In situ CAR Therapy Using oRNA™ Lipid Nanoparticles Regresses Tumors in Mice

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Abstract

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.

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 (Formulated oRNA Cell-based Evaluation): 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. Select IRESs from this screen drove high levels of CAR expression in primary human T cells, which translated to tumor regression in a human PBMC-engrafted NALM6 tumor-bearing mouse model. Tumor regression was dose-dependent and was well tolerated. oRNA-enabled isCAR therapies promise a transient, re-dosable and scalable immune cell therapy, without requiring immunodepletion, for the treatment of cancer.

in situ CARs: Potential to Revolutionize CAR-T Cell Therapy

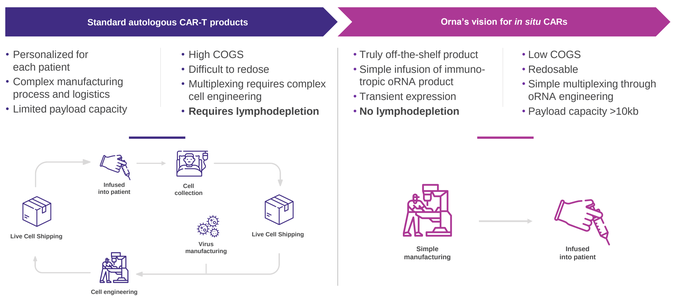


Figure 1: Schematic showing Orna's vision to revolutionize cell therapy

isCAR Product Design and Concept

- Rapid clinical validation is the key focus of the isCAR platform
- CD19⁺ target cells (B cell malignancies or normal B cells) are preferred to limit biological risks and enable rapid clinical PoC
- Orna's isCAR drug discovery efforts are focused on 3 key elements:
  - Well-tolerated immunotropic LNP
  - Immune-active IRES
  - CD19 CAR has that has been shown to be safe and effective in clinical trials

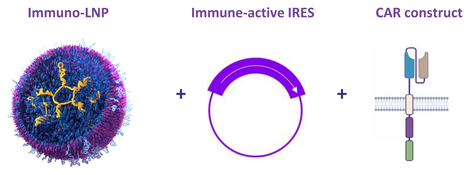


Figure 2: Schematic and description of the CD19 isCAR Product Concept

oRNA Expression is Durable and Tunable

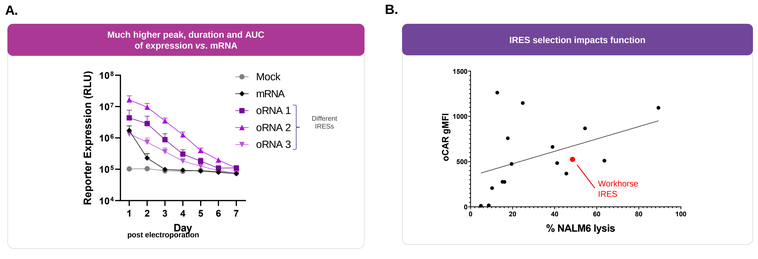


Figure 3: IRES-dependent oRNA reporter expression is more durable that cap-translated mRNA. A) Human T cells were activated and then electroporated with oRNA expressing Luc via three distinct IRES sequences or treated with standard modified mRNA encoding the same Luc protein. The kinetics of luciferase expression was measured over 7 days using a luminometer. B) Human T cells were activated and then electroporated with oRNA constructs coding an anti-human CD19 CAR. Each construct used a different IRES to drive oRNA CAR (oCAR) expression. oCAR expression on T cells was measured via FACs and reported as the mean fluorescence intensity (MFI). The graft shows the correlation of oCAR MFI to in vitro NALM-6 tumor lysis. Our base IRES (workhorse) is shown in red. These data show that CAR expression is durable and tunable.

CD19 isCAR POC: Antigen Dependent Tumor Regression in Humanized Mice

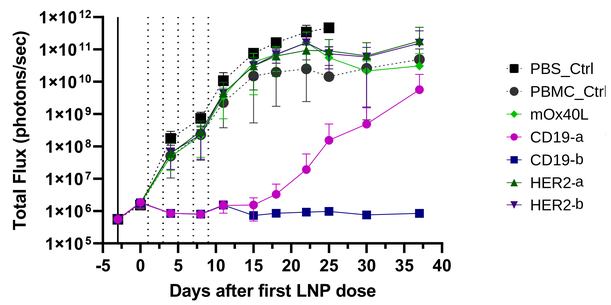


Figure 4: First POC demonstrated *in vivo* with CD19 isCAR. NSG mice were inoculated with NALM/6-Luc tumor cells. Human PBMCs were engrafted 4 days post inoculation. Tumor growth was monitored by *in vivo* luminescence using an IVIS spectrum. Animals were treated 4 times (dotted lines) with vehicle or LNPs that carried payloads for mOX40L, anti-human CD19-oCAR with configuration a or b, or HER-2 oCARs with configuration a or b. Data show that mice treated with CD19 oCAR-b have durable tumor control, even upon withdrawal of treatment. However, mice treated with LNLP-oCAR-a showed rebound tumor growth after withdrawal of treatment. Animals treated with vehicle, LNP-mOX40L or LNP-HER2 oCAR (a or b) do not control tumor growth suggesting that CD19 isCAR tumor regression is antigen dependent. These data are generated using our workhorse IRES.

Orna's Lipid Screening Platform

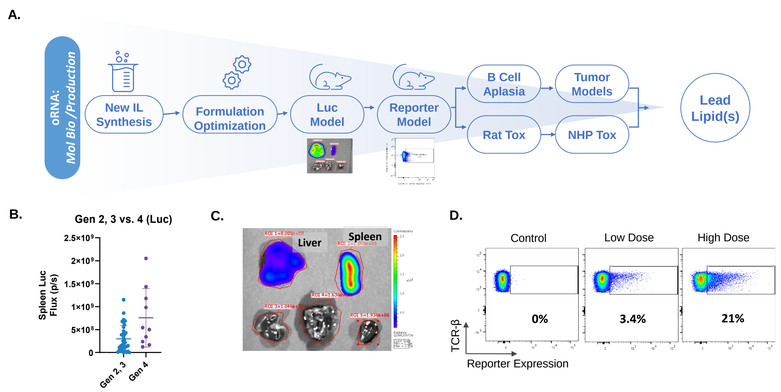


Figure 5: Orna has an internal lipid screening pipeline to identify well tolerated immunotropic lipids. A) Schematic showing Orna's lipid screening platform. B) Data from mice 24 hours after LNP treatment. Data show luciferase expression in the whole spleen using Generation 2, 3, or 4 ionizable lipids. C) Orna lipids show increased targeting of oRNA to the spleen. Data are IVIS images of whole organs harvested 24 hours post-LNP treatment. D) FACs dot plots show the expression of an mOX40L reporter on the surface of mouse TCR-β+ T cells harvested 24 hours post LNP treatment at a low or high dose of LNP-oRNA

Immunotropism is Maintained Across Species

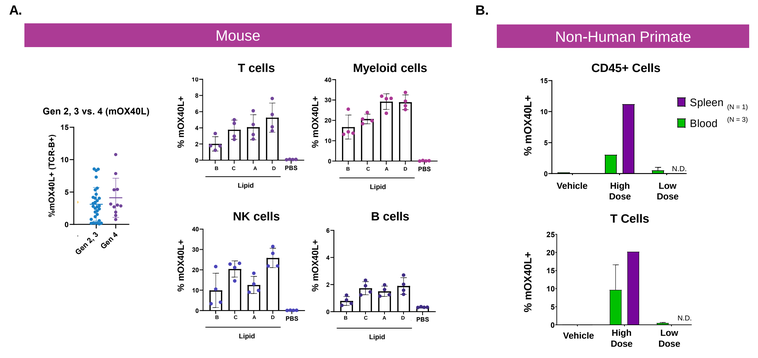


Figure 6: Immunotropic Lipids Target Multiple Immune Subsets in Mice and NHPs. Lipid nanoparticles incorporating unique ionizable lipids were formulated with reporter oRNA (mOX40L). Reporter expression on immune cell subsets was analyzed by FACs. A) C57BL/6 mice were treated i.v. with lipid nanoparticles formulated with distinct ionizable lipids and mOX40L oRNA. The graphs show mOX40L expression in immune cell subsets with various lipid classes 24 hours post-treatment. B) Non-human primates were treated with LNPs formulated using ionizable lipid B and mOX40L oRNA. Animals were treated with two doses of LNP-oRNA. mOX40L was analyzed by FACs on the surface of CD45+ leukocytes and CD3+ T cells in the blood and spleen. Data show immune delivery is preserved across species.

Dose-Dependent Anti-Tumor Efficacy after isCAR i.v. Dosing

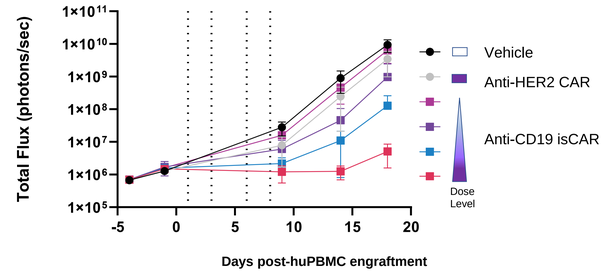
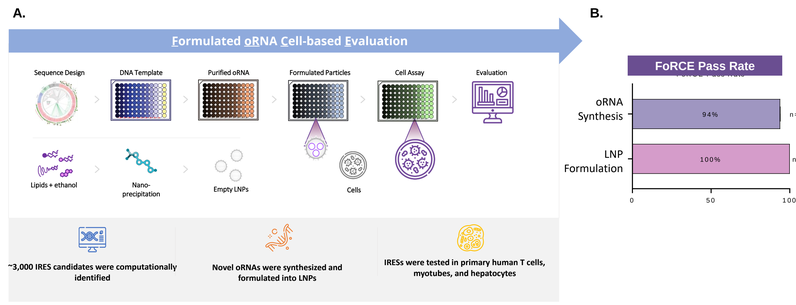


Figure 7:Tumor control after isCAR i.v. dosing is dose dependent. NSG mice were inoculated with NALM/6-Luc tumor cells. Human PBMCs were engrafted 4 days post-inoculation. Tumor growth was monitored by *in vivo* luminescence using an IVIS spectrum. Mice were treated with 4 doses of LNP-oCAR or vehicle at the doses shown above (dotted lines, scale). The test articles include vehicle (PBS), LNP-CD19 oCAR or LNP-HER2 oCAR. Data show a dose dependent decrease in tumor burden as LNP-oCAR dose increases. These data were generated using our workhorse IRES.

FoRCE™ platform enables interrogation of the IRESome



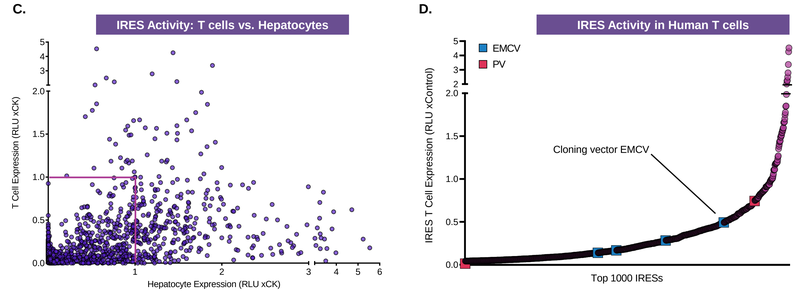


Figure 8: Development of the Formulated oRNA Cell-based Evaluation (FoRCE™) high-throughput screening system to investigate the IRESome. The completely automated process begins with a plate of DNA templates which are then linearized, *in vitro* transcribed, purified, and encapsulated into LNPs with an extremely high success rate. By screening ¬3000 viral UTRs, we were able to identify hundreds of novel IRESs that are active in the context of oRNA. Many of these IRESs demonstrate increased activity when compared to the field standard cloning vector EMCV sequence

Conclusions and Next Steps

- **isCAR offers a transient, off the shelf autologous treatment option for cancer patients**
- **This platform eliminates manufacturing challenges and wait times for patients and eliminates the need for lymphodepletion**
- **Orna has shown first POC for *in vivo* CD19 isCAR in a tumor bearing humanized NALM-6 model using test lipids and the workhorse IRES**
- **Orna has established a lipid screening pipeline to identify immunotropic lipids**
- **Orna's immunotropic lipids show improved localization to the spleen and show immunotropism is conserved in mice and NHPs**
- **CD19 isCAR is dose titratable**
- **Further improvement of isCAR efficacy will be achieved by combining our lead candidates in our lipid and FoRCE screening platforms**