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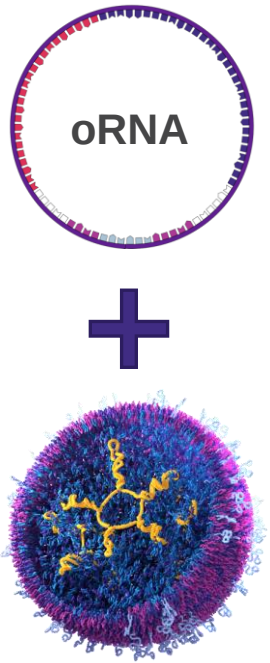
In situ CAR Therapy Using oRNA

MAY 18, 2023

oRNA and LNPs combine to make a broad platform



Immunotropic delivery

- **Oncology** *(in situ CAR)*
- **Autoimmune diseases**
- **Cancer vaccines**

Solid organ delivery

- **Genetic diseases** *(protein replacement)*
- **Gene editing**
- **Regenerative Medicine**

Other classes of delivery

- **Vaccines**
- **Antibodies** *(in situ mAbs)*
- **Proteins** *(in situ)*

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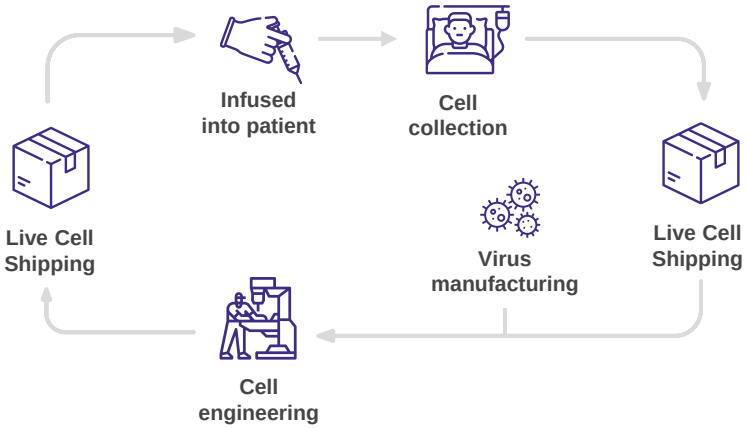
in situ CARs: Potential to Revolutionize CAR-T Cell Therapy

Standard autologous CAR-T products

- Personalized for each patient
- Complex manufacturing process and logistics
- Limited payload capacity

- High COGS
- Difficult to redose
- Multiplexing requires complex cell engineering
- **Requires lymphodepletion**



Orna's vision for *in situ* CARs

- Truly off-the-shelf product
- Simple infusion of immuno-tropic oRNA product
- Transient expression
- **No lymphodepletion**

- Low COGS
- Redosable
- Simple multiplexing through oRNA engineering
- Payload capacity >10kb

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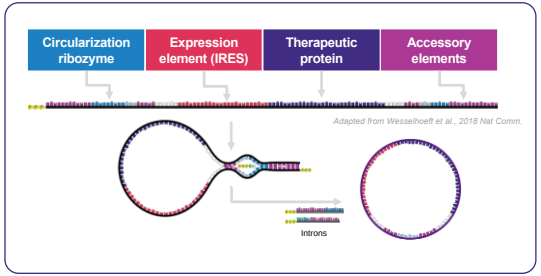
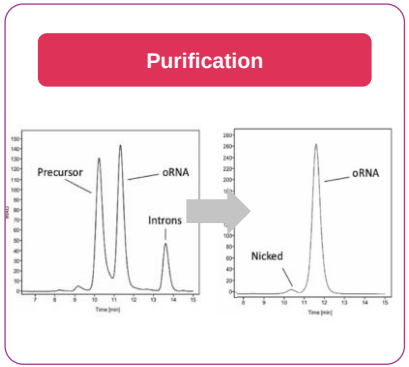
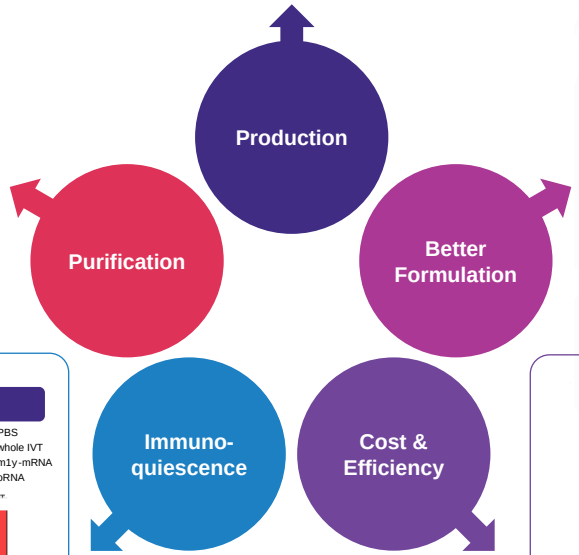
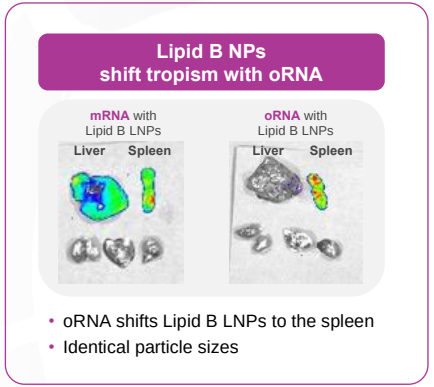
Iyer et al 2018 *Frontiers in Medicine*

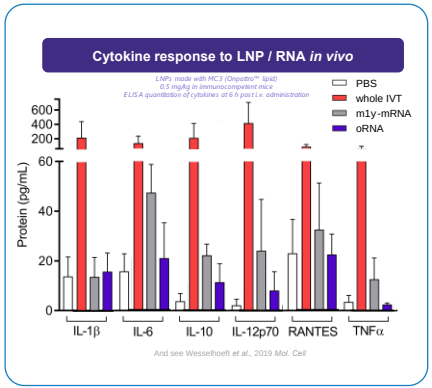
oRNA advantages

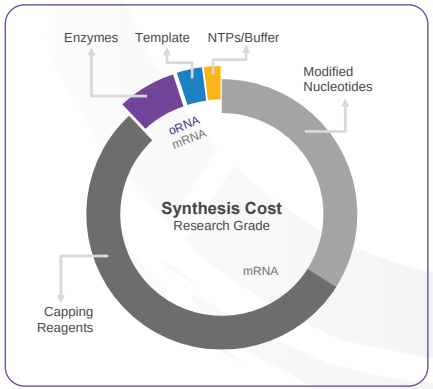












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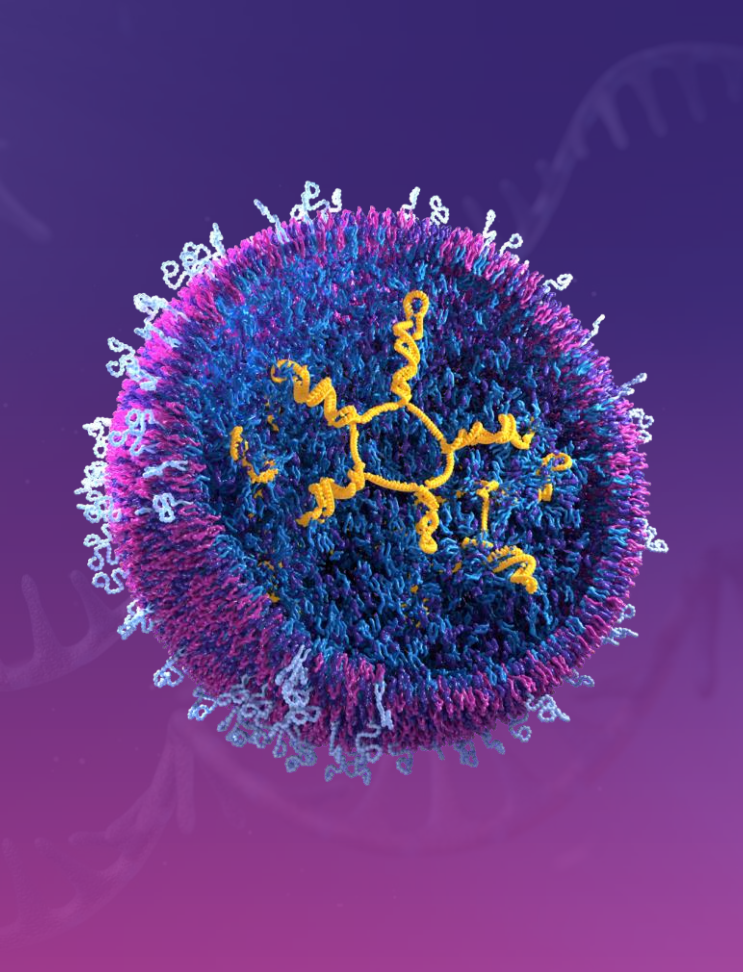

Lipid Nanoparticle (LNP) Technology

- LNPs are clinically and commercially validated delivery vehicles for long (coding) and short RNAs
- Classic LNPs have 4 lipid components and a payload
- The most important lipid is the ***ionizable lipid***, which determines cell uptake and payload escape from the endosome

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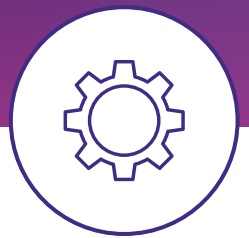
Orna’s Platform



Production

Expression

Delivery

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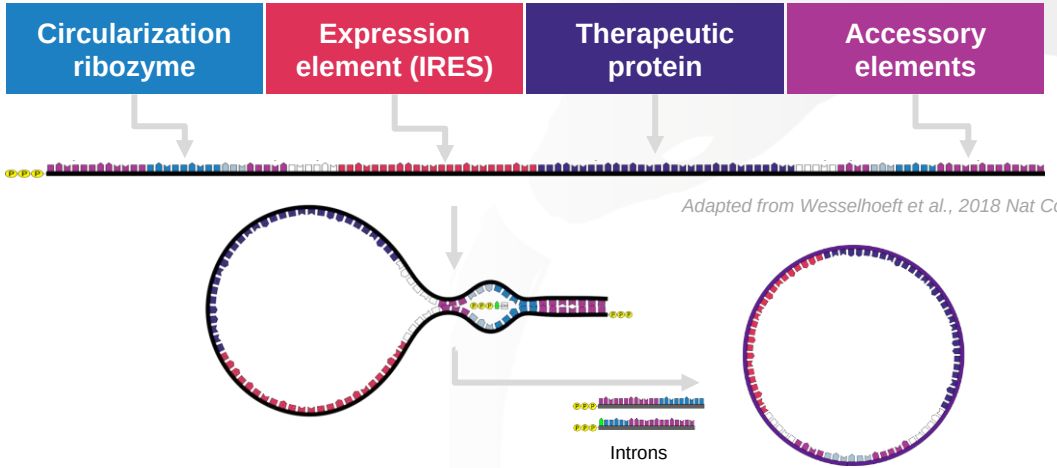
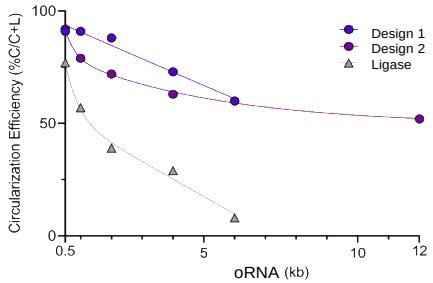
Production: oRNA Self-Circularizes

- Production is by *in vitro* transcription of a linearized plasmid
- **No** modified nucleotides
- Co-transcriptional circularization *via* a proprietary, autocatalytic split ribozyme
- All circles are full-length
  - Only full-length transcripts can reconstitute the ribozyme
  - No *N-1* molecules
- Payload capacity over 10 kb, with robust circularization efficiency
- Circular topology allows for robust purification

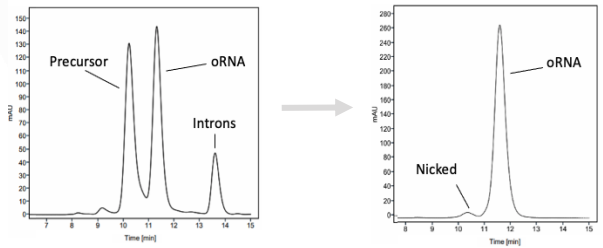


Adapted from Wesselhoeft et al., 2018 Nat Comm.

Circularization efficiency



Purification



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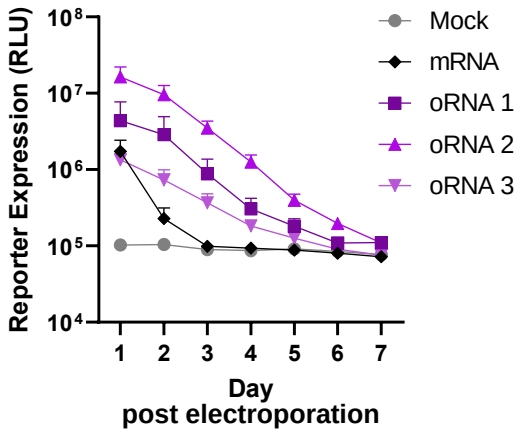
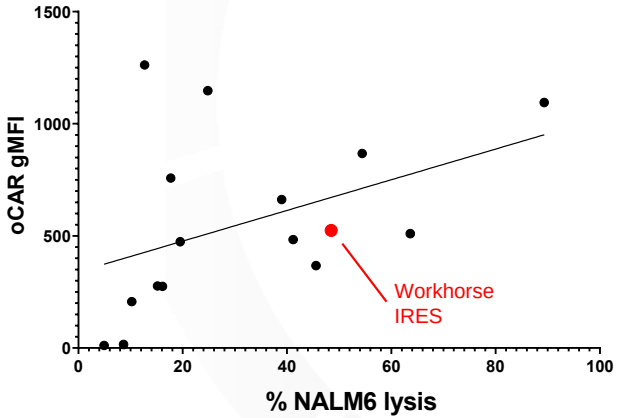
IRES Impacts Protein Expression and Function in T cells

Much higher peak, duration and AUC of expression *vs*. mRNA

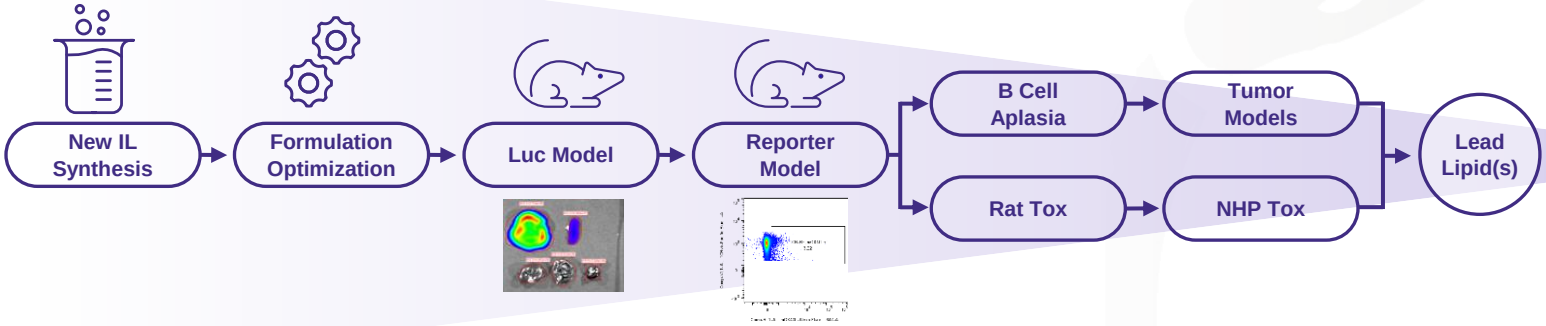


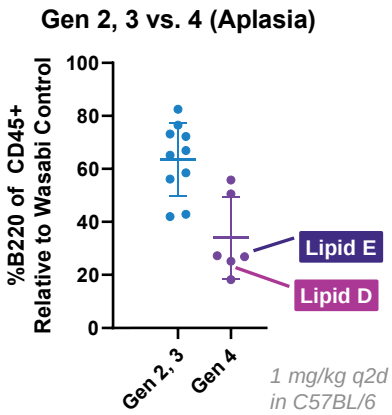
IRES selection impacts function



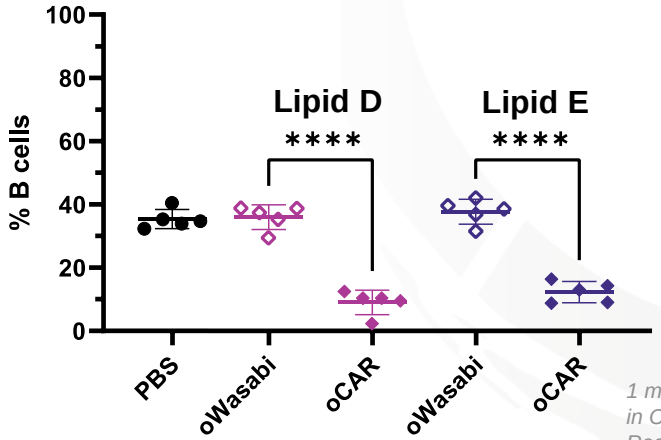
Lipid synthesis & screening pipeline

oRNA: *MoI Bio / Production*





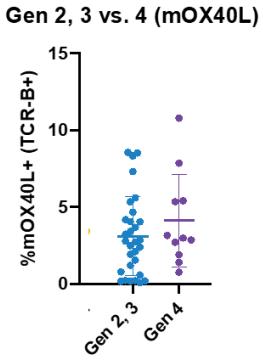
1 mg/kg q2d in C57BL/6 Read on d8

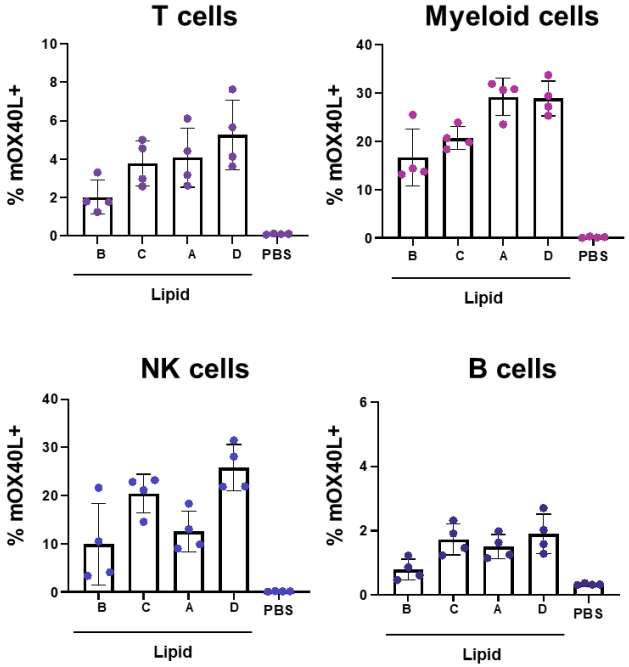


1 mg/kg q2d in C57BL/6 Read on d8

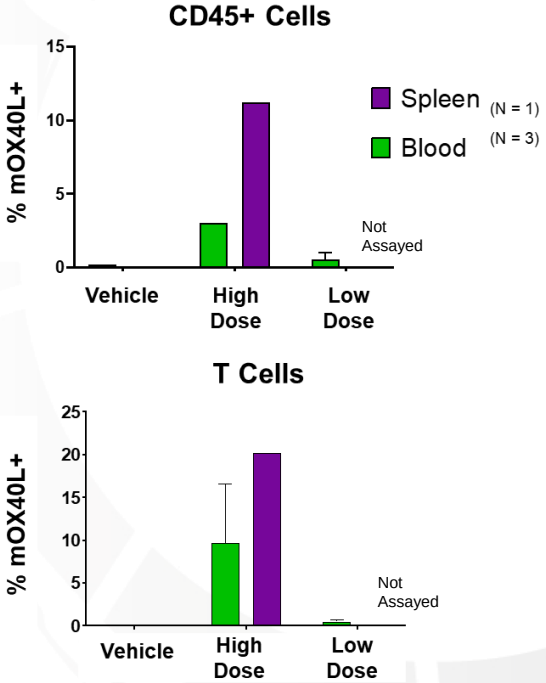
LNP ImmunoTropism is Maintained Across Species

Mouse





Non-Human Primate



In situ CAR (isCAR™) Platform

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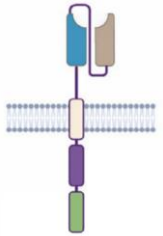
CAR oRNA design

The oRNA has three components for optimization:

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

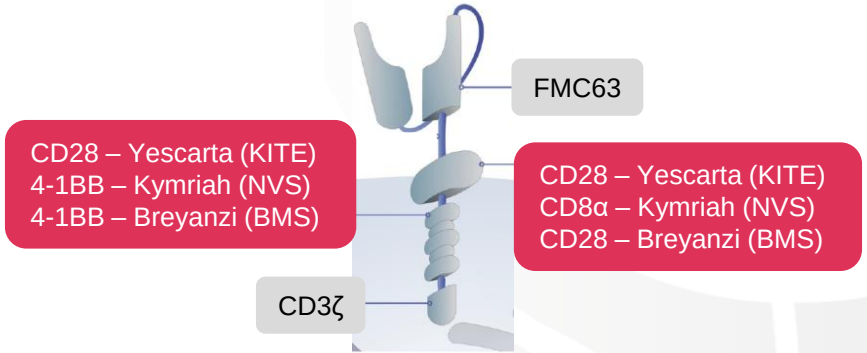
1. Natural, full-length IRES sequences from the FoRCE™/IRESome screen
   - Screened & validated in human T cells
2. Clinically validated CAR amino acid sequence for POC (FMC63)
   - For anti-CD19 CARs: Yescarta / Kymriah / Breyanzi
3. CAR oRNA sequence optimized for CAR functional expression

CAR protein



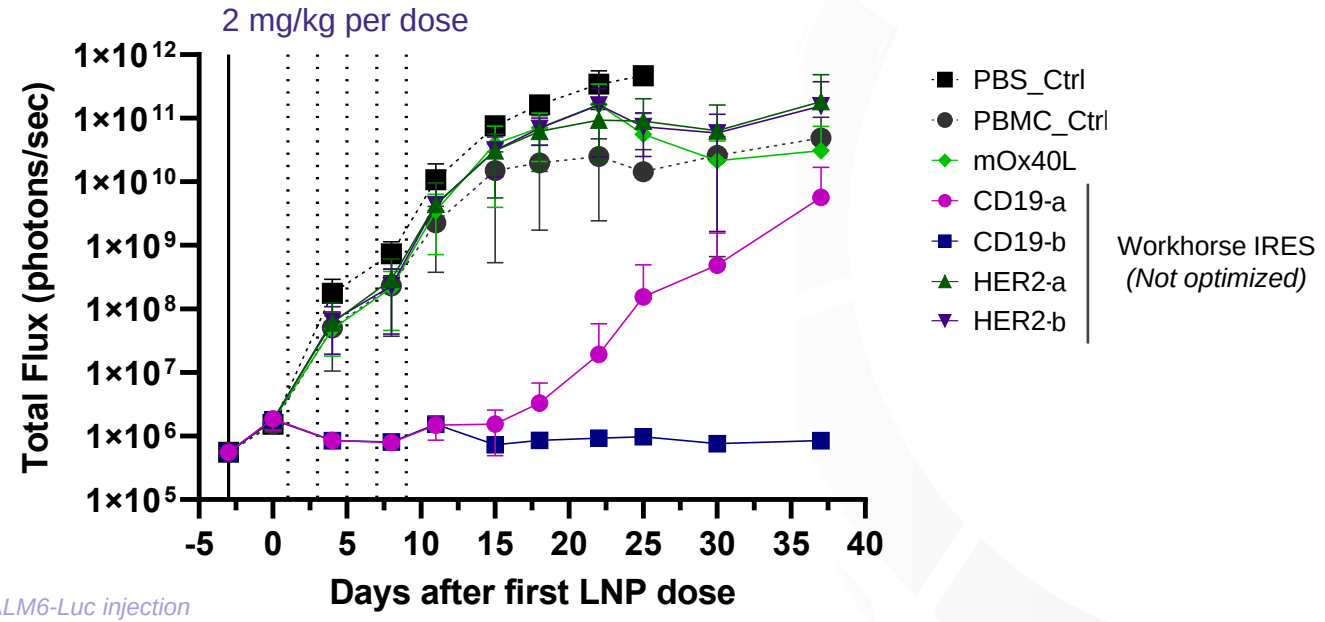
IRES & CAR sequence

Clinical CAR



First *in vivo* POC for isCAR in hematologic malignancies

Antigen-dependent tumor regression/elimination



NSG mice
d-4 1e6 NALM6-Luc injection
d0 1e7 huPBMC engraftment

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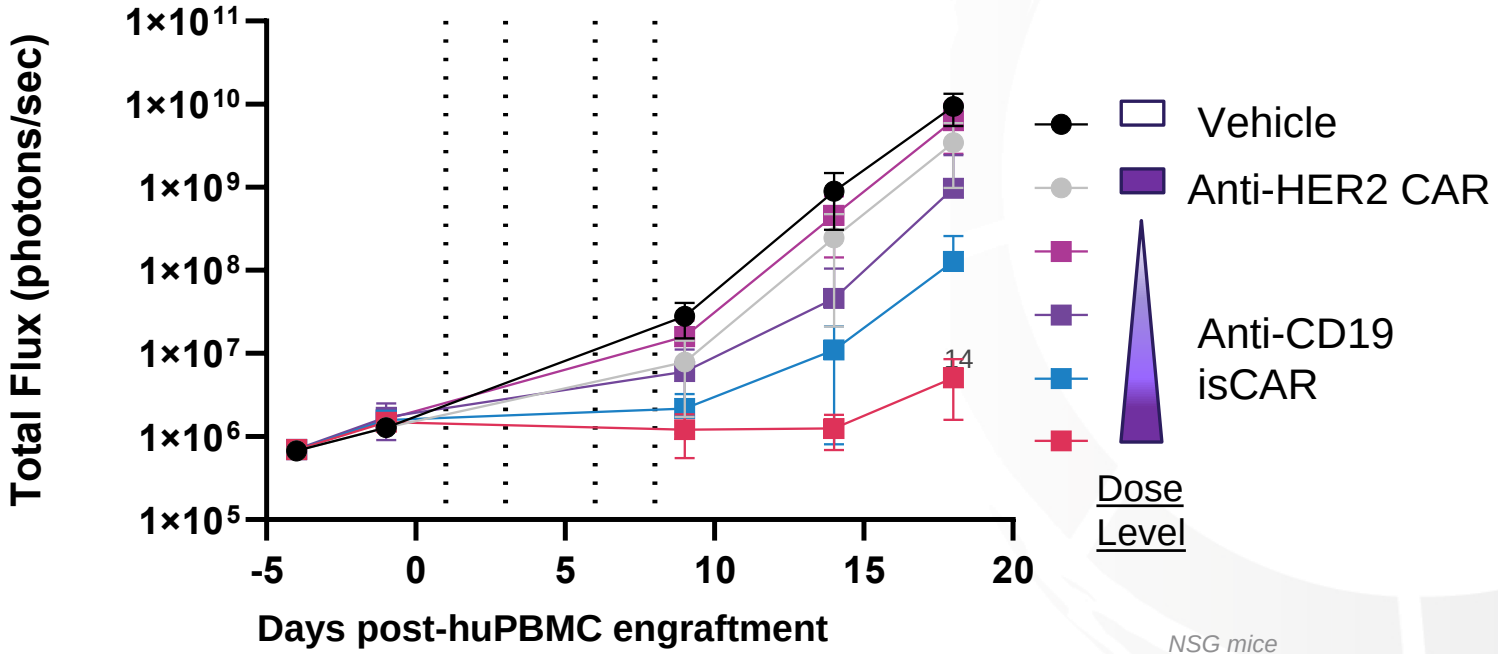
isCAR exhibits dose-responsiveness

- Non-Optimized IRES and oCAR construct and Generation 4 lipid used in this study
- Unstimulated PBMCs used for engraftment
- 4 Doses vs. 5 in previous slide

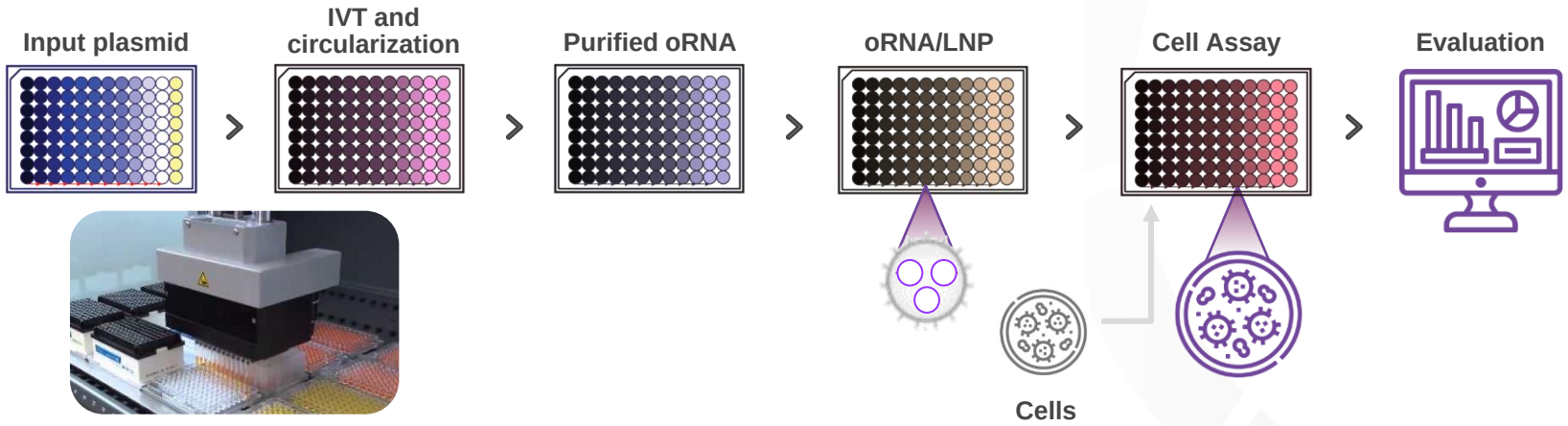


NSG mice
d-4 1e6 NALM6-Luc injection
d0 1e7 huPBMC engraftment

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

Formulated oRNA Cell-based Evaluation

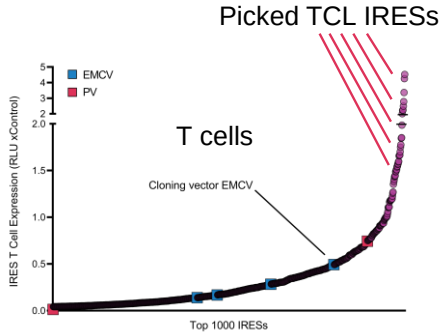


Arrayed oRNA can be created, purified, formulated and assayed by the hundreds

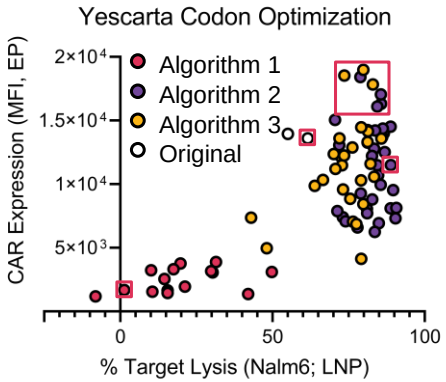
Critical capability for IRES screening and oRNA optimization

Combining IRES and CO sequences drives higher CAR expression

IRES

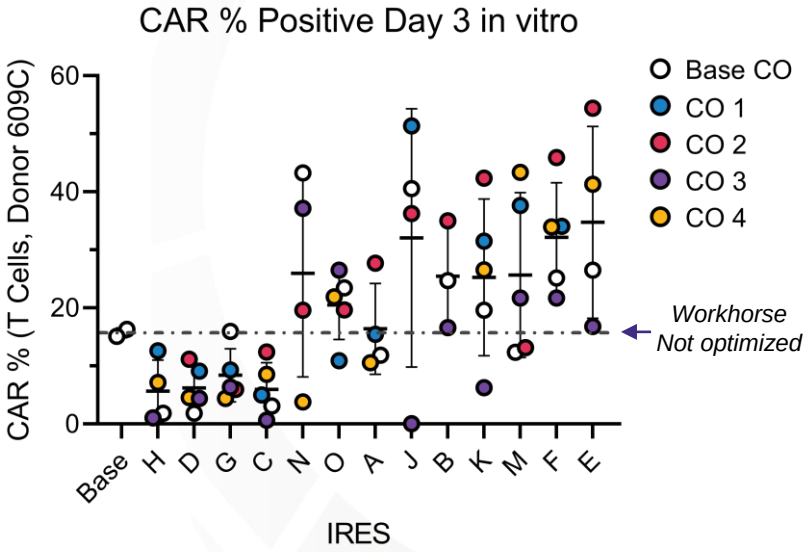


Codons



Assess CAR expression

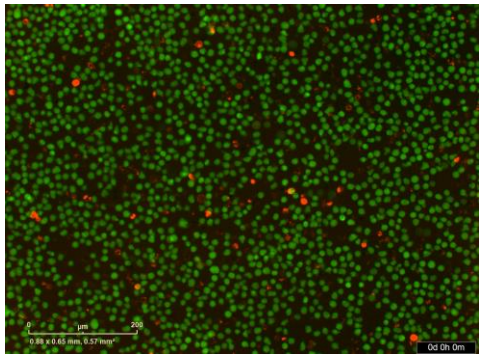
Combinations of lead IRES and codon sequences boost CAR expression in vitro



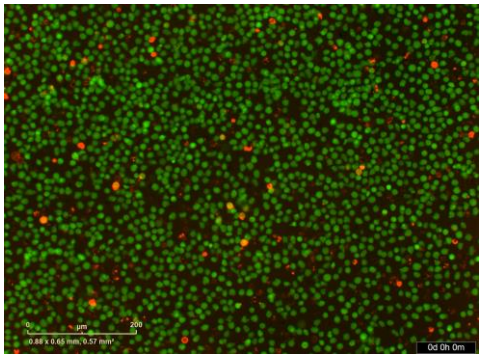
Constructs Found In IRES and Codon Optimization Screens Outperforms Workhorse CD19 CAR Construct

Nalm6
Annexin V

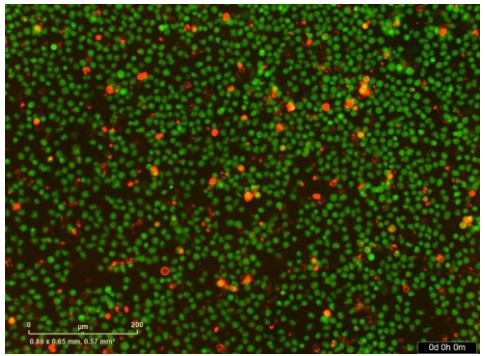
HER2 oCAR



Benchmark oCAR Construct



Optimized oCAR Construct



- These assays represent LNP-X transfected, activated PBMCs in coculture with NALM6 cells
- These data show a lead oRNA construct for ORN-101 (three leads were tested)
- The Old CD19 CAR construct contains our workhorse CK IRES and a non-optimized CAR sequence
- New oCAR constructs combine hits form our IRES and CodOp screens
- These data show that the New oCAR Construct outperforms the Old

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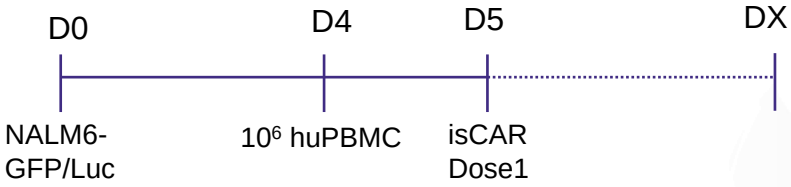
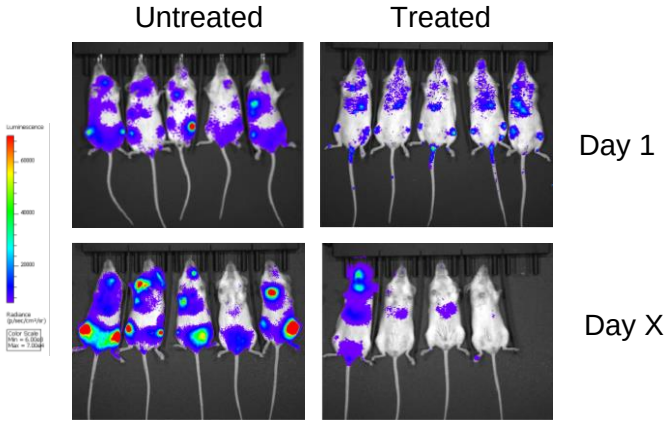
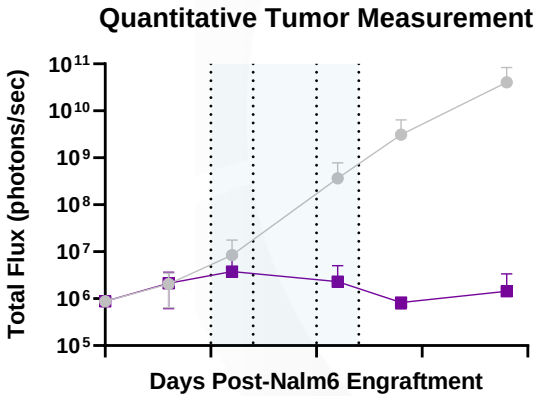
In Vivo Humanized NALM6 Tumor Efficacy Model







- *NSG or NSG MHC Class I/II KO immunodeficient mice*
- *Add human immune cells (PBMCs)*
- *In vivo imaging (IVIS) technology allow quantitative in life tumor monitoring*

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Newly identified IRES/CO constructs significantly improve in vivo potency

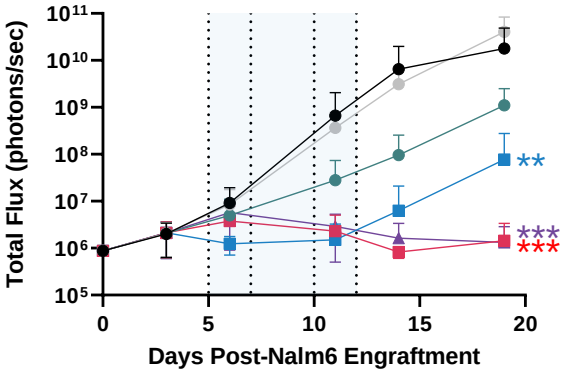
- *These data repeat across 2 donors for doses as low as 0.3 mg/kg*
- ***IRES/CO C** shows anti-tumor activity at doses as low as **0.1 mg/kg***

NSG mice
d0 1e6 NALM6-Luc injection
d4 1e7 huPBMC engraftment

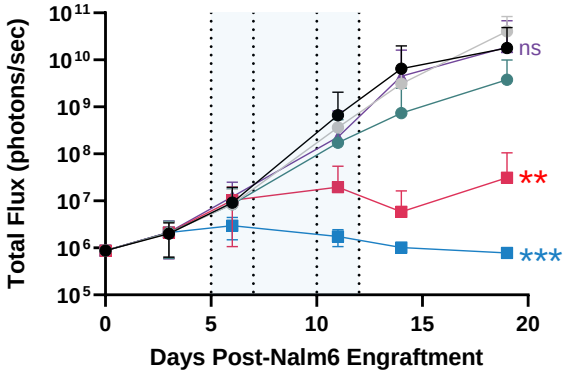
Newly Identified (FoRCE™)

Vehicle | HER-2 Ctr (1 mg/kg) | Base IRES | IRES/CO **A** | IRES/CO **B** | IRES/CO **C**

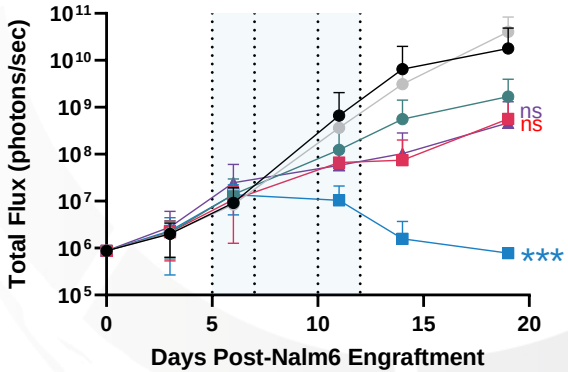
1.0 mg/kg



0.3 mg/kg

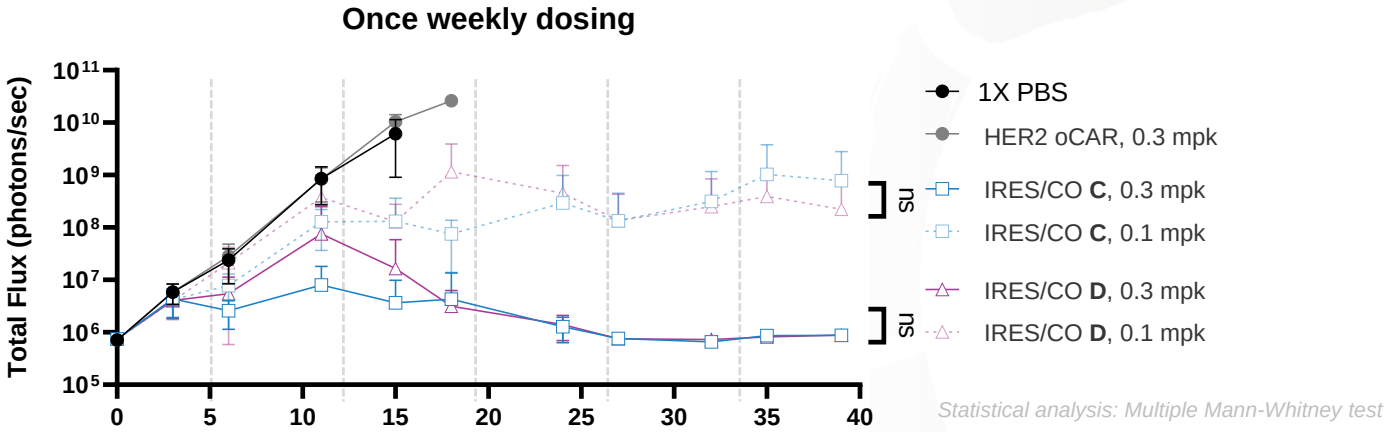


0.1 mg/kg



Multiple Mann-Whitney Test with Holm-Sidak Correction
**P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001*

isCAR is efficacious using weekly dosing schedule



***1X/Wk**: Days 5, 12, 19, 26,33*
NSG MHC I/II KO
D0 NALM6
D4 huPBMC

Efficacy observed across multiple donors and multiple experiments
Dosing can be sustained for at least 5 cycles

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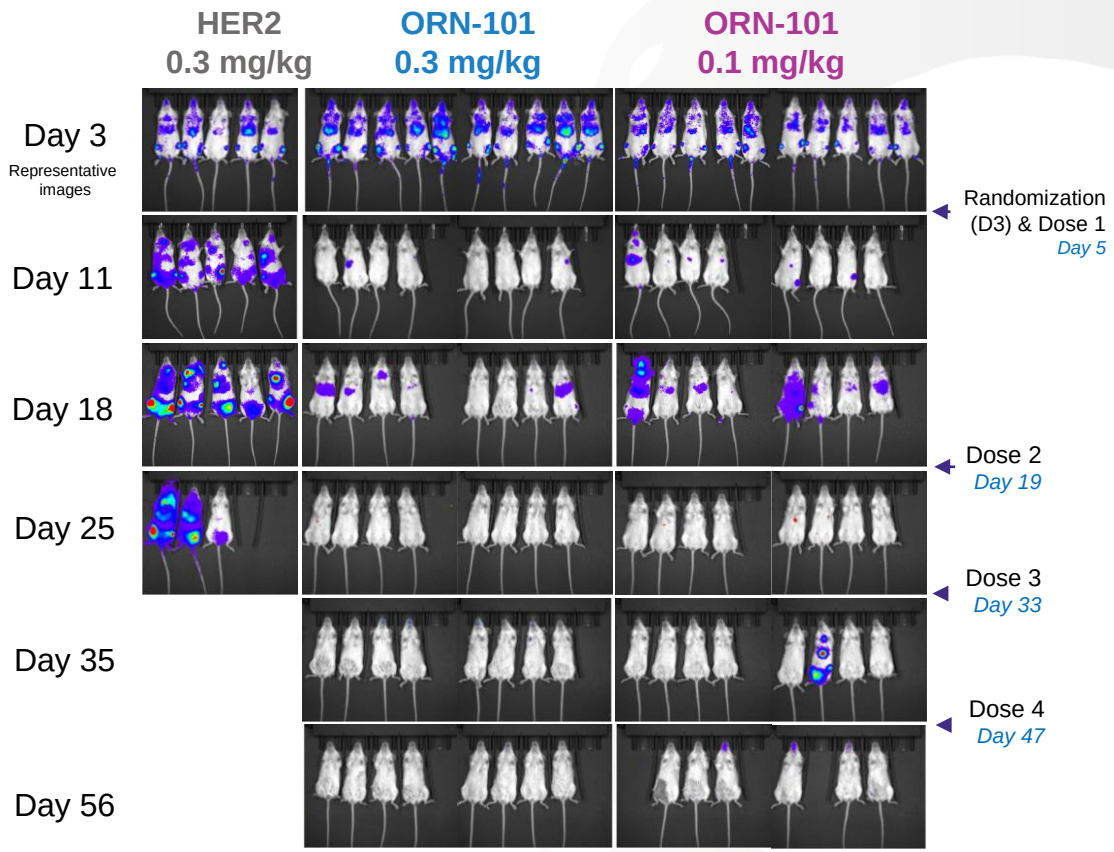
Tumor Control Observed with Bi-weekly Dosing

- Animals treated with oRN-101 at 0.3 and 0.1 mg/kg show tumor control for up to 56 days
- LNP-HER2 treated animals succumb to tumors at By Day 30
- Lower and less frequent dosing increases the therapeutic window of the isCAR



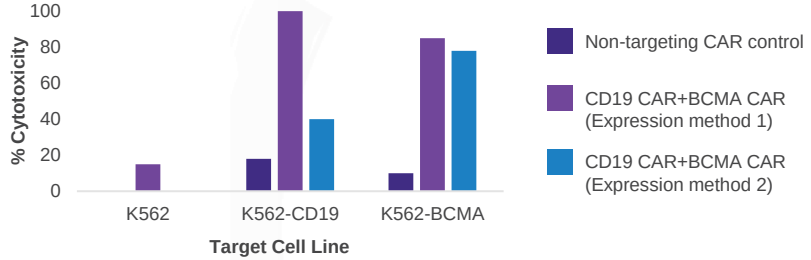
Nalm6: Day 0
PBMC: Day 4
IVIS: 2x weekly
q2w: Day 5, 19, 33, 47

ELN2583
David S, Ramya E, Akinola E

oRNA capacity & IRES diversity empowers multi-targeting

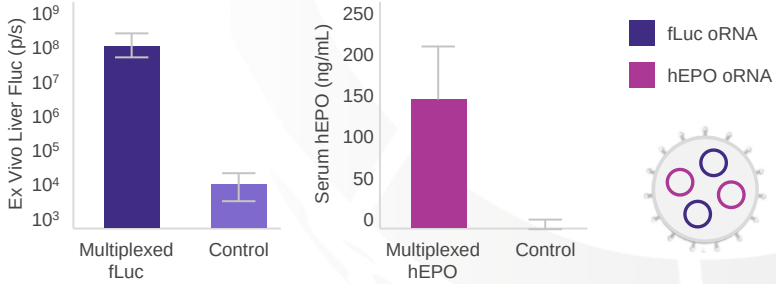
Functional *cis* bi-targeting

- Expression of *cis*-multiplexed anti-CD19 / anti-BCMA CARs oRNA kills both CD19⁺ and BCMA⁺ cell lines
- Capacity for two proteins – full CARs, not a bispecific
  - Ribosome skipping or two orthogonal IRESs



Trans expression bi-targeting

- Co-formulation of secreted (EPO) and non-secreted (fLuc) oRNA reporters in LNPs
- Robust liver delivery and expression *in vivo* of each oRNA



Acknowledgements

Molecular Biology | Delivery Science | Biology

CMC | PreClinical Safety

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