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

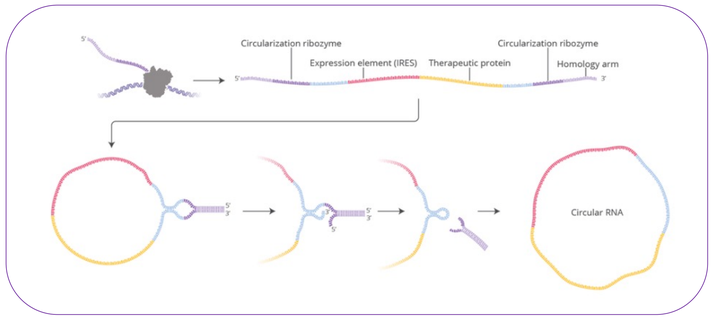
Tatiana Fontelonga, Elena Minissale, Allie Lundberg, Sharmistha Kundu, Kristen Ott, Trent Stevens, Yessica Wiryawan, Ian Langer, Rahul Vungutur, Corey Ciullo, Junghoon Yang, Emily Fitzgerald, Thomas M. Barnes, Nelson Chau, Robert Mabry, Allen Horhota, R. Alexander Wesselhoeft, Shobu Odate

Orna Therapeutics, Cambridge MA, 02139

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 (11kB) of the dystrophin coding region has made systemic 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-coding circular RNA technology (oRNA™) that, coupled with our LNP delivery system, exhibits improved durability, protein expression, non-liver 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.

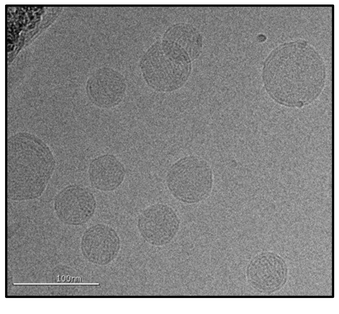
oRNA™ Technology

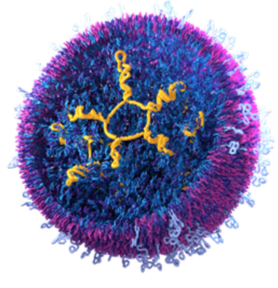


Orna's technology relies on a self-circularizing mechanism to co-transcriptionally create full-length circles robustly and efficiently. The main features used to create oRNA circles include a circularization ribozyme, a protein-coding sequence, an IRES element that drives expression of the coding sequence, and accessory elements to facilitate production and manufacturing steps. oRNA constructs formed using these methods can be produced rapidly at large scale and with high purity, without the need for chemical modifications or capping technologies used with traditional mRNA.

Lipid Nanoparticles

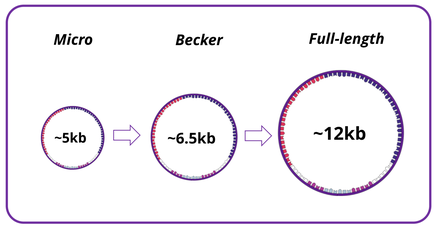
Cryo EM of Lipid Nanoparticles

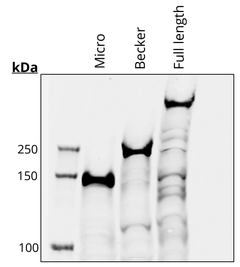




Lipid nanoparticles (LNPs) have now been clinically and commercially validated for delivery of both long (coding) and short RNAs. Most commonly, LNPs have 4 lipid components, including an ionizable lipid, helper lipids, PEG lipids, and cholesterol, as well as the nucleic acid payload. A key component 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





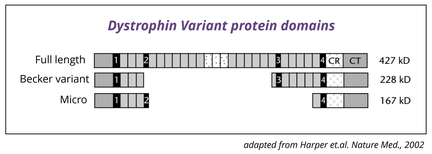
Translation and protein expression of oRNA™ constructs encoding micro, Becker variant and full-length dystrophin in a cell free translation (CFT) assay

Three dystrophin oRNA constructs of increasing size were placed in tube with a 1-step human IVT kit that includes HeLa cell accessory proteins capable of translating RNA into protein. The right panel shows a Western blot using a V5-tag antibody recognizing all three dystrophin constructs, demonstrating expression of micro, Becker variant and full-length protein. These data suggest intact circularization and translation capacity of our large sized oRNAs.

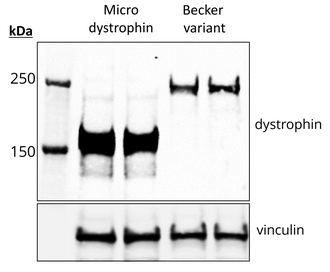
Expression of Partial Dystrophin in Human Primary Skeletal Muscle Myotubes

- *This specific* ***Becker variant*** has an internal deletion of about 46% of total 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

England, S.B., et.al., Nature, 1990



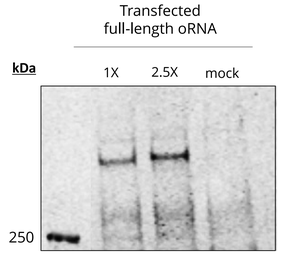
adapted from Harper et.al. Nature Med., 2002



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

Protein expression of both micro and Becker variant oRNA at 167 and 228kDa, respectively, using a western blot for V5-tag present in both constructs for specificity (duplicates). Vinculin used as a muscle specific loading control.

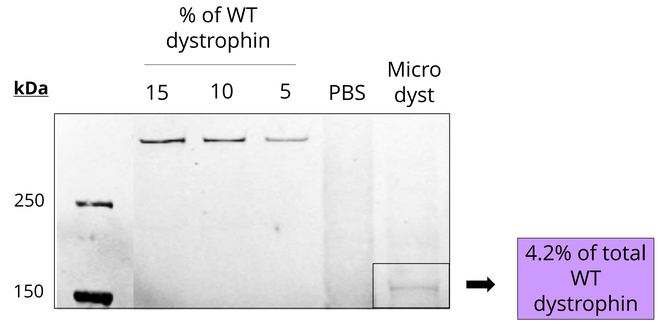
Expression of Full-Length Dystrophin in Human Primary Skeletal Muscle Myotubes



oRNA™ encoding full-length dystrophin was used to transfect primary human skeletal muscle myotubes in culture

Protein expression of full length (12kb) oRNA shown at 427kDa using a western blot for V5-tag present in the construct for specificity. Each lane corresponds to a different concentration with the last lane being mock treated.

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

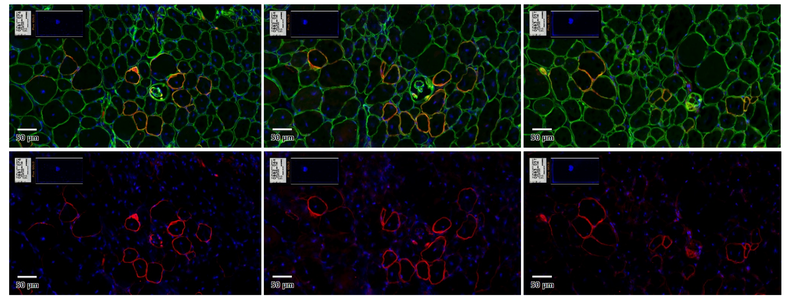


oRNA™ encoding micro-dystrophin was formulated into lipid nanoparticles and delivered systemically into the mdx mouse model of DMD (dystrophin null)

Protein expression in the quadriceps muscle was determined via western blot and quantified as a percent of full-length dystrophin expressed in a wild-type mouse quadriceps muscle. Expression from one IV dose determined to be 4.2%.

Micro-dystrophin localized to muscle sarcolemma

Laminin micro-dystrophin nuclei



oRNA™ encoding micro-dystrophin was formulated into lipid nanoparticles and delivered systemically via IV injection to the *mdx* mouse model of DMD (dystophin-null)

Three sections of the quadriceps muscle of treated *mdx* mice are shown above, sections were taken throughout the muscle to give a global view of micro-dystrophin expression distribution. The top panels have laminin-211 outlining the sarcolemma of the myofibers overlayed with micro-dystrophin (overlay in orange) with DAPI stain in blue showing individual nuclei. Bottom panels are replicates of the top without the laminin-211 outline to give a better view of micro-dystrophin expression at the myofiber (shown in red) with DAPI outlining nuclei.

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 sarcolemmal localization of systemically delivered micro-dystrophin in the *mdx* mouse model of DMD

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