Discovery of ABX1100, a CD71 Centyrin-Gys1 siRNA Conjugate Clinical Candidate for the Treatment of Pompe Disease

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Abstract

RNAi therapeutics present a significant potential to treat diseases by selectively decreasing the level of target genes especially in cases where targets have been intractable by other methods. Aro Biotherapeutics is pioneering a new approach to tissue specific delivery using proprietary Centyrin technology. Centyrins are ~10KDa proteins that are conveniently expressed in E. Coli with high affinity, specificity, superior stability and solubility properties compared to standard monoclonal antibodies or antibody fragments. Despite recent successes in the liver with ASGPR targeted GalNAc conjugates, extra-hepatic delivery of siRNA and other oligonucleotide classes remains a challenge. Using Aro's proprietary Centyrin platform technology, we have demonstrated potent functional delivery of siRNA and ASOs extrahepatically. In head-to-head studies, we have demonstrated comparable potency with significantly lower dose of Centyrin–siRNA or Centyrin–ASO conjugates vs mAb or Fab–oligonucleotide conjugates.

Rare diseases present a rich class of targets that have been challenging to address by standard mAb or small molecule platforms. Aro is building a set of tools to address these targets initially in muscle cells. Pompe disease is a lysosomal storage disease characterized by loss of function mutations in alpha acid glucosidase (GAA), an enzyme involved in the degradation of glycogen in muscle tissues. The current standard of care for Pompe disease is enzyme replacement therapy (ERT) using recombinant GAA. However, ERT effectiveness is limited and requires frequent high doses and long infusion times. An alternative strategy is inhibition of glycogen synthase 1 (GYS1) activity to normalize muscle glycogen by suppressing glycogen synthesis.

This poster will highlight Aro's clinical candidate, ABX1100, a CD71 (TfR1) Centyrin-Gys1 siRNA conjugate, as the first muscle targeted approach for treatment of Pompe disease.

Centyrin Overview

- 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
- Readily expressed in E. Coli as mono-specific and multi-specific proteins
- Approximately 1/15th size of standard monoclonal antibodies
- Site-specific covalent conjugation to drug payloads
- Extensive patent portfolio; strong IP position

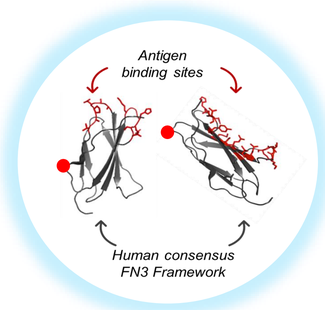


Figure 1: Aro's Centyrin

Ideal properties for targeted delivery of oligonucleotide therapies

Transferrin (CD71) Receptor

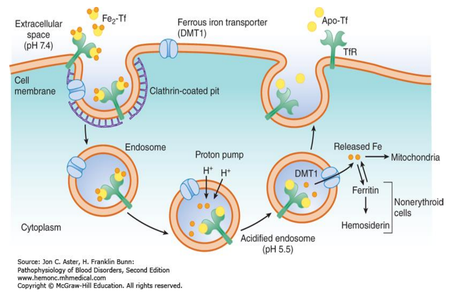


Figure 3A: Transferrin receptor 1 mediated endocytosis

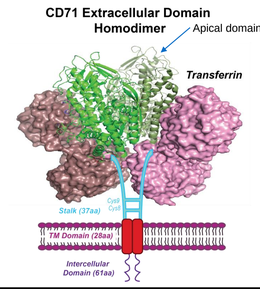


Figure 3B: Centyrin-targeting the Apical domain of CD71

Figure 2: Aro's Lead Identification Process

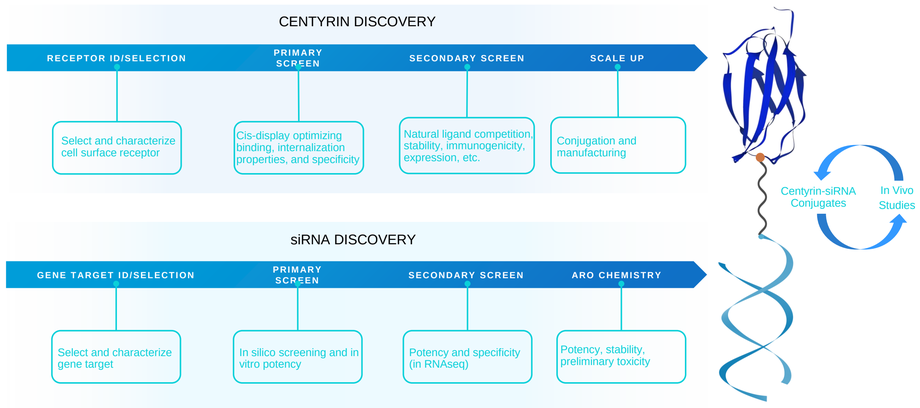
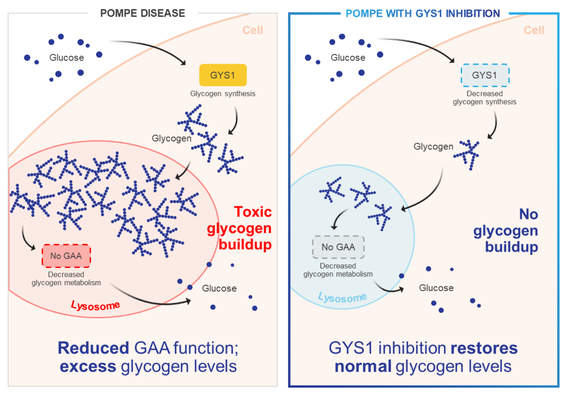


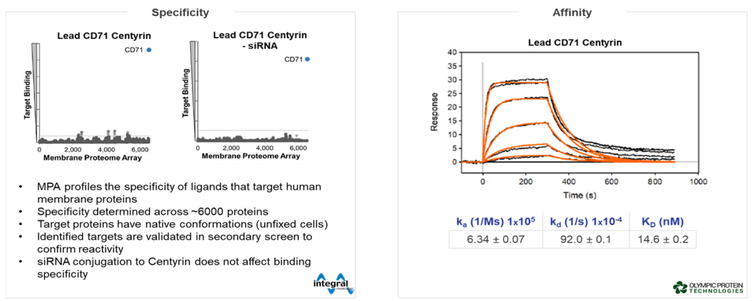
Figure 4: Gys1 is a Validated Target that Can Revolutionize the Treatment of Pompe Disease



- ~50% of LOPD pts fail to achieve durable response and/or discontinue enzyme replacement therapy
- Inhibiting Gys1 safely reduces muscle glycogen, validated by
  - ✓ *Human Genetic Data*
  - ✓ *Mouse Model Data*
  - ✓ *Clinical Data*

Inhibiting Gys1 treats Pompe disease at its source

Figure 5: CD71 Centyrin Selection



- MPA profiles the specificity of ligands that target human membrane proteins
- Specificity determined across ~6000 proteins
- Target proteins have native conformations (unfixed cells)
- Identified targets are validated in secondary screen to confirm reactivity
- siRNA conjugation to Centyrin does not affect binding specificity

Low Immunogenicity

Protein	Immune Response Index (RI)
PPD (Positive Control 1)	61.39
KLH (Positive Control 2)	32.05
Lead CD71 Centyrin	0.11
Lead CD71 Centyrin - siRNA	0.12

- 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
- Most approved biologics have RI value between 0-1

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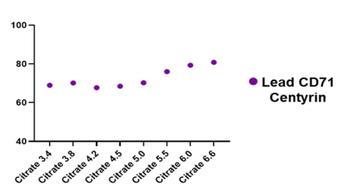
High Stability



- 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

Figure 6: Gys1 siRNA Selection

***In silico* screen**
Human-Cyno cross-reactivity
Minimal miRNA/off-target effects (OTE)
1000 > ~150 sequences

***In vitro* screen**
Synthesize ~150 sequences
Transfect modified siRNAs - In vitro gene KD, Tolerability (anti-proliferative)
~100 > ~10 lead sequences

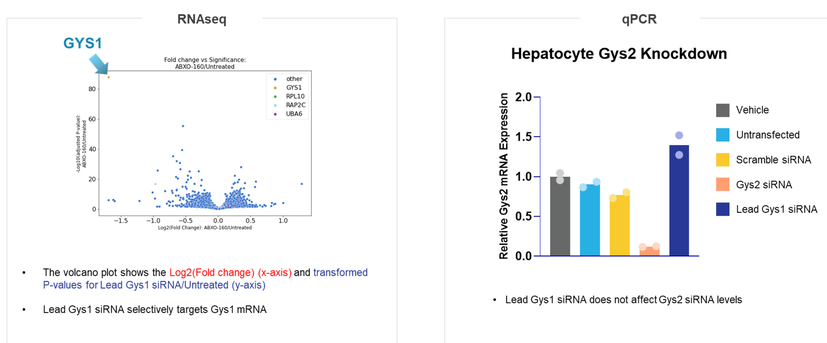
***In vivo* optimization**
Conjugate synthesis
Optimize for potency/stability
RNA seq (OTEs)
~5 candidates

Mouse/NHP efficacy
Rat tolerability

1-2 Leads

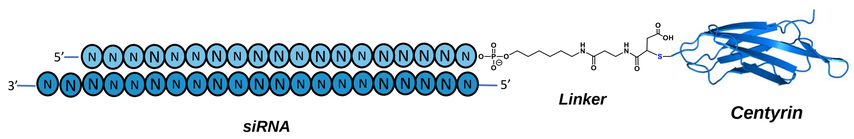
siRNA	qPCR KD (H358 cells)	
	EC50 (pM)	% Emax
ABXO-160	0.78	80.1
ABXO-124	2.64	70.5
ABXO-008	3.84	70.0
ABXO-123	4.04	75.5
ABXO-203	5.70	74.9
ABXO-114	6.71	75.5

Figure 7: Secondary siRNA Screens Ensure siRNA Target specificity



- The volcano plot shows the Log2(Fold change) (x-axis) and transformed P-values for Lead Gys1 siRNA/Untreated (y-axis)
- Lead Gys1 siRNA selectively targets Gys1 mRNA
- Lead Gys1 siRNA does not affect Gys2 siRNA levels

Figure 8: ABX1100 (CD71 Centyrin-Gys1 siRNA Conjugate)



- Aro's siRNA-linker employs proprietary and clinically validated designs
- Centyrin-siRNA conjugates use site-specific cysteine conjugation
- Aro's conjugation process is reproducible, generates site-specific Oligonucleotide to Centyrin Ratio (OCR1) conjugates with >95% purity
- Centyrin conjugation onto siRNA does not interfere with loading of the antisense to Ago2

Figure 9: ABX1100 Murine Surrogate Demonstrates Dose-dependent Activity in Muscle with No Effect in Liver (Pompe Mouse Model)

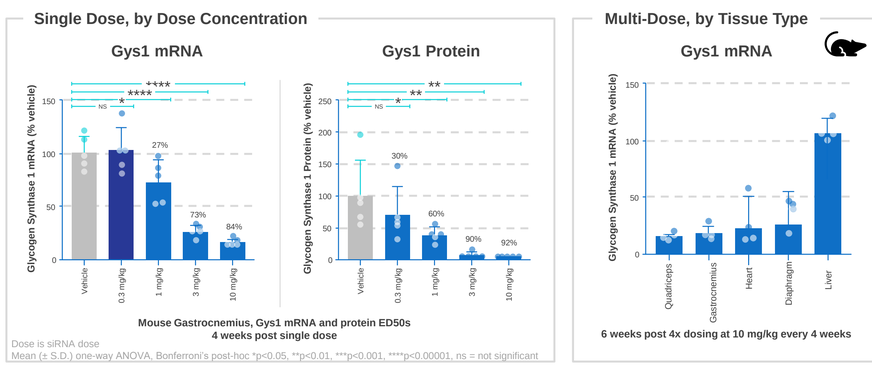


Figure 10: ABX1100 Murine Surrogate Greatly Reduced Gys1 mRNA, Gys1 Enzyme Activity and Muscle Glycogen in Pompe Mouse Model

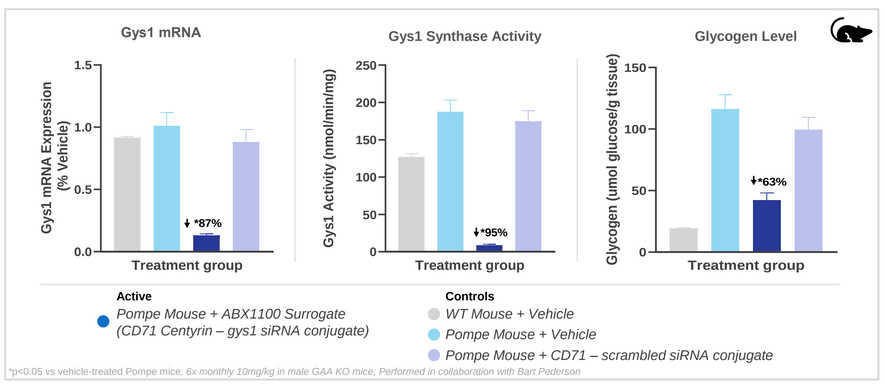
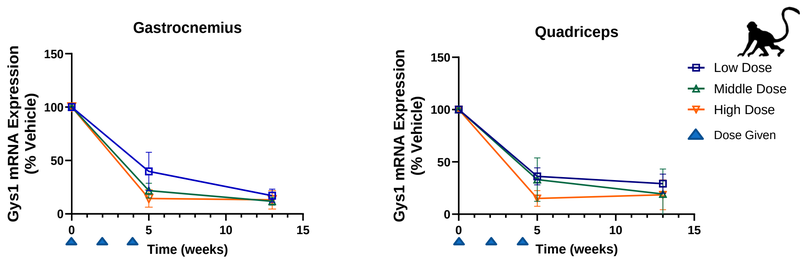
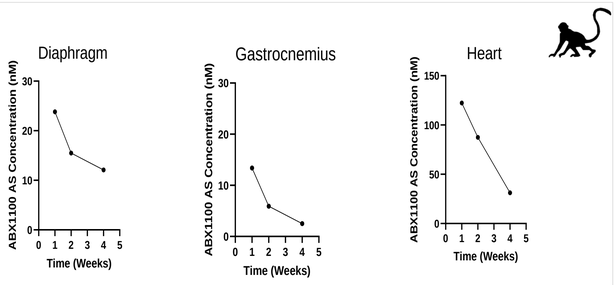


Figure 11: In Non-GLP NHP Tox Study ABX1100 Demonstrates Robust and Durable Effect in Muscles with No Adverse Safety Findings



- No ABX1100-related mortality, in life AE's, histology or laboratory findings; NOAEL 250 mg/kg
- Durable reduction of Gys1 mRNA in muscles lasting 8 weeks post last dose
- Rapid plasma clearance, and long siRNA half life in muscle; PK/PD model established

Figure 12: Pharmacological Levels of ABX1100 Achieved in Muscle Tissue After Single Dose in NHP



NHPs were injected IV with a single dose at 50mpk of ABX1100. At various time points post dose animals were euthanized, and various tissues were removed for an assessment of anti-sense siRNA levels. **The half life of the siRNA ranged from 2-4 weeks across the various tissues.** While high levels of siRNA were detected in liver (data not shown), there was no knockdown of mRNA in liver.

Summary of Findings From a 5-Week NHP GLP Tox Study

- ✓ NO ABX1100-related mortality
- ✓ No in life adverse events observed
- ✓ All microscopic pathology findings were considered non-adverse
- ✓ No ABX1100 related effects noted across hematology, coagulation, clinical chemistries or urinalysis
- ✓ Pathologist declared NOAEL at highest dose tested which yields a wide safety margin
- ✓ ABX1100 is stable in monkey plasma both in vitro and in vivo

Summary

- ABX1100, a CD71 Centyrin-Gys1 siRNA conjugate currently in Phase 1 clinical trials
- Received Orphan Drug Designation (ODD) and Rare Pediatric Disease Designation (RPDD)
- Phase 1 dose escalation study in normal human volunteers completed; initial data expected 2H 2024

- A novel and first targeted siRNA approach for reducing muscle glycogen synthesis, and the pathologic accumulation of glycogen in Pompe patients
- Highly specific CD71 targeting Centyrin and Gys1 siRNA identified
- Efficient, homogeneous, truly site-specific siRNA conjugation demonstrated
- Excellent and durable in vivo efficacy in Pompe mouse model and NHP demonstrated
- No in-life, clinical chemistry or histological toxicities seen with the clinical lead siRNA or Centyrin in mouse, Rat and NHP
- Pharmacological drug levels in muscle tissue achieved after single and repeat IV dosing
- ABX1100 highly effective at decreasing the Gys1 mRNA, protein expression in various skeletal muscles and cardiac tissue but had no pharmacological activity in liver
- Demonstrated long pharmacodynamic half-life in muscle, supports the potential for infrequent dosing in humans
- Had no adverse effects in a 5-week, repeat dose NHP GLP toxicology study, supporting first in human study, currently ongoing

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