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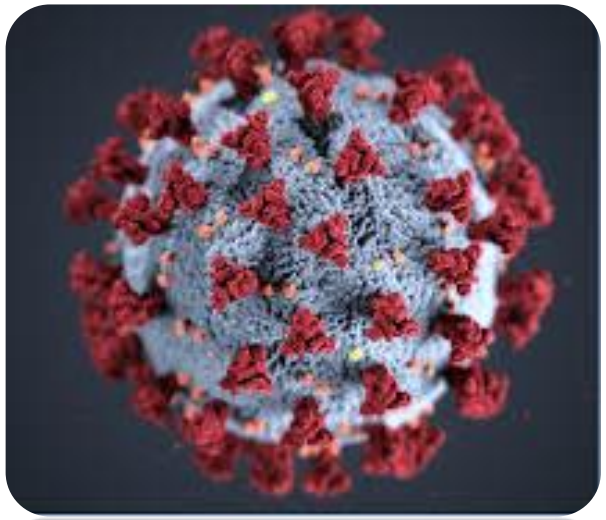
DNA vaccines against SARS-CoV-2



Joel Maslow MD PhD MBA
Chief Medical Officer
jmaslow@genels.us

DNA vaccines against SARS-CoV-2 and beyond

Outline of talk

- Review of DNA vaccines for SARS-CoV-2
- DNA vaccines: challenges and strengths
- DNA vaccine encoding for multiple SARS-CoV-2 antigens
- Heterologous booster regimens
- Role in future pandemics

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Approved vaccine platforms against SARS-CoV-2

Each with unique AE's and/or logistic challenges

- mRNA
- Adenoviral
- Nanoparticle, subunit
- Inactivated ± adjuvant
- Live attenuated
- DNA

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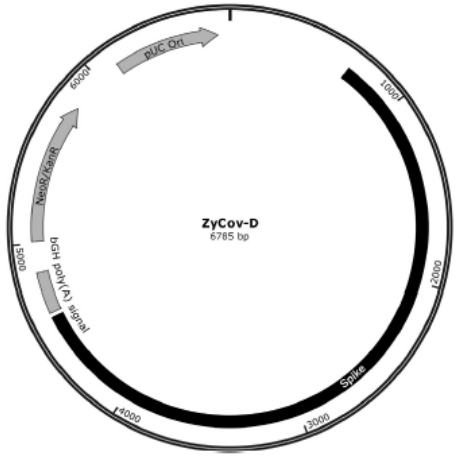
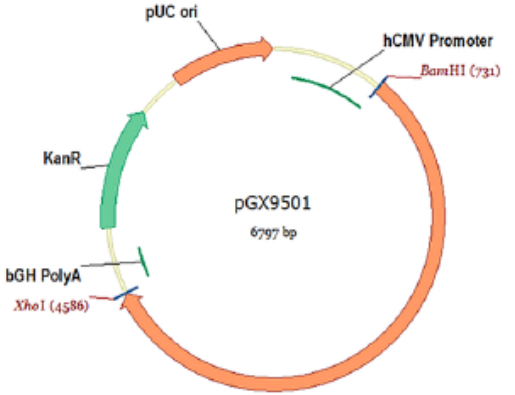
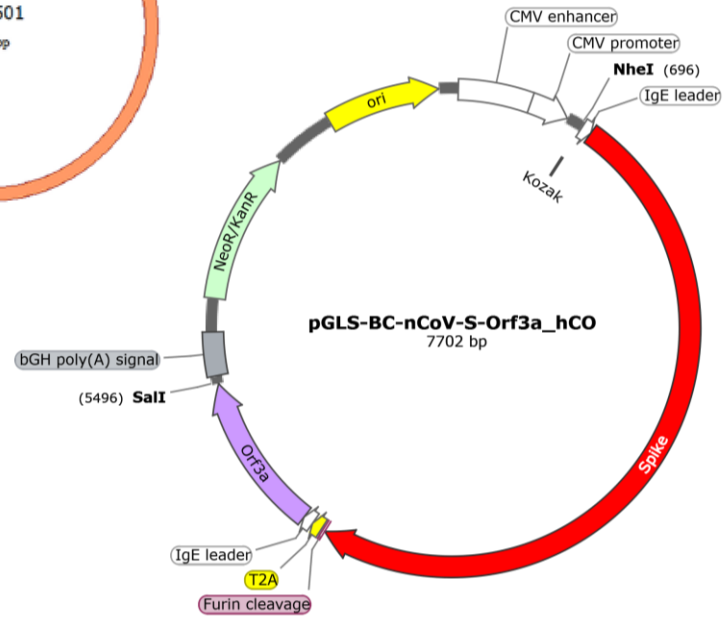
DNA vaccines against SARS-CoV-2

Phase 2 or beyond

- ZyCoV-D
  - Spike
  - Approved in India
  - ID using needle free injection system (NFIS)
  - 2 mg, 0-4-8 weeks (6 mg total)
- INO-4800
  - Spike
  - Phase 3 terminated
  - ID followed by EP
  - 2 mg, 0-4 weeks (4 mg total)
- GLS-5310
  - Spike + ORF3a
  - Phase 2
  - ID followed by application of suction
  - 1.2 mg, 0-8 weeks (2.4 mg total)







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ZyCoV-D

INO-4800

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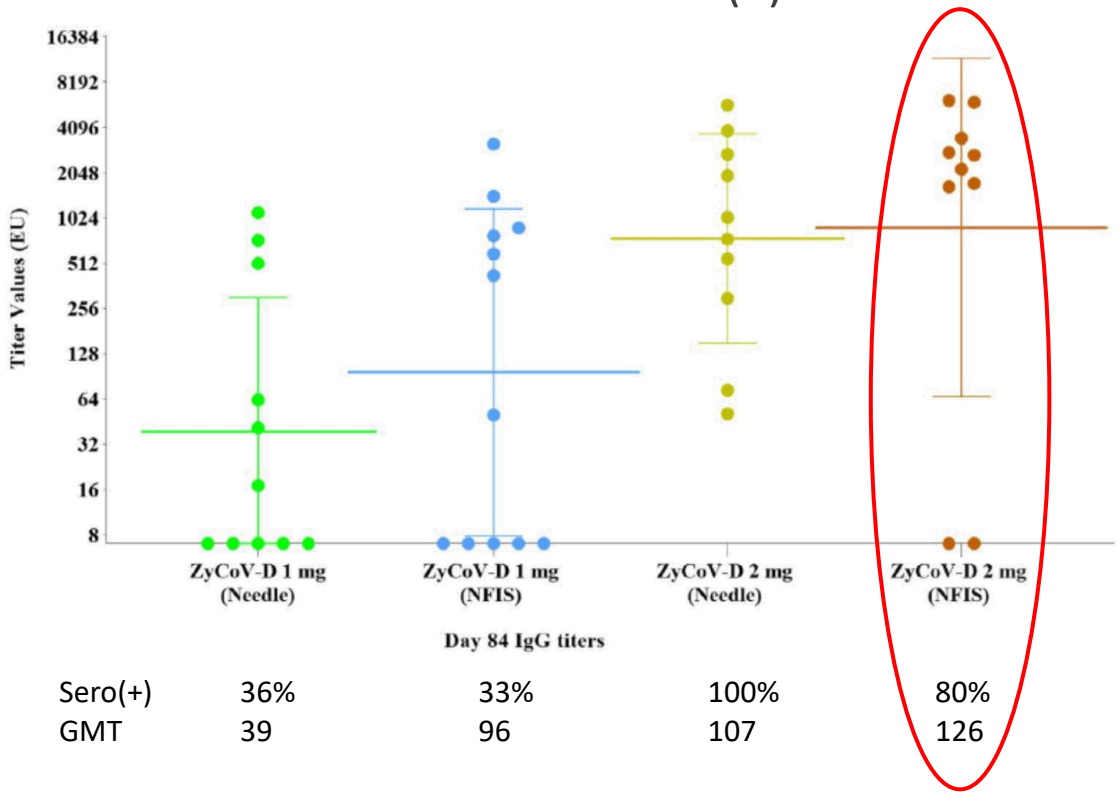
ZyCoV-D: Phase 1

- 1 mg vs 2 mg ID ± NFIS
  - 12 per group
  - Censored for sero(+) at baseline



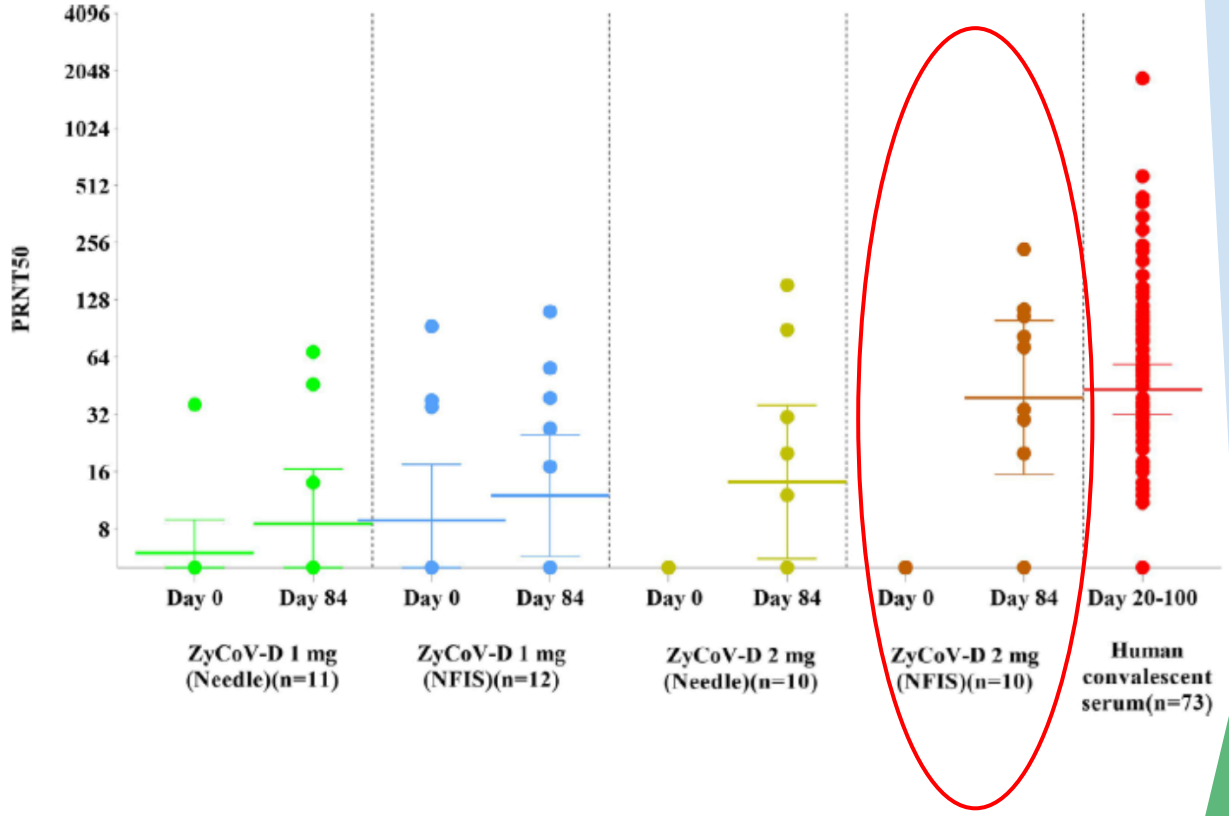
Sero(+)	36%	33%	100%	80%
GMT	39	96	107	126

EClinicalMedicine 38 (2021) 101020

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine



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ZyCoV-D: Phase 1

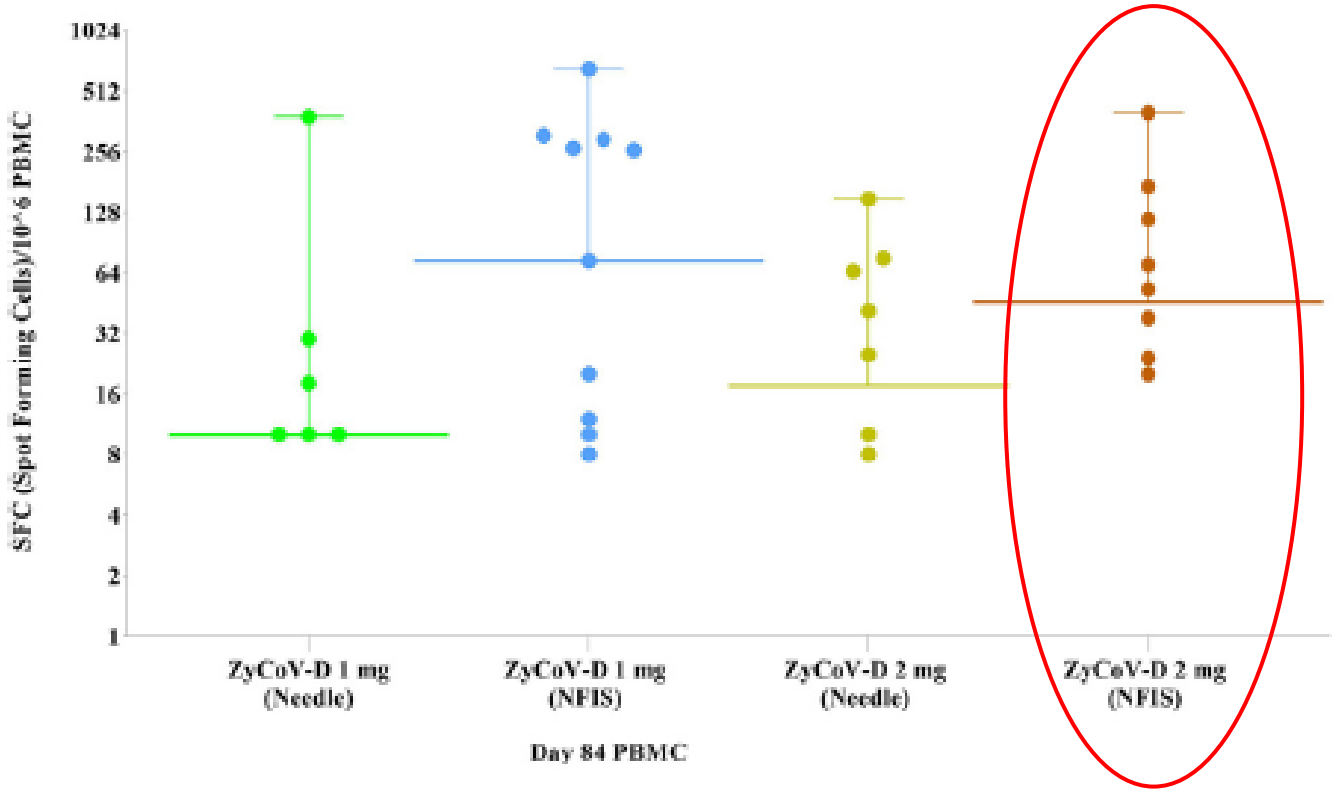
EClinicalMedicine 38 (2021) 101020

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

- Summary
  - Immune response – dose dependent
  - Neutralizing antibodies: post vaccine comparable to convalescent sera
  - T cell responses : 45.5 SFU/10^6 cells post vaccination



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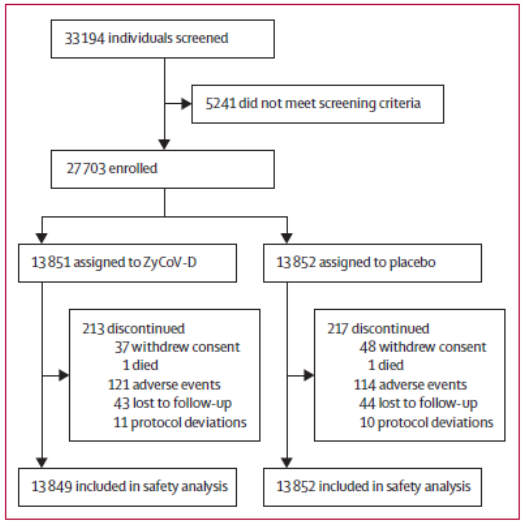
ZyCoV-D: Phase 3

Efficacy, safety, and immunogenicity of the DNA SARS-CoV-2 vaccine (ZyCoV-D): the interim efficacy results of a phase 3, randomised, double-blind, placebo-controlled study in India

Lancet 2022; 399: 1313–21

- Study design
  - Enrolled 27,703, randomized 1:1 to vaccine or placebo
  - 2 mg administered ID with NFIS on Days 0-28-56
  - Outcome: prevention of symptomatic infection
- Results
  - Efficacy: 66.6% (cases per group: 20 vaccine, 61 placebo)
  - Immune responses comparable to Phase 1



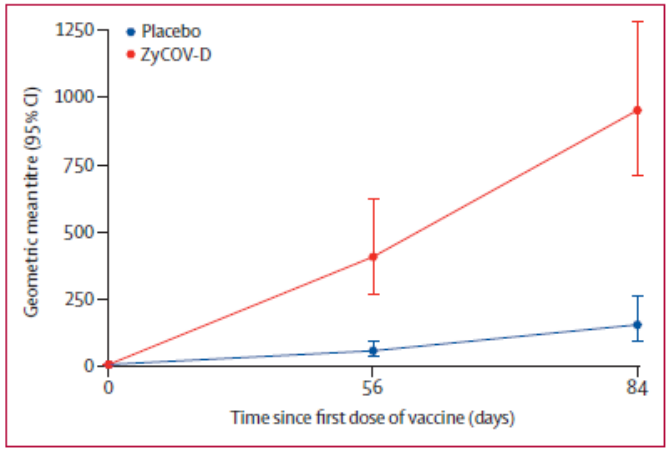


Figure 2: IgG comparison of geometric mean titre of ZyCoV-D and placebo at days 0, 56, and 84

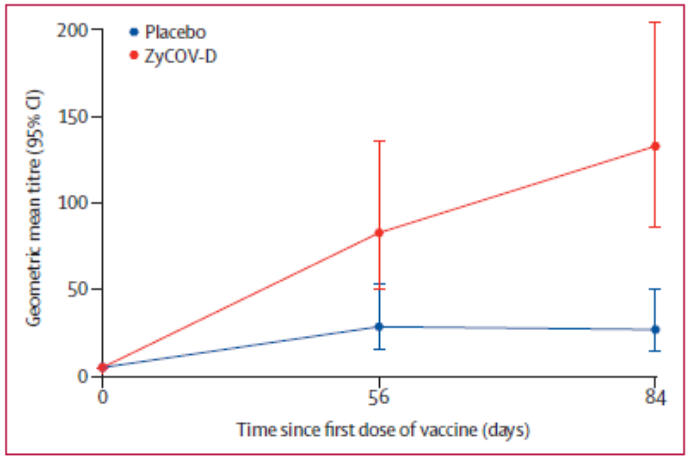


Figure 3: NAB($PRNT_{50}$) comparison of geometric mean titre of ZyCoV-D and placebo at days 0, 56, and 84

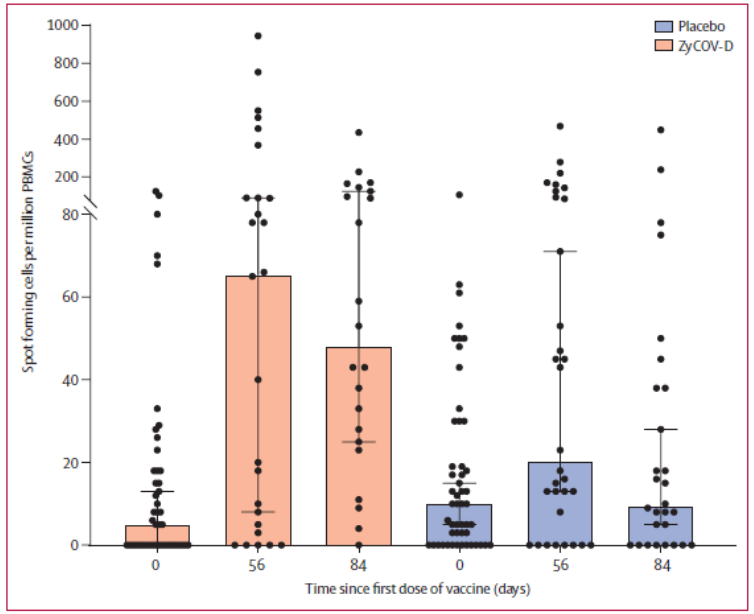


Figure 4: Cellular response (IFN-γ) to ZyCoV-D and placebo at days 0, 56, and 84

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INO-4800: Phase 1

- 1 mg vs 2 mg ID + EP
  - 20 per group

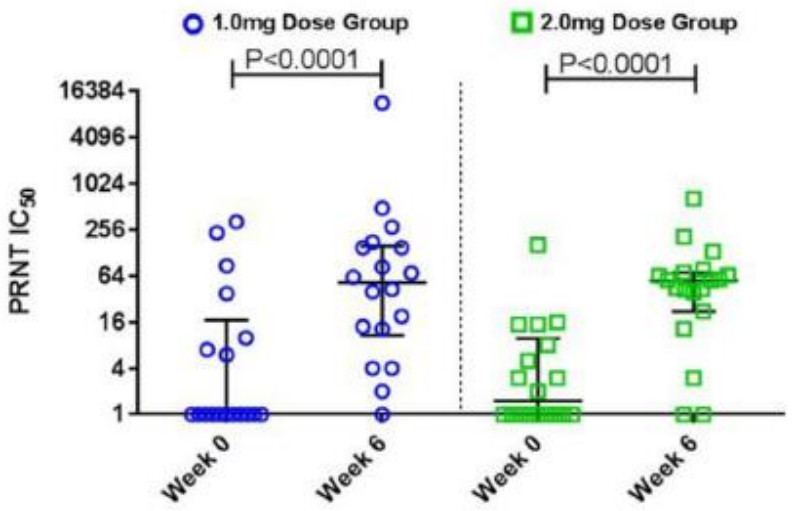
EClinicalMedicine 31 (2021) 100689

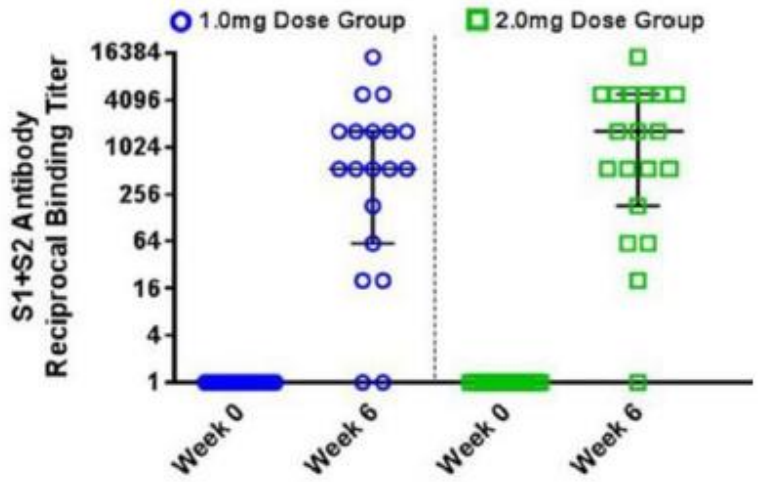
Contents lists available at ScienceDirect

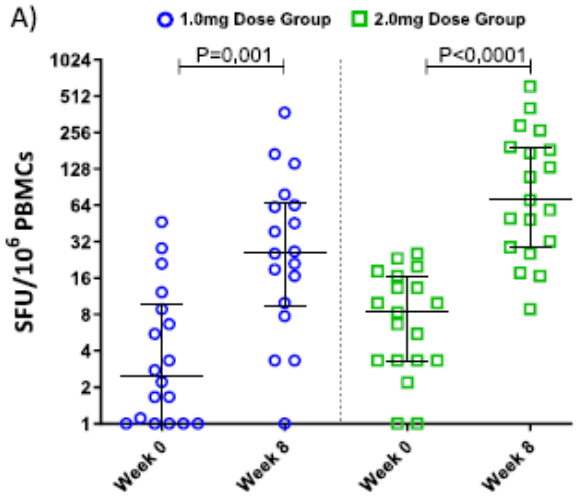
EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, Phase 1 clinical trial

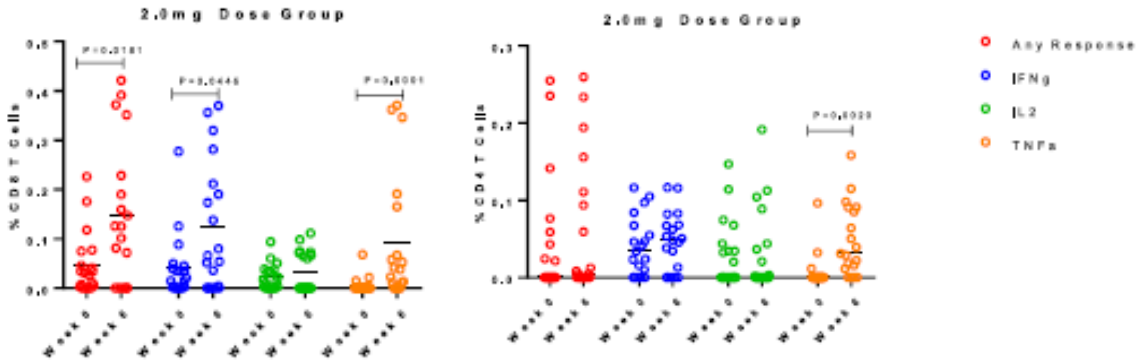






Summary

- Possible dose dependence
- Immune responses similar to ZyCoV-D
- T cell responses ~50 SFU/10^6 cells over baseline



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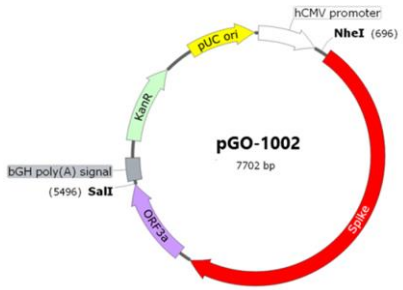
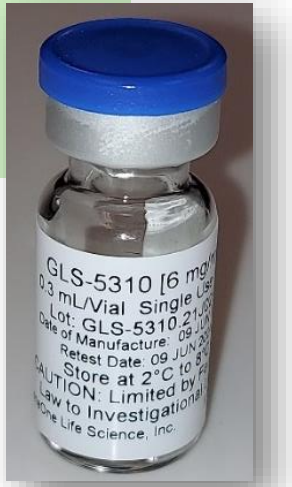


GLS-5310 DNA vaccine

CoV2-001 Phase I

NCT04673149



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CoV2-001 Study Design

- GLS-5310 administration
  - ID (Mantoux) injection in volar aspect of forearm
  - Followed by application of suction using GeneDerm
- Immunology at 4 weeks post-2nd vaccination

Phase	Group	N	Route	Dose	Vaccination
1	1a	15	ID	0.6 mg	Week 0 – 8
	1b	15	ID	1.2 mg	Week 0 – 8
	1c	15	ID	1.2 mg	Week 0 – 12

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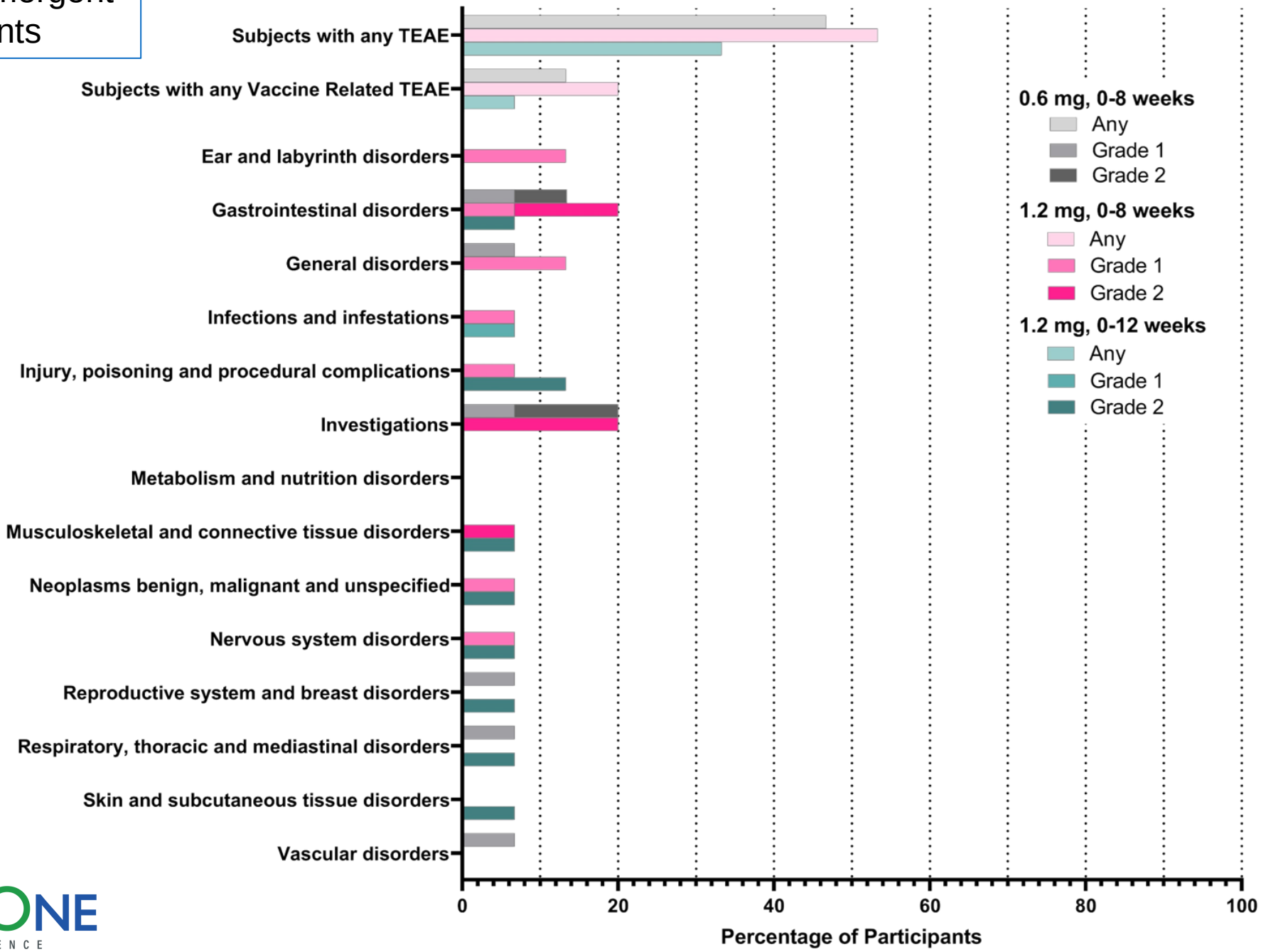
Treatment emergent Adverse events



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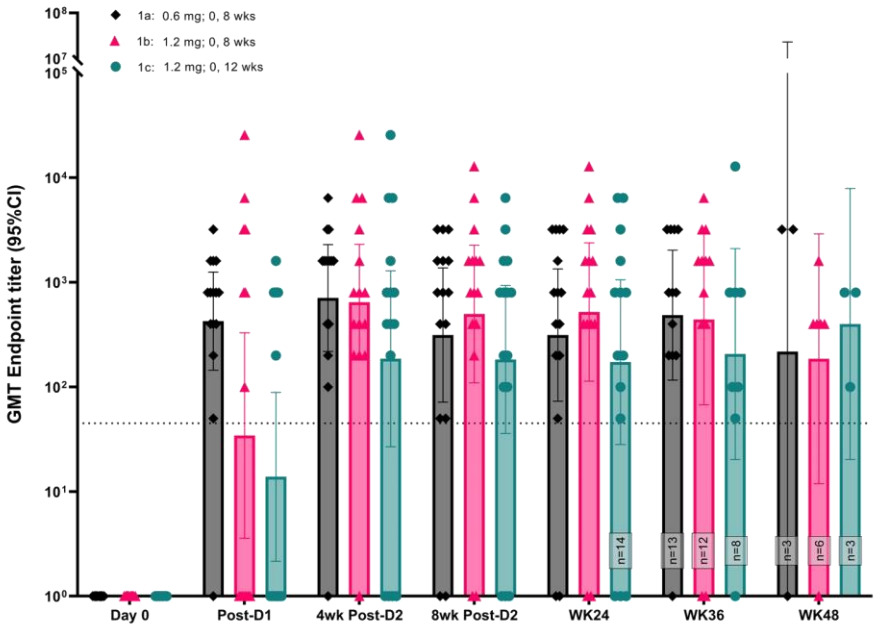
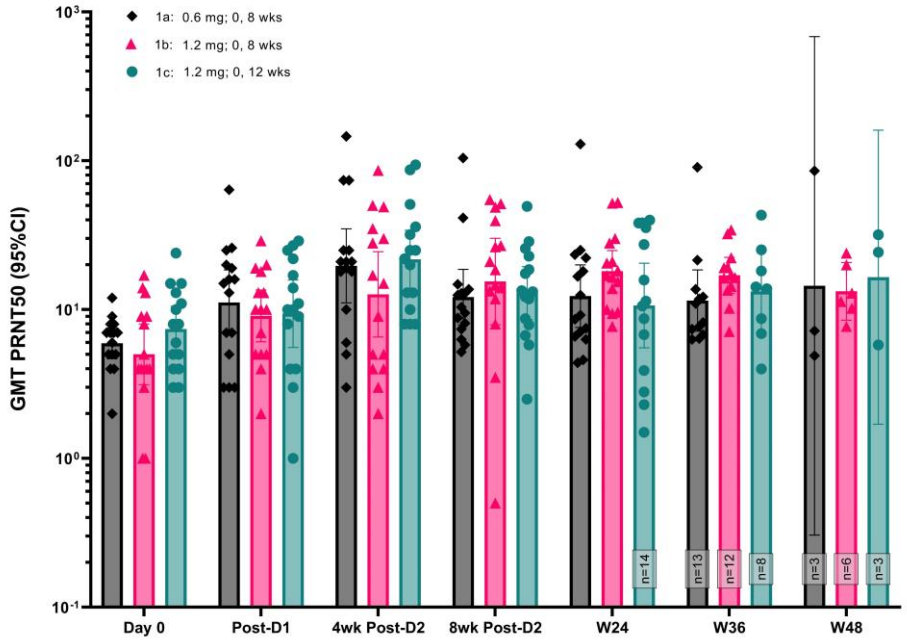
GLS-5310: B cell responses

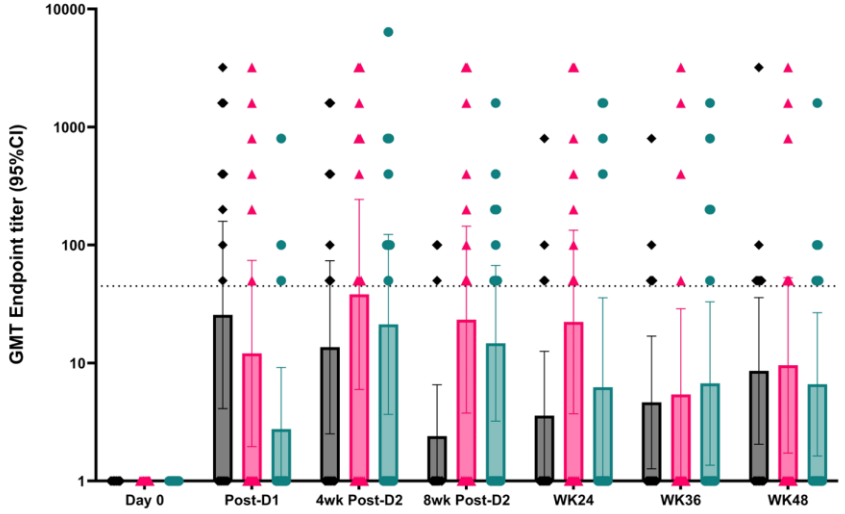
Through 48 weeks



Spike ELISA: 95% seroconversion



PRNT50



ORF3a ELISA: 80% seroconversion

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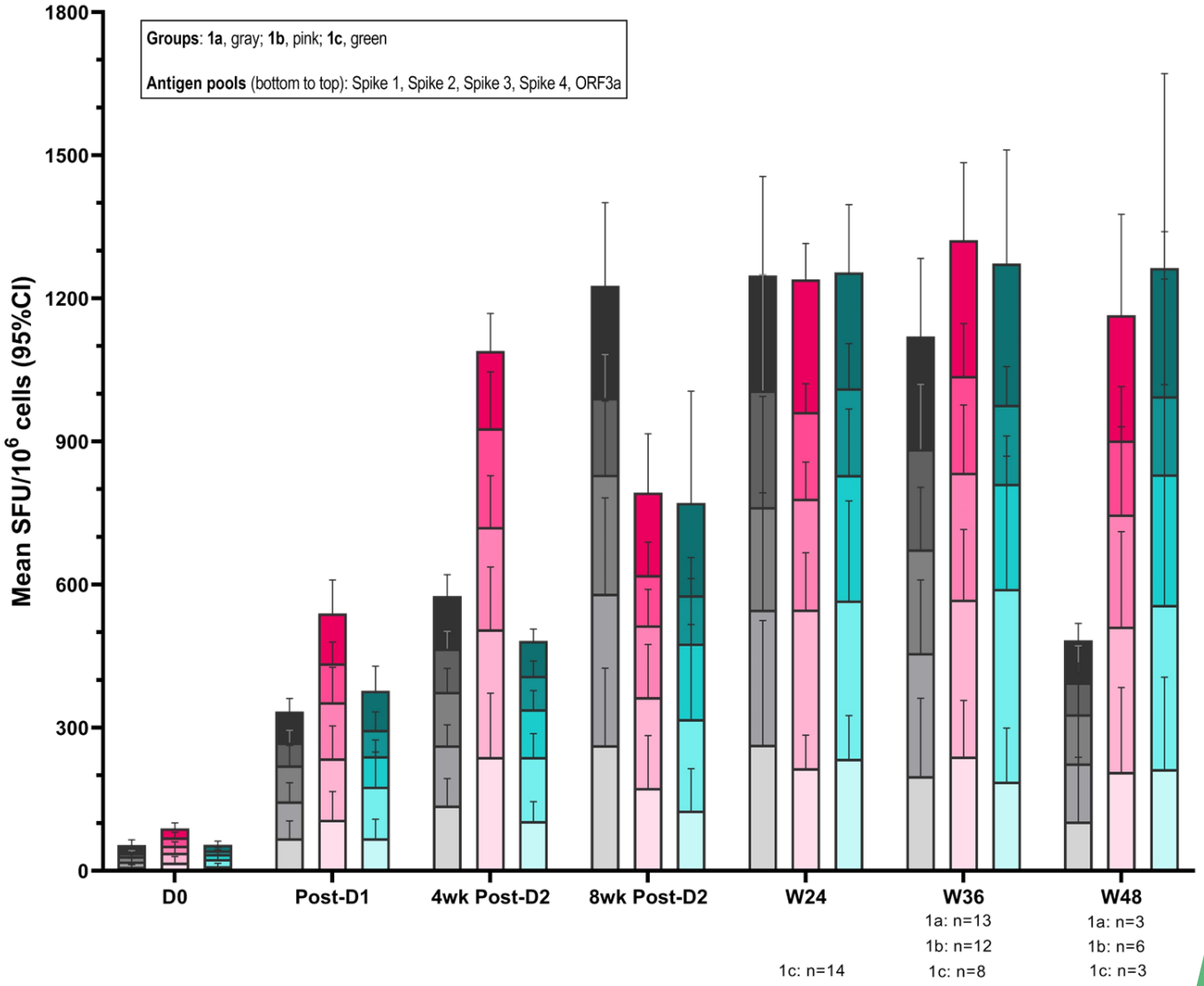
GLS-5310 T cell responses & summary

Stable through 48 weeks

- Immune responses
  - Dose independent
  - Stable through 48 weeks
- T cell responses
  - Increasing through to 6 months
  - Peak ~ 1200 SFU/10^6 cells

T cell responses

- 97% T cell responders
- All seronegative subjects had high T cell responses



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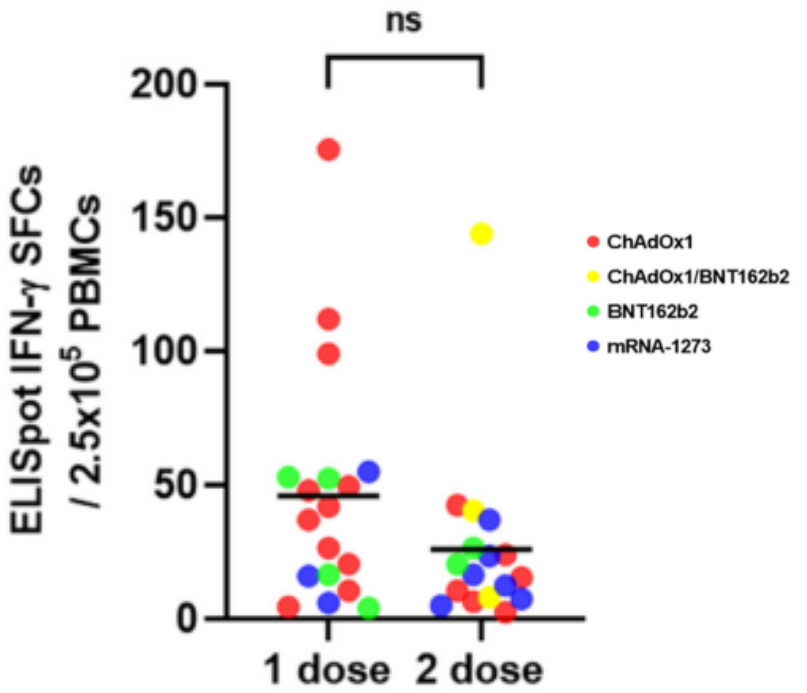
DNA vaccines vs other platforms

- B cell responses
  - Reported GMTs: binding Abs & neuts similar for 3 DNA vaccines
  - Compared to other platforms
    - ~ 2 logs lower than mRNA vaccines
    - ~ 1 log lower than adenoviral vaccines
- T cell responses
  - Reported values by ELISpot (Hwang)
    - Similar between mRNA and Adeno vaccines
    - Post-vaccination ~ 100 SFU/10^6 cells
  - DNA vaccines
    - ZyCoV-D, INO-4800: ~50 SFU/10^6 cells
    - GLS-5310: ~1200 SFU/10^6 cells



(Hwang 2022 Vaccines)

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DNA vaccines

Challenges & Strengths

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DNA vaccines - strengths

- Rapid production: design to clinic in < 2 months
- Thermal stability
  - Stable at 4°C for 2-3 years
  - Stable at 25°C for ≥ 1 year
  - Stable at 37.5°C for 3 months
    - Reduces logistic cost and complexity
    - Increased shelf-life
- *In vivo* stability
  - DNA is injected “naked” without need for lipid nanoparticle
    - Reduces manufacturing cost, complexity, AE’s
- Non-reactogenic
  - DNA does not trigger innate immune activation
    - No need for modified nucleosides

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Challenge 1: Low levels of Neutralizing antibodies

- Neutralizing antibody responses – lower than mRNA
  - Neuts ~ 100-fold lower than mRNA
- Can adjuvants increase antibody responses of DNA vaccines?
  - A variety of adjuvants have been explored
    - Those used in **human trials** are highlighted
  - Interleukins
    - **IL-12**, IL-15, IL-18, **IL-28**, IL-33, IL-2, MDA7/IL-24
  - Chemokines
    - CCL28, CCL19, ISG15
  - Co-stimulatory surface proteins
    - CD40, CD40L, CD63, CD80/86
  - Nanoparticle formulations & other
    - Liposome ± Mn^{++}, CaPhos, O-2'-hydroxypropyl trimethyl ammonium Chloride chitosan, LNP TLR4, LNP MANα1-2MAN
    - **GM-CSF**, Polysaccharides, polyinosinic-polycytidylic acid, Montanide, amiloride
    - Plasmid encoded proteins: caspase-1, CpG, HSV gD, ADA

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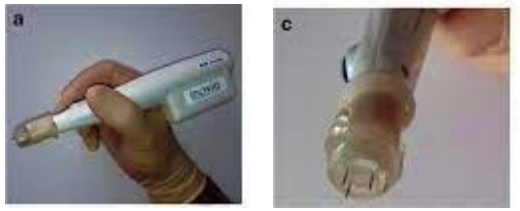
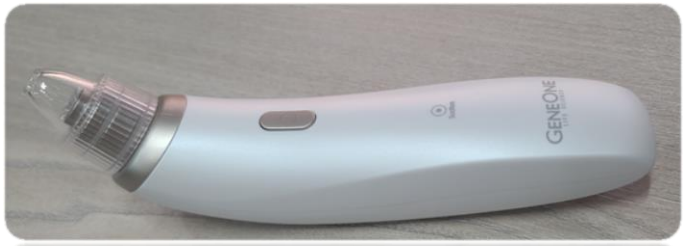
Challenge 2: Device required to induce *in vivo* transfection

- Electroporation
  - Electric current generated across electrodes inserted in skin
    - 200V, 0.1-0.2 mA electric current



- Needle-free injection system
  - Microdroplets forced through skin
  - Spring-loaded, mechanical
    - Uses pre-loaded cartridges

- Suction
  - 80 kPa pressure, 15 sec
  - Battery operated

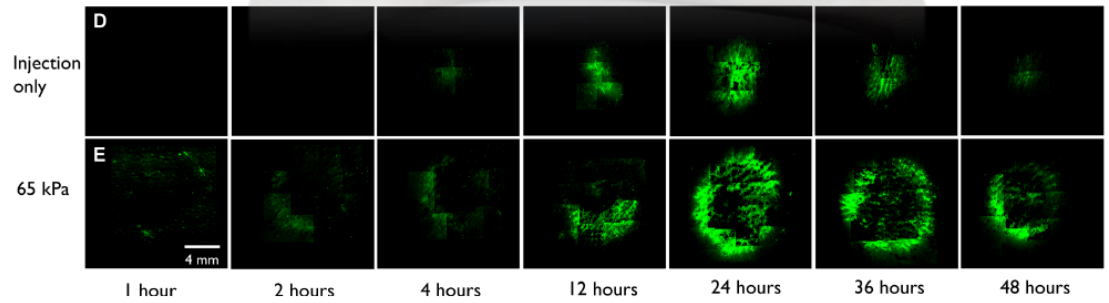


SCIENCE ADVANCES | RESEARCH ARTICLE

BIOENGINEERING

Novel suction-based in vivo cutaneous DNA transfection platform

Lallow *et al.*, *Sci. Adv.* **7**, eabj0611 (2021) 5 November 2021



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Comparison of in vivo transfection devices

	EP	NFIS	Suction
			
Training needs	++++	+++	+
Pain	+++	++	+
Device cost	High	Medium	Low
Disposables (per vacc)	~$5	~$1	~$0.25

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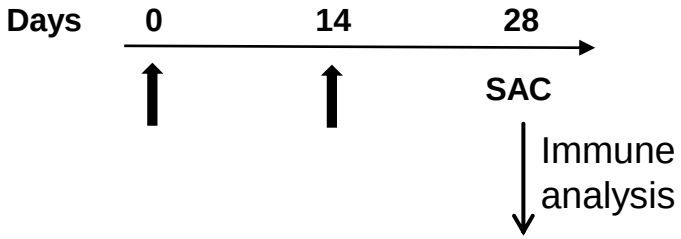
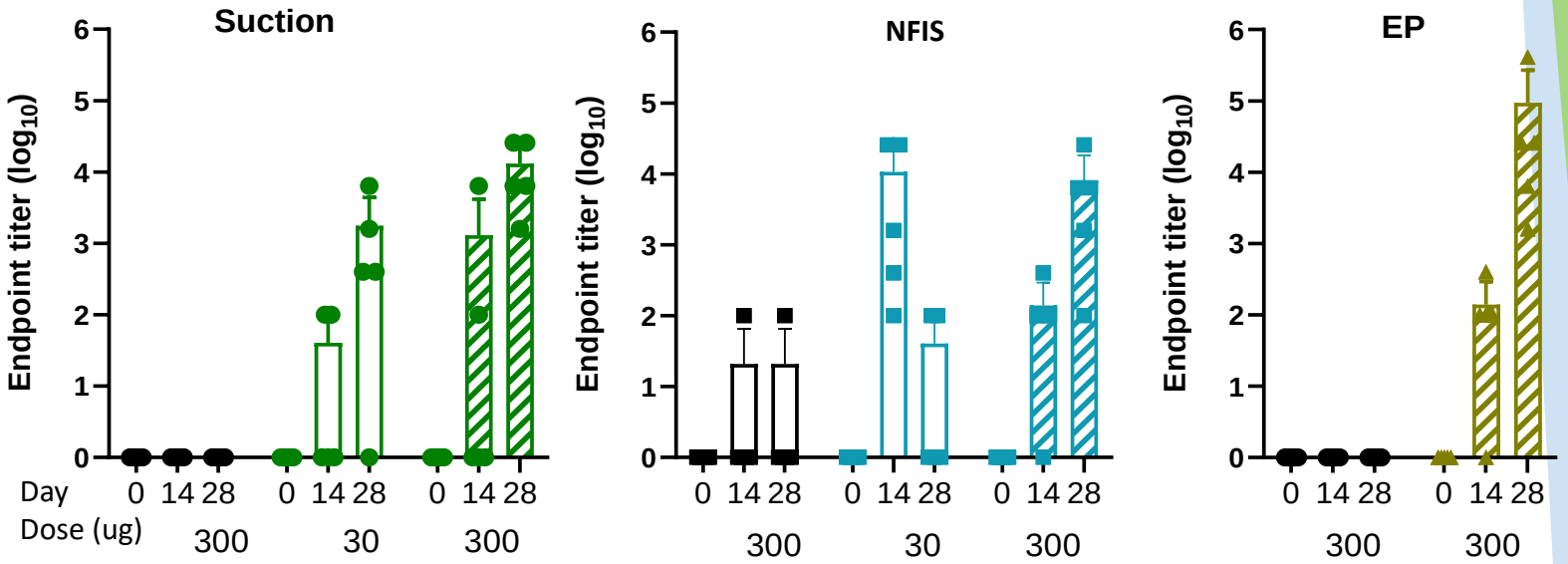
Delivery device comparison

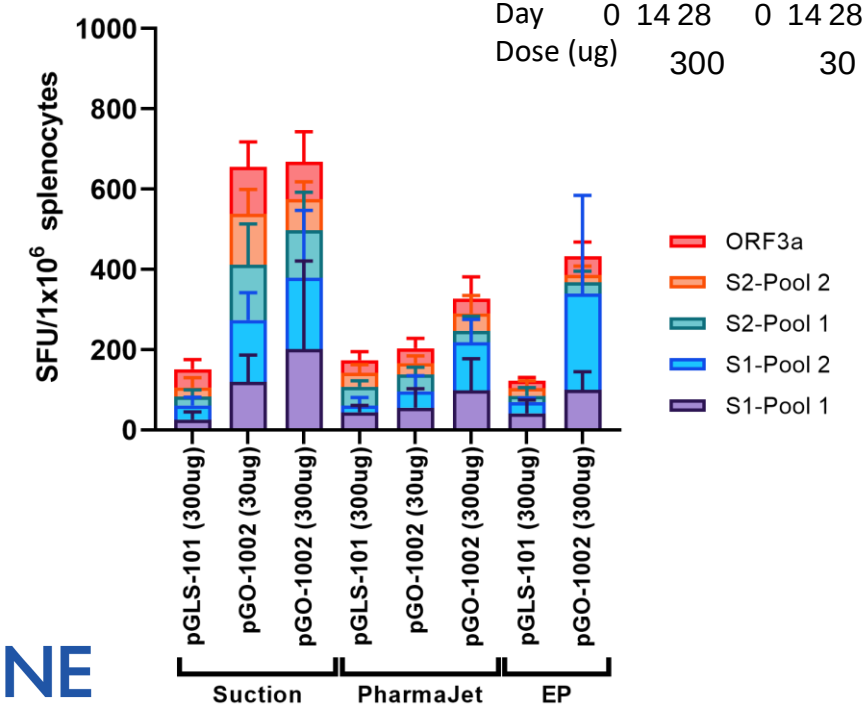
Rats

Rat immunization schedule (n=5/group)



Binding Antibody Titers





GeneDerm (suction) delivered vaccine

- Dose independent
  - Equivalent response 30 vs 300 ug
- Antibody responses equivalent to EP, PJ
- T cell responses 1.5x to 2.5x greater

frontiers

Frontiers in Virology

Immune responses of a novel bi-cistronic SARS-CoV-2 DNA vaccine following intradermal immunization with suction delivery

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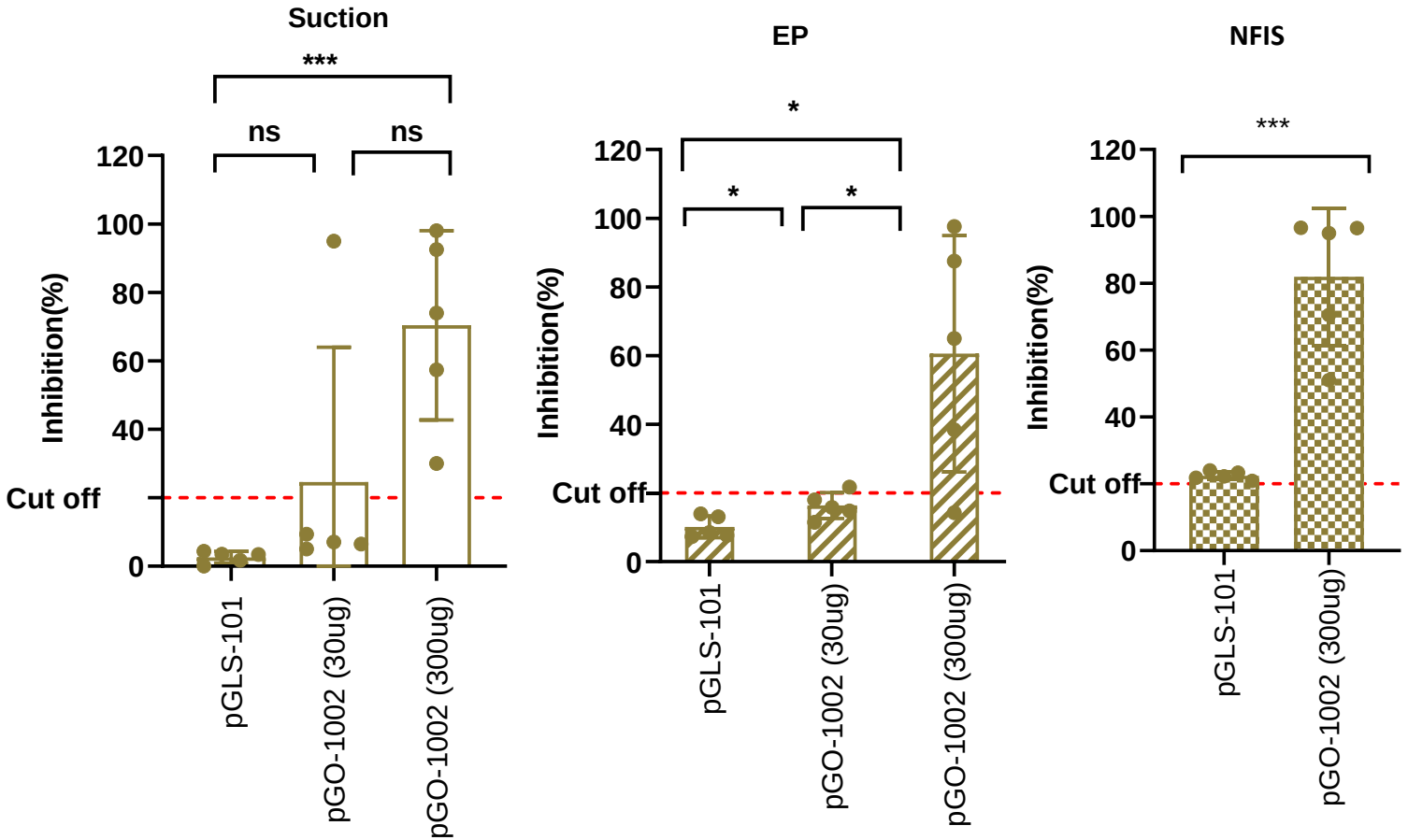
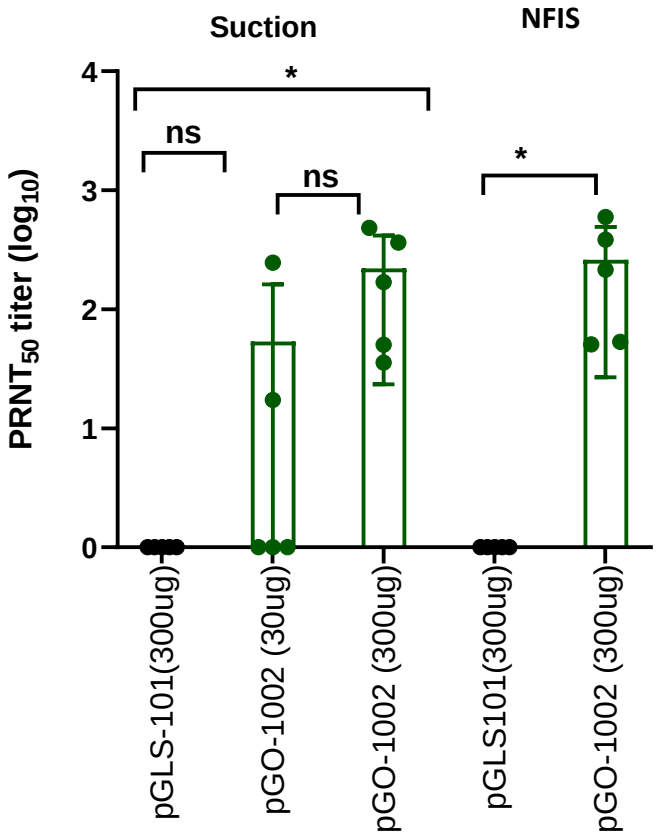
Characterization in rats: cont'd





Neutralization response

- Neutralization similar for all devices

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Mono vs Multiple antigenic targets

GLS-5310: SARS-CoV-2 vaccine expressing both S and ORF3a

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Reasons to target multiple antigens

- SARS-CoV-2 genetic evolution
  - Significant genetic evolution in “real time”
    - Greatest changes in RBD of spike
    - Resulting in increased transmissibility, immune escape
  - MERS-CoV did not have same level of evolution
    - However, documented case #’s are orders of magnitude lower (3,000 vs 650M)
- Immune paradigm
  - Neutralizing antibodies – prevent infection
  - T cell responses – limit level of illness

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T cell responses correlate with less severe disease

- Data from MERS (Korea, Saudi Arabia) showed that
  - Those with severe disease had highest neutralizing antibody titers
  - Appearance of neutralizing antibodies did not result in viral clearance
- T cell immunity correlated with better outcomes
  - MERS-CoV: survival correlated with CD8+ response (Zhao 2017)
  - SARS-CoV-2: correlation between T cell response and outcome
- Longevity of immune responses: T cell > B cell
  - SARS-CoV
    - T cell responses present at 6 years post-infx
    - Antibodies start to decline after 6-9 months
  - MERS-CoV
    - Continued shedding in severe disease despite NAbs
    - Recurrence of disease despite Nabs
  - SARS-CoV-2
    - Antibody responses short lived (4-6 months) post-infection

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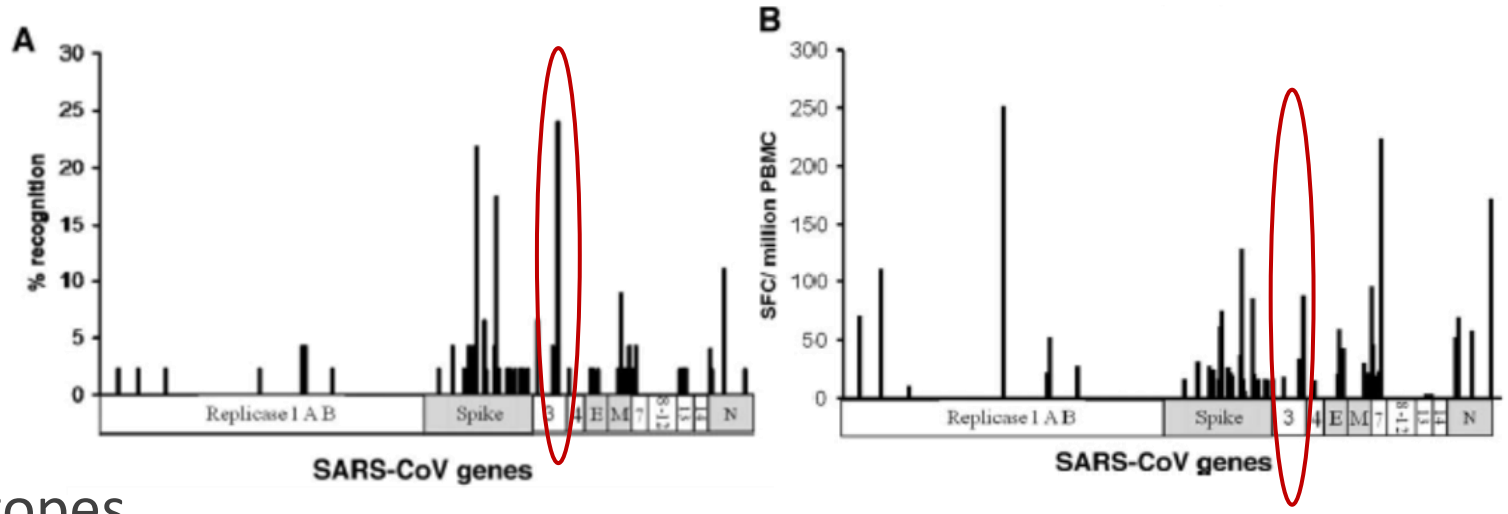
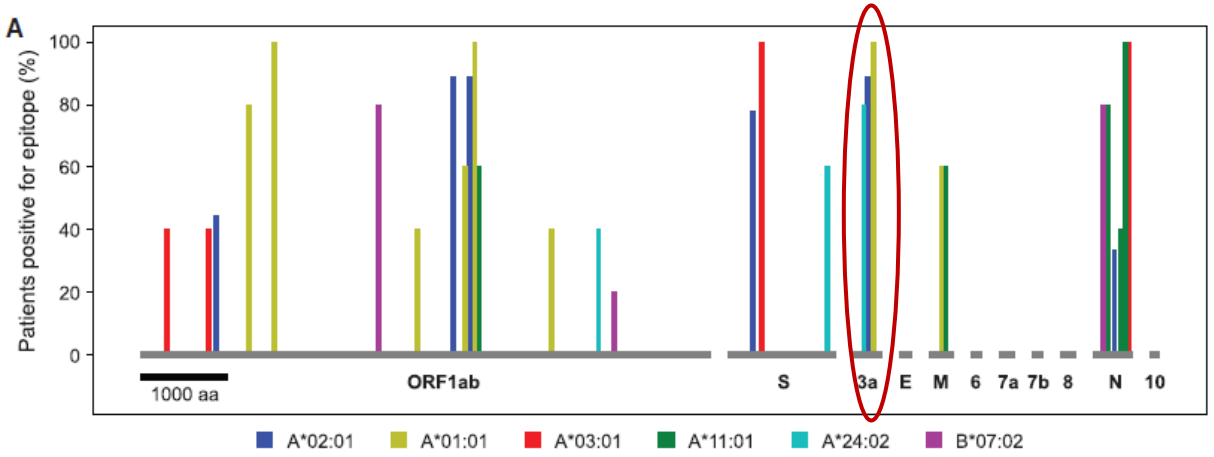
ORF3a – Immunodominant T cell antigen

- SARS-CoV (Li 2008)
  - ORF3 T cell responses
    - High percentage of patients
    - High magnitude of response
- SARS-CoV-2
  - Multiple ORF3a T cell epitopes
  - Conservation of T cell epitopes among variants





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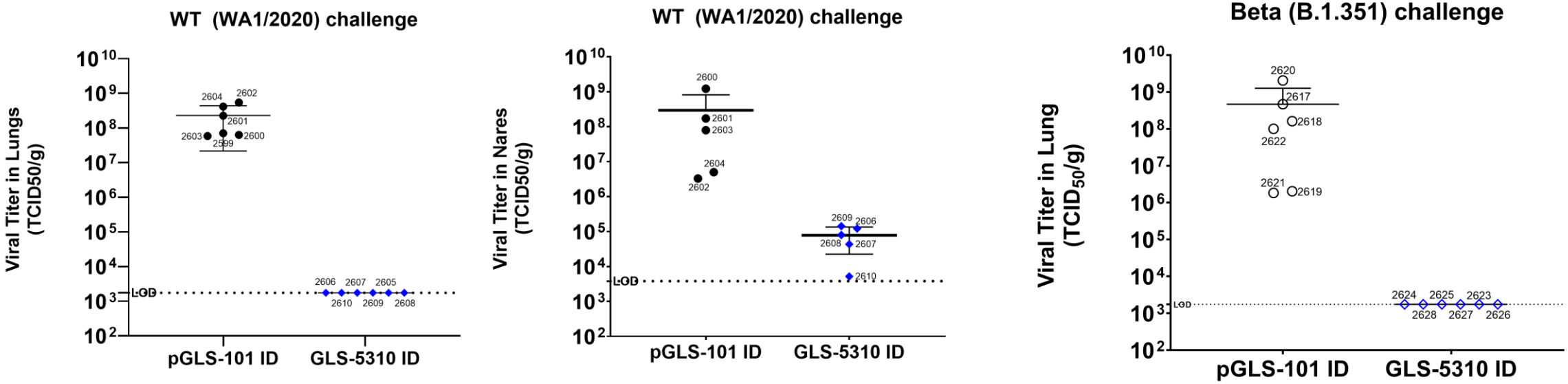
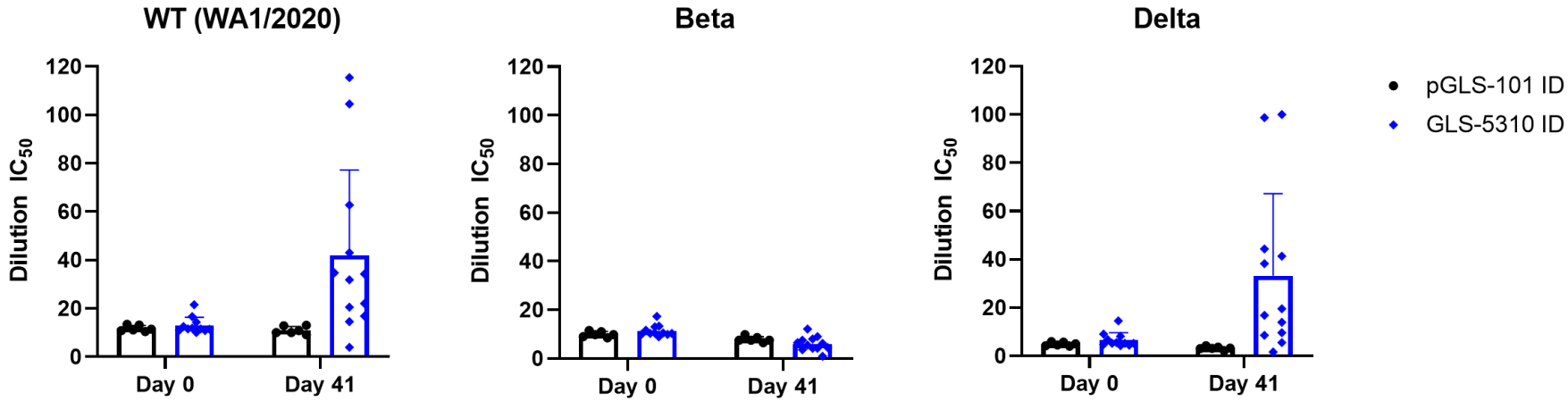
GLS-5310 protects hamsters against variants

Live virus Challenge of Hamsters



ACE2—RBD Binding Inhibition of Hamster Immune Serum



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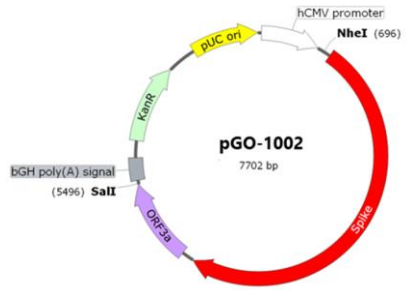
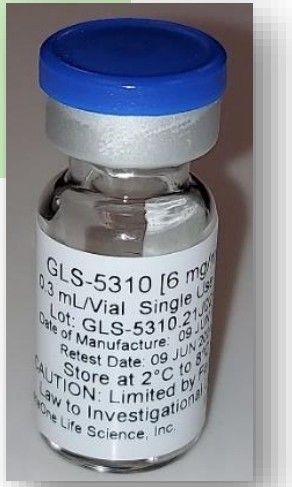
Protects hamsters irrespective of binding inhibition



Heterologous boost

mRNA revaccination in GLS-5310 vaccinated

NCT04673149



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mRNA boost following GLS-5310 DNA primary series

CoV2-001 (Phase 1)

- 32 of 45 persons received a recently-EUA-approved mRNA vaccine during study
  - 1 prior to Week 24 visit
  - 13 prior to Week 36 visit
  - 18 prior to Week 48 visit
- Immune responses
  - IgG titers: 3.07 log increase (1,187-fold)
    - Post-mRNA GMT ~$10^{5.5}$
  - Neutralization: ~2.04 log increase (110-fold)
    - Post-mRNA GMT ~ 10^{3}
  - T cell responses: avg 2.9-fold increase
    - Post-mRNA 3220 SFU/10^{6} cells

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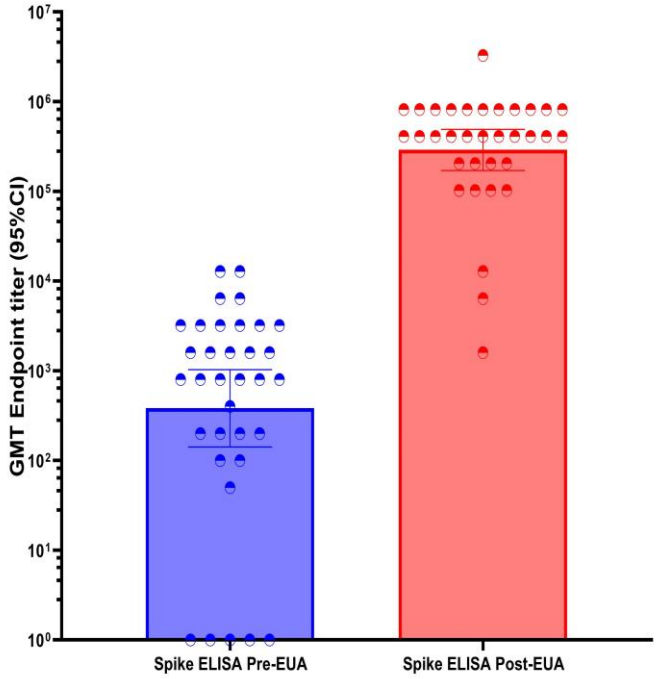
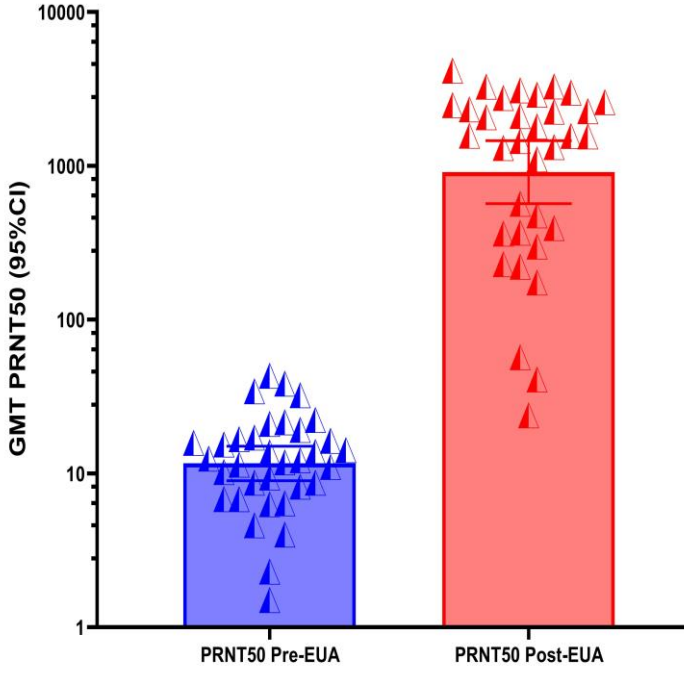
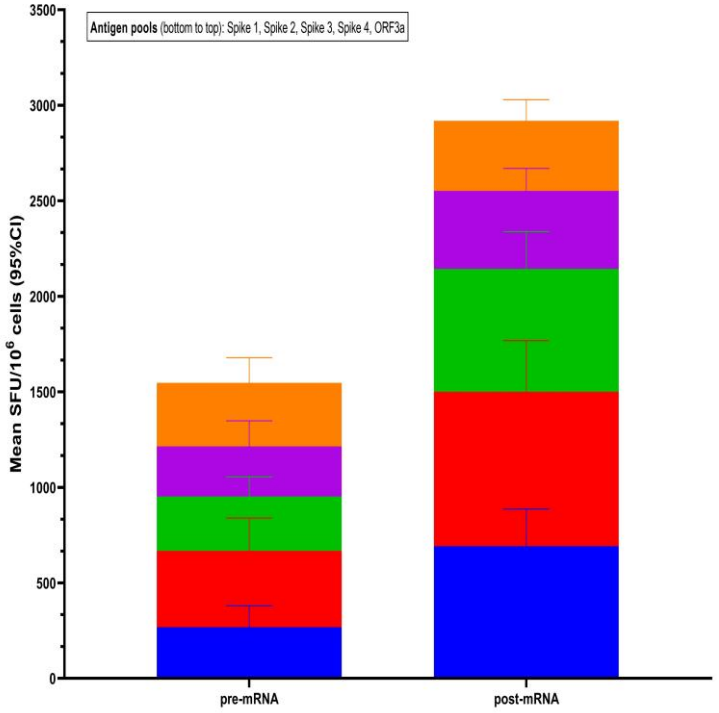
mRNA boost following GLS-5310 vaccination



Binding antibodies



Neutralizing antibodies



T cell responses

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The potential role of DNA vaccines in future pandemics

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Heterologous Prime – Boost

- Potential for DNA in heterologous vaccination regimens
  - Prior studies have examined DNA-adeno, DNA-protein prime-boost regimens in animals
  - SARS-CoV-2 data suggests heterologous prime-boost may yield superior breadth of immune response
- Phase 1 study (CoV2-001) showed
  - DNA primary series with mRNA boost yielded
    - High binding antibodies
    - High neutralizing antibodies
    - Further increase in T cell responses
- Current pilot in US (NCT05182567)
  - To assess DNA boost following mRNA or Adeno primary vaccination

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DNA vaccines in future pandemics

- Rapid design to manufacturing scale up
- Ideally suited where distribution logistics is challenging
  - Avoids cold-chain requirements for -20°C or -80°C
  - Prolonged stability at ambient temp
- Vaccine scale up in place
  - VGXI – opened 120,000 sf facility
- Device availability
  - Device scale up in place (NFIS, GeneDerm)
  - Ease of use (NFIS, GeneDerm)
  - Low and medium cost device
  - Disposable scale up in place (NFIS, GeneDerm)

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Thank you

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