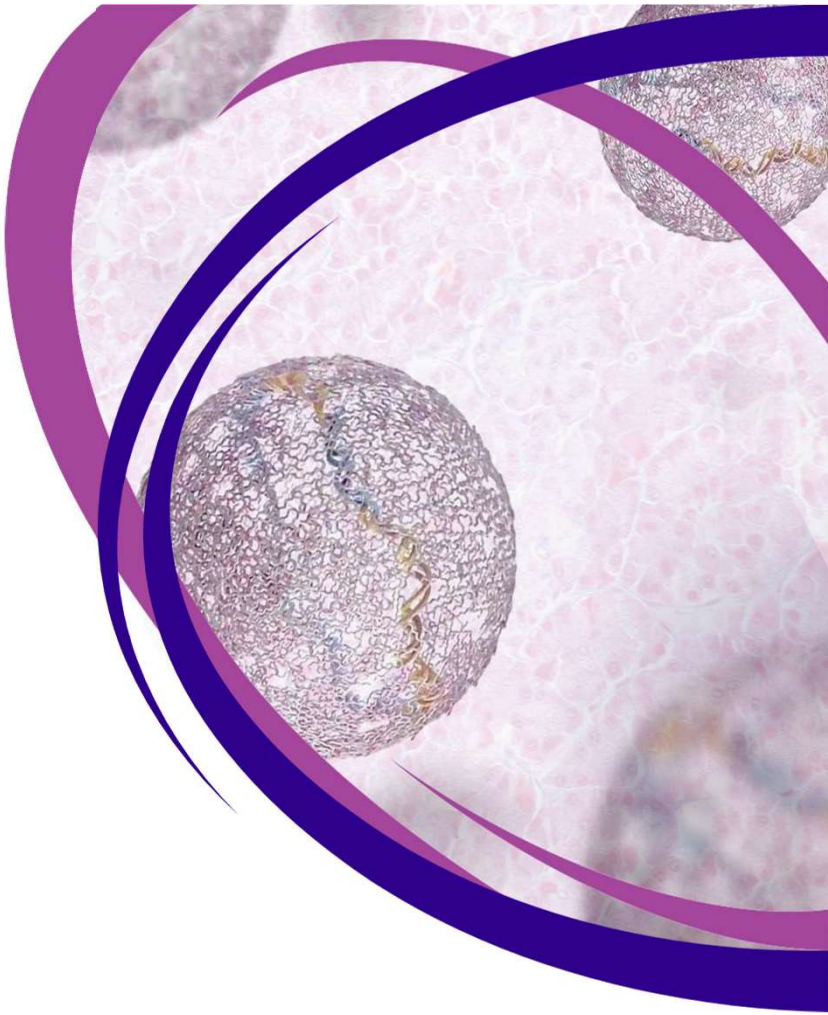
PLACCINE Nucleic Acid Vaccine Platform

Preclinical Proof of Concept Using a SARS-CoV-2 pDNA Construct

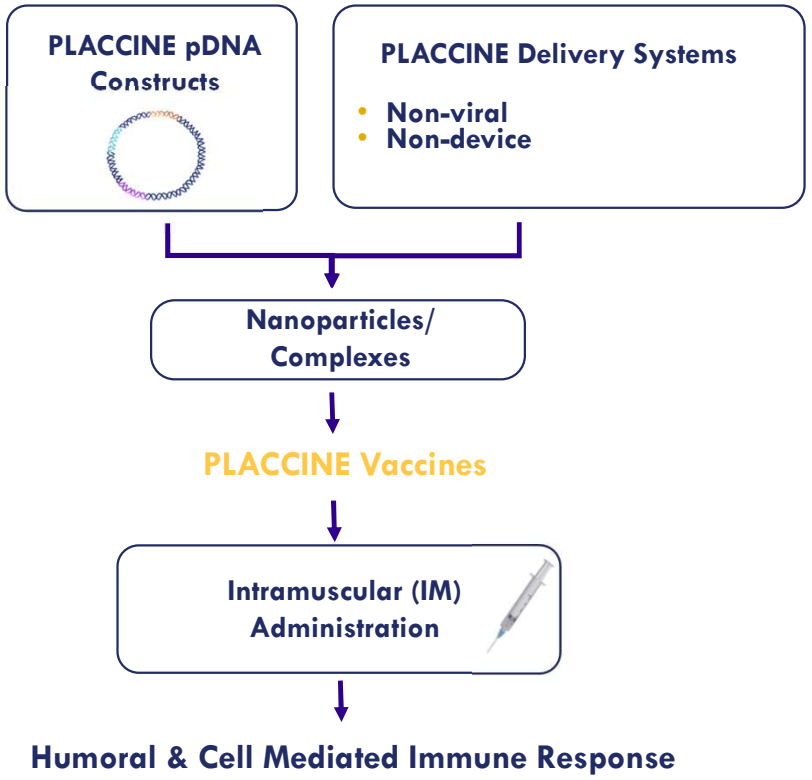


World Vaccine & Immunotherapy Congress
Nov 28 – Dec 1, 2022

PLACCINE – A New Class of Nucleic Acid Vaccines

Executive Summary

- Plasmid DNA (pDNA)-based modality targeting multiple antigens from a single vector
- Independent of virus or device for delivery
- Preclinical POC achieved using SARS-CoV-2 as benchmark
  - Humoral and cellular responses & protection in NHP & mice
  - Protection activity comparable to a commercial mRNA vaccine
  - >6-month stability at 4°C (ongoing)
- Potentially longer durability than mRNA (ongoing)
- Simple, rapid, and scalable manufacturing
- Patent portfolio in infectious diseases and cancer vaccines



Current Vaccines Despite some Success have Significant Limitations

PLACCINE Technology is Designed to Address these Limitations

mRNA

- Short-lived responses requiring frequent boosts
- Poor stability at working temperatures

Protein

- Challenges in manufacturing & subunit mixtures
- Poor cytolytic CD8 responses

pDNA Well-suited to overcome these Limitations

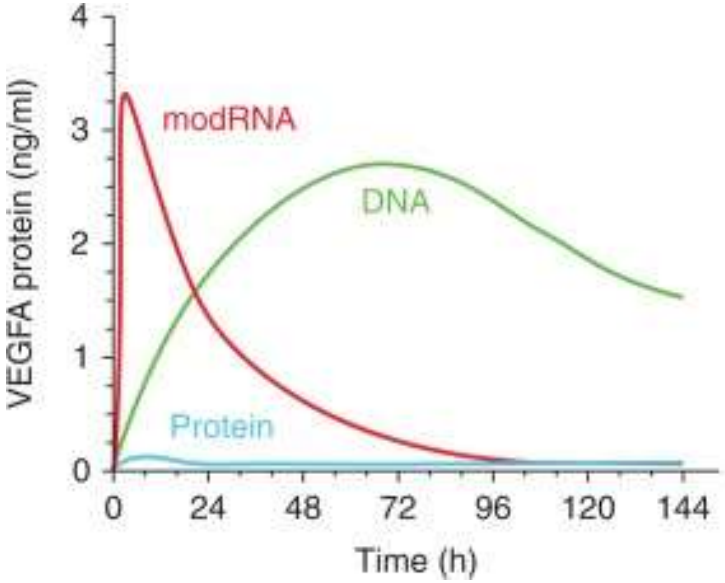
- Longer duration of antigen expression/exposure
- Strong CD8 response
- Stability at $\geq 4^{O}$ C

pDNA limitation: Insufficient delivery relying on viruses or devices (e.g., electroporation, jet)

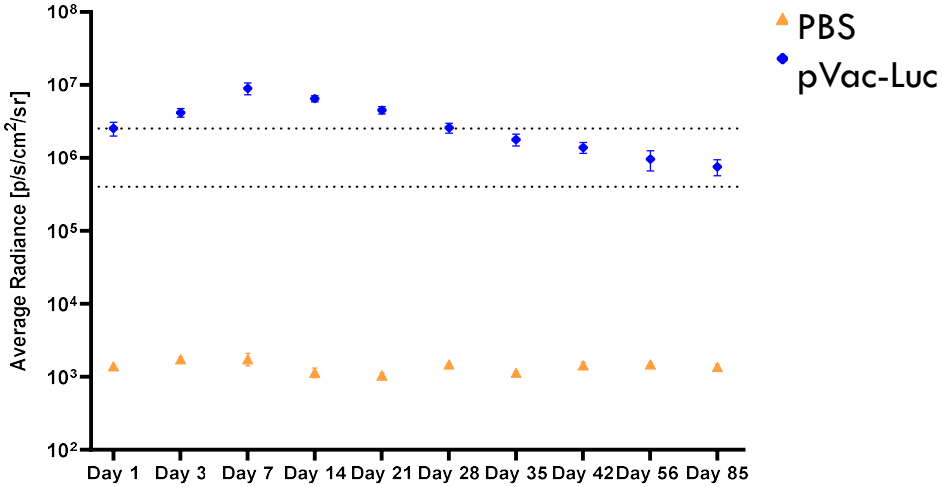
PLACCINE Approach

- Leveraging the DNA advantages (multi-cistronic, durability, CD8 response, shelf-life)
- Delivery without virus or device

pDNA Yields More Durable Antigen Expression than the Protein or Modified mRNA



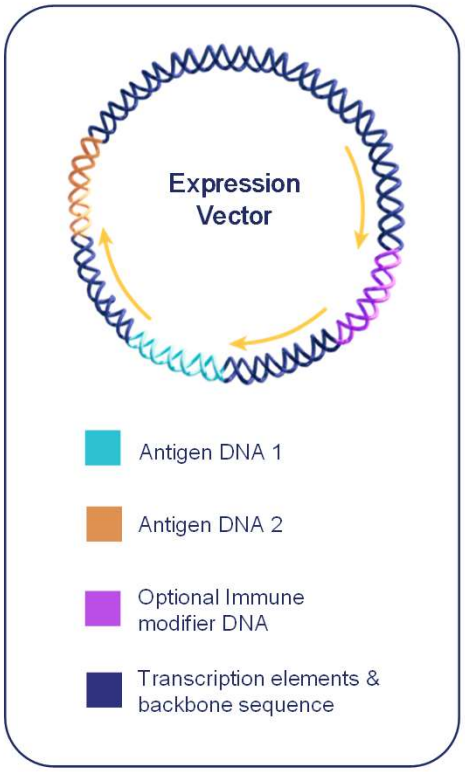
Chien KR Cold Spring Harb Perspect Med 2015;5:a014035



PLACCINE Formulation

PLACCINE Technology Platform

Multicistronic or Single Antigen Vector Formulations Independent of Virus or Device



Up to 4 antigens have been successfully incorporated and expressed

PLACCINE pDNA Constructs

PLACCINE Delivery Systems

-Synthetic Delivery Systems

- **Non-viral**
- **Non-device**

Nanoparticles/Complexes

PLACCINE Vaccines

Intramuscular (IM) Administration

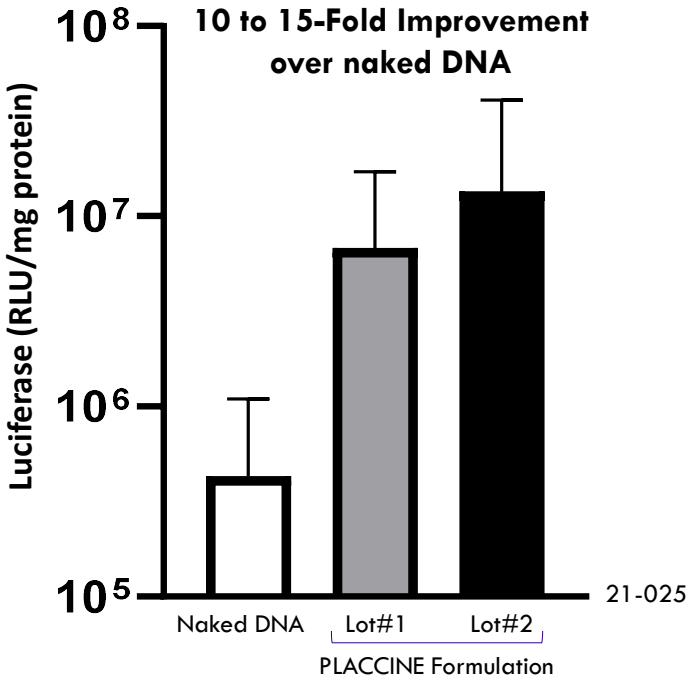
Potential Administration Routes

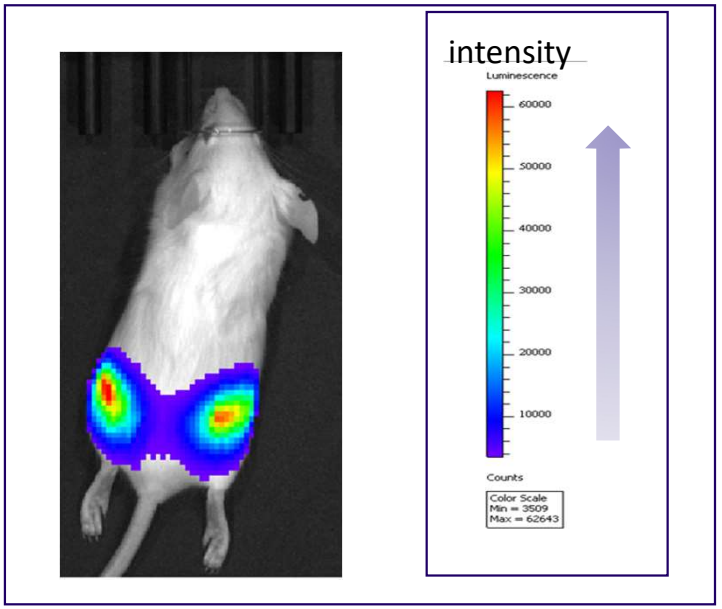
- Subcutaneous (SC)
- Intradermal (ID)
- Intranasal (IN)

Humoral & Cell Mediated Immune Response

A PLACCINE Formulation for Intramuscular Delivery without a Device/Virus

Early Antigen Expression (24 hr) and Bio-distribution in Mouse Muscle Tissue (day 7)

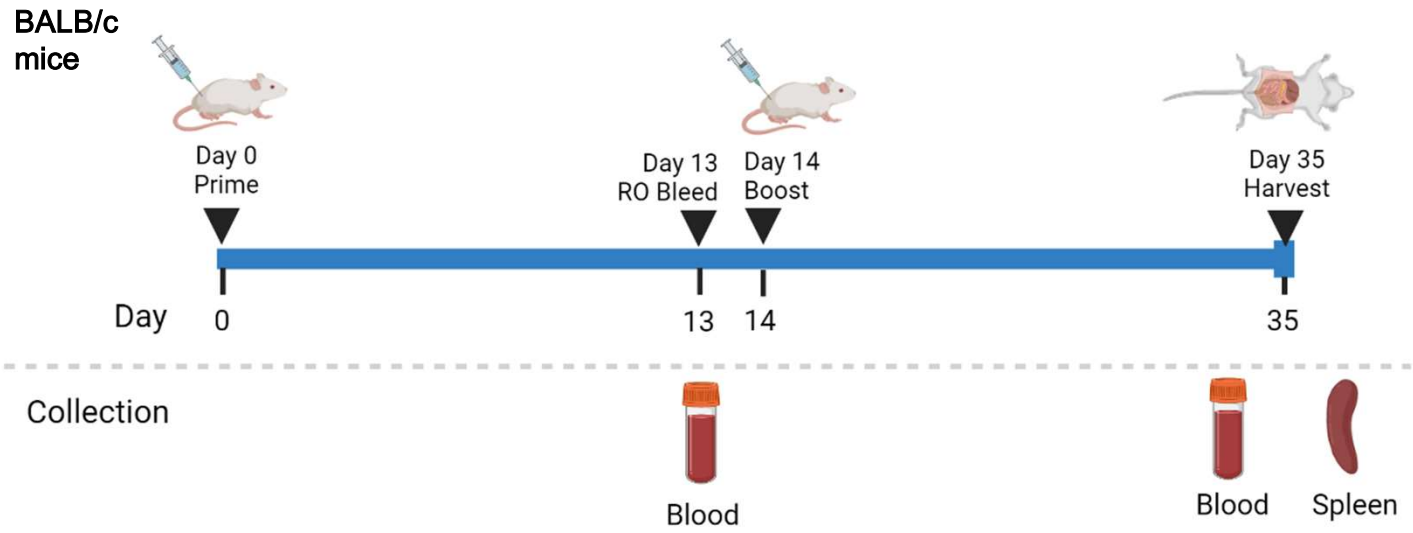




Mouse Studies

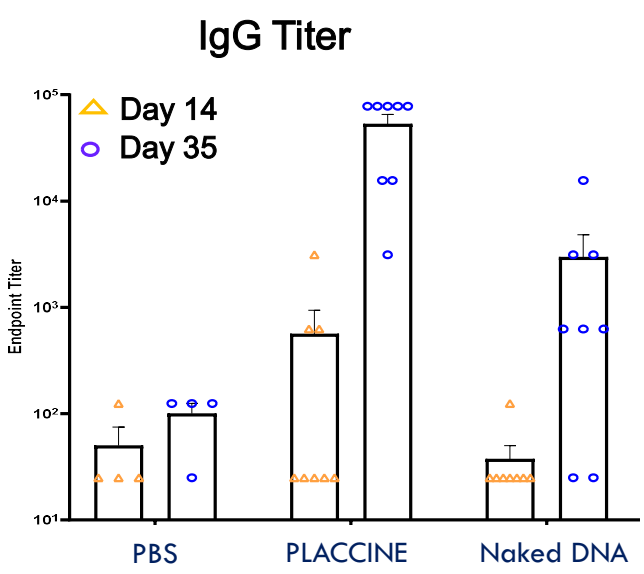
Standard Vaccine Regimen for In Vivo Studies

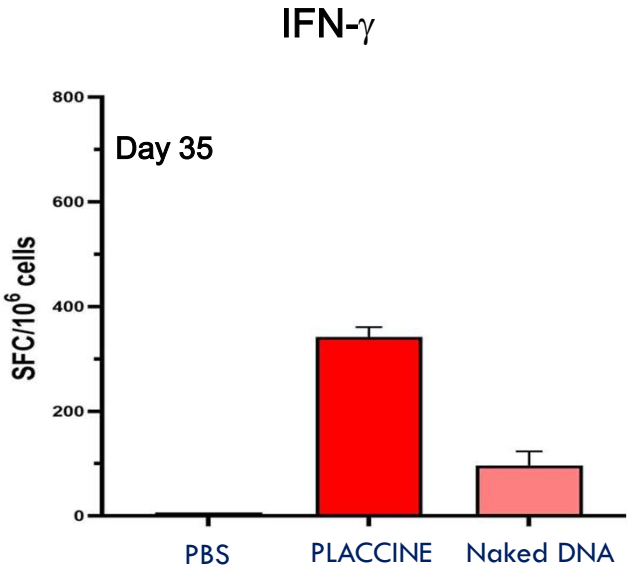
- Prime Day 0
- Bost Day 14
- Bleed for IgG Day 14, 35
- Spleen (IFN-γ) Day 35



Higher Immunogenicity of PLACCINE Formulation than Naked pDNA

- DNA vector **pVac-9**
- Spike antigen D614G
- Formulation PLACCINE
- 125 μg DNA

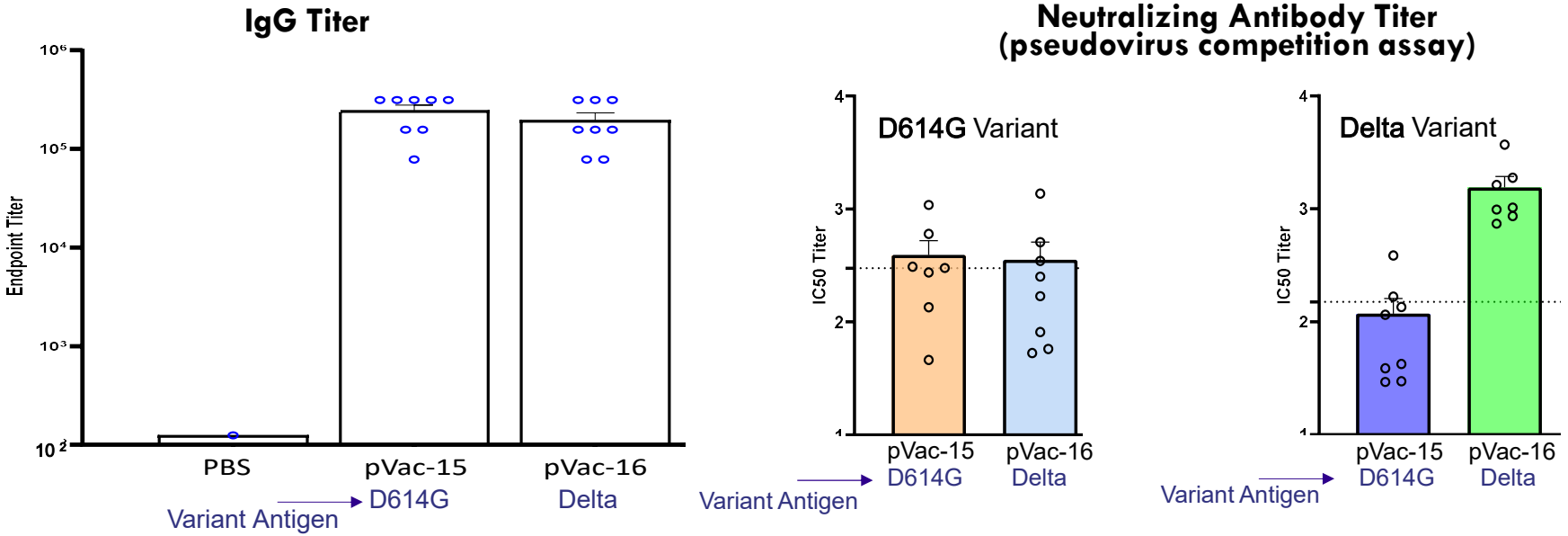




Immunogenicity of Single Antigen PLACCINE Vectors - IgG and nAB titers

Viral Mutation Warrants Vaccine Effectiveness Against Multiple Variants

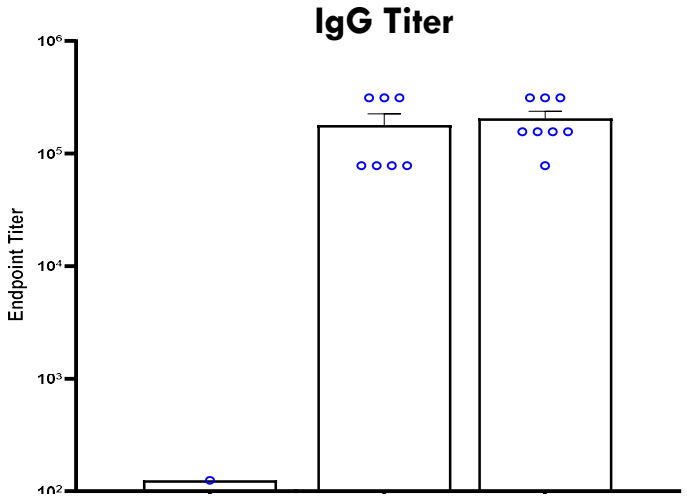
- Optimized vectors **pVac-15, pVac-16**
- Spike antigen D614G, Delta
- Formulation: PLACCINE
- 125 µg DNA
- IgG & nAB titer (day-35)
- Balb/c mice

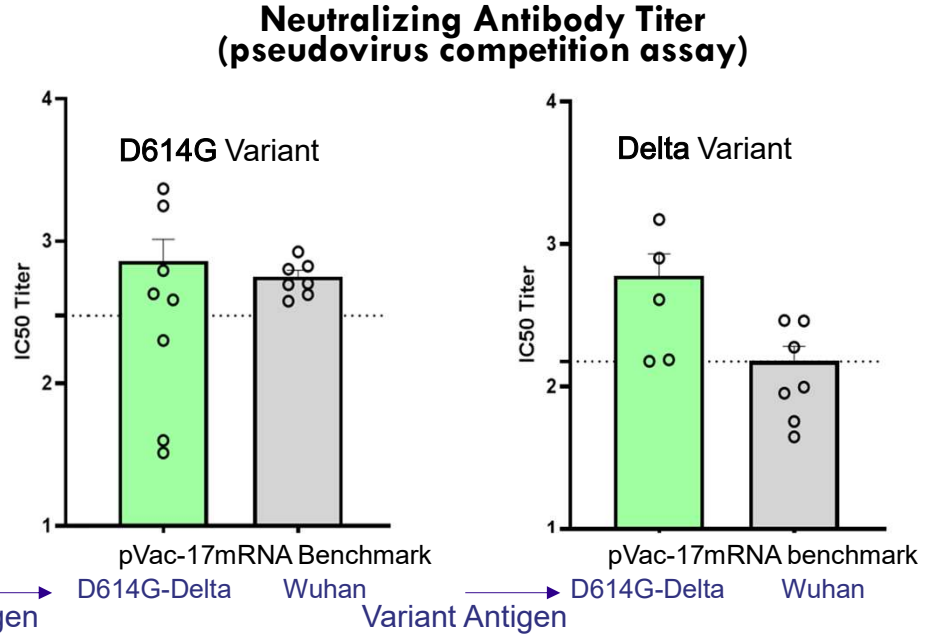


Immunogenicity of a Multi-variant PLACCINE Vaccine

A Bivalent Vaccine is Well Suited for a Mutating Virus

- Multicistronic vector **pVac-17**
- Spike antigen D614G, Delta
- Formulation: PLACCINE
- 125 µg DNA
- IgG & nAB titer (day 35)

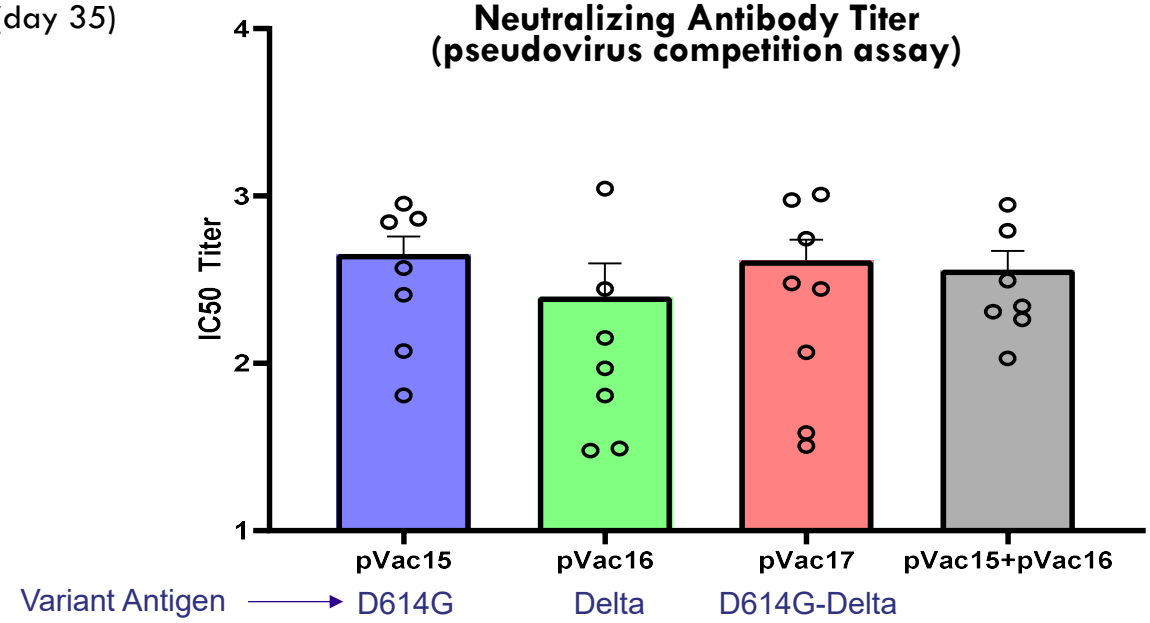




Single pDNA Multivariant Vaccine as Effective as Vaccine Mixture

Executive Summary

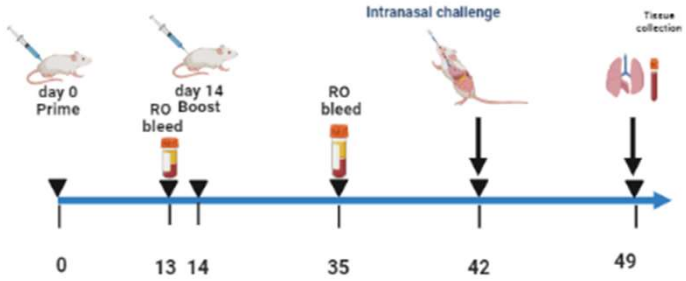
- Vectors **pVac-15** (D614G)
  pVac-16 (Delta)
  pVac-17 (D614G+Delta)
- Formulation: PLACCINE
- 125 µg DNA
- nAb titer (day 35)

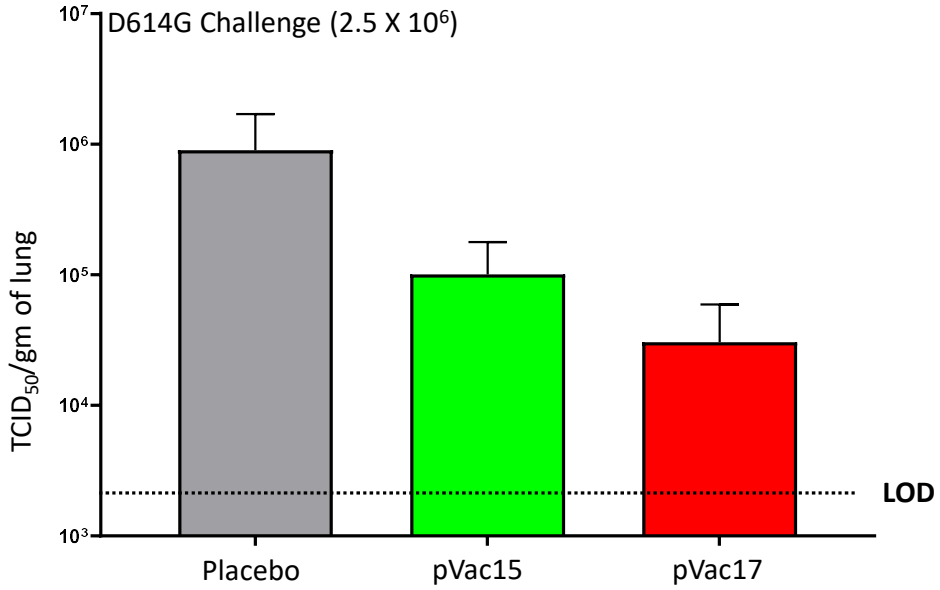


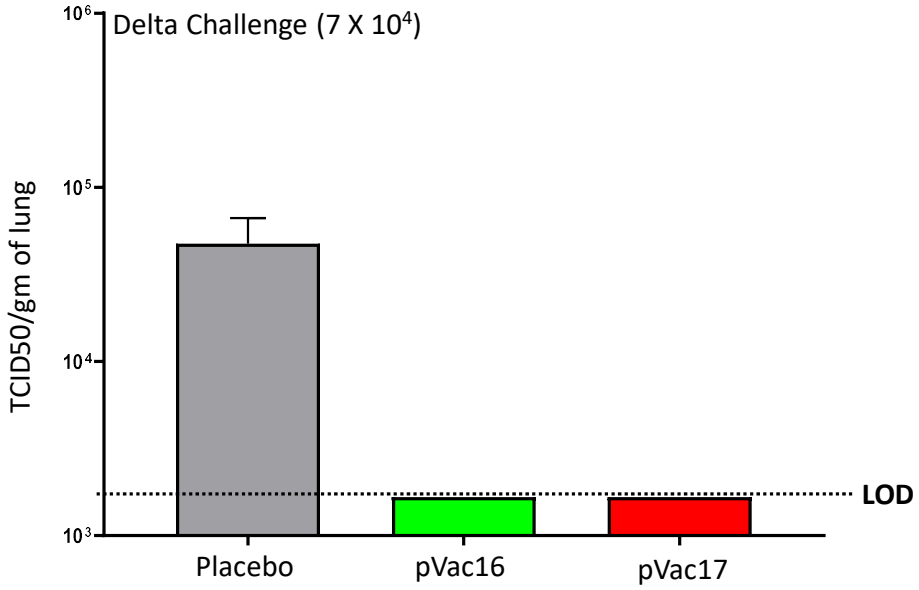
PLACCINE Vaccines are Protective Against Viral Challenge - hACE2:K18 Model

- **pVac-15** - D614G
- **pVac-16** - Delta
- **pVac-17** - D614G - Delta
- Formulation: PLACCINE
- Dose - 125 ug DNA

TCID50 Tissue Culture Infection Dose
(7 days post challenge)

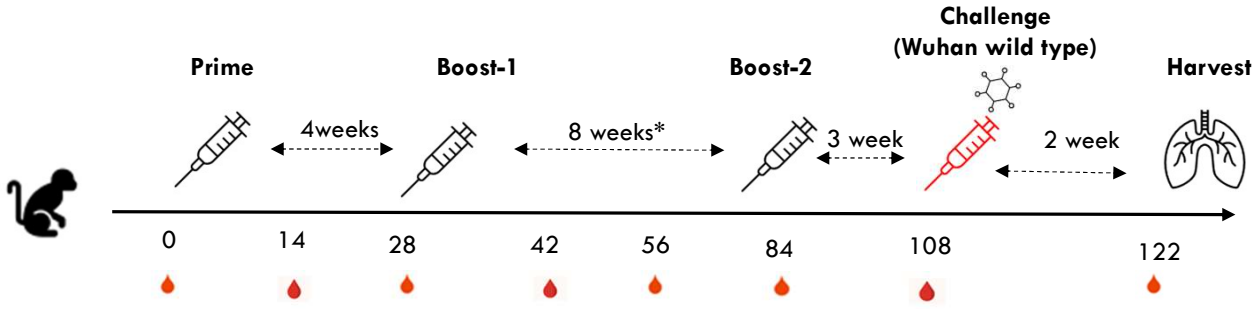






NHP Studies

NHP Study Protocol



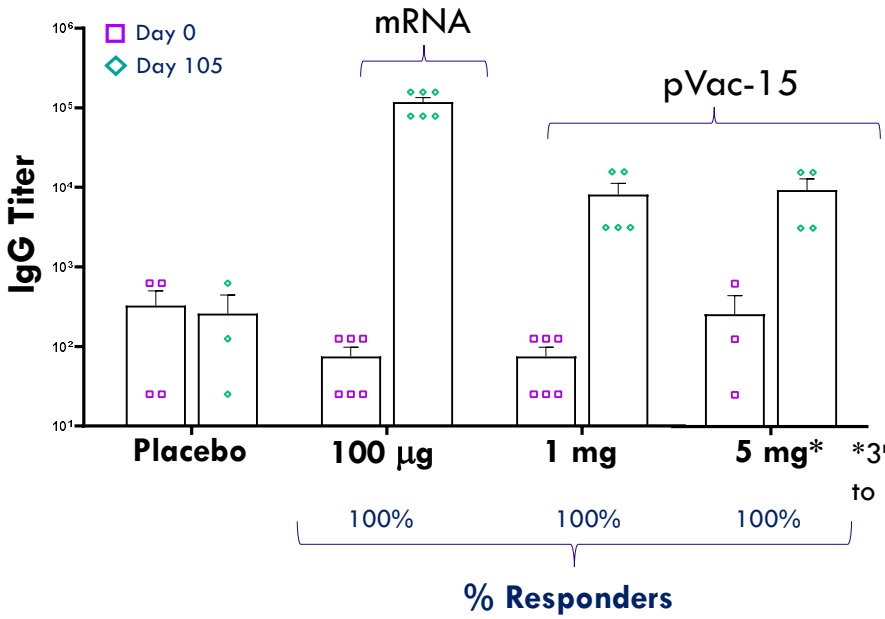
* Due to the time lag for sample analysis and data review the second boost was given 8 weeks after the first boost

@ Post Challenge Samples Collection Scheme				
	Day-2	**Day-4**	**Day-7**	**Day-14**
Tissue	BAL, nasal swabs	BAL, nasal swabs	BAL, Nasal swabs, IgG	BAL, Nasal swabs, PBMC
Assay	Viral load*	Viral load*	Viral load* IgG	Viral load* IgG, T-cell (PBMC)

Binding IgG Titers after Complete Vaccination (Prime and Two Boosters)

100% of PLACCINE Subjects Showed IgG Response

- Single antigen vector — **pVac-15** (D614G) in PLACCINE
- Comparator mRNA — **Commercial mRNA Vaccine** (LNP)
- Dosing schedule — Day 1, 28, 84
- IgG titer — Day 105 (21 days after 3rd dose)

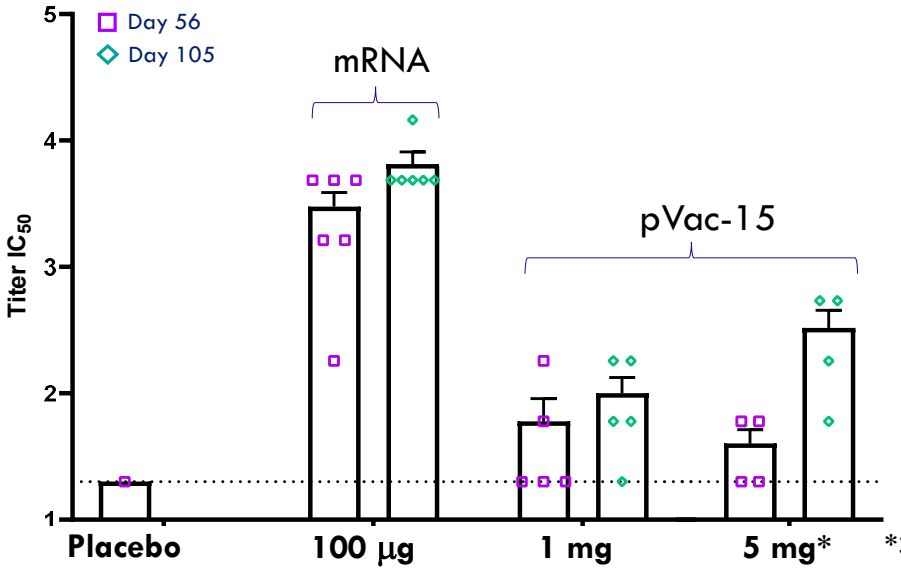


*3rd dose for this group was changed to pVac-16 at 2 mg dose

Neutralizing Antibody Titers after Vaccination

90% of PLACCINE Subjects Showed Neutralizing Ab Response

- Single antigen vector — **pVac-15** (D614G) in PLACCINE
- Comparator mRNA — Commercial mRNA Vaccine (LNP)
- Dosing schedule — Day 1, 28, 84
- nAB titer — Day 105 (21 days after 3rd dose)



*3rd dose for this group was changed to pVac-16 at 2 mg dose

Additional Immune Analysis to Better Define the PLACCINE Technology

In Progress

Additional Humoral and B Cell Analysis

- Functional antibody analysis
  - Antibody-dependent cellular toxicity (ADCC)
  - Antibody-dependent cellular phagocytosis (ADCP)
- Avidity testing
- B cell mapping
- Pseudovirus neutralization antibody assay

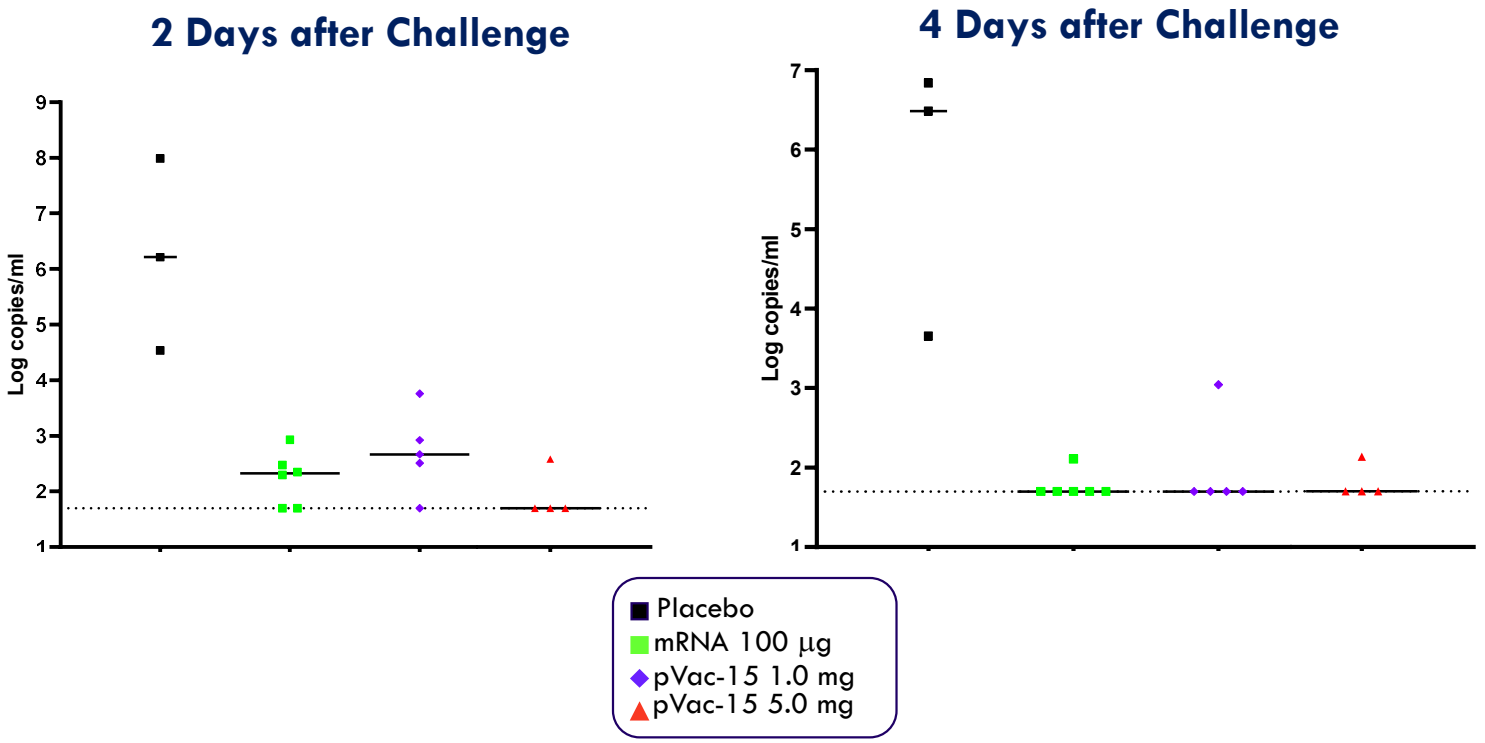
Induction of CD4 and CD8 Cytokine Producing Cells

- T cell Phenotype Analysis by Flow Cytometry

Viral Clearance by PLACCINE is Comparable to mRNA Vaccine

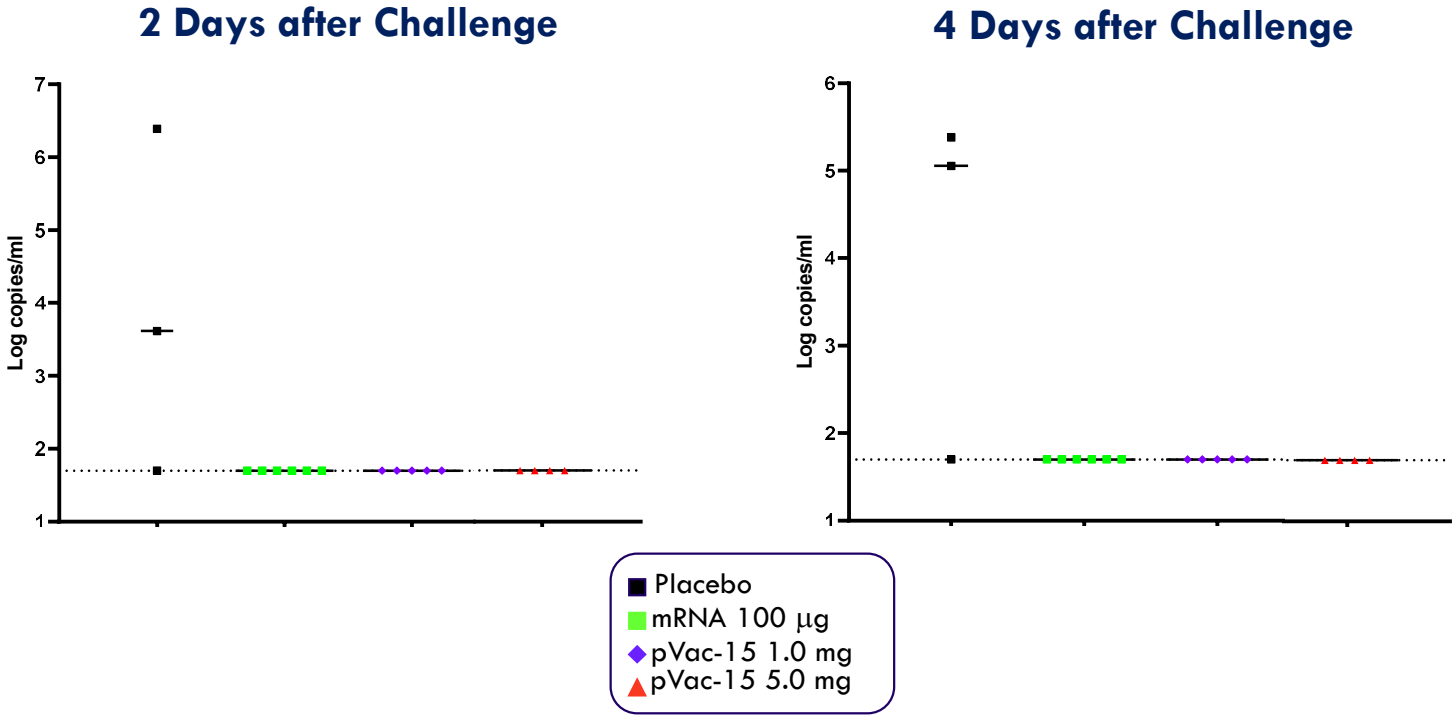
Genomic RNA- Bronchoalveolar Lavage

Challenge dose: $1x10^6$ $TCID_{50}$



Viral Clearance by PLACCINE is Comparable to mRNA Vaccine

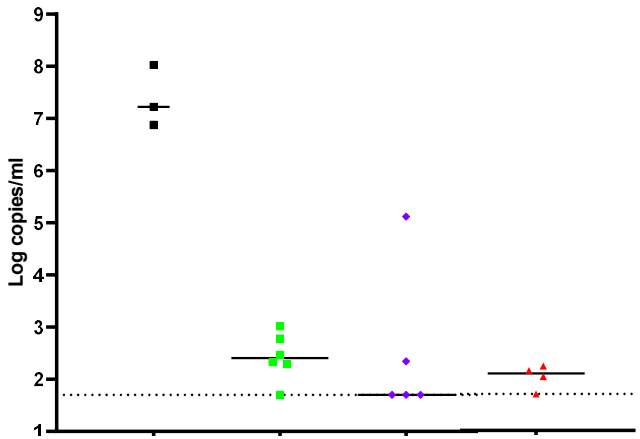
Sub-genomic RNA- Bronchoalveolar Lavage



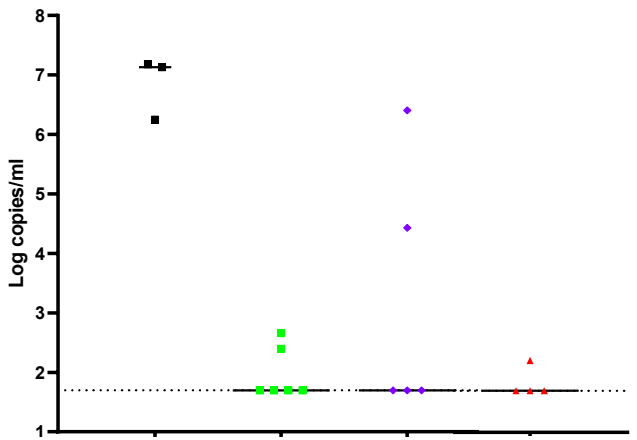
Viral Clearance by PLACCINE is Comparable to mRNA Vaccine

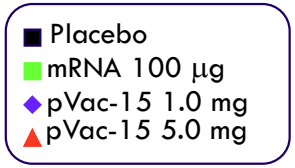
Genomic RNA- Nasal Swab

2 Days after Challenge



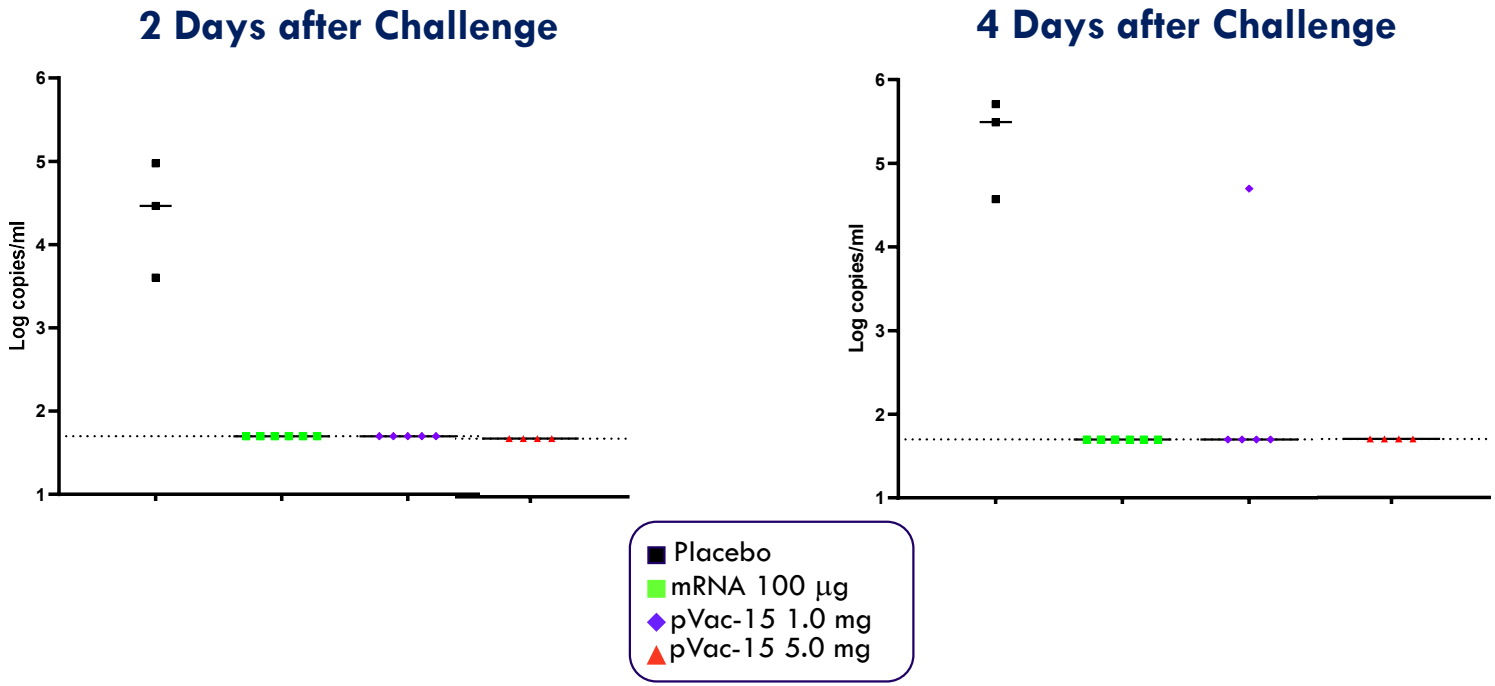
4 Days after Challenge





Viral Clearance by PLACCINE is Comparable to mRNA Vaccine

Sub-genomic RNA- Nasal Swab



Viral Clearance from BAL & NS after Challenge- TCID50 Assay

Clearance Efficiency Comparable to mRNA Vaccine

Challenge dose: 1 x 10^6 $TCID_{50}$

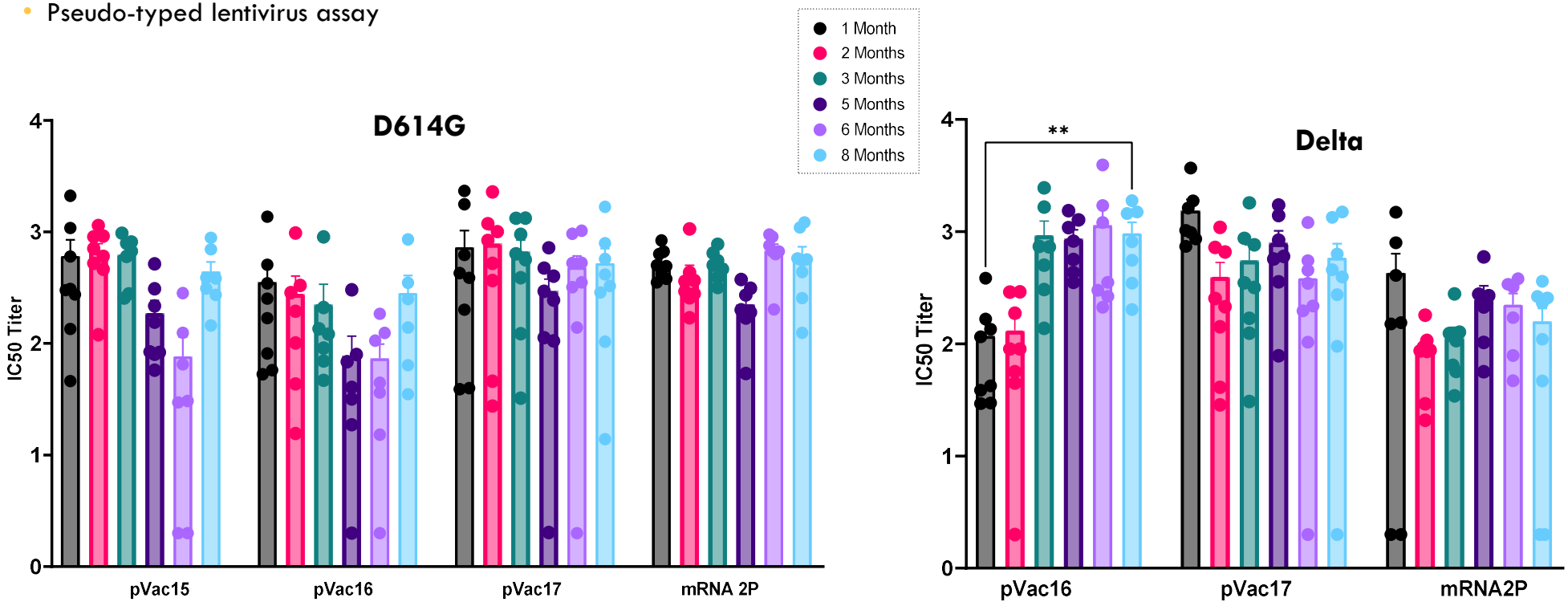
Group	Vaccine	Viral Load					
		LOG $TCID_{50}$/ml			LOG $TCID_{50}$/ml		
		Bronchoalveolar lavage			**Nasal swab**		
		Day-2	Day-4	Day -7	Day-2	Day-4	Day -7
1	Placebo	6.20 3.20 5.20	4.37 4.37 <2.7	3.70 <2.7 <2.7	5.37 6.37 5.20	4.70 5.20 5.70	4.20 3.70 3.37
2	mRNA (100 μg)	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7 <2.7
3	pVac 15 (1mg)	<2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 4.70 <2.7	<2.7 <2.7 4.20 5.37 <2.7	<2.7 <2.7 <2.7 <2.7 <2.7
5	pVac-15/pVac-16 (5mg, 1mg)	<2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7	<2.7 <2.7 <2.7 <2.7

<2.7 means below the lower limit of detection

Durability of Neutralizing Antibody Response

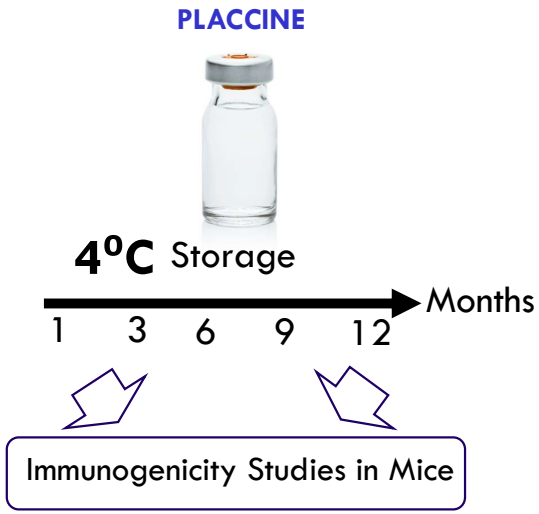
Eight-month Durability in Mice

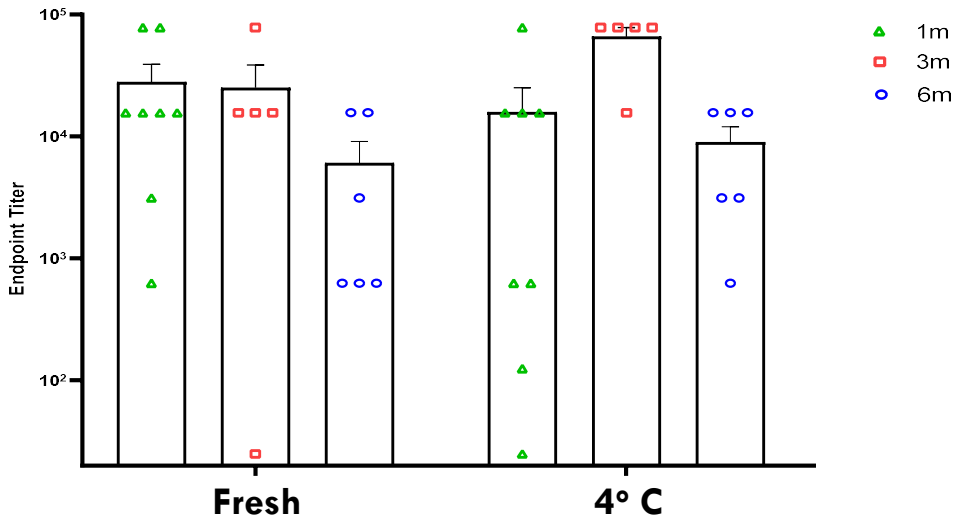
- Vectors: **pVac-15** (D614G), **pVac-16** (Delta), **pVac-17** (D614G+Delta)
- Formulation: PLACCINE
- 125 ug DNA
- Pseudo-typed lentivirus assay



PLACCINE is Stable at 4°C for Six Months or Longer

Vector: **pVac -17** (D614G-Delta)
Formulation: PLACCINE





PLACCINE – Summary

- PLACCINE- a new class of vaccines leveraging Inherent pDNA advantages including:
    - Multivalency, durability, cytotoxic responses, storage stability and rapid production and scale up
- Independent of virus or device for delivery for better safety compliance.
- Preclinical POC in NHP and mice using SARS-CoV-2 benchmark.
    - Potent IgG, nAB, or T-cell responses
    - >95% protection from live viral challenge
    - Comparable immunogenicity to a commercial mRNA vaccine
    - Better breadth of immune response than the commercial mRNA vaccine.
- >6-month stability at $4^{o}C$, a shelf-life advantage over mRNA vaccine
- Preclinical data warrants application to other pathogens (flu vaccine and SARS-CoV-2 booster vaccine - BARDA proposal)

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