Developing an mRNA-Encoded Antibody Platform to Accelerate Therapies to Clinic

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1 INTRODUCTION

The manufacture of therapeutic antibodies requires expensive production, often challenging purification, lengthy stability optimization and complex protein characterization that, despite continual improvement, keeps the cost of treatment in the clinic high. Alternatively, leveraging mRNA nanomedicine approaches to express biologics circumvents many of the manufacturing challenges and instead relies on *in vivo* production of antibodies within a patient, improving both the cost of sophisticated antibodies and time to clinic.

Crucially, recent work demonstrates that therapeutic antibodies translated *in vivo* from mRNA can be readily detected within hours following infusion into pre-clinical models and can persist up to several days or weeks. Peak levels of circulating mRNA-encoded antibodies are comparable to infused recombinant equivalents dosed to patients and have been shown to be within favourable therapeutic ranges in recent Phase I trials (*August et al, 2021, Nat. Med. 27:2224*).

Here we outline the basis of an mRNA-LNP platform to encode and express therapeutic antibodies *in vivo* to bypass costly and lengthy manufacturing. We set out an example roadmap to demonstrate how to encode a standard-of-care anti-HER2 antibody – Trastuzumab - using LNP-encapsulated modified mRNA and validation steps using preclinical models to demonstrate both robust PK/PD kinetics and anti-tumour potency.

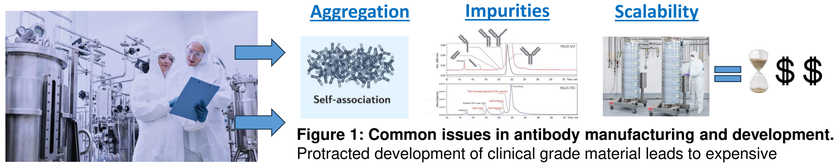


Figure 1: Common issues in antibody manufacturing and development. Protracted development of clinical grade material leads to expensive antibodies and lengthens drug-to-patient timelines.

2 mRNA-ENCODED TRASTUZUMAB: A CASE STUDY

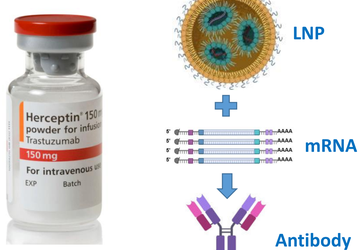
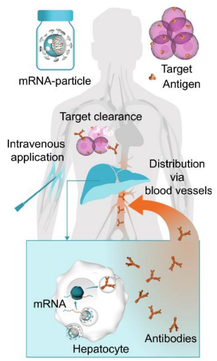


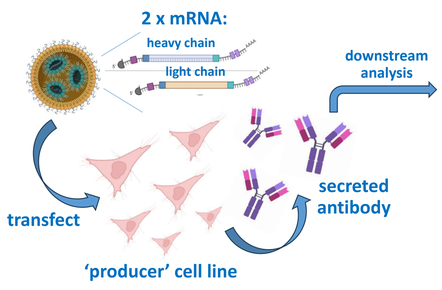
Figure 2: Harnessing mRNA-LNP nanotechnology. Lipid nanoparticles (LNPs) are used to deliver mRNA encoding therapeutic antibodies for in vivo expression.



Schlake et al, 2018 Cell Mol Life Sci

Figure 3: Turning patients into drug factories. Encoding either standard of care antibodies for cancer indications - such as Trastuzumab - or novel immune cell engagers using mRNA - would circumvent the need for complex antibody manufacturing and instead rely on hepatic antibody production within patients followed by systemic distribution via blood circulation.

3 FINE TUNING mRNA-ENCODED ANTIBODIES *IN VITRO*



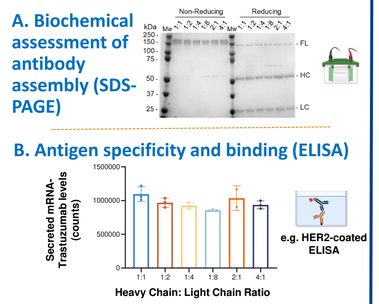
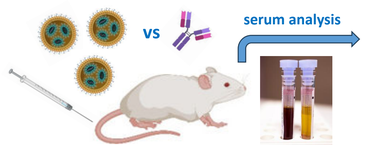


Figure 4: Design and characterization of mRNA-encoded antibodies. To express full length antibodies, heavy and light chains are encoded by two distinct pseudo-uridine modified mRNAs containing fully optimized 5'-UTR , 3'UTR, signal peptide and poly A tail sequences. Optimal mRNA sequences and HC:LC ratios can be optimized in cell lines using both biochemical analysis (A) and antigen-specific ELISAs as shown in B for mRNA-encoded Trastuzumab.

4 BENCHMARKING mRNA-ENCODED ANTIBODY PHARMACOKINETICS *IN VIVO*



PK/PD profiling:

- **Compare protein equivalent vs mRNA-encoded antibodies**
- **Dose finding and repeat administration**

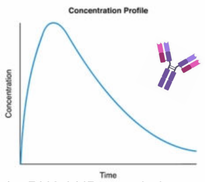
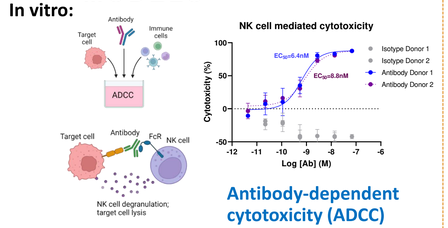


Figure 5: Interrogating mRNA-encoded antibody serum pharmacology. Kinetics of mRNA-LNP encoded antibodies infused intravenously can be monitored over time to determine PK values and optimal dosing.

5 FUNCTIONALLY VALIDATING mRNA-ENCODED TRASTUZUMAB IN PRE-CLINICAL ONCOLOGY MODELS

In vitro:



In vivo:

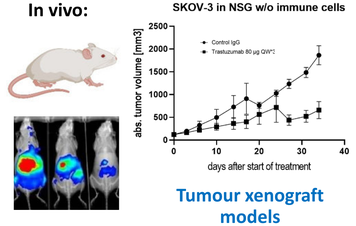
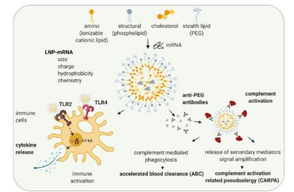


Figure 6: Efficacy of mRNA-encoded antibodies. mRNA-encoded antibodies – such as Trastuzumab - can be functionally validated in translationally-relevant efficacy models as per standard recombinant antibodies (example data shown above).

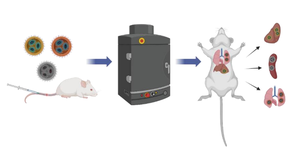
6 DE-RISKING mRNA-LNP STRATEGIES FOR *IN VIVO* EXPRESSION OF ANTIBODIES

A. Predicting immunogenicity of mRNA drugs



Vlatkovic et al, 2021 Biomed. 9:530

B. Biodistribution



Interrogating custom LNP-mRNA targeting using whole-animal imaging

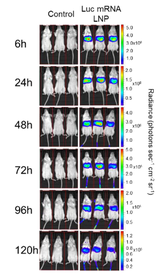
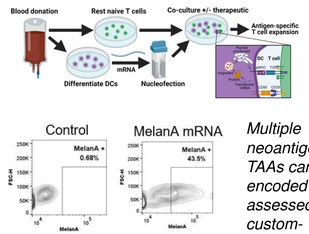


Figure 7: De-risking mRNA-encoded antibodies. A. Immunogenicity and anti-drug responses to mRNA-LNPs can be tested in the early phases of mRNA-antibody development to minimize late-stage failure. Additionally, mRNA-Luc is used to interrogate biodistribution of mRNA-LNPs (**Vernal Biosciences**) *in vivo* following IV infusion as shown in B.

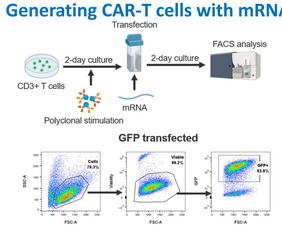
7 HARNESSING mRNA NANOTECHNOLOGY AS AN ALTERNATIVE STRATEGY FOR IMMUNOTHERAPY

mRNA cancer vaccines



Multiple neoantigens or TAAs can be encoded and assessed using custom-designed mRNA

Generating CAR-T cells with mRNA

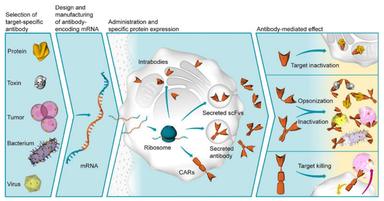


mRNA-encoded CARs can be rapidly generated via electroporation

Figure 8: mRNA nanomedicines in oncology. Similar mRNA approaches can be extended to generate neoantigen or tumour-associated antigen vaccines as well as encoding chimeric-antigen receptors for *in vivo* CAR-T or CAR-M generation. MelanA and GFP are used as respective examples of each approach.

8 SUMMARY

- Our ambition is to generate a transformative mRNA-based platform for *in vivo* expression of biologics to accelerate therapies into the clinic and to patients.
- Leveraging our discovery expertise and extensive biologics platforms plus end-to-end capabilities, CRL can explore the potential for mRNA-encoded biologics across multiple therapeutic areas.



Schlake et al, 2019 Mol Ther .27:773-778

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