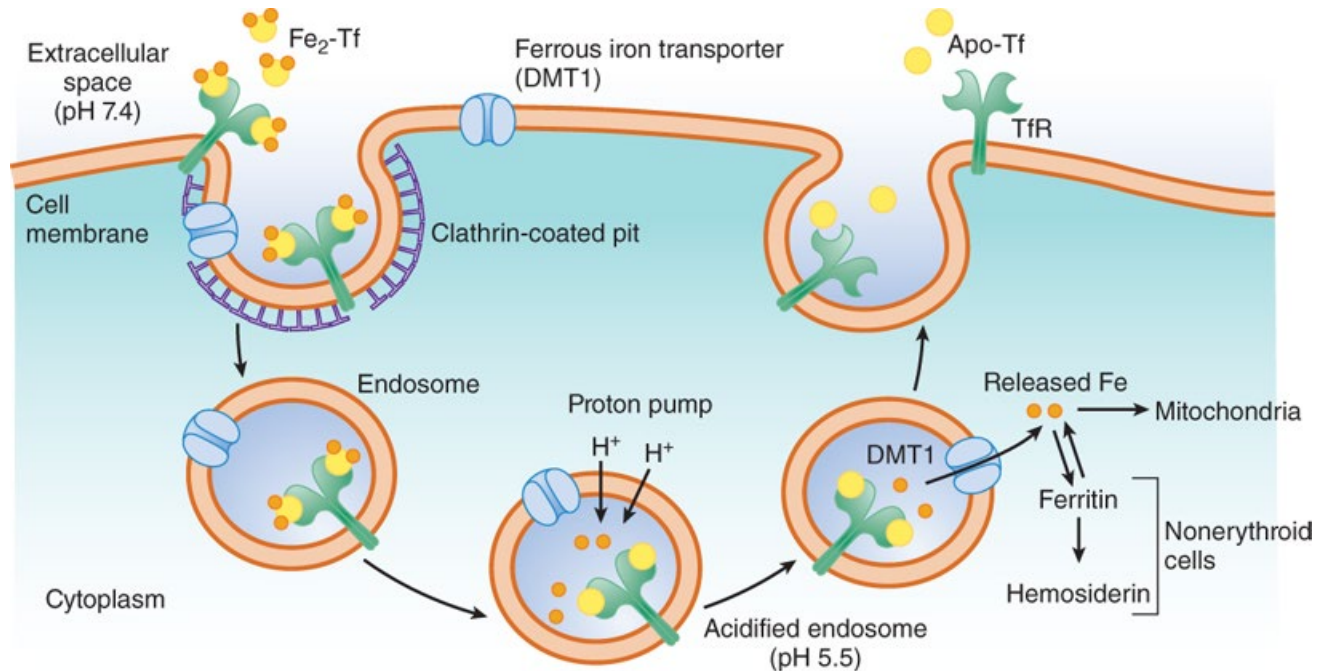
** Klein, et al, Centyrin ligands for extrahepatic delivery of siRNA, Molecular Therapy (2021), <https://doi.org/10.1016/j.ymthe.2021.02.015>



- EGFR Centyrin conjugated to CTNNb1 siRNAs and evaluated in A431 tumor xenograft model**
- Conjugate results in up to ~70% kd of CTNNb1 mRNA

Aro is developing an industry-leading position in targeting CD71

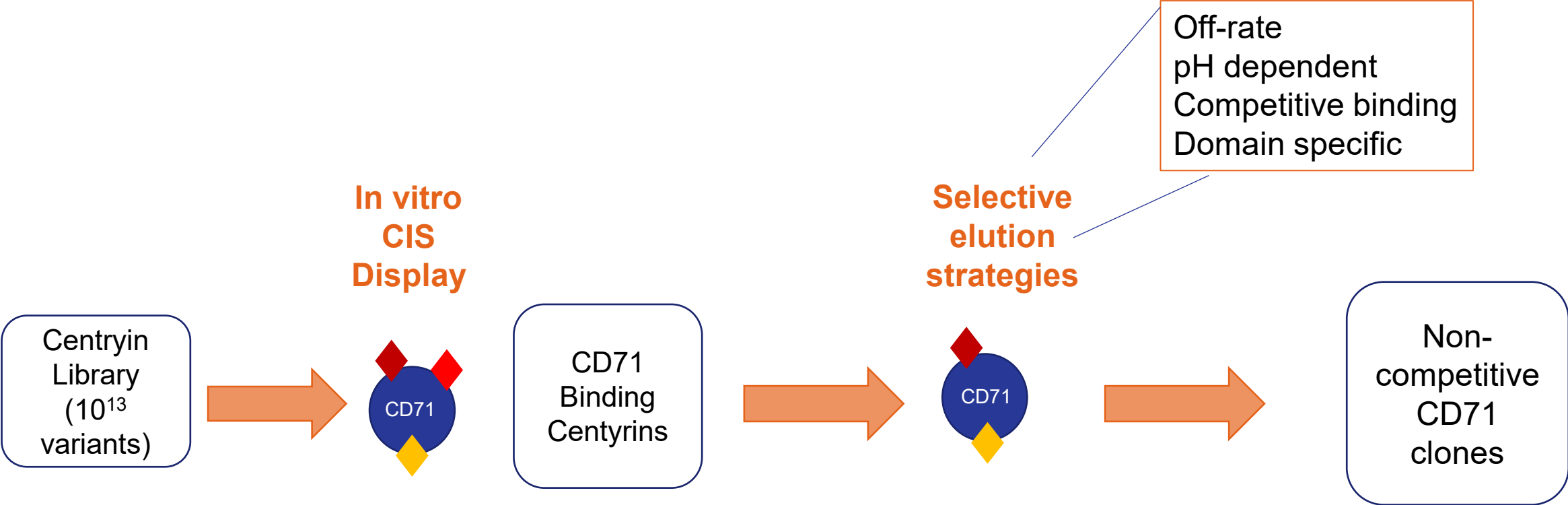
Customized CD71 Centyrins for different tissues to address a broad set of diseases



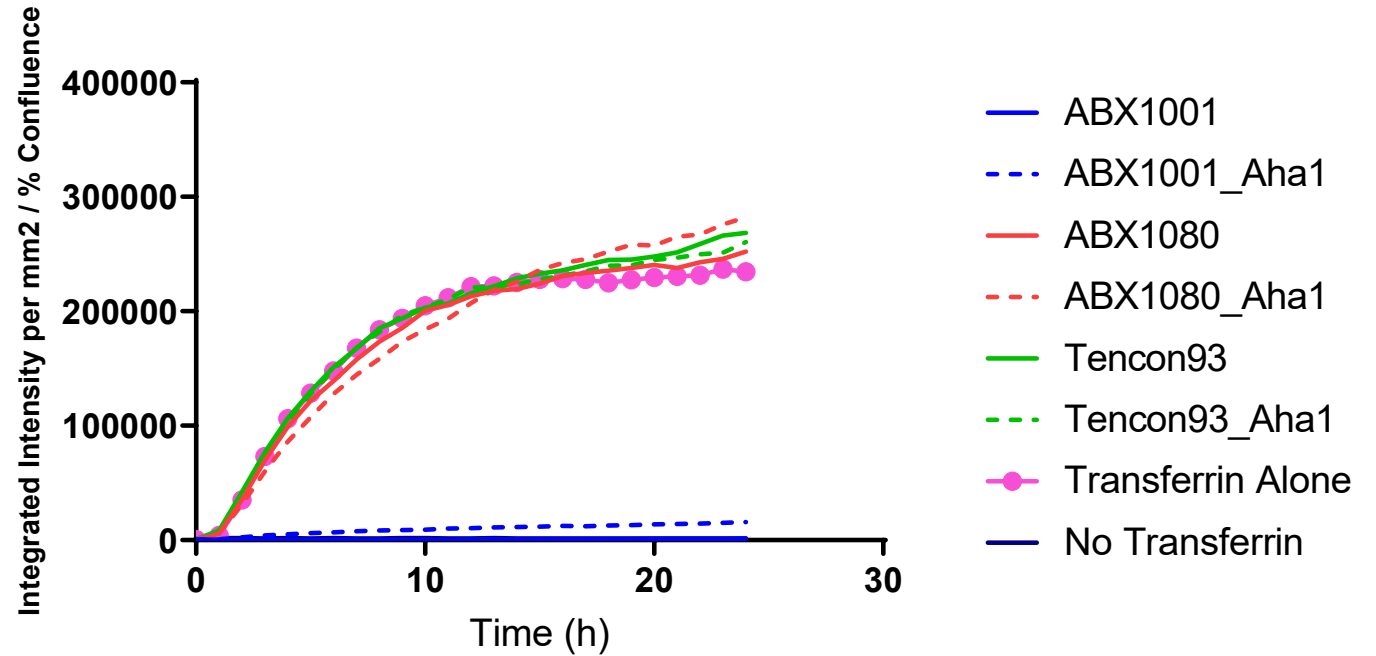
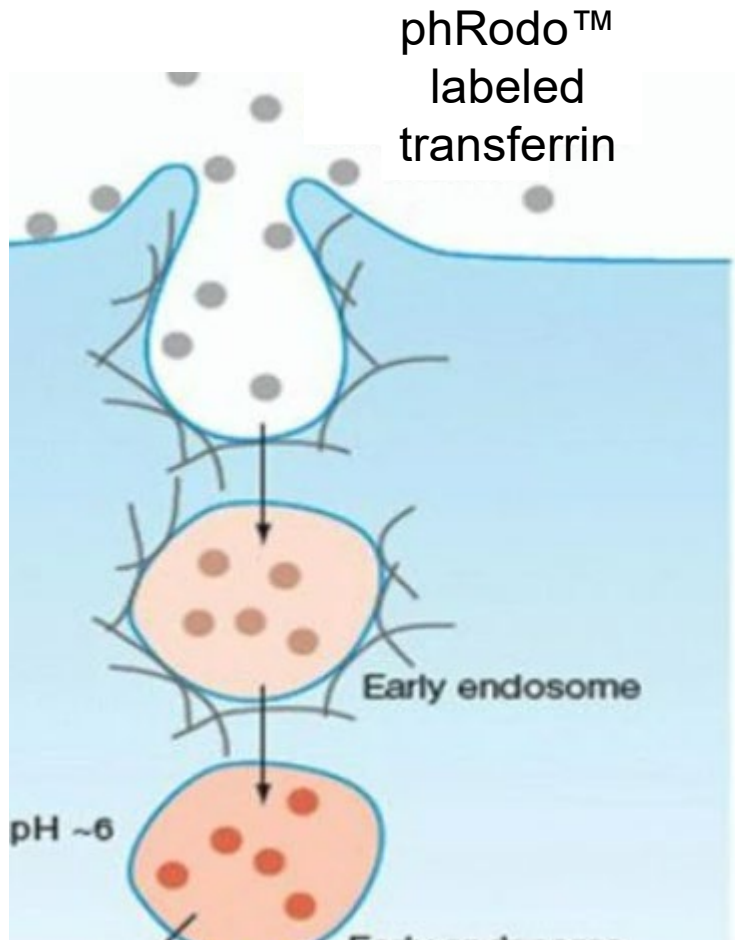
Source: Jon C. Aster, H. Franklin Bunn:
Pathophysiology of Blood Disorders, Second Edition
www.hemonc.mhmedical.com
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- Essential and ubiquitously expressed receptor responsible for iron transport into cells
- Efficient internalization on muscle, tumor cells, proliferating immune cells and endothelial cells at blood brain barrier
- We have generated a large diversity of CD71 Centyrins to enable efficient and customized targeting of various CD71+ cell types

CD71 Centyrins bind to multiple epitopes with a range of affinities



Selected CD71 Centyrins do not compete for transferrin uptake

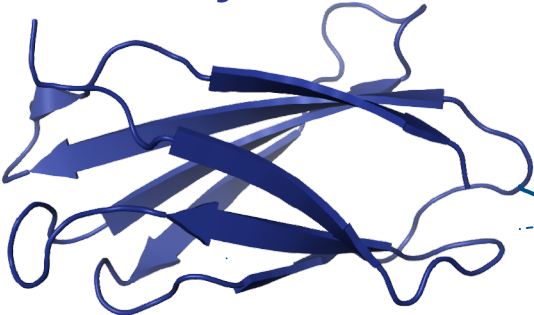


Human SKBR3 cells

Centyryn Oligonucleotide Platform

1

Proprietary Tissue Targeting
Centyryn



2

Established
Bioconjugation
Chemistry

3

Proprietary
Oligonucleotides



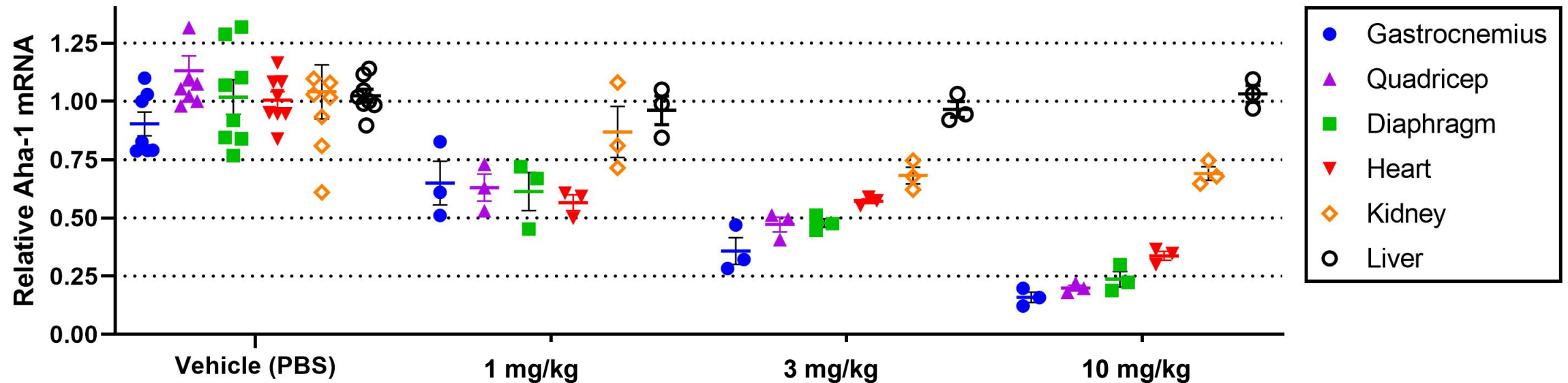
Robust and selective gene knockdown in skeletal and cardiac muscle

Tool CD71 Centyrin-AHA1 siRNA conjugate

Up to 80% gene knockdown observed 2 weeks after single dose

No / minimal gene knockdown observed in liver / kidney

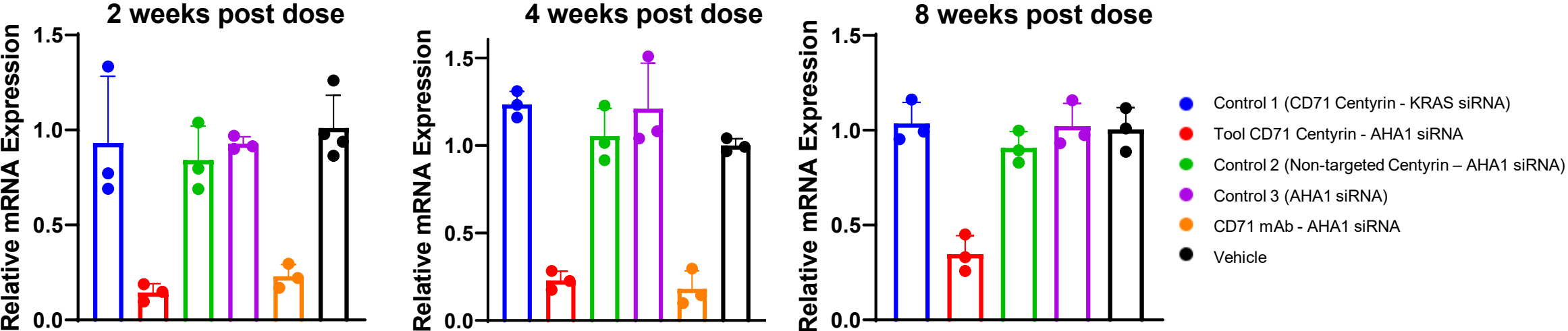
Strong dose-response relationship observed



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

Tool CD71 Centyryn conjugate drives sustained gene knockdown at fraction of mAb conjugate dose

AHA1 Knockdown, 10mg/kg siRNA, Gastrocnemius

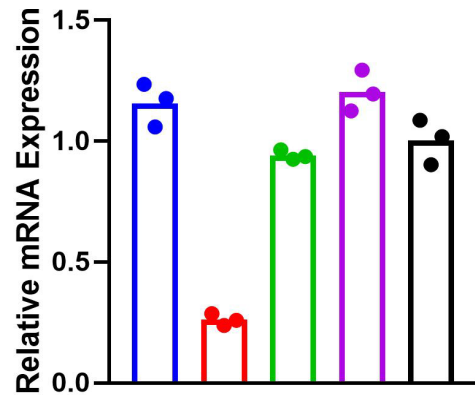


POC study with AHA1 housekeeping gene
C57/B6 mice received single dose of conjugates

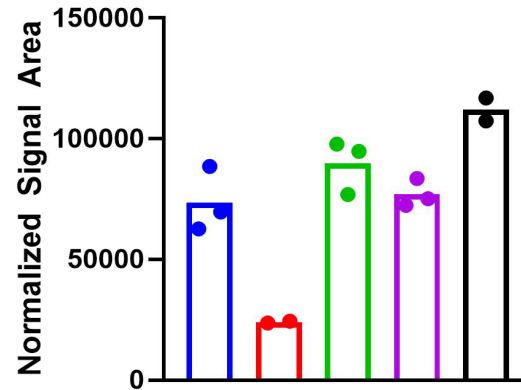
	Centyryn – siRNA conjugate	mAb – siRNA conjugate
AHA1 knockdown wk2	86%	77%
AHA1 knockdown wk4	77%	82%
AHA1 knockdown wk8	65%	N/A
siRNA dose (mg/kg)	10 mg/kg	10 mg/kg
Conjugate dose (mg/kg)	~18 mg/kg	~120 mg/kg

In vivo mRNA and protein knockdown are well correlated

AHA1 mRNA Knockdown
(Quadricep, 4 wks)

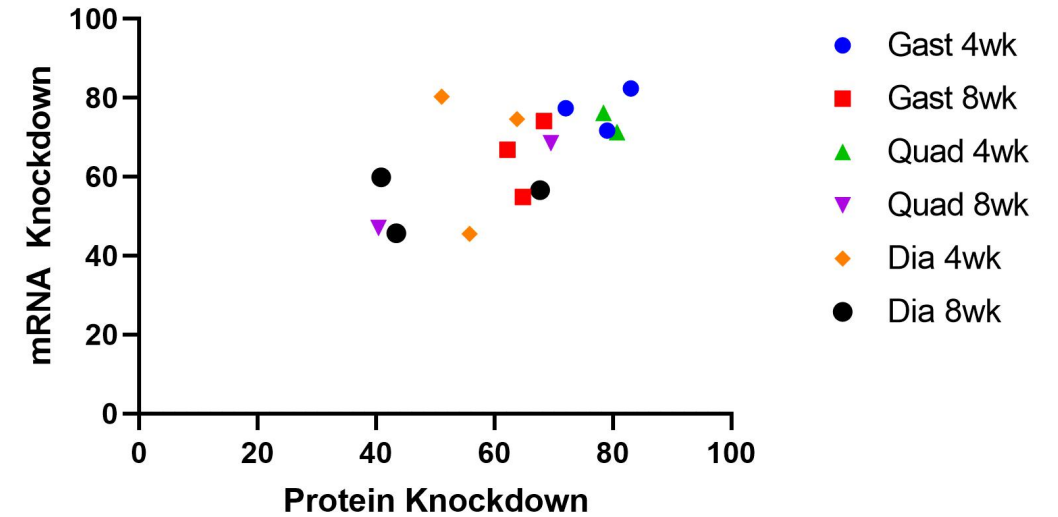


AHA1 Protein Knockdown
(Quadricep, 4 wks)

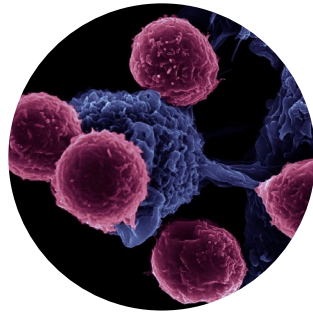


- Control 1 (CD71 Centyrin - KRAS siRNA)
- Tool CD71 Centyrin - AHA1 siRNA
- Control 2 (Non-targeted Centyrin - AHA1 siRNA)
- Control 3 (AHA1 siRNA)
- Vehicle

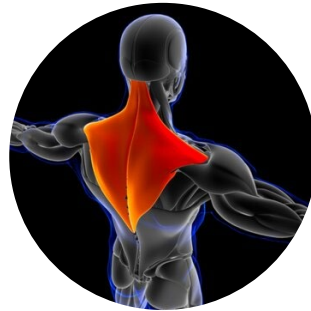
Long Term PD Study 2104081
Skeletal Muscle Correlation
4wk, 8wk post dose



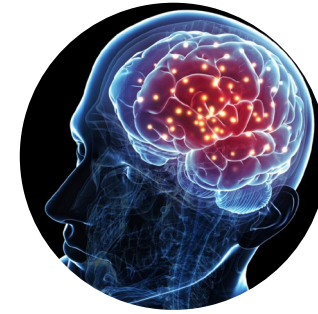
We are exploring Centyrin – siRNA conjugates across a broad range of disease areas



Immune Cells



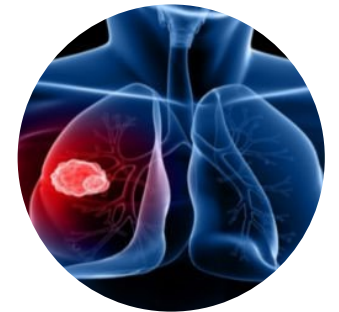
Skeletal Muscle



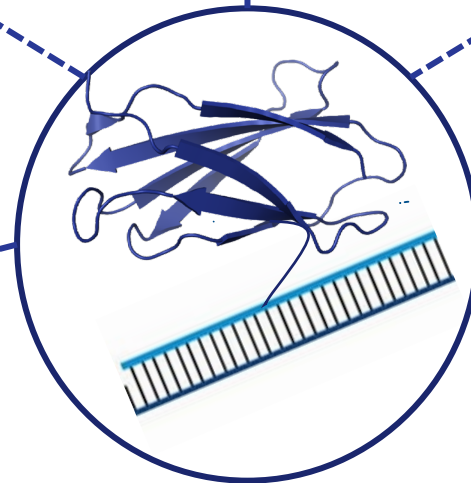
CNS



Heart



Tumors



In Vivo POC achieved with Centyrin – siRNA drug conjugates



Thank You

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