Title: PPMO's as Therapeutic Modulators for Myotonic Dystrophy Type 1

Author: Ashling Holland, Miguel Varela, Arnaud F. Klein, Richard Raz, Andrey Arzumanov, Michael J. Gait and Matthew J.A. Wood
Name: Ashling Holland
Email: ashling.holland@dpag.ox.ac.uk
Institution: Department of Physiology, Anatomy and Genetics, University of Oxford
Country: England

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. DM1 is caused by the pathological microsatellite (CTG) repeat expansion in the 3'untranslated region of the *DMPK* gene. Mutant RNAs containing the repeat CUG expansions are retained in the nucleus as foci and sequester proteins required for the regulation of mRNA splicing and translation. These perturbations result in a multisystemic disorder characterised by myotonia, progressive muscle weakness, cardiac arrhythmias, cataracts and impaired endocrine and nervous system function. To date there is no cure for DM1. Antisense oligonucleotides (ASOs) are a promising genetic therapy for RNA gain-of function diseases like DM1. A leading strategy for an enhanced delivery system is modulation of ASO chemistry though peptide conjugation. This allows for tissue-specific delivery while directly targeting CUG repeat expansions that can interfere with abnormal sequestration and binding of RNA-binding proteins like MBNL1.

This study investigates the therapeutic ability of an arginine-rich cell-penetrating peptide, pip6a, conjugated to an ASO targeting the CUG repeat in DM1 patient cells and in the HSA^{LR} mouse model of DM1. Pip6a-PMO treatment *in vitro* can correct molecular abnormalities by liberating MBNL1 and normalising splicing profiles in patient cells. Single intravenous administration of pip6a-PMO can partially correct mis-splicing events in gastrocnemius muscle. Repeat administration of PPMO successfully normalises mis-splicing events in skeletal muscle, has reduced foci, illustrates redistribution of MBNL1 and fully corrects myotonia phenotype. This treatment also has positive long lasting effects, up to 6 months, in HSA^{LR} mouse skeletal muscle.

This work provides insights into the application of arginine-rich cell-penetrating peptide coupled to PMOs for treatment of myotonic dystrophy type 1. Pip6a-PMO demonstrates an encouraging therapeutic potential for DM1 patients.