Development and characterisation of a SARS-CoV-2 RNA vaccine expressing three linked-RBD domains

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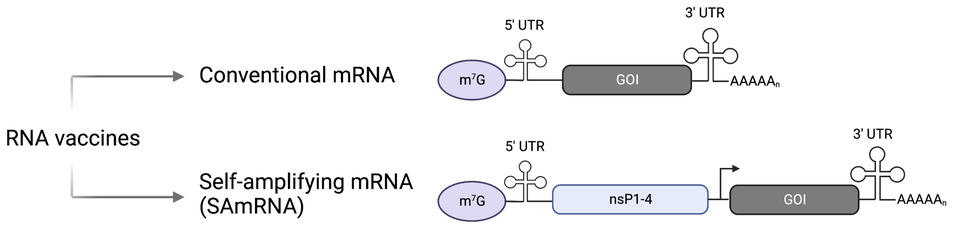
Introduction

- SARS-CoV-2, the causative agent of the current global COVID-19 pandemic, has resulted in over 767 million confirmed cases and over 6.9 million deaths[1].
- Different vaccine platforms have been approved for use worldwide to sustainably control the pandemic, with the leading vaccines such as BNT162b2[2] and mRNA-1273[3] making use of the mRNA-based technology.
- Compared to conventional mRNA vaccines, self-amplifying mRNA (SAmRNA) vaccine platform has the self-replicative properties conferred by the alphavirus replicase genes which may induce potent immune responses at a lower dose.
- As the virus continues to spread, a more transmissible and infectious variant can potentially emerge that escapes vaccine immunity.

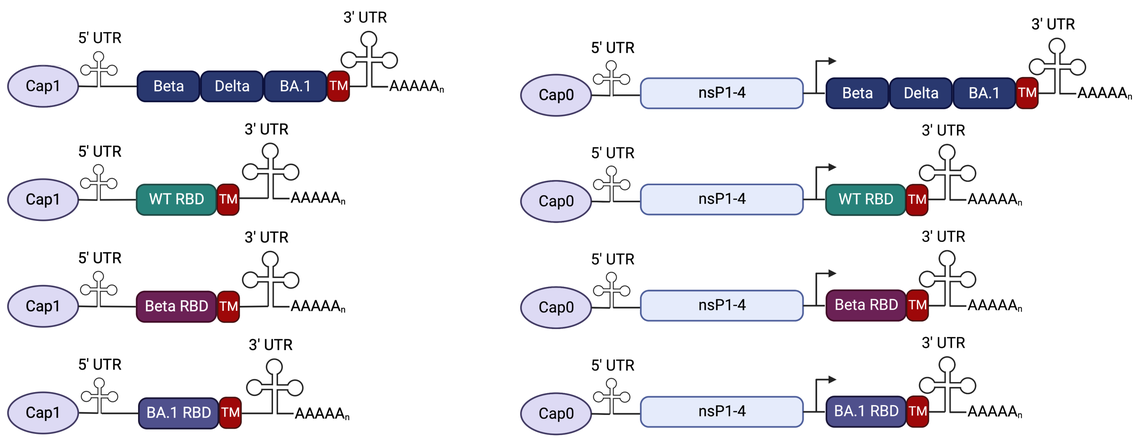


Hypothesis/Aims

We aim to investigate the potency and the neutralisation breadth of COVID-19 vaccines by focusing the response at an individual RBD or three different linked-RBDs using either a typical modified mRNA or an alphavirus SAmRNA.

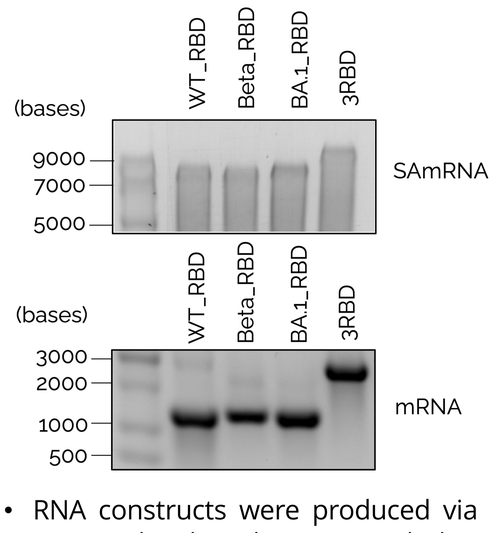
Methods

- Our single RBD RNA vaccines individually encode the RBD of the **ancestral strain** (referred to as wildtype (WT)), **B.1.351 strain (Beta)** or **Omicron BA.1 strain** of SARS-CoV-2 attached to a transmembrane domain anchor on the C-terminus.
- For our linked-RBDs approach, the 3RBD antigen was designed to tether together the RBDs of **B.1.351 (Beta), B.1.617.2 (Delta)** and **Omicron BA.1** variants with a short flexible linker and a C-terminal transmembrane domain.

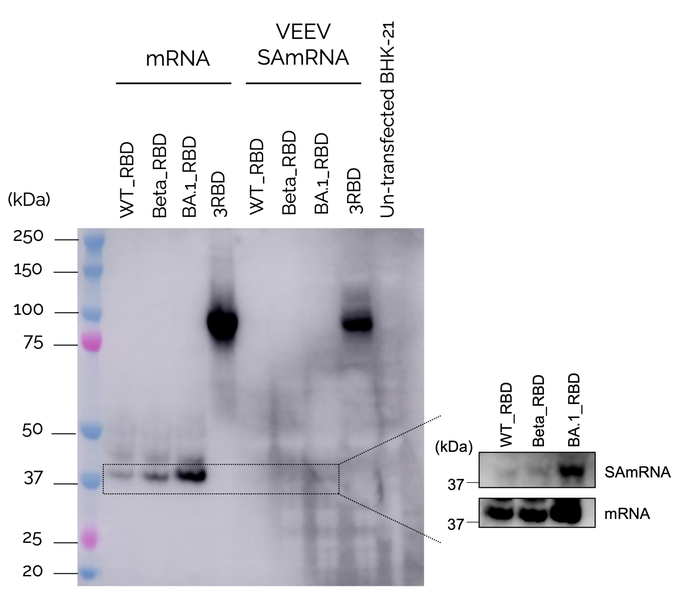


- Our various vaccine antigens are expressed either as a **conventional mRNA** or in a Venezuelan equine encephalitis virus-derived[4] (VEEV) **SAmRNA** expression vector.

Vaccine antigens are robustly expressed *in vitro*

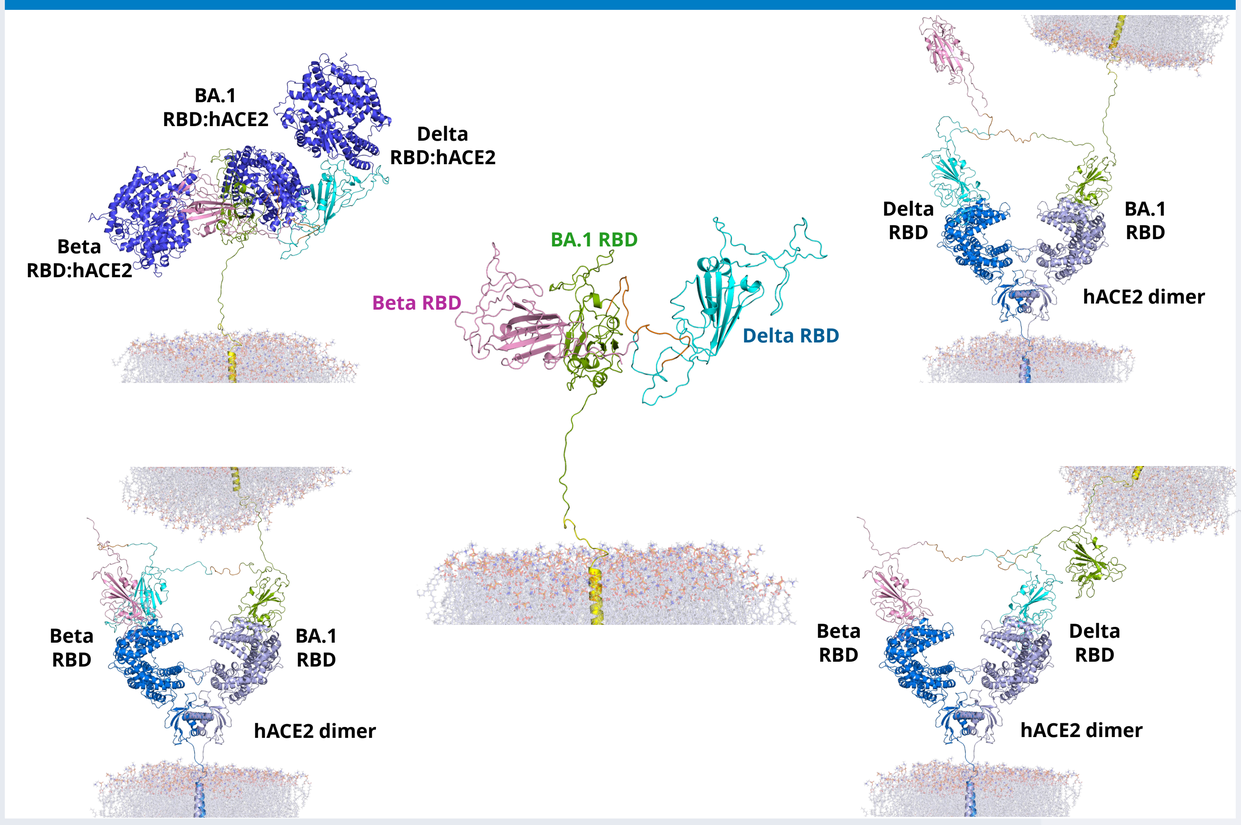


- RNA constructs were produced via enzymatic *in vitro* transcription using T7 RNA polymerase.
- The plasmid templates for our vaccine antigens were optimised for cap, codons, poly (A) and pseudouridine for maximum mRNA expression or native structured RNA in a VEEV-derived SAmRNA expression vector.

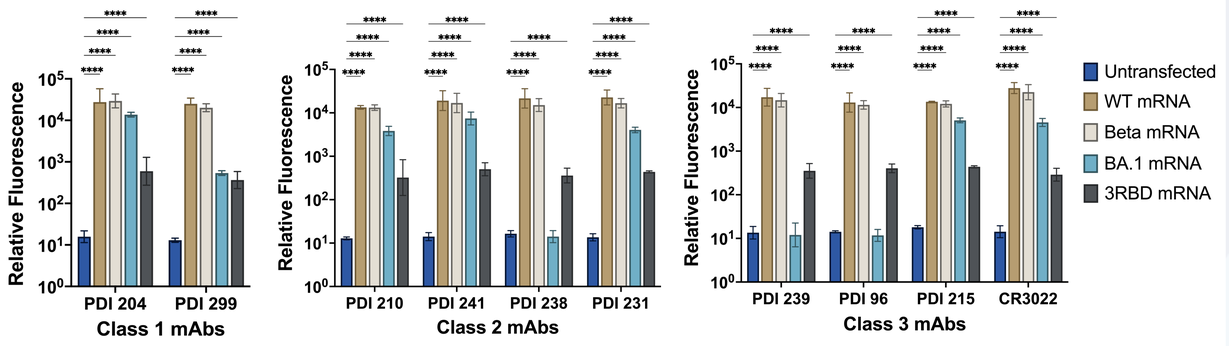


- *In vitro* expression of the purified RNAs encoding either the single RBDs or 3RBD antigen were investigated in BHK-21 cells.
- Vaccine antigens in the cell lysates of the transfected cells were readily detected via Western blot.

Modelling of the novel 3RBD antigen and its binding to hACE2 receptor

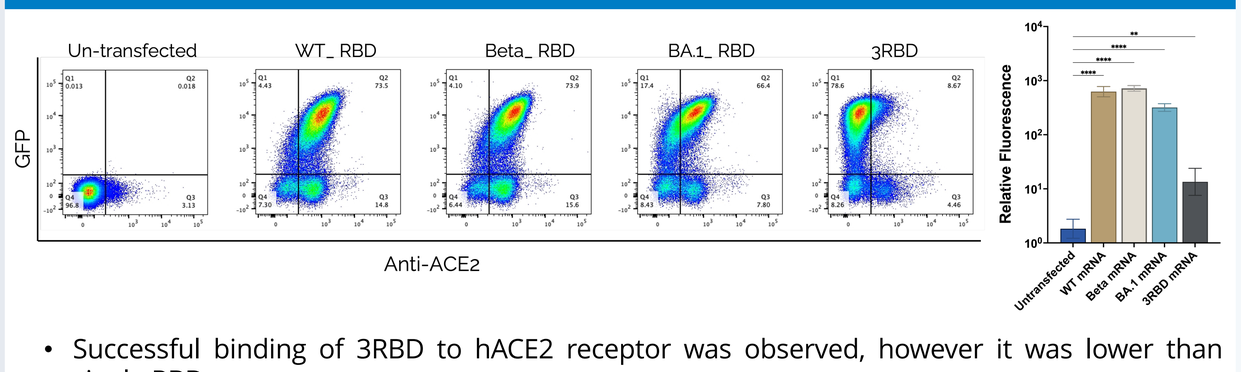


Monoclonal antibodies (mAbs) confirm neutralising epitopes on the vaccine antigens



- Different classes of human mAbs recognise different neutralising epitopes that are distributed widely across the RBD surface[5].
- The pattern of recognition of these mAbs matches their neutralisation profiles against the SARS-CoV-2 Spike variants[5].

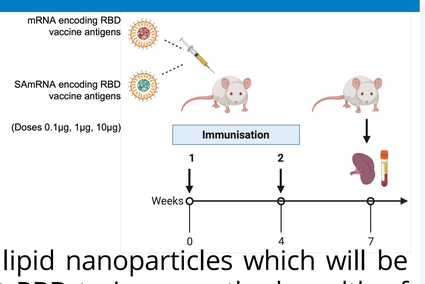
3RBD antigen binds to hACE2 *in vitro*



- Successful binding of 3RBD to hACE2 receptor was observed, however it was lower than single RBDs.
- 3RBD antigen was able to retain a conformation that allows receptor recognition.

Conclusion and Future direction

- We were successful in producing and purifying our RBD mRNA and SAmRNA vaccines with the expected chemical integrity.
- We have validated the antigenicity of our vaccine antigens, especially our novel polyvalent 3RBD antigen, and their binding to hACE2 receptor *in vitro.*



- Our current work has formulated our vaccine mRNAs into lipid nanoparticles which will be administered to mice to investigate the ability of polyvalent RBD to increase the breadth of neutralising humoral and cell-mediated immunity.

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