Systemic Delivery of Circular RNA Encoding Partial Dystrophins and Expression in Skeletal Muscle

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Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is a devastating, lethal muscle disease caused by mutations in the DMD gene that result in the absence of dystrophin protein expression, triggering rapid, severe muscle wasting. The large size (115kB) of the dystrophin coding region has made systems like full-length protein (427kDa) replacement unachievable to date. Becker muscular dystrophy is a milder dystrophy where patients express truncated dystrophin protein and exhibit increased lifespans, suggesting that expression of shortened versions of dystrophin may induce partial function and slow disease progression. Current therapies are targeting the re-expression of truncated versions of dystrophin (micro-dystrophin) via gene replacement or exon skipping strategies using adeno-associated virus (AAV) delivery systems. However, these methods are hampered by reduced cloning capacity and (viral) immunogenicity that limits redosing capabilities. At Orna Therapeutics, we are developing a protein therapeutic using circular RNA technology (oRNA™) that, coupled with our UHP delivery system, exhibits durability, protein expression, non-lyer tissue distribution, and multi-dosing capabilities. In addition, our platform offers unprecedented payload capacity that can accommodate significantly large constructs, opening new opportunities in therapeutic areas such as muscle disease.

oRNA™ Technology

Orna’s technology relies on a self-templating mechanism to co-transcriptionally create full-length circular RNAs robustly and efficiently. A key component is the choice of ionizable lipid, which can influence cell specificity. Each lane corresponds to a different concentration with the last lane being mock treated. Vinculin used as a muscle specific loading control.

Lipid Nanoparticles

Cryo EM of Lipid Nanoparticles

Lipid nanoparticles (LNPs) have now been clinically and commercially validated for delivery of both long (coding) and short (RNA). Mouse commercial LNPs have 4 lipid components, including an immunostable lipid, helper lipid, FEG lipids, and cholesterol), as well as the nucleic acid payload. A key component is the choice of ionizable lipid, which can influence cell uptake and payload escape from the endosome, allowing for ultimate activity of the RNA cargo inside a cell.

Cell Free Translation of partial and full-length dystrophin

Expression of Partial Dystrophin in Human Primary Skeletal Muscle Myotubes

• This specific Becker variant has an internal deletion of about 46% of full dystrophin protein
• It is associated with a very mild muscular dystrophy
• This construct prompted the production of micro dystrophins (amenable to AAV packaging) as a therapeutic for DMD

Expression of Full-Length Dystrophin in Human Primary Skeletal Muscle Myotubes

• oRNA™ encoding micro-dystrophin and the Becker variant were formulated into lipid nanoparticles and used to transfect primary human skeletal muscle myotubes in culture

Systemic Delivery and Expression of micro-dystrophin in mdx mouse muscle

Conclusions

Using our high capacity oRNA™ technology, in combination with our proprietary lipid nanoparticles we have successfully shown:

• Expression of circular RNA encoding micro (167kDa), Becker (228kDa) and full-length (427kDa) dystrophin in primary human skeletal muscle myotubes

• Expression (4.2% of WT dystrophin expression) and correct sarcomerolocalization of systemically delivered micro-dystrophin in the mdx mouse model of DMD (dystrophin-null)

Our oRNA™ technology represents a scalable, durable protein replacement therapy with a cargo capacity capable of expressing large constructs that can one day provide a currently unmet therapy for Duchenne Muscular Dystrophy.