Nonclinical Studies in Non-Human Primates on ABX-1100: A Centyrin:Gys1 siRNA Conjugate for the Treatment of Pompe Disease

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ABSTRACT:
Pompe disease is caused by deficiency of acid alpha-glucosidase (GAA), a glycogen degradative enzyme in lysosomes, resulting in membrane-bound glycogen accumulation in multiple tissues. This glycogen storage disease is characterized by progressive skeletal muscle weakness, respiratory distress, and in the early onset form, cardiomyopathy. The standard, and only approved, treatment of the disease is enzyme replacement therapy (ERT) with human recombinant GAA (rhGAA) to restore glycogen degradation in lysosomes. While ERT therapy extends life span, residual symptoms remain, with poor muscle uptake and immunogenicity limiting efficacy. We examined a novel Centyrin protein - short interfering ribonucleic acid (siRNA) conjugate termed ABX1100 which targets CD71 (transferrin receptor type 1, TfR1) and GYS1, a key enzyme involved in glycogen synthesis. To support clinical development, we have assessed stability of ABX1100 in serum, tissue and serum pharmacokinetics, Gys1 mRNA decreases and safety in non-human primates. ABX1100 was shown to be stable in serum in vivo and achieved pharmacologic levels of drug in skeletal muscle to mediate Gys1 mRNA knockdown. While clearance in plasma was rapid, ABX1100 levels in tissue persisted for ~4 weeks with a decrease in Gys1 mRNA that extended to 8 weeks post last dose. There were no safety issues noted at the highest dose tested in the GLP toxicology study which supports clinical development of ABX1100.

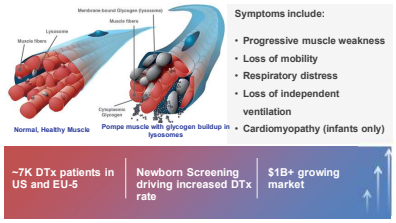

Figure 1. Pompe disease pathology and symptoms. The absence of lysosomal GAA leads to accumulation of glycogen in lysosomes resulting in skeletal and cardiac muscle pathologies

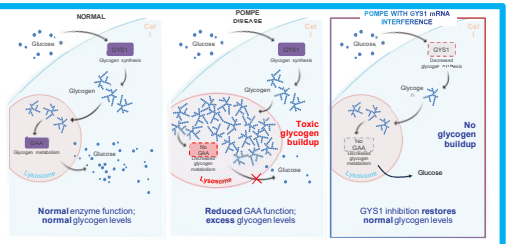

Figure 2. Inhibition of *Gys1* mRNA and protein expression is a new approach to reducing toxic glycogen accumulation in Pompe disease. By inhibiting glycogen synthesis with the Centyrin-*Gys1* siRNA conjugate (ABX1100), less glycogen will be available to accumulate in lysosomes.

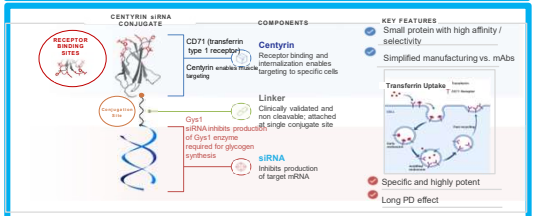

Figure 3. CD71 Centyrins* target siRNAs to tissues via receptor binding & internalization. CD71 centyrin conjugated to *Gys1* siRNA binds to transferrin receptor (CD71) leading to internalization and expected inhibition of *Gys1* expression. *Aro's proprietary platform for delivering oligonucleotides

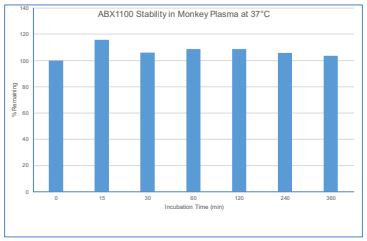

Figure 4. ABX1100 is stable in monkey plasma over 6 hours
1μM of ABX1100 was incubated in monkey plasma in vitro at 37ºC for various times and analyzed by mass spectrometry. There was no degradation of the Centryin or separation of the Centryin from the siRNA detected.

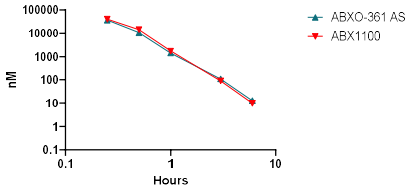

Figure 5. The ABX1100 Centyrin:siRNA conjugate is stable in plasma in vivo in NHP. Non-human primates were injected IV with a single dose of ABX1100 at 90 mpk (based on siRNA) and plasma levels of ABX1100 and the antisense strand (ABXO-361 AS) were measured by mass spectrometry. Bioanalytical data suggest that all detectable antisense must be part of the ABX1100 conjugate. There is no evidence for the separation of the anti-sense siRNA from the Centyrin. The plasma t1/2 was 0.68 hours.

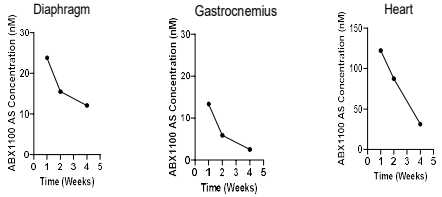

Figure 6. Pharmacologic levels of ABX1100 achieved in muscle tissue after a single dose in NHP Non-human primates were injected IV with a single dose at 50mpk of ABX1100. At various time points post dose animals were euthanized and various tissues were removed for an assessment of anti-sense siRNA levels. 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.

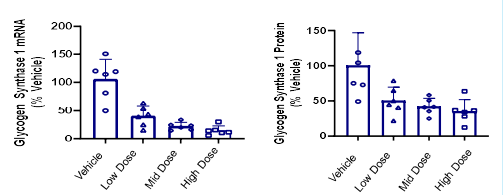

Figure 7. Dose dependent decreases in Gys1 mRNA and protein in the gastrocnemius in a NHP GLP toxicology study. Animals were dosed IV at a low, mid and high dose level on days 0, 15 and 29. On day 34 animals were euthanized and tissues removed for analysis. mRNA levels of Gys1 were measured by qPCR and Gys1 protein levels were measured using an ELISA assay.

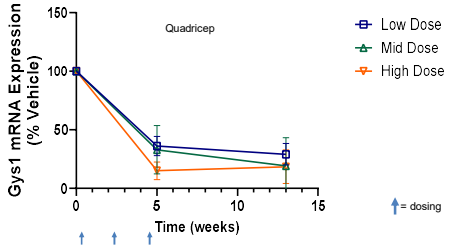

Figure 8. ABX1100 exerts a long term pharmacodynamic effect on Gys1 mRNA levels. NHP were dosed IV with 3 doses at a low, mid or high dose. Eight weeks after the last dose animals were euthanized and Gys1 mRNA levels were assessed in the quadricep by qPCR.

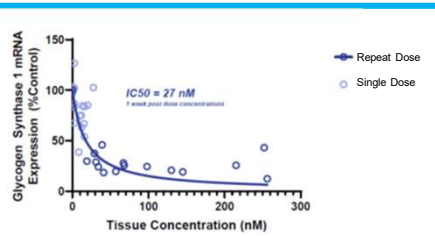

Figure 9. Correlation between skeletal muscle ABX1100 siRNA concentration and Gys1 mRNA expression in NHPs. NHPs were treated with a single dose or 3 doses with ABX1100 and tissue levels of both siRNA and Gys1 mRNA were measured one week post the last dose. Data are pooled across 2 studies. The IC50 in tissue of 27nM agrees well with other in vitro preclinical studies with ABX1100

SUMMARY OF SAFETY FROM A 5-WEEK NHP GLP TOXICOLOGY 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**

Summary and Conclusions

- ABX1100 a Centyrin:Gys1 siRNA conjugate currently in phase I clinical trials was assessed in a number of nonclinical NHP pharmacodynamic and toxicology studies
- ABX1100 is stable in monkey plasma both in vitro and in vivo
- ABX1100 IV dosing achieved pharmacologic drug levels in muscle tissues after single and repeat dosing
- ABX1100 was highly effective at decreasing the expression of Gys1 mRNA and protein in various skeletal muscles and cardiac tissue, but had no pharmacologic activity in liver
- ABX1100 has a long pharmacodynamic half life in muscle which supports the potential for infrequent dosing in humans
- ABX1100 had no adverse effects in a 5 week, repeat dose non-human primate GLP toxicology study, supporting a First in Human study in normal volunteers, currently ongoing
- ABX1100 is a novel approach for reducing muscle glycogen synthesis, and thereby the pathologic accumulation of glycogen in patients with Pompe Disease