

DNA Vaccine Platform for SARS-COV-2

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Pros of DNA construct

- Can be manufactured more easily than vaccines composed of inactivated pathogen, subcellular fraction, or recombinant protein (CDC)
- Almost all plasmids can be manufactured in essentially the same way, substantial economies of scale can be achieved (CDC)
- DNA is very stable and resists extreme temps and therefore its storage, transportation and distribution in vaccine form will be more practical and less expensive
- Possible to change the sequence of antigenic protein or add heterologous epitopes by introducing mutations to the plasmid DNA and increase our understanding of immune response to antigens

Pros of DNA construct cont'd

- DNA-mediated immunization is easy to use because once the protein coding sequences are cloned into a suitable expression vector, the direct introduction of the plasmid vector (into mice for example) allows experimental assessment of the immune response and its consequences without further experimental steps such as preparation of a recombinant protein as antigen
- Straightforward and requires only simple molecular biologic techniques which are practical for many labs around the world
- DNA-mediated immunization can be used in countries that cannot implement more complicated and expensive strategies
- DNA is non-infectious, a potential safety benefit over attenuated viral vaccines

Cons of DNA Construct

- Delivery methods can vary- some require a special device that provides the electrical pulse while others use DNA plasmid as a transportation vehicle for the vaccine
- Risk that it can cause permanent change to the cell's natural DNA sequence
- Many aspects of immune response caused by DNA vaccines that are not yet fully understood.
- DNA vaccines usually encode one protein from the pathogen so they may not be so good if you need to make an immune response against multiple proteins to get protection but can be dealt with by mixing multiple vaccines together

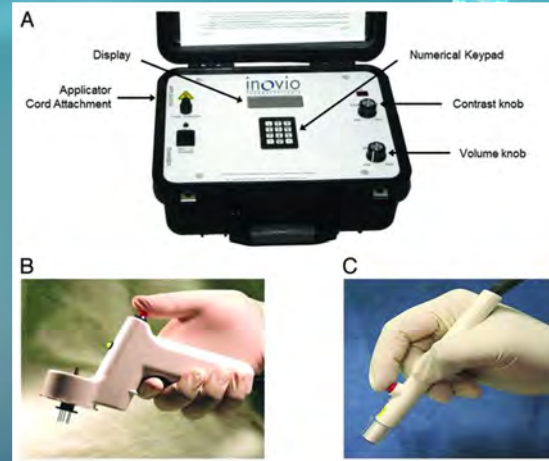
Route of Administration

Dermal Electroporation:

- The application of brief electric pulses to tissue in order to permeabilize cell membranes in a transient and reversible manner. The temporary formation of pores facilitates the fast transport of the DNA molecule via passive diffusion
- It has been evaluated in several animal models and has demonstrated favorable immunogenicity

Devices:

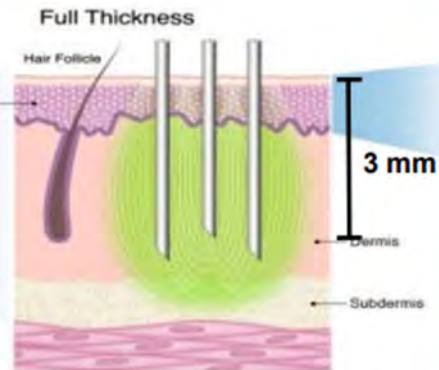
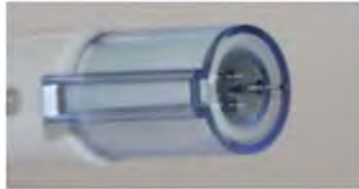
- Hand-held CELLECTRA® smart devices
- Surface Electroporation
 - Increased comfort and lowered cost



Route of Administration

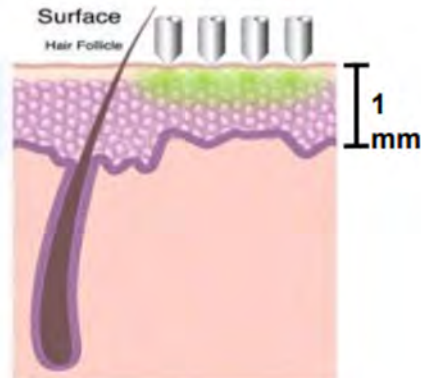
CELLECTRA®-3P

- Intradermal – minimally invasive
- 3mm electrodes
- In clinical use



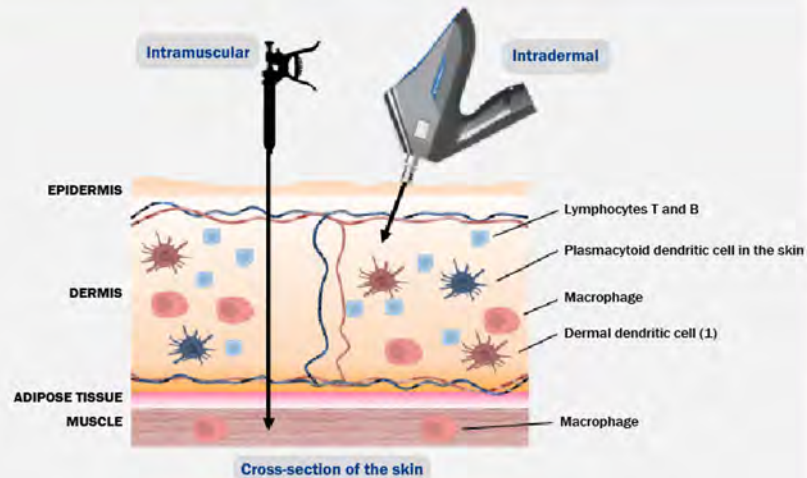
Surface EP (SEP)

- Surface
- Noninvasive
- 4x4 electrode array
- Specifically targets epidermis
- In late-stage preclinical development



EP-mediated immune response enhancement

Routes of administration



(1) Dermal dendritic cells bear high amounts of MHC-II molecules, are very potent antigen presenting cells, present antigen cross-linked to lymphocytes Th1/Th2 + cytotoxic lymphocytes and have a high ability to migrate to lymph nodes.

Intradermal Electroporation

PROS	CONS
Skin is an easily accessible area for injection	Skin holds limited volume
Shallower needle depth/less invasive	Power source and Powered injector needed
Reduced pain level	Longer duration of injection
Higher immune response	Injection site reactions--discoloration, swelling, itching
Slow and gradual local absorption/more time to capture the antigen	-

Proposed Preclinical Studies - Toxicology

- Single and repeat dose testing
- Dose dependence
- Good laboratory practice (GLP) guidelines to be followed as outlined by FDA
- Clinical observation of endpoints must be assessed
 - Health biometrics
 - Regularly monitor injection site
 - Pathology of other major body organs, especially the lung
- Developmental and reproductive toxicity studies

Proposed Preclinical Studies - Immunology

Important to characterize the immune response

- ELISA for IgG, IgM, and IgA antibodies after each vaccination
- Flow cytometry
 - Spleen (after euthanasia)
 - Monitor count of immune cell population at time intervals
- Assays for clinical testing

Proposed Preclinical Studies - Efficacy

- Viral neutralization assay
- ELISA for antibodies (as mentioned under Immunology)

Preclinical Study Design

- Primate studies: Rhesus macaques
 - Good for understanding vaccine immune response since they are large animal models
 - Anatomical and physiological relevance to humans
- Non-primate studies: Mice
 - Genome similar to human genome (99%)
 - Well characterized immune system
 - Very useful for understanding mechanism of immune protection and contribution of IgA, IgC, etc.
 - Inexpensive to work with

TARGET PRODUCT PROFILE SUMMARY

PRODUCT TARGET	Minimum Acceptable Result	Ideal Results
INDICATION FOR USE	Minimal: Prevent Infection Severity; Reduce the Number of Severe Cases	Preferred: Prevent Infection; Reduce the Number of Cases
TARGET POPULATION	Adults, aged 19-70	All Adults and adolescent patients 12 years and older.
CONTRAINDICATION	Minimal: Prior Allergy to Vaccine Components; immunosuppressive disorders	Preferred: None
SAFETY/REACTOGENICITY	Minimal: statistically lower or equivalent risk of SAE in treatment group than control.	Preferred: Minor AEs (no SAEs that cannot be explained as a result of the vaccine)
EFFICACY	Minimum:50%	Preferred: 85%
DOSE REGIMEN	Minimal: Two doses, spaced by 4 weeks	Preferred: Single dose is found to be effective without second dose.
DURATION OF PROTECTION	Minimum: 3 months	Preferred: 1 year, optimally lifetime.
ROUTE OF ADMINISTRATION	Collectra 2000 dermal injection and electroporation	Non-invasive needle (surface EP) or Dermal Patch
COVERAGE	Minimal: Monovalent	Preferred: Multivalent, with boosters for new variants
PRODUCT STABILITY/STORAGE	Product must be stored at <-80C and must be stored in specialized freezers	Stable at room temperature >1 yr; at 37C more than one month, 5 year refrigerator life.

TARGET PRODUCT PROFILE SUMMARY

PRODUCT TARGET	Minimum Acceptable Result	Ideal Results
CO-ADMINISTRATION WITH OTHER VACCINES	Vaccine must be taken alone	Able to be co-administered
PRODUCTION	Minimal: 100 Million Doses/year	Optimal: 120 Million Doses per year
REGISTRATION AND PREQUALIFICATION	EUA, WHO prequalification, BLA, FDA approval	
POSTMARKETING SURVEILLANCE	SAEs and efficacy (including novel variants)	

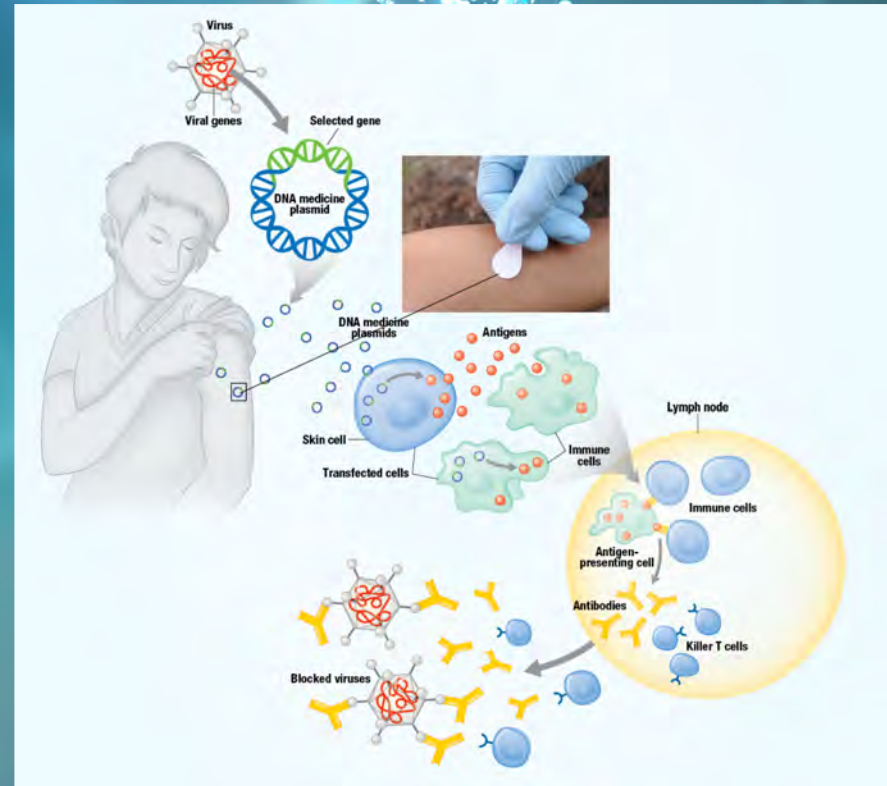
INDICATION FOR USE

Minimal: Prevent Infection Severity: Reduce the Number of Severe Cases

Preferred: Prevent Infection: Reduce the Number of Cases

INDICATION FOR USE

- Minimal: reduces mortality and morbidity
- Preferred: reduces spread via infection prevention.
- Evidence for this guideline:
 - In non-human primates, it was demonstrated that DNA COVID-19 vaccines have been shown to:
 - Clear virus from lungs and nasal sections of the respiratory tract more quickly
 - reduce the viral load
 - This suggests that beyond just reducing the detrimental effects of the disease, the effects of “viral shedding” and therefore disease spread could be reduced as well.
 - Source: Inovio press release



TARGET POPULATION

Adults, aged 19-70

All Adults and adolescent patients 12 years and older.

TARGET POPULATION

- Minimal: Adults, aged 19-70
 - This includes a part of the aging population, which is critical as they are more susceptible.
- Preferred: All Adults and adolescent patients.
- Data used for this guideline:
 - The Inovio MERS trial included those 19-70.
 - The Zydus Phase III trial involves patients 12 years and older.
 - The phase I/IIa Inovio trial included those 19-50 in part A and 19-64 in part B of the trial (which happens after part A).
- Thus, as there is limited evidence for use in children, it would seem appropriate to limit the scope of the target population to that which has been used in studies involving a similar technology.

CONTRAINDICATION

Minimal: Prior Allergy to Vaccine Components; immunosuppressive disorders

Preferred: None

- Minimal: Prior Allergy to Vaccine Components; immunosuppressive disorders
- Preferred: No Contraindications
- Data used:
 - "hypersensitivity or severe allergic reactions to vaccines or drugs" was one of the exclusion criteria in the **phase I Inovio trial**, so currently the safety of those with possible hypersensitivity reactions is not known.
 - The **Cydus Zydila phase III Trial** also excluded those with **immunosuppressive/immunodeficiency** disorders from trial participation. Thus, the safety of a vaccine for those with these disorders might not be assured.

SAFETY/REACTOGENICITY

Minimal: statistically lower or equivalent risk of SAE in treatment group than control.

Preferred: Minor AEs (no SAEs that cannot be explained as a result of the vaccine)

- Minimal: Mild AEs
- Preferred: No AEs
- Evidence:
- The MERS (INO-4700) trial had no SAEs
- “The vaccination regimen was well-tolerated with no vaccine-associated severe adverse events (SAEs).”
- The Inovio 4800 phase I trial had no SAEs.
- The Zydus cadila trials had no SAEs.
- From available data, we can expect that reasonably, our product will be safe, in addition to being effective.
- If we have SAEs, like the AstraZenica Vaccine, we would hope that at a minimum, the risk of an SAE is less in treatment than control.

EFFICACY

Minimum: 50%

Preferred: 85%

EFFICACY

- Minimal: 50%
- Preferred: 85%
- Evidence:
 - The Inovio DNA MERS vaccine, using the same technology was reported to have 92% neutralizing antibody responses and 100% binding. **Immune responses were detected in 85% of patients.**
 - The lowest overall efficacy for an approved vaccine is currently 66.1% (Johnson and Johnson, although US efficacy is 85.9% after 28 days and 81.7% against variants).
 - **Unmet demand in many countries:** However, for LMICs, offerings are limited as the US-based vaccines are not currently being exported and thus a 50% efficacy could be enough to become marketable if the product is easily deployed and affordable.
 - Further, there is **still unmet demand for the vaccine in the US** and the FDA claims that "To ensure that a widely deployed COVID-19 vaccine is effective, the primary efficacy endpoint point estimate for a placebo-controlled efficacy trial should be **at least 50%**".
- **The WHO listing policy also claims 50% efficacy is required:** "the primary efficacy endpoint point estimate for a placebo-controlled efficacy trial should be **at least 50%**"
- The minimum of 50% will meet this standard.

DOSE REGIMEN

Minimal: Two doses, spaced by 4 weeks

Preferred: Single dose is found to be effective without second dose.

DOSE REGIMEN

- Minimal: Two doses, spaced by 4 weeks
- Preferred: Single dose is found to be effective without second dose.
- Evidence (most follow a 2 dose regimen):
 - Inovio SARS-CoV-2 Trial was designed around a 2 dose schedule, 4 weeks apart.
Inovio MERS trial tested both a 2 and 3 dose schedule.
 - Zydus Cadila uses a 3 dose regimen
 - Sputnik V, Pfizer, Moderna, Sinovac, and AstraZenica vaccines all follow a 2 dose regimen.
 - The Janssen Vaccine follows a single dose regimen (but **they tested both single and two dose regimens in their phase I/IIa trial**).
- Thus, we will attempt to pursue a two dose regimen, in line with what was previously done using similar technology and competitive with other currently available vaccines. We will also test a single dose regimen.

DURATION OF PROTECTION

Minimum: 3 months

Preferred: 1 year, optimally lifetime.

DURATION OF PROTECTION

- Minimal: 3 Months
- Preferred: 1 year (optimally lifetime)
- Evidence:
 - Inovio DNA Zika Vaccine protected 100% of NHP from infection for **13 weeks**, using the same DNA technology in a challenge trial.
 - DNA based vaccines may higher safety with boosters (more than technologies that have vector hypersensitivity risk and dose dependent toxicity).
 - Therefore, **higher duration** of protection might be possible with DNA-based **booster shots (which would extend duration of protection)**.
 - This is important as protection seems to “wain rapidly” in those who have recovered from COVID-19. Source: Inovio Phase I trial article
- NHP demonstrated **4 month** duration for inovio INO-4800 COVID-19 vaccine
- The Inovio **MERS vaccine had immune responses that were durable for 1 year**, for 85% of participants.
- Therefore, we would hope that at a minimum we would have a duration as short as the DNA Zika Vaccine (3 months), but perhaps as long as 1 year (MERS vaccine).

ROUTE OF ADMINISTRATION

Cellectra 2000 dermal injection and electroporation

Non-invasive needle (surface EP) or Dermal Patch



ROUTE OF ADMINISTRATION

Electroporation:

- the application of brief **electric pulses to tissue** in order to **permeabilize cell membranes** in a transient and reversible manner.

CELLECTRA-3P

- has been evaluated in **several animal models and in the clinic**
- 3mm penetration depth

Surface EP:

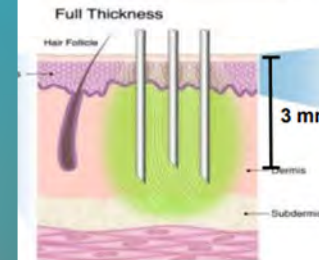
- Minimally invasive needles increase **comfort**
- **"Virtually undetectable scratch"** to deliver vaccine
- Inovio touts these as Needle-free

Newer patch delivery systems:

- Technology available to **both deliver electrical pulses necessary through patch, inject vaccine, and measure peptide markers of immune response.**
- **Lower cost:** "At 95% vaccination coverage, microneedle patch vaccination was estimated to cost \$1.78 per measles case averted (range: \$1.35–\$2.25) compared with an estimated cost of \$2.98 per case averted (range: \$2.24–\$3.73) using subcutaneous vaccinations."
- For an LMIC, this may be a better option than traditional needles, if proven effective.
 - **Simpler** means of delivering vaccine,
 - **cheaper**, and
 - with **potentially lower risk of injury** on application.
- Simpler delivery & lower cost optimal for LMICs

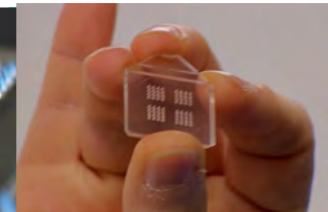
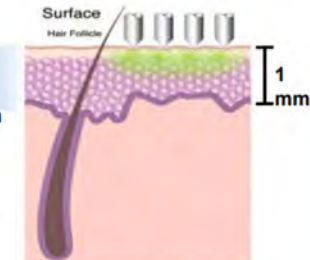
CELLECTRA®-3P

- Intradermal – minimally invasive
- 3mm electrodes
- In clinical use



Surface EP (SEP)

- Surface
- Noninvasive
- 4x4 electrode array
- Specifically targets epidermis
- In late-stage preclinical development



COVERAG
E

Minimal: Monovalent

Preferred: Multivalent, with boosters for new variants

- Minimal: Monovalent
- Preferred: Multivalent, with boosters for new variants
- Evidence:
 - DNA vaccine technology allows “combining multi[ple] antigens into [a] single vial”
 - Thus, with multiple antigens, the vaccine could protect against multiple variants/strains.
 - DNA Technology **proven to work against D614G variant (key in transmissability)**:
 - “[Inovio SARS-CoV2 DNA vaccine] generated neutralizing antibody responses against early virus and the G614 mutant variant.” Source: Inovio Press Release
 - The D614 mutant variant is found in the 3 most common strains (UK, Japan/Brazil, and South Africa) and increases transmissability.
 - **Boosters can be added without the limitation of viral vector reactions**, increasing cellular/humoral immune responses. Safety data suggests doing so will not increase toxicity and may increase duration of protection.

**PRODUCT
STABILITY/STORAGE**

Product must be stored at $<-80^{\circ}\text{C}$ and must be stored in specialized freezers

Stable at room temperature >1 yr; at 37°C more than one month, 5 year refrigerator life.

**PRODUCT STABILITY &
STORAGE**

- Minimal: Product must be stored at $<-80^{\circ}\text{C}$ and must be stored in specialized freezers
- Preferred: Stable at room temperature >1 yr; at 37°C more than one month, 5 year refrigerator life.
- Evidence for this guideline:
- "The stability characteristics mean that our DNA drug product is non-frozen and can be stored for 4.5+ years at $2-8^{\circ}\text{C}$,
- room temperature (RT) for 1 year and
- 1 month at 37°C ,
- while maintaining potency at temperatures upwards of 60°C . "
 - Source: Immunogenicity study for Inovio MERS vaccine candidate, Nature communications.
- We can assume that our candidate will be likewise shelf stable and suitable for hot climates.

**Room Temp (25°C)
Storage >1 year;
 37°C >2 months**



**Refrigerated
($2-8^{\circ}\text{C}$) Storage
 >4 years**



CO-ADMINISTRATION WITH OTHER VACCINES

Vaccine must be taken alone

Able to be co-administered

- Minimal: Vaccine must be taken Alone
- Preferred: Vaccine able to be co-administered (evaluated in phase IV)
- Data needed to allow for co-administration:
 - Current CDC guidance suggests that without more data on currently available vaccines, it is difficult to ascertain the safety of co-administration.
- Other DNA technology vaccines tend not to include this as part of the phase III design, so we will similarly evaluate this in a phase IV study.
 - Zydus excludes patients who have taken a vaccine in the study period (instead of evaluating this as a factor in the model, and having a group of co-administered vaccinations in the study). Inovio doesn't explicitly exclude it, but it is not part of the experimental design, so it is difficult to know the safety of coadministration.
- With follow-up studies (in phase IV), co-administration could be proven safe, although current guidance suggests that co-administration should be safe:
 - Tables 3-3 and 3-4 suggest that **inactivated viruses may be co-administered, while live/attenuated may require vaccination** on different dates. MMR and antibody/antigen based products also require spacing.
 - They also state: "**With some exceptions, simultaneously administering the most widely used live and inactivated vaccines has produced seroconversion rates and rates for adverse reactions similar to those observed when the vaccines are administered separately**"

CO-ADMINISTRATION WITH OTHER VACCINES

TABLE 3-3. Guidelines for spacing of live and inactivated antigens

Antigen combination	Recommended minimum interval between doses
Two or more inactivated ^{(a),(b)}	May be administered simultaneously or at any interval between doses
Inactivated and live ^(c)	May be administered simultaneously or at any interval between doses
Two or more live injectable ^(c)	28 days minimum interval, if not administered simultaneously

PRESENTATION

Minimal: 0.5mL, single dose

Preferred: ~10µL in a microneedle patch after dipping in solution.

PRESENTATION

- Minimal: Liquid in single dose form. 0.5mL dosage.
- Preferred: Although there are lyophilized DNA vaccines that have been developed (which would eliminate the need for needles), the patch requires very small quantities (~10µL) of solution and cost effective.
- More:
 - Several lyophilized DNA vaccines exist, as shown in this review in the year 2000
 - <https://pubmed.ncbi.nlm.nih.gov/21374306/>
 - A recent gonorrhea vaccine using a recombinant plasmid in a lyophilized form has been tested as part of a vaccine regimen in 2020.
 - <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3706567/>
 - An MMR patch vaccine only required ~2µL per needle (5 needles in total). The apparatus was dipped in solution and then applied.
 - <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3706567/>

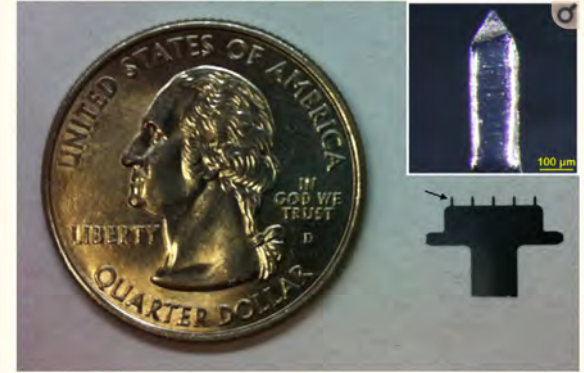


Fig. 1

[Open in a separate window](#)

A five-needle microneedle array next to a U.S. quarter coin with a diameter of 24 mm. The arrow points at one of the microneedles mounted on the holder. Inset: a single microneedle coated with measles vaccine in a trehalose-based coating formulation.

3 | *J Appl Microbiol.* 2020 Aug 6; doi: 10.1111/jam.14815. Online ahead of print.

Enhancement of immune responses by co-administration of bacterial ghosts-mediated *Neisseria gonorrhoeae* DNA vaccines

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Affiliations = expand
PMID: 32770820 DOI: 10.1111/jam.14815

Abstract

Aim: *Gonorrhoea* remains a leading public health burden and the development of vaccine against *gonorrhoea* becomes more urgent. Here, a novel *Neisseria gonorrhoeae* DNA vaccine delivered by *Salmonella enteritidis* ghosts was developed and the immune responses of the vaccine candidates were evaluated.

Methods and results: *Neisseria gonorrhoeae* *mspA* gene was cloned into the pVAX1 vector. The constructed recombinant plasmid pVAX1-*mspA* was loaded into the *S. enteritidis* SE ghosts to produce SE ghosts (pVAX1-*mspA*). Then, the immune responses induced by SE ghosts (pVAX1-*mspA*) alone and co-administered with SE ghosts (pVAX1-porB) were evaluated in mouse model. Co-administered SE ghosts (pVAX1-*mspA*) and SE ghosts (pVAX1-porB) could elicit significantly higher levels of specific IgG antibody responses and lymphocyte proliferative responses than the control groups. Furthermore, the group co-administered SE ghosts (pVAX1-*mspA*) and SE ghosts (pVAX1-porB) had the highest bactericidal antibody titres.

Conclusions: Co-administration of SE ghosts (pVAX1-*mspA*) and SE ghosts (pVAX1-porB) elicited significant specific humoral and cellular immune responses.

Significance and impact of the study: This study demonstrates the potential of co-administration of SE ghosts (pVAX1-*mspA*) and SE ghosts (pVAX1-porB) as an attractive vaccination regimen for *gonorrhoea*.

Keywords: *Neisseria gonorrhoeae*; bacterial ghosts; co-immunostimulatory immune responses; vaccine.
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PRODUCTION

Minimal: 100 Million Doses/year

Optimal: 120 Million Doses per year

- Minimal: 100 million doses/year
 - Preferred: 120 million doses/year
- Evidence:
 - Inovio plans to manufacture 100 doses in 2021
 - Zydus Cadila plans to manufacture 120 Million doses
 - Reuters suggests 100 million doses per year from Zydus Cadila:
 - Inovio's earlier estimates stated 1M doses by the end of 2020, but production has been slower than anticipated.

PRODUCTION

- FDA:
 - The study protocol and assessment at the 6 month mark will provide the data needed for an EUA (emergency use authorization) application.
 - This decision will require approval from a session of the VRBPAC (Vaccines and Related Biological Products Advisory Committee).
 - Following approval, an FDA inspection of the manufacturing facility will commence and the process for a BLA (Biologics License Application) will begin (the first steps in the path towards FDA approval).

The decision for approval may require input from the VRPAC as well.
- WHO:
 - Simultaneously, we will apply for WHO listing under the WHO Emergency Use Assessment and Listing procedure.
 - Monitoring of SAEs & incidence rates will continue prior to and after approval. SAEs will be reported via the (vaccine adverse event reporting system (VAERS) to ensure even rare vaccine injury related to the IP does not occur.
 - FDA may mandate specific phase IV studies, which may affect final approval of the drug.
- Reference: Ch 4, fundamental aspects of vaccinology, p.51-52.

- Post-marketing surveillance will assess:
- Adverse event reporting (via VAERS)
 - SAE definition: events that causes/extends hospitalization, death, threatens life, or causes a significant or long-term disability.
 - Examples in the moderna trial included Facial Swelling, Rheumatoid Arthritis, B-cell lymphoma, and pulmonary embolism.
 - The company will make a determination of relatedness to the vaccine in the VRBPAC report (in addition to the physician).
- AEs can include adverse reactions to the vaccine (including suspected adverse reactions and those that were not detected in preclinical toxicology studies).
- Additional phase IV studies will evaluate efficacy on new variants, including P.1 (Brazil/Japan variant), B.1.351 variant (South Africa), and B.1.1.7 Variant.

VAERS Home

Home | Report an Adverse Event | [en Español](#)

Report an Adverse Event to VAERS

VAERS is a passive reporting system, meaning it relies on individuals to send in reports of their experiences. Anyone can submit a report to VAERS, including parents and patients.

Healthcare providers are **required by law** to report to VAERS:

- Any adverse event listed in the [VAERS Table of Reportable Events Following Vaccination](#) that occurs within the specified time period after vaccinations
- An adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine

Healthcare providers are strongly **encouraged** to report to VAERS:

- Any adverse event that occurs after the administration of a vaccine licensed in the United States, whether it is or is not clear that a vaccine caused the adverse event
- Vaccine administration errors

[Click here for guidance to healthcare providers on reporting adverse events to VAERS after COVID-19 vaccination](#)

Phase 3 Trial Design

Estimated duration of the trial

- 3-month enrollment period
- 15-month study duration

Inclusion Criteria

1. Adults aged 19 or older
2. Deemed healthy by study investigator
3. Able and willing to comply with all study procedures
4. History of laboratory results to confirm previous diagnosis of SARS-CoV-2 infection (will not affect study participation, but information collected as part of study inclusion)

Phase 3 Trial Design

Exclusion Criteria

1. Pregnant or breastfeeding, or planning to become pregnant during the study period
2. Laboratory testing of SARS-CoV-2 infection shows positive for serum antibodies
3. Confirmed immunodeficiency disorders
4. History of following medical conditions (or any other illness or condition that the investigator suggests may affect the study or the participant):
 - a. Diabetes mellitus
 - b. Cardiovascular diseases
 - c. Respiratory diseases
 - d. History of severe allergic reactions
5. Participation in other clinical trial in the past 3 months
6. Healthcare workers who are actively providing medical care to SARS-CoV-2 patients

Phase 3 Trial Design

Immunization Schedule

Day 0

1. baseline data collected from participants
2. Participants randomized to two arms
 - a. Single Shot Participants - Administered first dose of vaccination or placebo
 - b. Two Shot Participants - Administered first dose of vaccination or placebo

Day 28

1. Two Shot Participants - Administered second dose of vaccination or placebo

Phase 3 Trial Design

Primary Endpoint - Vaccine Efficacy

Outcome measured by the following:

1. Measurement of occurrence of disease:
 - a. At least two of any of these symptoms: Fever, chills, rigors, sore throat, headache, loss of sense of taste and/or smell, etc.

OR

At least one respiratory sign or symptom: cough, shortness of breath, or radiographic evidence of pneumonia

- a. AND

A positive SARS-CoV-2 test using at least one nasopharyngeal swab, nasal swab, or saliva sample (or respiratory sample, if the participant was hospitalized)

Phase 3 Trial Design

Primary Endpoint - Vaccine Efficacy

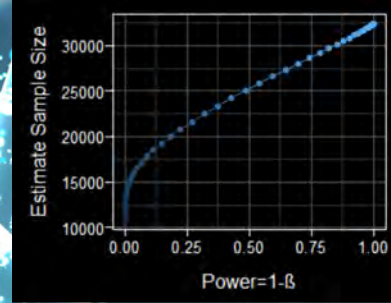
Outcome measured by the following:

1. Measurement of immunologic response:
 - a. Test for presence of SARS-CoV-2 antibodies
2. Other outcome measurements:
 - a. Self-reported symptoms: Participants will have an online portal to log daily symptoms related to SARS-CoV-2 (if any)
 - b. Medical records of participants will be captured if hospitalized for illness caused by SARS-CoV-2
 - c. Participants testing positive for SARS-CoV-2 will be followed for 14 days to assess symptom severity

The comparisons between control and treatment group will occur beginning 7 days after vaccination.

Phase 3 Trial Design (Sequential Wedge)

- Vaccine efficacy was assessed using a sequential stepped wedge trial design with 5 looks in total (4 interim analyses(IA) and one final analysis). Interim analyses can save money and lives, so we feel that this was a superior choice than a standard stepped wedge. If the trial is found to be remarkably successful or poor, there is a chance for earlier approval of the vaccine.
- As it is a stepped wedge design, it is robust to higher intraclass correlation($ICC = \sigma_e^2 / (\sigma_e^2 + \sigma_c^2)$), which is likely in a 470 site multinational trial where variability between clusters is significant when compared to the variability within a cluster.
- The stepped wedge also allows for delayed start of sites, which can be helpful for sites where logistics and approval in the given country are more difficult.
- In sequential model allows for efficacy and futility testing in several interim analyses, guided by a set of predefined test statistic boundaries. At each IA, the trial can be stopped if the treatment is found to have high efficacy.
- A stepped wedge trial was designed using a target power of $1-\beta=0.9$, $\alpha=0.025$, $OR_{treat}/OR_{ctrl}=1.5$, $odds(disease)=0.006973636$, number of steps=5, $ICC=0.1$, $\sigma_e^2=0.00007$, $\sigma_c^2=0.00686$, clusters=470 (these last three parameters were derived from the power calculation of a standard stepped wedge design).
 - The ICC is not currently known in all locales for our phase III study, but we took the most moderate values from the original methods paper. We will adjust as the phase I data makes clear the variability estimates.
- We estimate that approximately 33 persons will be needed for each treatment arm (4 in total, for a total of 132 persons) in each cluster.
- We estimate that we will need 62040 individuals in total, for all four arms. If we only choose to look at a two dose regimen, this could be cut by precisely half (31020). This number is similar to the estimates derived for the Moderna($n=30,420$), Zydus Cadila($n=28216$), and pfizer trials($n=43,548$).
- Reference: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5632563/>



	Single dose	Two dose
Treatment	15510 (33 per cluster)	15510(33 per cluster)
Placebo Control	15510(33 per cluster)	15510(33 per cluster)

Phase 3 Trial Design

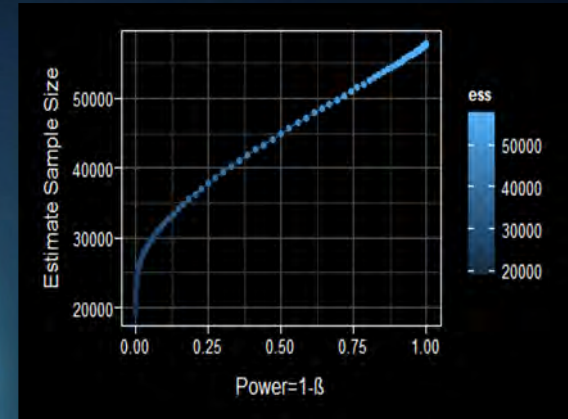
Secondary Endpoints

- HR>0.80 (20% risk reduction) Prevention of mortality while diagnosed with COVID-19, during the trial period (59 per cluster, n=55460 for all four arms, with C=470 clusters)
- Average Reduction in average hospital stay by at least 24 hours (n=65536 required, 70 per cluster, for all four arms)

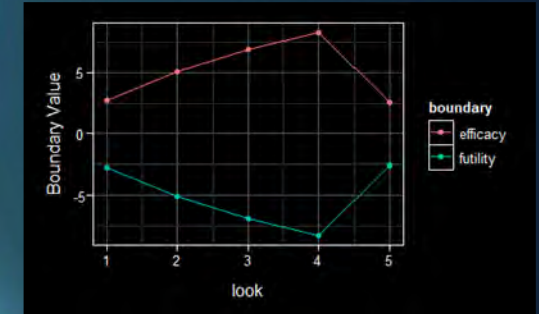
Primary and secondary outcomes were assessed 7 days following the last dose of the regimen. These interim analyses used O'Brien-Fleming boundaries as early stopping criteria. If, at the interim analysis, it is determined that the treatment is successful enough (or so futile) that it exceeds the interim analysis boundary, then participants will be unblinded and the trial will end with a final outcome of the trial determined as a success or failure of the primary outcome.

SAEs, if determined to be related to the vaccine under investigation, will also cause a pause in the trial until an investigation is completed. The DSMB will determine if any changes need to be made in protocol or if enrollment should discontinue.

Duration difference sample size estimate was calculated using 11.5 days (CDC source suggests a median duration of 10-13 days), with 470 clusters, and difference of $SM=\delta=1$ day), using $1-\beta=0.9$, $\alpha=0.025$, and $ICC=0.01$, $\sigma=4.9$ days.



Sample size calculation for mortality & early stopping boundaries



Phase 3 Trial Design

- **Safety Sample Size**
- **SAE data is limited:** Sample size requirements for safety outcomes will be determined when there is sufficient data to estimate incidence of a serious adverse event (using the phase I trial data). The only similar trials had no SAEs (Inovio SARS-COV2 and MERS vaccines and Zydus Cadila). The INO-4800 preclinical trial also reported that there were no known instances of “vaccine-induced immunopathology” in MERS NHP & mouse trials or in the SARS DNA vaccine mouse trials. All of this is reassuring that the product will **likely have a great safety profile but makes it difficult to estimate the capacity to detect safety events without more data.**
- **Sample size likely sufficient, according to Plotkin's Vaccines:** Plotkin's vaccines suggests **at least 3000 persons for trials involving new vaccines in order to assess safety, to be assessed 5-7 days post vaccination. We have 20 times this amount and are following this timeline with 7 days.** This should give confidence that this trial, with postmarket monitoring, will be able to be shown to be safe.
- **Non-inferiority Analysis shows sample size is roughly double that which is required:** Using precise numbers from the Oxford-AstraZenica phase III trial safety data, we performed a non-inferiority group sequential using hazard rates for sample size determination, using power=0.9, $\alpha=0.025$, $h_{trt}=79/10673/6$ (rate of incidence per month in a 6 month trial), $h_{ctrl}=84/10002/6$, a 1% loss rate of subjects in treatment and control, and O'Brien-Fleming Analog boundaries. We determine that the sample size required to detect such a difference is $n=8568$ per treatment arm, meaning **$N=34272$ total subjects required for all four arms. We have nearly doubled this amount**, so we can be fairly confident that we have sufficient sample size to determine rate differences as small as 0.1% between monthly rates.
- We also state that some uncommon events, including “rare adverse events” (e.g. Stevens-Johnson Syndrome, with an incidence of 0.4-1.2 cases per million, or Anaphylaxis) and “unexpected adverse events” are so rare that they can only be readily detected in a phase IV trial.

Power and Sample Size Summary									
Maximum Number of Stages	5								
Alternative Hypothesis	h1 - h2 < (NIM)								
Alpha Spending Function	O'Brien-Fleming Analog								
Beta Spending Function	O'Brien-Fleming Analog								
Number of Simulations	10000								
Computer-Generated Random Seed	7556316								
Target Power	Sim Power	N1	N2	N	h1	h2	NIM	Target Alpha	Sim Alpha
0.9	0.9058	8568	8568	17136	0.00123	0.0014	0.001	0.025	0.0291

“Although vaccine-induced immunopathology has been raised as a potential concern for SARS and MERS vaccine candidates, and possibly for SARS-CoV-2 vaccines, these concerns are likely vaccine-platform dependent and, to-date, no evidence of immune pathogenesis has been reported for MERS DNA vaccines in mice or non-human primate models or SARS DNA vaccines in mice.”
 “Immunogenicity of a DNA vaccine candidate for COVID-19 | Nature Communications” <https://www.nature.com/articles/s41467-020-16505-0>

Phase 3 Trial Design

Definition of regional groupings

WHO regions: WHO Member States are grouped into 6 WHO regions: African Region, Region of the Americas, South-East Asia Region, European Region, Eastern Mediterranean Region, and Western Pacific Region. See [map](#) and the Notes sheet in each spreadsheet for more information.

World Bank income groups: WHO Member States are grouped into 4 income groups (low, lower-middle, upper-middle, and high) based on the World Bank list of analytical income classification of economies for the fiscal year, which is based on the Atlas gross national income per capita estimates (released July annually). See the Notes sheet in each spreadsheet for more information.

World Bank regions: countries are separated into 6 World Bank regions, separating out high-income countries as a 7th group: High Income, East Asia and Pacific, Europe and Central Asia, Latin America and the Caribbean, Middle East and North Africa, South Asia and Sub-Saharan Africa. See the Notes sheet in each spreadsheet for more information.

WHO regions and World Bank income categories: WHO Member States are grouped into low and middle-income countries (LMIC) by WHO region (the 6 WHO regions are used), separating out high-income countries within each of these regions into a 7th group. See the Notes sheet in each spreadsheet for more information.

UN Sustainable Development Goals (SDGs) regions: Countries, areas and territories are classified into groups as defined by the UN for the reporting of SDG indicators in 2017. See also the Notes sheet in each spreadsheet for more information.

UN Millennium Development Goals (MDGs) regions: Regions are classified as "developing" regions and "developed" regions. The developing regions are further broken down into the subregions. See also the Notes sheet in each spreadsheet for more information.

LOWER-MIDDLE INCOME ECONOMIES (\$1,036 TO \$4,045)

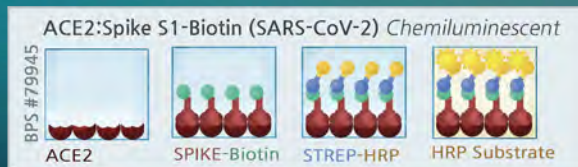
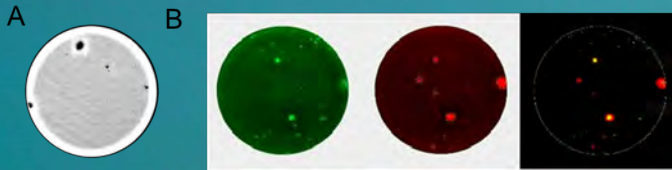
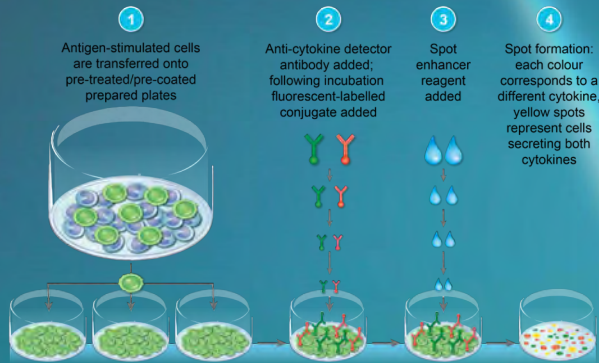
[50]

Angola	Honduras	Papua New Guinea
Algeria	India	Philippines
Bangladesh	Kenya	São Tomé and Príncipe
Benin	Kiribati	Senegal
Bhutan	Kyrgyz Republic	Solomon Islands
Bolivia	Lao PDR	Sri Lanka
Cabo Verde	Lesotho	Tanzania
Cambodia	Mauritania	Timor-Leste
Cameroon	Micronesia, Fed. Sts.	Tunisia
Comoros	Moldova	Ukraine
Congo, Rep.	Mongolia	Uzbekistan
Côte d'Ivoire	Morocco	Vanuatu
Djibouti	Myanmar	Vietnam
Egypt, Arab Rep.	Nepal	West Bank and Gaza
El Salvador	Nicaragua	Zambia
Eswatini	Nigeria	Zimbabwe
Ghana	Pakistan	

Incidence calculation

- We obtained a list of lower-middle income countries, grouped by the WHO using worldbank data, then took weekly notification case counts (measured per 100,000 persons) from the european CDC data and averaged the first eight weeks of the year and multiplied by (52/2) to get a 6 month incidence proportion.
 - This sixth month incidence calculation was employed in the moderna trial.
- As our trial is distributed across many LMICs, we performed an average of the LMIC incidence proportion of 0.006973636 (0.6973636% of the population in LMICs, on average). This is used as the risk of the control group in the trial. This was done because the vaccine regimen will take up to 4 weeks and each month efficacy is assessed (5 times in total).

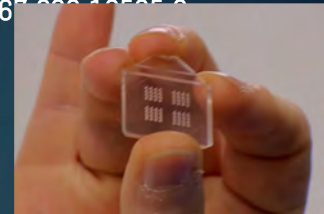
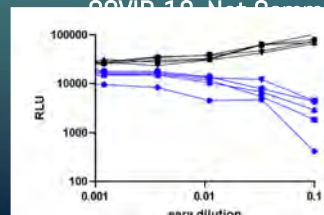
Phase 3 Trial Design



Immunogenicity Assays:

- Both assayed 7 days after each dose, at interim analyses, and at final analysis.
- Cellular immunity:
 - Interferon gamma (IFN- γ) ELIspot assay, measured as SFC/ 10^6 PBMCs. Cross-reactivity will be assessed to MERS, SARS viruses to determine if response is specific to SARS-nCoV2.
- Humoral immunity:
 - wt SARS-CoV2 virus neutralization assay (p.3, Invio phase I immunogenicity study)
 - ACE2 inhibition assay (surrogate for neutralization, p.4, 2nd col, Invio phase I immunogenicity study)
 - Basic idea: if there is a neutralization antibody present, the biotin and chemiluminescent antibody won't be able to fluoresce. Therefore, if a regimen is successful in neutralizing the Spike-ACE2 interaction, there will be a decrease in fluorescence in a dilution assay (as seen in the graph at bottom).
- Immune Biomarker monitoring of patients using dual-use, electroporating patch

Reference: Smith, T.R.F., Patel, A., Ramos, S. et al. Immunogenicity of a DNA vaccine candidate for COVID-19. *Nat Commun* 11, 2601 (2020) <https://doi.org/10.1038/s41467-020-18505-0>

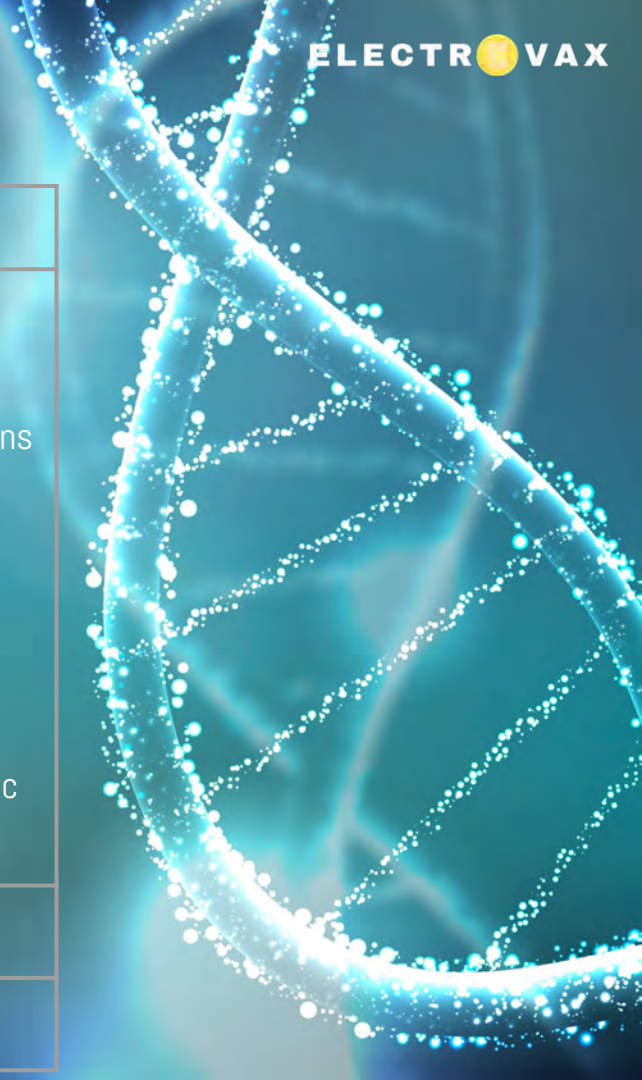


Priority Groups

Phase	Groups
Phase 1a	Healthcare personnel
Phase 1b	<ul style="list-style-type: none"><li data-bbox="316 405 1271 740">● Frontline essential workers (teachers, restaurant workers and taxi drivers, fire fighters, police officers, corrections officers, food and agricultural workers, postal workers, manufacturing workers, grocery store workers, public transit workers, and those who work in the educational sector (teachers, support staff, and daycare workers.)<li data-bbox="316 757 1286 852">● people aged 75 years and older because they are at high risk of hospitalization, illness, and death from COVID-19.

Priority Groups cont'd

Phase	Groups
Phase 1c	<ul style="list-style-type: none"><li data-bbox="357 303 1271 401">● People aged 65—74 years because they are at high risk of hospitalization, illness, and death from COVID-19.<li data-bbox="357 423 1323 576">● People aged 16—64 years with underlying medical conditions which increase the risk of serious, life-threatening complications from COVID-19.<li data-bbox="357 598 1309 871">● other essential workers such as people who work in transportation and logistics, food service, housing construction and finance, information technology, communications, energy, law, media, public safety, and public health.
Phase 1d	Populations living in high density areas
Phase 1e	People with disabilities



Phase IV Study Design - Post-Marketing Product Surveillance

Estimated duration of the trial

- 36 months study duration

Number of Participants:

- Depends on efficacy and adverse event accidents from phase III

Outcome Measures:

- Incidence rates of unexpected adverse events and adverse drug reactions
- Incidence rate of serious adverse events and serious adverse reactions (life-threatening)
- Efficacy of boosters for Novel Variants not tested in original phase III trial.
- Coadministration with common vaccines
- Flexibility: Interchangeability analyses with other COVID-19 vaccines

Phase IV Trial Design

Inclusion Criteria

1. 12 years and older
2. Subjects who have signed the informed consent form for the study
3. Subjects who are administered the DNA vaccine in the United States

Exclusion Criteria

1. Subject who have any known hypersensitivity to the drug or its ingredients
2. Participation in other clinical trial in the past 3 months

Questions?