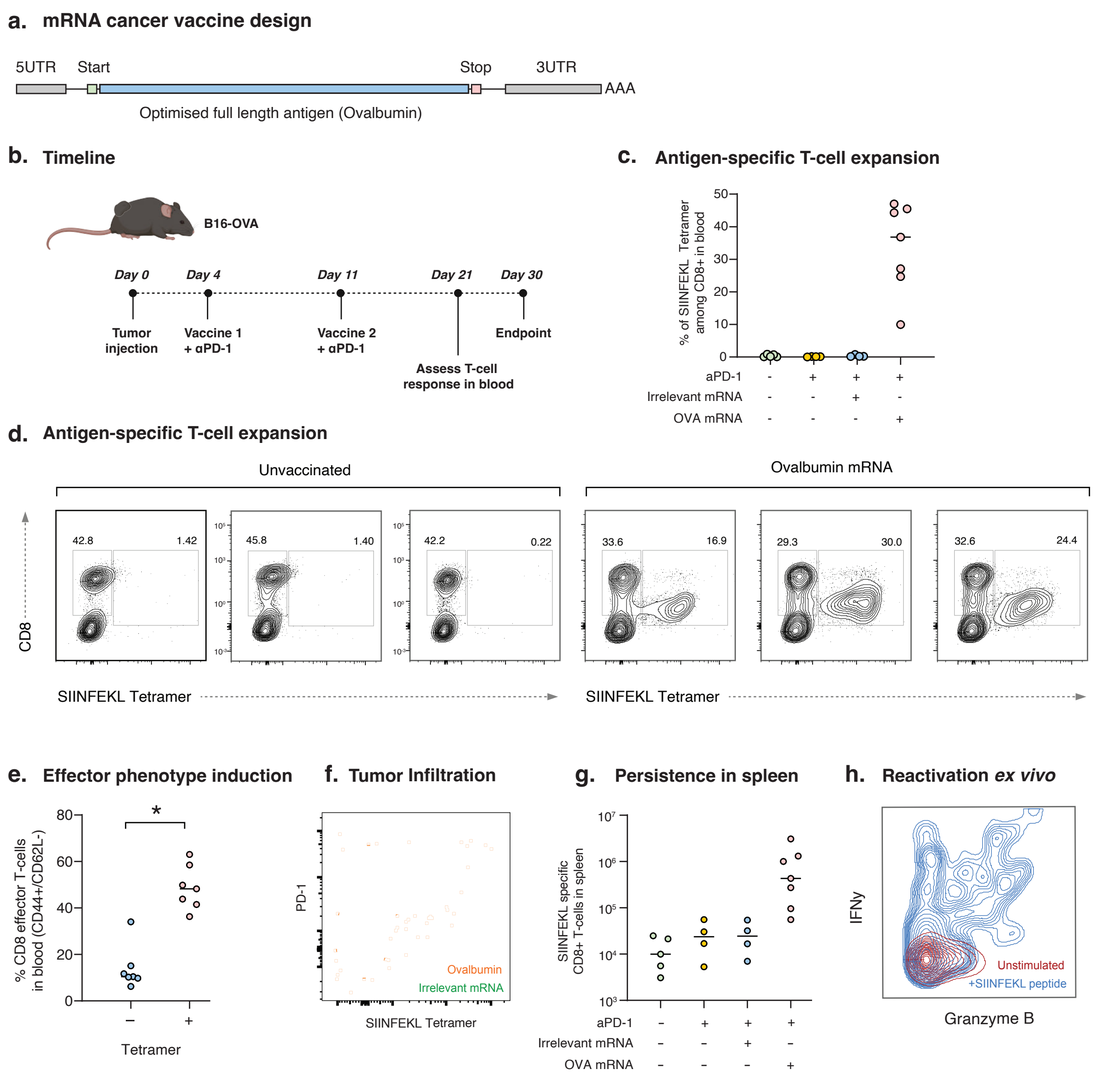
# mRNA Cancer Vaccines; Design, Delivery and Performance

Liam O'Brien[1], Fathima Nooru-Mohamed[1], Kylie Taylor[1], Maria Victorova[1], Tim Mercer[1] and Seth Cheetham[1]
AIBN, University Of Queensland, AUSTRALIA




## BACKGROUND

Messenger (m)RNA vaccines represent one of the most promising platforms for cancer immunotherapy, with emerging clinical responses in lung[1], pancreatic[2] and skin cancer patients[3]. Often administered with immune checkpoint inhibitors including anti-PD-1, mRNA vaccines broaden and intensify anti-tumour T cell responses and have the potential to exert clinical activity across additional, diverse tumour types. Preclinical studies including those with model antigens are crucial in optimising target selection strategies, backbone design, defining adjunct therapies and responsive tumour types. Expertise encompassing spanning mRNA science, immunology and oncology is required to complete such studies. BASE is establishing Australia's first centre for mRNA cancer immunotherapies , supported by programmatic funding from the Medical Research Future Fund. We have established in-house design, production, and evaluation capabilities for preclinical mRNA cancer vaccines.

## AIMS

We have established a platform for mRNA vaccine development integrating:

- mRNA design and codon optimization for human and mouse using mRNArchitect
- *In vitro* transcription (IVT) synthesis of high purity, capped, and polyadenylated mRNA.
- Optimized formulation compatible with clinically relevant lipid nanoparticle (LNP) delivery systems.
- Efficacy and mechanism evaluation *in vivo* using immunocompetent mice

## METHODS

Mice bearing B16 melanoma tumours engineered to express the model antigen ovalbumin (OVA) in-house were treated with PD-1 blockade immunotherapy +/- 2 rounds of intramuscular mRNA- LNP vaccination encoding full-length OVA protein or irrelevant control (Figure 1a-b). Both vaccines contained N1-methylpseudouridine-modified mRNA. CD8+ T cell responses were assessed in blood 10 days after the 2nd round of vaccination, and in spleen and tumour at study endpoint using tetramer staining and flow cytometry for activation markers. Reactivation capacity for OVA-specific T cells was also assessed *in vitro* using SIINFEKL peptide and intracellular staining for effector cytokines.

## RESULTS

Intramuscular OVA mRNA-LNP vaccination in combination with anti-PD-1 (αPD1) immunotherapy led to robust expansion of effector CD8+ T cells recognizing the immunodominant CD8-restricted SIINFEKL peptide from OVA (Figure 1c-d), demonstrating mRNA uptake, translation and T cell priming *in vivo*. Vaccine-expanded T cells displayed an effector phenotype in peripheral blood (Figure 1e), infiltrated tumours and uniformly expressed PD-1 suggesting activation (Figure 1f). High numbers of SIINFEKL-specific T cells were found in spleens at study endpoint (Figure 1g), and restimulation of these cells with SIINFEKL peptide induced Interferon (IFN)γ and Granzyme B production (Figure 1h). This confirmed that OVA mRNA vaccination generated functional, tumor-relevant CD8+ T cells with the capacity to kill target cells. Intramuscular OVA mRNA vaccination in combination with αPD1 resulted in tumour control in 5 out of 7 mice (Figure 1i), in contrast to treatment with αPD1 alone or vaccination with irrelevant mRNA (50% control, mCherry), suggesting targeted mRNA vaccination exerts superior clinical effects.

## DISCUSSION

UQ BASE facility has end-to-end capabilities for mRNA design, production and LNP formulation for ready-to-use research material and is establishing Australia's first mRNA cancer vaccine centre. mRNA vaccines produced by BASE caused specific T cell expansion to a model antigen and facilitated tumour control in an immunocompetent mouse model. Capacity for individualized neoantigen prediction from patient samples through analysis of tumour vs. PBMC whole-exome and RNA sequencing data has also been established through implementation of recently described bioinformatic pipelines[4].

## REFERENCES

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**Figure 1. Vaccination with Ovalbumin mRNA LNPs facilitates specific CD8+ T cell expansion, activation, tumour infiltration and memory formation. (a**) An mRNA vaccine encoding the model antigen Ovalbumin was codon-optimized and produced at BASE facility, UQ. (b) 2x10$^5$ B16 melanoma cells expressing full-length Ovalbumin were subcutaneously injected in C57Bl/6 mice before immunotherapy timecourse (UQ AEC 2025/AE000037 and 2024/AE000526). Schematic created using biorender.com. **(c)** Percentage of CD8+ T cells in peripheral blood 10 days post-vaccination (timecourse d21) positive for SIINFEKL tetramer (i.e. responding to OVA vaccine). Flow cytometry plots show CD3+ T cells gated from size/singlet/live/CD45+ (d). **(e)** Effector (CD44+/CD62L- ) CD8+ T cell proportions in OVA-vaccinated mice among CD8+ split by vaccine-responsive (Tetramer+) vs. all other specificities (Tetramer-). **(f)** CD3+ T cells in tumour gated as in (b) from OVA vs. irrelevant (mCherry) RNA-vaccinated mice. **(g-h)** CD8+ T cells from spleens of vaccinated mice degranulated and produced IFNγ after restimulation with 5μg/mL SIINFEKL peptide overnight. **(i)** Tumour protection induced by Ovalbumin mRNA vaccine. Tumours measured using digital callipers 3 times per week. Researchers measuring tumours were blinded to treatment groups at time of measurement. Numbers on plots show responders/total mice in treatment group. Wilcoxon test used to assess significance in e; *p<0.05. (Credit: Dr Liam O'Brien)