1222 *IN SITU* CAR THERAPY USING ORNA™ LIPID NANOPARTICLES REGRESSES TUMORS IN MICE

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Background LNP-mediated delivery of long coding RNA has been clinically validated for vaccines and gene editing. We have been developing a novel, synthetic, circular coding RNA platform (oRNA technology) which exhibits significant improvements in production, expression and formulation compared to mRNAs. Lacking the cap structure of mRNA, our oRNA technology uses a proprietary sequence-based IRES element to initiate protein translation in target cells. At the same time, *ex vivo* generated chimeric antigen receptor (CAR) T cell therapies have had tremendous success in treating hematologic malignancies, yet manufacturing, safety and efficacy challenges remain. At Orna Therapeutics, we are combining oRNA technology with novel immunotropic LNPs to address these challenges, by creating off-the-shelf "autologous" *in situ* CAR (isCAR™) therapies.

Methods Orna's immunotropic LNPs show preferential biodistribution to the spleen, with oRNA reporter expression detected in multiple immune cell subsets, including T cells, macrophages and NK cells. Delivery to immune cells is preserved across mice, rats and non-human primates. *In vitro*, expanding human T cells expressing an anti-human CD19 CAR oRNA show potent and sustained cytotoxicity and pro-inflammatory cytokine production compared to controls. To maximize protein expression, we developed FoRCE (**F**ormulated **o**RNA **C**ell-based **E**valuation)[AB1] [AW2] : a robust high-throughput platform that enables parallel arrayed synthesis, purification, lipid nanoparticle (LNP) formulation, and cell-based screening of oRNAs. We applied FoRCE to almost 3,000 unique oRNAs containing UTRs extracted from viral genomes and discovered hundreds of IRESs that drive translation from synthetic oRNA in primary human T cells, hepatocytes, and myotubes.

Results Select IRESs from this screen drove high levels of CAR expression in primary human T cells. This elevated CAR expression translated to signficantly improved tumor regression in a human PBMC-engrafted NALM6 tumor-bearing mouse model. Tumor regression was dose-dependent, and the novel imunotropic LNP was well tolerated. oRNA-enabled isCAR therapies promise a re-dosable and scalable immune cell therapy.

Conclusions This off-the-shelf treatment dose not require leukapheresis or lymphodepletion, and the transient expression of isCAR may provide better management of cytokine release syndrome (CRS) and complexities associated with tumor lysis as compared to conventional autologous cell therapy. Future opportunities exist to expand targeting strategies and leverage a payload capacity (up to 12 kb) well beyond the current cell therapy delivery space.

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