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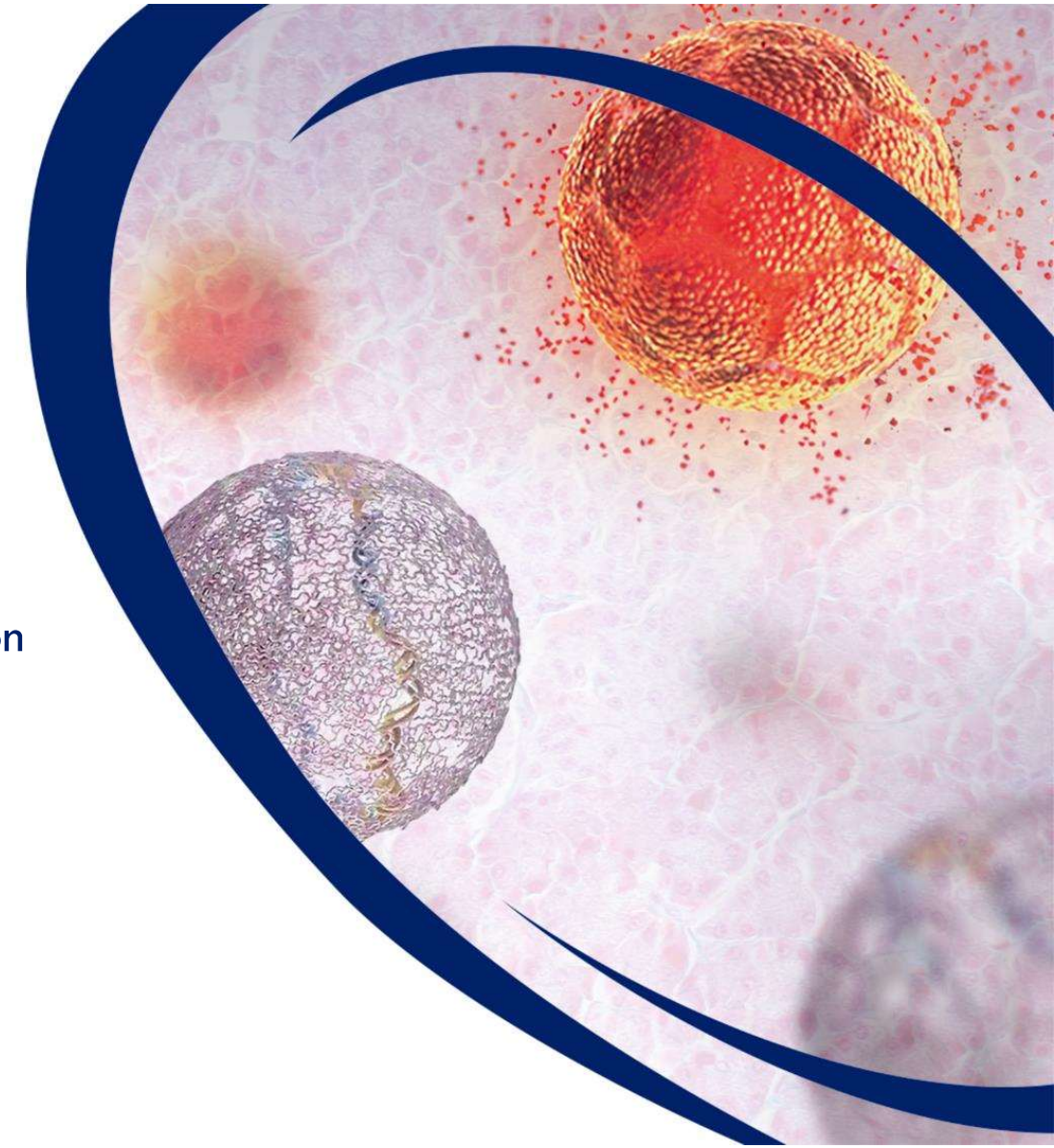
The Immunogenicity of DNA Vaccines based on Multicistronic Vectors and Synthetic Delivery Systems

International Vaccines Congress
October 18-19, 2021



New Vaccines Technologies

New approaches to safe, efficacious and rapid vaccines are warranted by:

- Emergence of new pathogens and uncontrolled outbreaks (flu, Zika, Ebola, Covid)
- Current unmet need (RSV, CMV)

Current vaccine strategies

- Inactivated and live-attenuated
- Adjuvanted subunit
- Viral vector
- mRNA & DNA

DNA approach represents an attractive alternative to live attenuated and subunit vaccines

- Capacity to triggering both antibody-mediated and cell-mediated immunity
- Safety
- Durable antigen expression
- Low-cost and generic production process

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A Multicistronic Formulated DNA Approach to Vaccines

We are developing a next generation DNA vaccine platform that comprises:

- A multicistronic plasmid with capacity to simultaneously express multiple antigens and immune agents
- A synthetic DNA delivery system with simple chemistry, high efficiency, safety compliance, and low cost

Potential benefits of a formulated multicistronic plasmid DNA vaccine

- Breadth of immune response
- Durability of antigen exposure
- Storage stability $\geq 4^{\circ}$ C
- Cost of manufacturing

Multiple antigens or antigen variants of SARS-CoV-2 being developed for proof-of-concept studies; comparisons will be made to marketed C-19 mRNA vaccines.

Additional pathogens to consider following the POC studies

This presentation describes the initial progress in our approach and planned future studies

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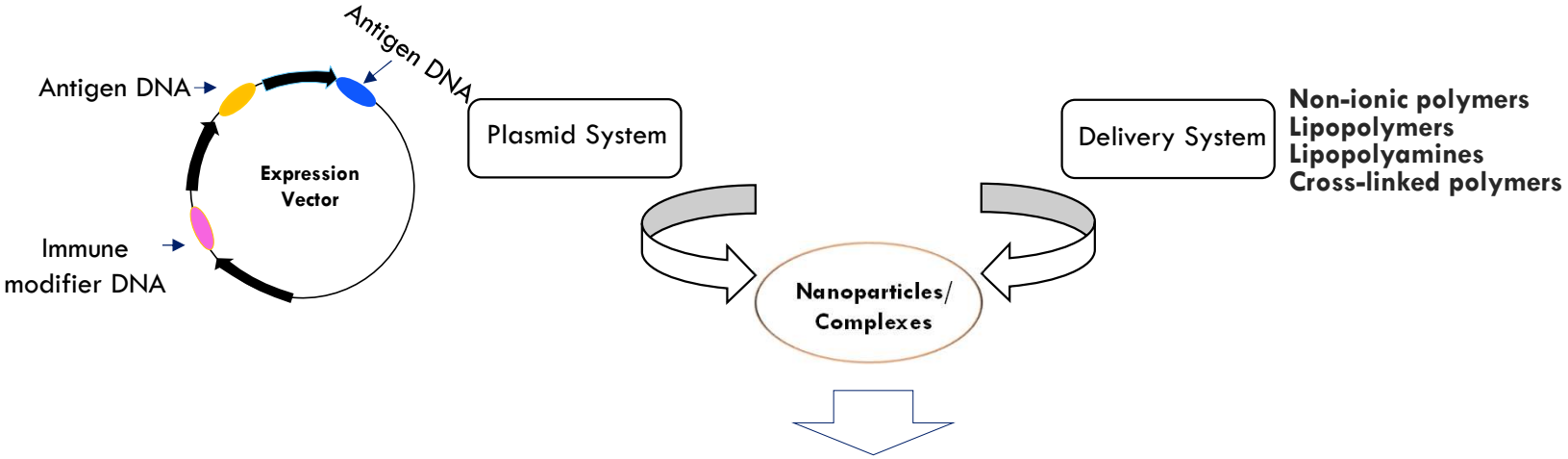
The Multicistronic Formulated DNA Vaccines Platform

The PLACCINE Platform



PLACCINE Vaccines

Potential Administration Routes

Intramuscular	Subcutaneous	Intradermal	Inhalation

Humoral & Cell Mediated Immune Response

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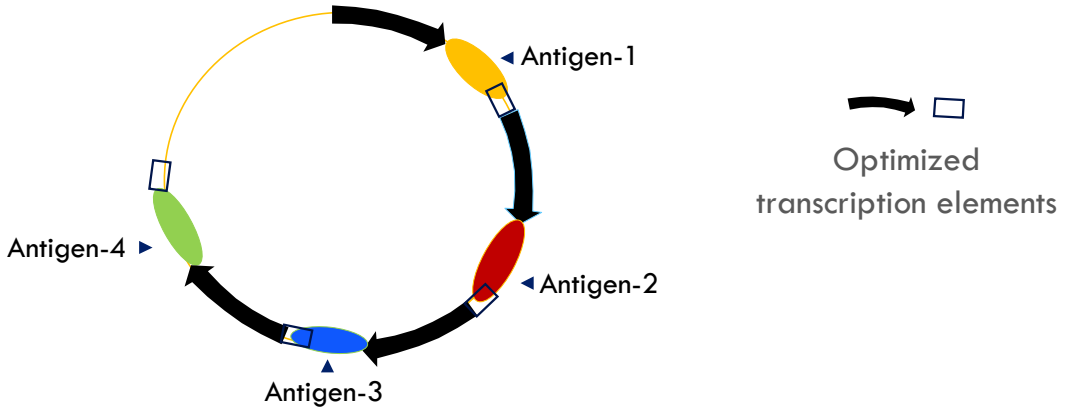
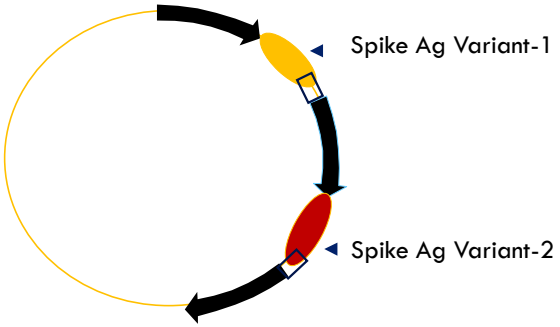
Multicistronic Vector Cassette

- A template backbone cassette with optimized transcriptional elements
- Flexible cloning sites for rapid gene incorporation/removal
- Up to four gene sequences have been cloned in the current plasmid cassette



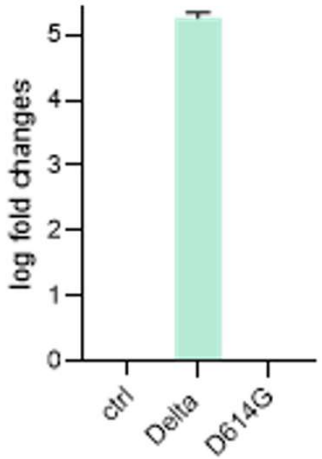
Multicistronic Vector Cassette

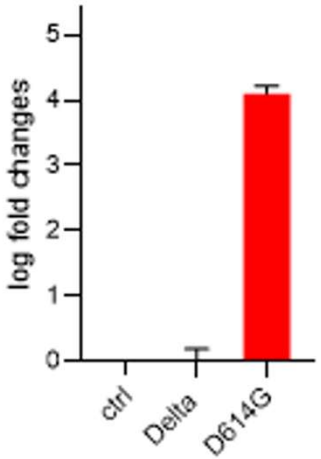
A Multicistronic Vector Expressing with Two or More SARS-CoV-2 Antigen Variants



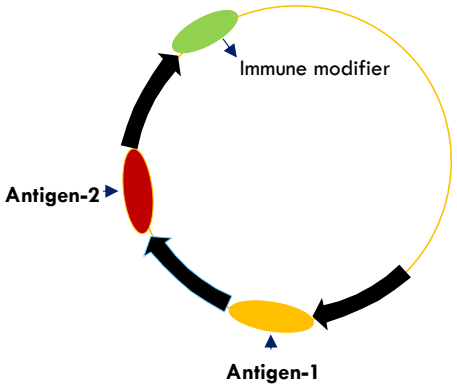
Two-Variant Multicistronic Vector

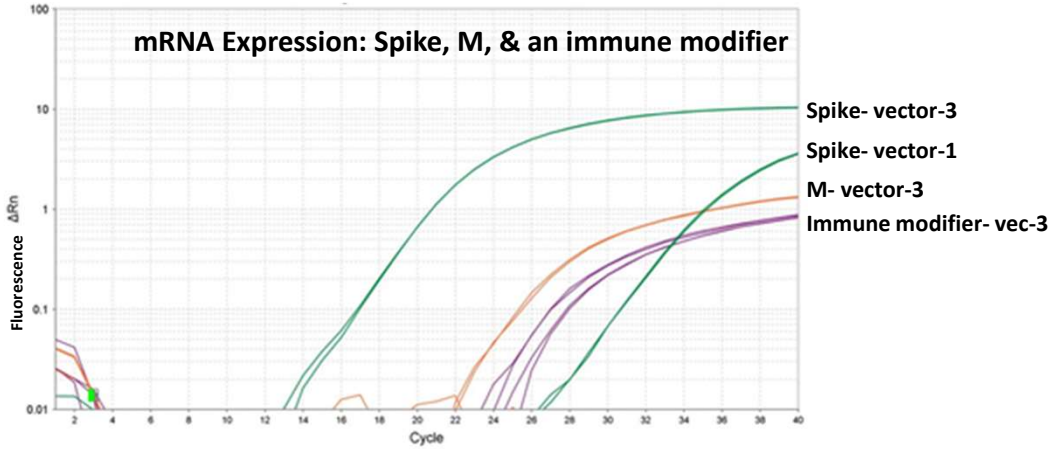
Distinguishing between D614G and Delta variant mRNA expression by RT-PCR





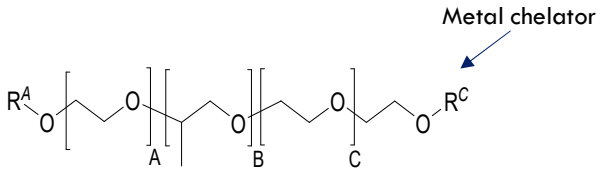
A Multicistronic Vector Expressing Two Different SARS-CoV-2 Antigens & An Immune modifier



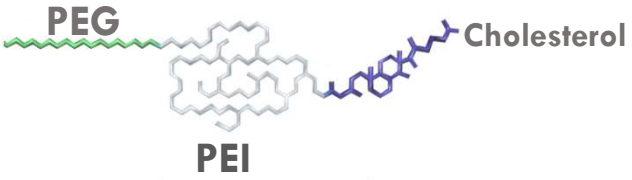


Vector-3: a multicistronic vector expressing Spike antigen (—), M antigen (—), and an immune modifier (—).

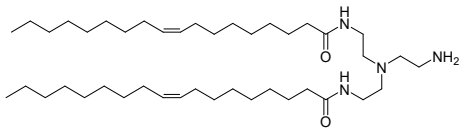
Synthetic Delivery Systems for DNA Vaccines



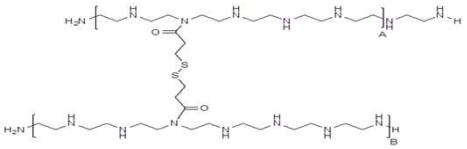
Functionalized Poloxamer



Lipopolymer PPC



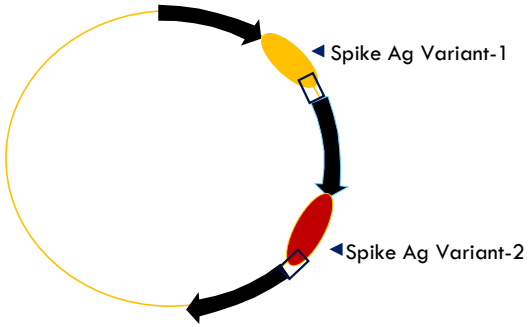
Lipopolyamine



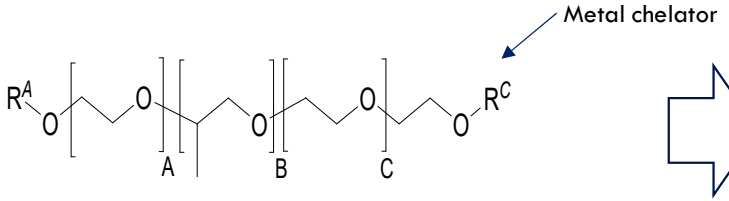
Crosslinked polymer

Current Focus of a Multicistronic Vaccine Development

- Two antigen variants of SARS-CoV-2 spike antigen
- Formulated with a functionalized poloxamer and delivered intramuscular
- IgG and T-cell responses
- Neutralizing antibodies
- Challenge studies



Two-Variant Multicistronic Vector



Functionalized Poloxamer

Intramuscular

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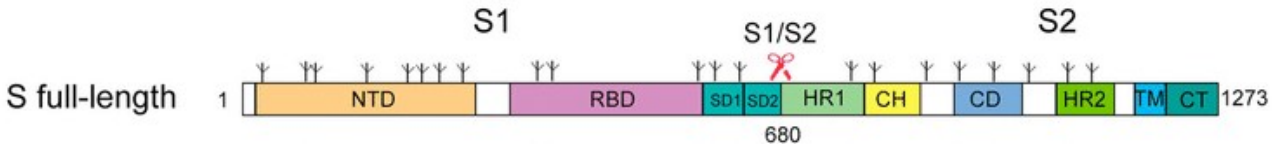
Target Antigen(s) for SARS-CoV-2 Vaccine Vectors

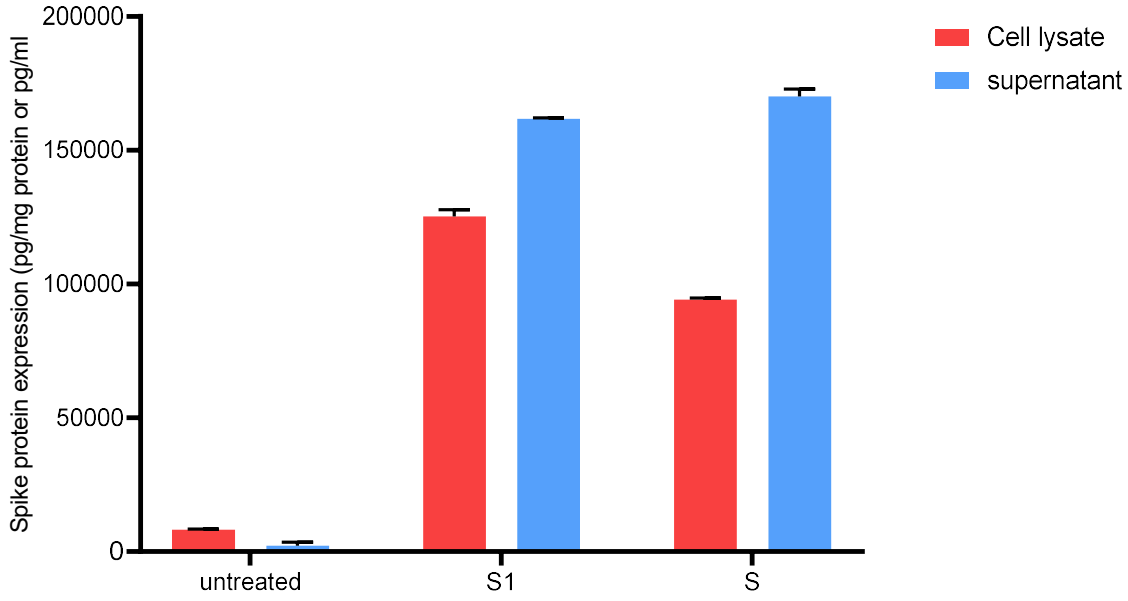
Spike Protein

- The S protein is a trimeric Class I fusion protein, extensively glycosylated
- The protein is composed of two functional subunits, responsible for receptor binding (S1) and membrane fusion (S2)
- Almost 90% of the plasma neutralizing antibodies target the spike receptor-binding domain (RBD)
- The mutation G614 has determined a transmission advantage over viruses with D614



Evidence of Spike Antigen Expression from DNA Vector

Full-length or S1 Spike Antigen DNA



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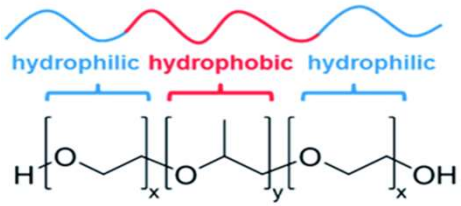
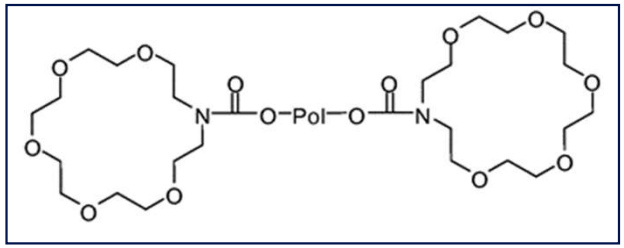
A Functionalized Poloxamer for DNA Delivery

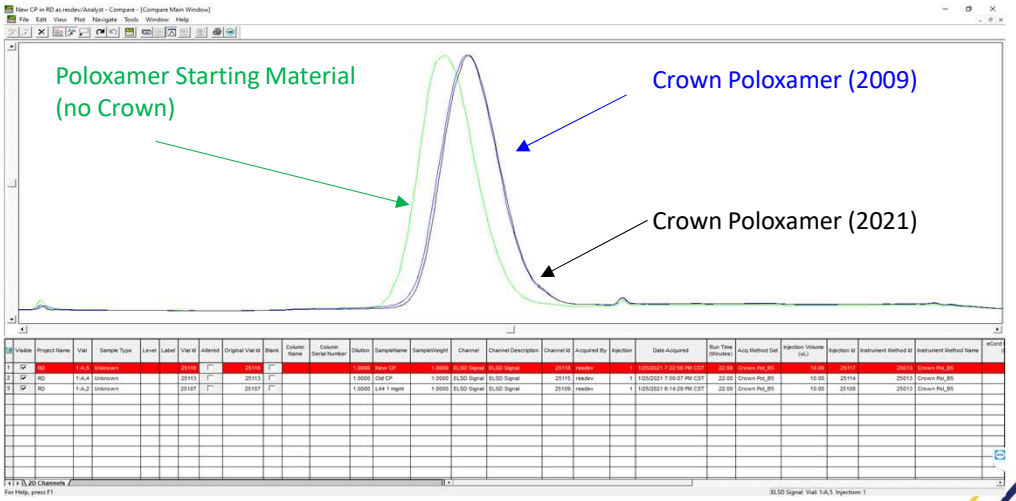
- Poloxamer is a functionalized amphiphilic triblock poloxamer of ethylene oxide and propylene oxide
- Crown poloxamer is functionalized backbone by covalent coupling of metal chelator (crown) to the terminal ends
- Poloxamers are safe for human use and have a rapid clearance
- Formulation with poloxamer is independent of plasmid size, a limitation of the LNP systems

Poloxamer



Crown-Poloxamer





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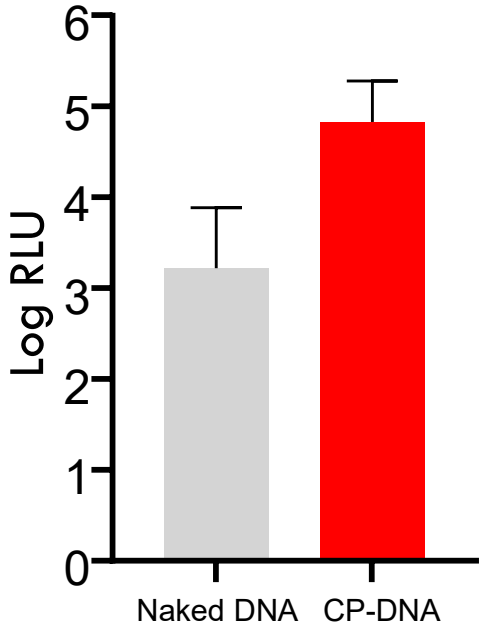
Improved Muscle Gene Transfer with IM CP Formulation

Muscle Luciferase Expression



25 μg DNA in saline or poloxamer formulation was injected into mouse tibialis muscle, tissue harvested 24 hours after the injection, and analyzed for luciferase expression.

CP delivery increases the early antigen expression (24hrs) by 40 - fold

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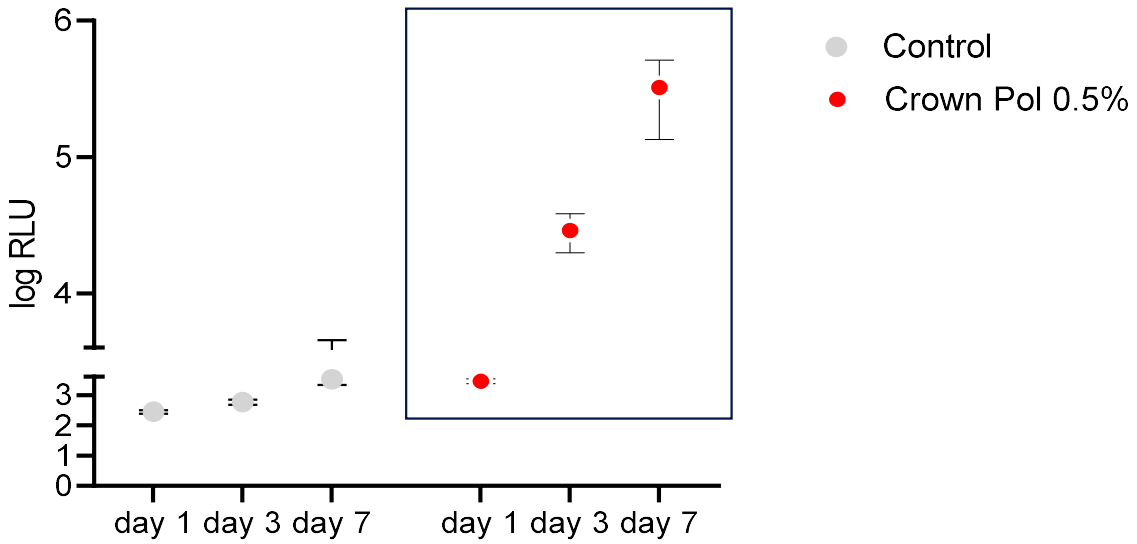
Durable Muscle Gene Transfer with IM CP Formulation

Serum Alkaline Phosphatase (SEAP) Expression



antigen (SEAP) expression increases overtime (day 1 → 7)

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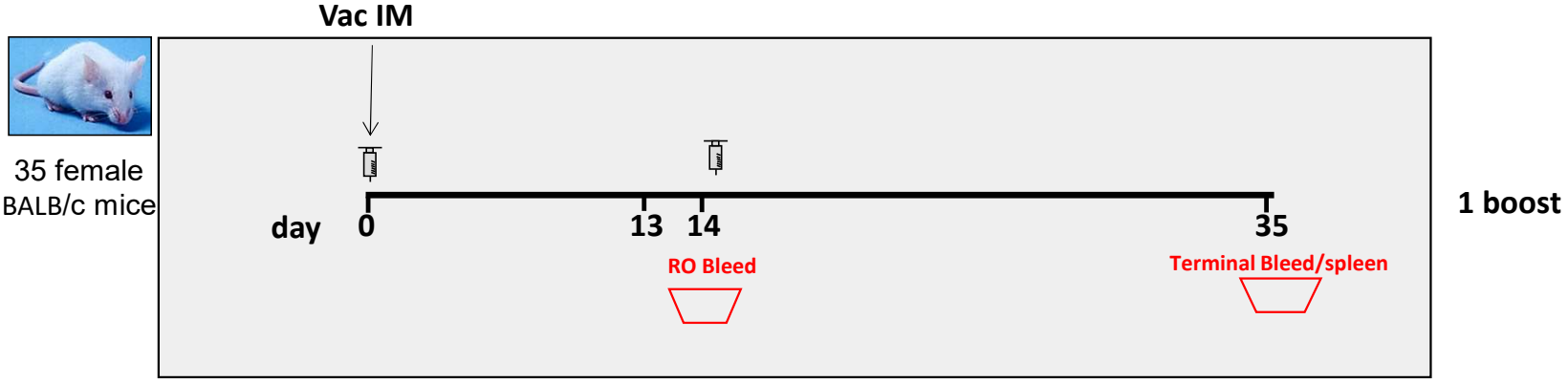
Immunogenicity Studies

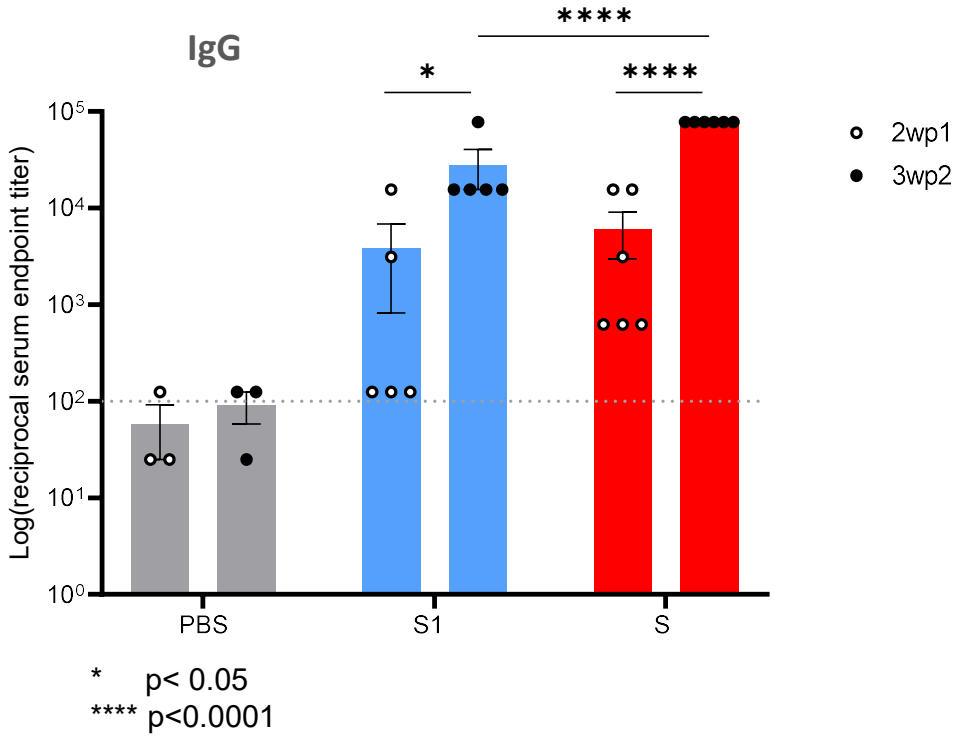
A Typical Study Design

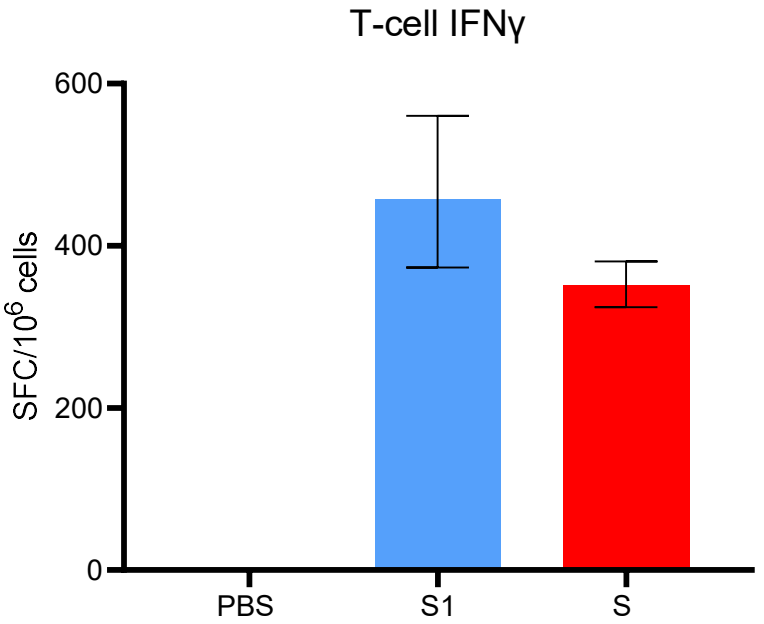


Optimizing booster frequency and booster interval around this design

Evidence of IgG and T-cell Responses to Formulated DNA Vaccine

Immunization with Full-length or S1 Spike Antigen DNA





The vaccine elicits a B-and T-cell response, antigen specific
S vaccine shows an increased IgG response (2wp2)

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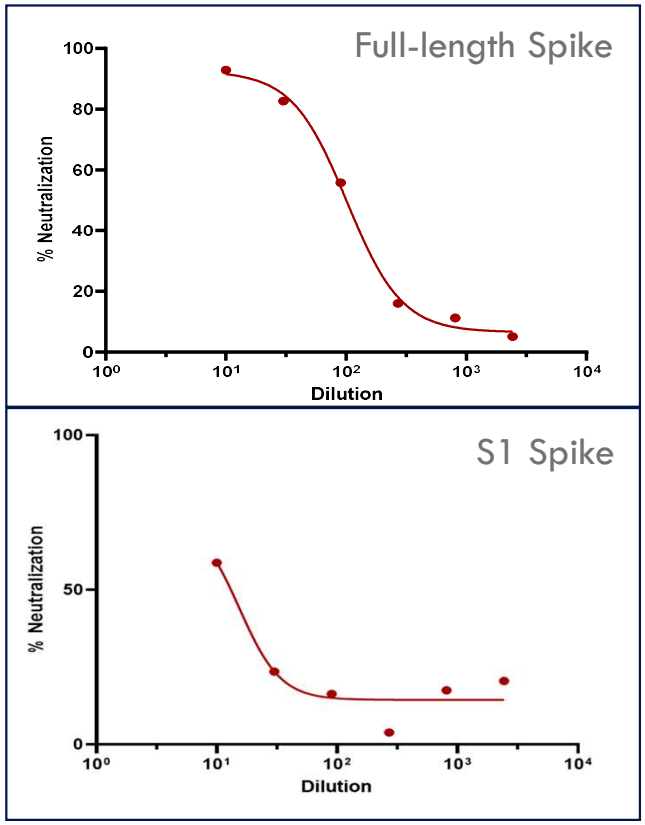
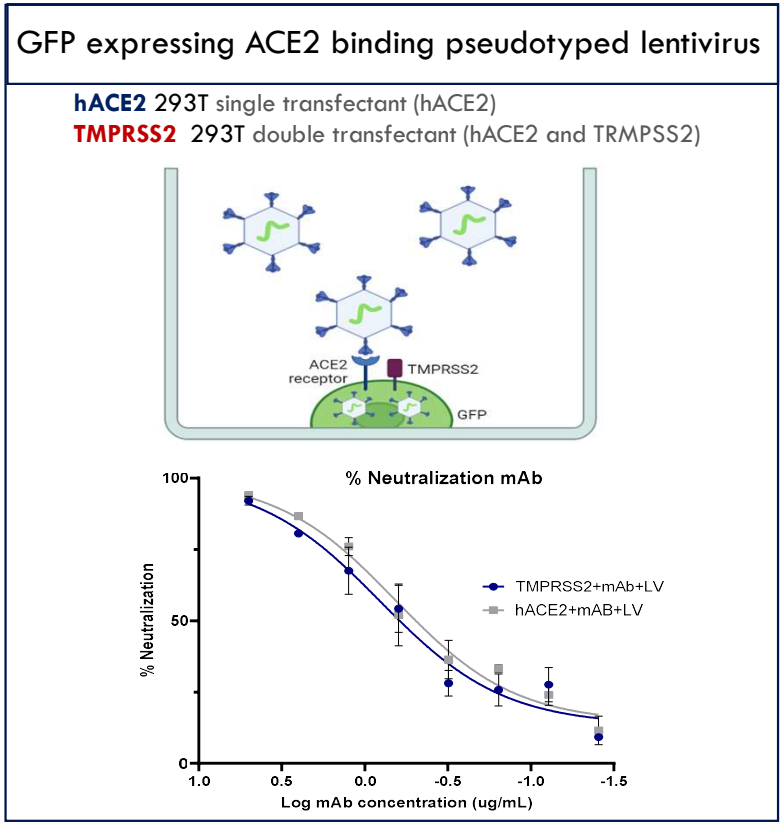
Evidence of Neutralizing Antibodies in Immunized Mice Sera

Blockage of pseudo-virus cell entry





Partial block of pseudovirus uptake by S1 as compared to full length spike S

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Conclusions & Ongoing/Future Studies

Conclusions

- Our preliminary data show evidences of immunogenicity against SARS-Cov2 antigen
- Plasmid DNA expressing S antigen stimulates a specific immune response
- Full-length spike determine an increased immunogenicity and neutralization response compared with S1
- CP indicates a lead delivery system for pDNA IM administration

Current & Future Focus

- Optimization of antigen, vector, delivery system, regimen for IgG and T-cell responses
- Neutralization antibodies against the targeted variants
- Response durability
- Dose response, safety toxicity, and biodistribution
- Challenge studies
- Stability studies

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Future Prospects

Proof of concept

- Immunogenicity and protection against multiple variants
- Comparable efficacy to approved vaccines and advantages in:
  - Response durability $\geq$ 6months
  - Safety
  - Shelf-life at $\geq$ 4C

DNA vectors and synthetic carriers are inherently suitable for the target profile

- Multiple antigens from a single plasmid
- High delivery efficiency, acceptable safety, expression durability, better shelf-life, rapid generic and cost-effective manufacturing

POC with SARS-CoV-2 will pave the vaccine path for:

- Targeting different antigens/variants of a same pathogen or of different pathogens from a single vector
- Incorporating a multitude of additional immune elements (modifiers) in a single plasmid to address a specific vaccine need
- Rapid development path for handling pandemics

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