in Situ CAR Therapy Using oRNA Lipid Nanoparticles Regresses Tumors In Vivo

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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. We have been combining oRNA technology with novel immunotropic LNPs to address these challenges, by creating off-the-shelf, yet "autologous" in situ CAR (isCAR™) therapies.

Our 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 the FoRCE (Formulated oRNA Cell-based Evaluation) platform: 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 ~3,000 unique oRNAs containing UTRs extracted from viral genomes and discovered hundreds of IRESs that drive translation from synthetic oRNA in primary human cells across diverse tissues. Select IRESs from this screen drove high levels of CAR expression and cytotoxicity in primary human T cells that were significantly elevated compared to modified mRNA and earlier IRESs. Optimizing the IRES and coding sequences translated into a 20-fold increase in efficacy in mice treated with the corresponding LNP-oRNAs in a human PBMC-engrafted NALM6 tumor-bearing mouse model. The optimized LNP-oCAR enabled weekly dosing at clinically relevant dose levels producing well-tolerated, robust and reproducible efficacy across multiple PBMC donors. Tumor regression was dose-dependent. oRNA-enabled isCAR therapies promise a transient, re-dosable and scalable immune cell therapy without requiring immunodepletion for the treatment of cancer.

oRNA and LNPs combine to make a broad platform

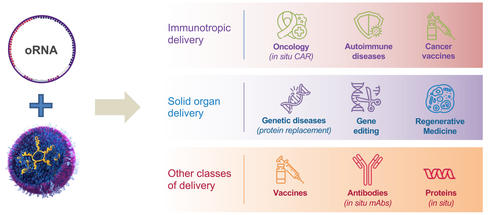

Figure 1: Orna has a versatile platform to address many therapeutic areas. oRNA will be delivered to patients using a classic 4 component LNP. Orna's first therapeutic application will be *in situ* CAR therapies for cancer patients.

in situ CARs: Potential to revolutionize CAR-T cell therapy

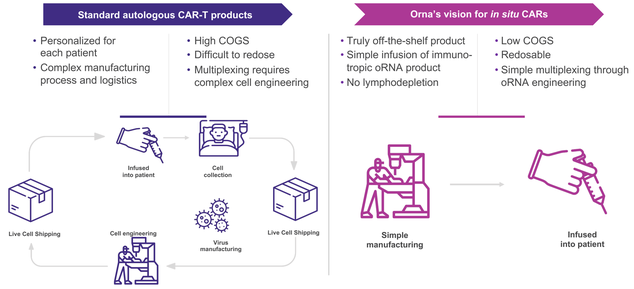

Figure 2: Schematic showing Orna's vision to revolutionize cell therapy. isCAR is scalable, transient and redosable. isCAR provides immediate access to patients through a simple i.v. infusion of an immunotropic LNP-oCAR. No lymphodepletion is required prior to treatment.

oRNA expression in human T cells in vitro

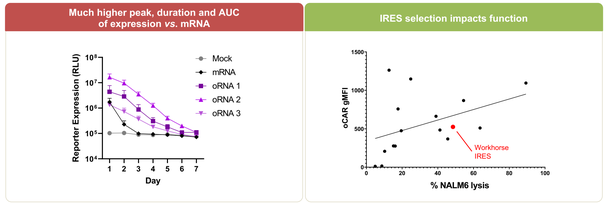

Figure 3: IRES-dependent oRNA reporter expression is more durable than cap-translated mRNA and allows tuning of protein expression. A) Human T cells were activated and then electroporated with oRNA expressing Luc via three distinct IRES sequences or treated with standard modified mRNA encoding the same 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.

CAR oRNA Design

The oRNA has three components for optimization:

1. IRES
2. CAR amino acid sequence
3. CAR nucleotide sequence

1. Natural, full-length IRES sequences from the FoRCE™/IRESome screen
   – Screened & validated in human T cells
2. Clinically validated CAR amino acid sequence for POC
   – For anti-CD19 CARs: Yescarta/Kymriah/ Breyanzi
3. CAR oRNA sequence optimized for CAR functional expression

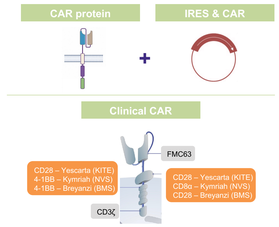

Figure 4: Description of the isCAR product concept and schematic of clinically approved CD19 CAR sequences.

In Vivo Tumor Efficacy Observed in Tumor Bearing Mice

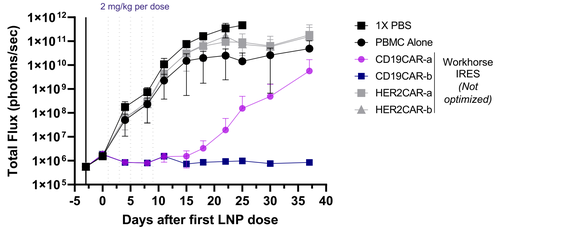

Figure 5: First POC demonstrated *in vivo* with CD19 isCAR. NSG mice were inoculated with NALM/6-Luc tumor cells. Human PBMCs were engrafted 4 days post inoculation. Tumor growth was monitored by *in vivo* luminescence using an IVIS Spectrum. Animals were treated 4 times (dotted lines) with vehicle or 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, showing CD19 isCAR tumor regression is antigen dependent.

FoRCE™ platform enables interrogation of the IRESome

- 3000 candidate IRES sequences; several thousand sequence clades
- Impractical to screen manually, especially through circularization and formulation
- FoRCE™ is an arrayed, automated screening platform to take plasmid DNA through IVT, formulation and cell-based readout

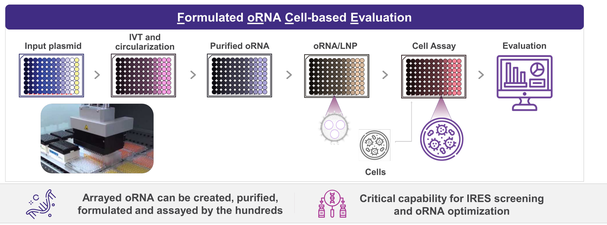

Figure 6: Description of the Formulated oRNA Cell-based Evaluation (FoRCE™) high-throughput screening system.

Construct Optimization Leads to Higher CAR Expression in T cells

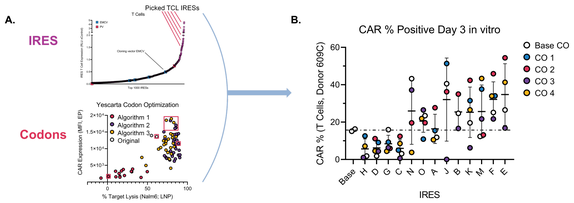

Figure 7: CD19 oCAR expression is improved through a combination of codon optimization and T cell active IRES selection. A) Screening of ~3000 viral UTRs identified hundreds of novel IRESs that are active in the context of oRNA. Many of these IRESs demonstrate increased activity when compared to the field standard cloning vector EMCV sequence. Algorithmic codon optimization of CD19 oCAR oRNA produced sequences that expressed higher than the non-optimized sequence and resulted in higher target cell lysis when delivered to T cells via LNP. B) Comparative CD19CAR expression in primary human T cells delivered the base CD19 oCAR construct, or a combined codon optimized + T cell IRES construct.

Optimized Constructs Improve In vivo Efficacy

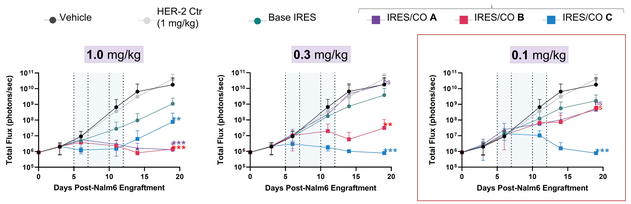

Figure 8: isCAR anti-leukemia efficacy is significantly improved with codon optimization and T cell active IRES: NSG mice were engrafted with Nalm6-fLuc B-ALL cells at D0. On D4, human peripheral blood mononuclear cells (PBMC) were engrafted, followed by LNP-oRNA dosing starting at D5. Animals received 4 doses, every other day, of LNP-CD19CAR ranging from 0.1 to 1 mg/kg, or LNP-HER2CAR at 1 mg/kg. Animals were imaged twice weekly to monitor Nalm6 burden.

Tumor control with q2w dosing using ORN-101

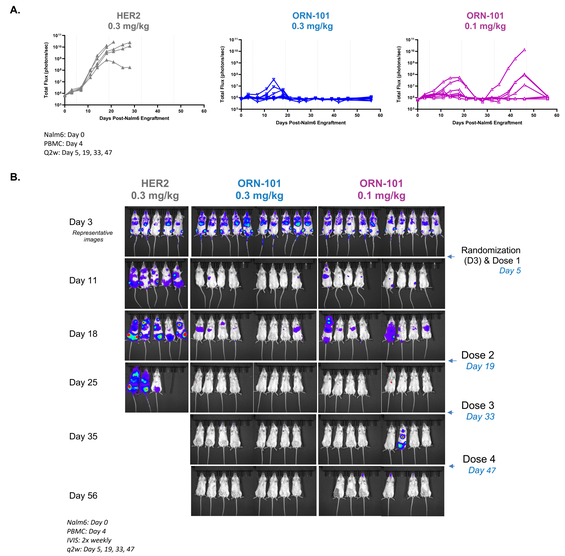

Figure 9: ORN-101 effectively controls leukemia growth when dosed every other week. NSG-MHC I/II double knockout mice were engrafted with Nalm6-fLuc B-ALL cells at D0. On D4, human peripheral blood mononuclear cells (PBMC) were engrafted, followed by LNP-oRNA dosing starting at D5. Animals received 4 doses, every other week, of LNP-CD19CAR at either 0.1 mg/kg or 0.3 mg/kg, or HER2CAR 0.3 mg/kg. Animals were imaged twice weekly to monitor Nalm6 burden. **A)** Nalm6 leukemia burden, based on tumor flux value, in ORN-101 or control treated individual mice. **B)** Representative images at indicated timepoints showing Nalm6 tumor flux in HER2CAR (0.3 mg/kg) or ORN-101 (0.3, 0.1 mg/kg) treated mice.

Conclusions

- **isCAR offers a transient, off-the-shelf autologous treatment option for cancer patients without requiring lymphodepletion**
- **The isCAR drug substance consists of a CD19 encoding oRNA encapsulated in an LNP**
- **oRNA expression is driven by an IRES sequence (internal ribosome entry site).**
- **Tumor bearing mice show tumor regression following i.v. treatment with LNP-CD19 oCAR.**
- **Orna's FoRCE screening platform enables interrogation of the IRESome to identify IRES sequences that drive high protein expression in T cells.**
- **Optimized LNP-oCAR constructs show improved *in vivo* efficacy and tumor control at doses as low as 0.1 mg/kg**
- **ORN-101 is efficacious at low doses when dosed every other week**
- **Lower and less frequent dosing enables a higher therapeutic window for ORN-101**