Silencing DMPK gene by ARO-DM1, an RNAi therapeutic, for Type 1 Myotonic Dystrophy

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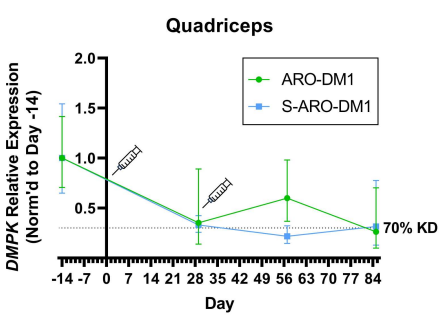
Overview:

- Pathogenesis of type I myotonic dystrophy (DM1) is driven by an expanded CUG trinucleotide repeat in the 3'-untranslated region of *DMPK* transcripts.
- Pathogenic transcripts of human *DMPK* sequester RNA splicing factors and thereby cause leading to myotonia, muscular dystrophy, cataracts, and cardiac conduction abnormalities.
- There is no approved drug for treating the root cause of DM1.
- To develop a therapeutic for DM1, we designed siRNA conjugates to silence *DMPK* mRNA in skeletal muscles
- ARO-DM1 and S-ARO-DM1 were identified as the best 2 conjugates, which target different positions of *DMPK* genes.
- In NHPs, *DMPK* mRNA expression in quadriceps and triceps was substantially decreased after two IV doses of ARO-DM1 or S-ARO-DM1, respectively.
- In the TREDT960i/HSA-rtTA mouse model of DM1 harboring a pathogenic *DMPK* transgene (*DMPK-CUG*), S-ARO-DM1:
  - Decreased the *DMPK-CUG* expression
  - Corrected the spliceopathies caused by overexpression of *DMPK-CUP* transgene

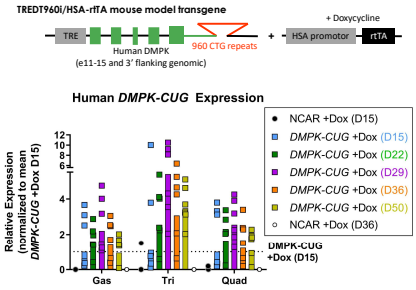
  When intravenously injected weekly at 20 and 40 mpk respectively.

Pharmacodynamic study of ARO-DM1 and S-ARO-DM1 in cynomolgus monkeys

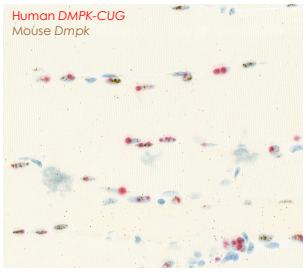


- After a single dose, muscular *DMPK* expression was reduced in quadriceps and triceps. Knockdown was maintained through Day 85 after a second dose on Day 29.
- ARO-DM1 and S-ARO-DM1 exhibited similar potency in quadriceps; however, in triceps, S-ARO-DM1 was slightly less potent.

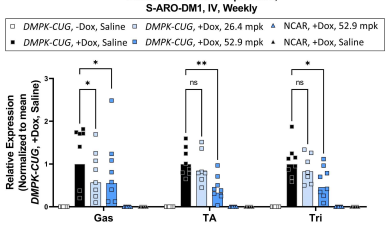
Pharmacological study of S-ARO-DM1 in TREDT960i/HSA-rtTA mouse model of DM1l



- *DMPK-CUG* expressing mice and noncarrier control mice (NCAR) were administered doxycycline by diet over 50 days.
- *In DMPK-CUG* expressing mice, *DMPK-CUG expression was induced* in skeletal muscles.
- *DMPK-CUG* expression plateaued by Day 22.



- Using RNAscope, human *DMPK-CUG* transcripts were detected in the myonuclei while endogenous mouse *Dmpk* was detected both in the myonuclei as well as in sarcoplasm.



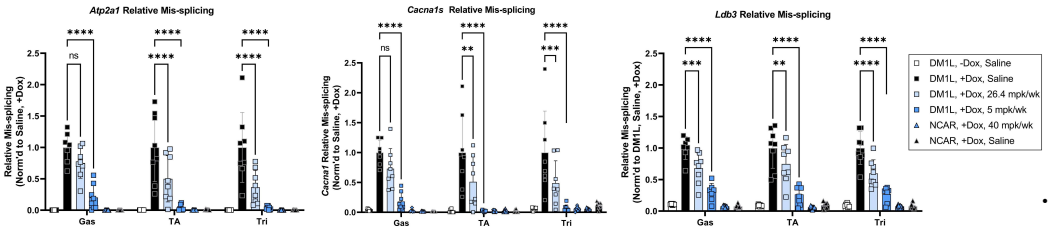
- S-ARO-DM1 was selected for pharmacological studies due to its sequence complementarity to *DMPK-CUG* in the transgenic mice.
- Weekly administration of S-ARO-DM1 decreased *DMPK-CUG* expression induced by doxycycline.

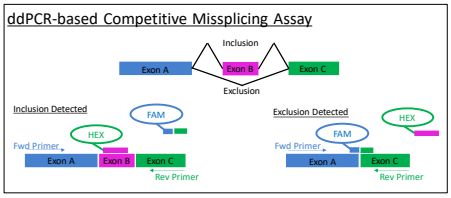
Conclusions

- ARO-DM1 and S-ARO-DM1 are potent RNAi therapeutics that silence skeletal muscle *DMPK* mRNA
- S-ARO-DM1 exhibited efficacies in reducing the expression of pathogenic *DMPK-CUG* transgene and correcting spliceopathies in the skeletal muscles of a mouse model of DM1
- ARO-DM1 phase 1/2a studies are in progress in patients with DM1

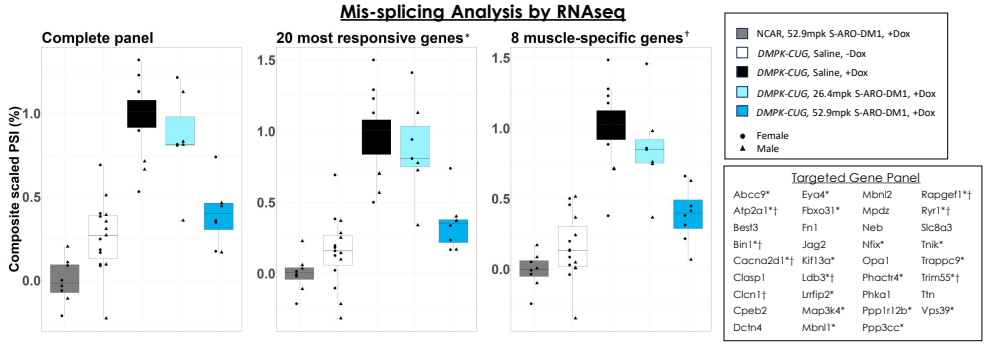
Analysis of Spliceopathies in the TREDT960i/HSA-rtTA mice treated with S-ARO-DM1

Relative Mis-splicing by ddPCR-based Competitive Probe Analysis





Mis-splicing Analysis by RNAseq



Targeted Gene Panel

Abcc9*	Eya4*	Mbnl2	Rapgef1*†
Atp2a1*†	Fbxo31*	Mpdz	Ryr1*†
Best3	Fn1	Neb	Slc8a3
Bin1*†	Jag2	Nfix*	Tnik*
Cacna2d1*†	Kif13a*	Opa1	Trappc9*
Clasp1	Ldb3*†	Phactr4*	Trim55*†
Clcn1†	Lrrfip2*	Phka1	Ttn
Cpeb2	Map3k4*	Ppp1r12b*	Vps39*
Dctn4	Mbnl1*	Ppp3cc*	

- Spliceopathies were analyzed by 2 different assays using the mRNA from the gastrocnemius of DMPK-CUG and NCAR mice: ddPCR-based competitive mis-splicing and RNAseq analysis.
- Mis-splicing of 3 marker genes reported in the literature was detected by the ddPCR based assay.
- S-ARO-DM1 treatment corrected the mis-splicing of those 3 marker genes.
- Mis-splicing of a targeted 35 gene panel was examined by RNAseq.
- Composite Percent Splice In Index (PSI) was analyzed for the entire panel; the 20 most responsive genes to S-ARO-DM1, and 8 genes primarily expressed in skeletal muscles.
- S-ARO-DM1 treatment corrected the mis-splicing in all 3 panels.