# Nucleic Acid Vaccines and a Nonhuman Primate Model for Valley Fever

National Academies Forum on Microbial Threats:
The Impact and Control of Valley Fever

Discussion: Currently Proposed Vaccine Candidates

November 18, 2022

Deborah Fuller, PhD
Professor, Department of Microbiology
University of Washington
Associate Director of Research
Washington National Primate Research Center

# Disclosures

I am a co-founder of ***Orlance, Inc***. a biotechnology company that aims to commercialize gene gun delivered DNA and RNA vaccine technologies developed in my lab.

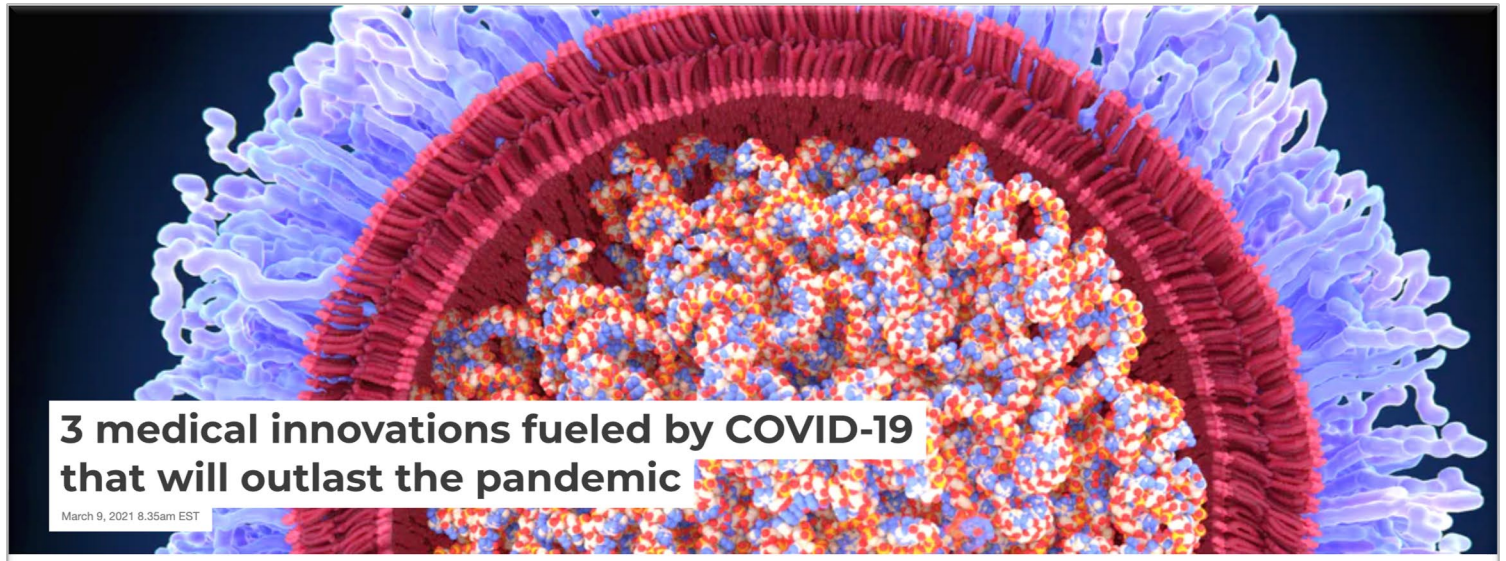
I am a scientific advisor for ***HDT Bio,*** a biotechnology company that aims to commercialize self amplified RNA vaccine and nanoparticle vaccine technologies tested in my lab.

# To develop a vaccine for Valley Fever we need:

- To know what types of immune responses provide protection
- To know what parts of the pathogen (or antigens) these immune responses need to target
- A vaccine strategy that can induce these responses → *Nucleic Acid Vaccines*
- An animal model that closely models human disease → *Nonhuman primates*

# Nucleic Acid Vaccines: Prospects for protection from Valley Fever

# Nucleic acid (DNA and RNA) vaccines: A revolution in modern medicine



## 3 medical innovations fueled by COVID-19 that will outlast the pandemic

March 9, 2021 8.35am EST

Gene-based vaccines had never been approved for humans before the coronavirus pandemic. Juan Gaertner/Science Photo Library via Getty Images

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*A number of technologies and tools got a chance to prove themselves for the first time in the context of COVID-19. Three researchers working in gene-based vaccines, wearable diagnostics and drug discovery explain how their work rose to the challenge of the pandemic, and their hopes that each technology is now poised to continue making big changes in medicine.*

### Genetic vaccines

**Deborah Fuller, Professor of Microbiology, University of Washington**

Authors

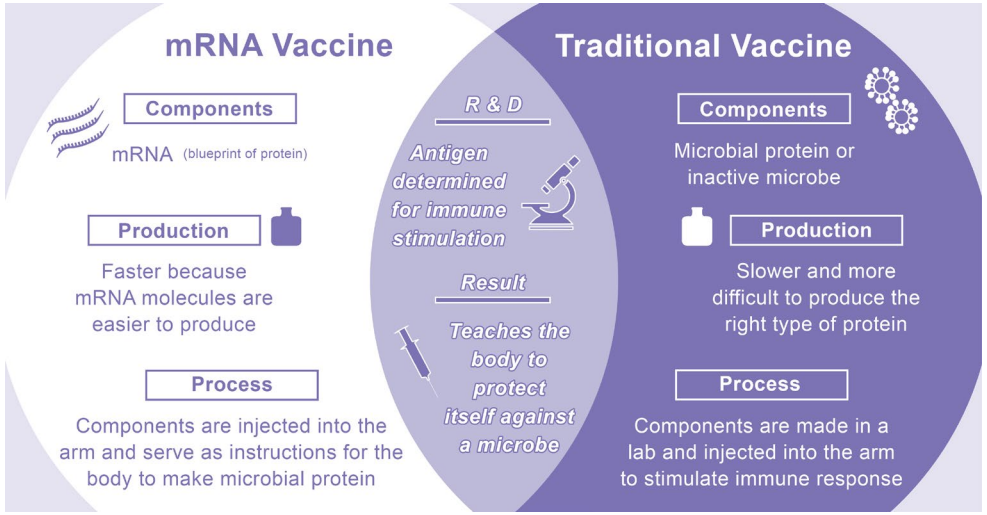
**Deborah Fuller**
Professor of Microbiology, School of Medicine, University of Washington

**Albert H. Titus**
Professor of Biomedical Engineering, University at Buffalo

**Nevan Krogan**
Professor and Director of Quantitative Biosciences Institute & Senior Investigator at the Gladstone Institutes, University of California, San Francisco

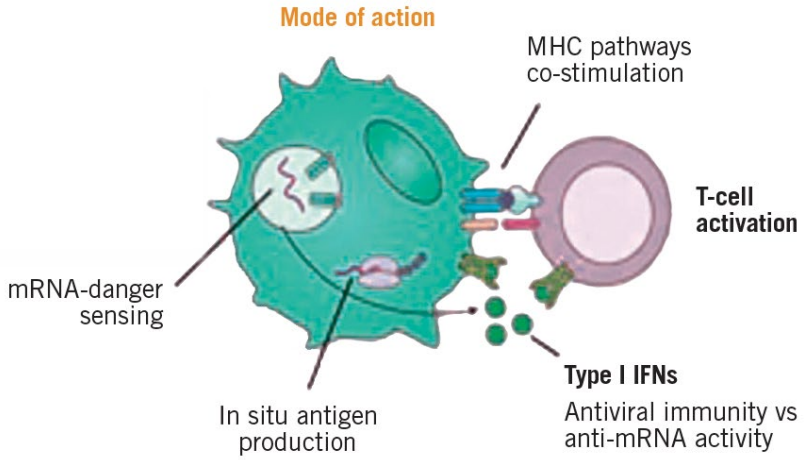
***Developing an effective mRNA vaccine was a turning point for COVID19 but COVID19 was also a turning point for nucleic acid vaccines***

# Key advantages of DNA and RNA vaccines




***Only the genetic sequence of an immunogen is needed to make a DNA or RNA vaccine***

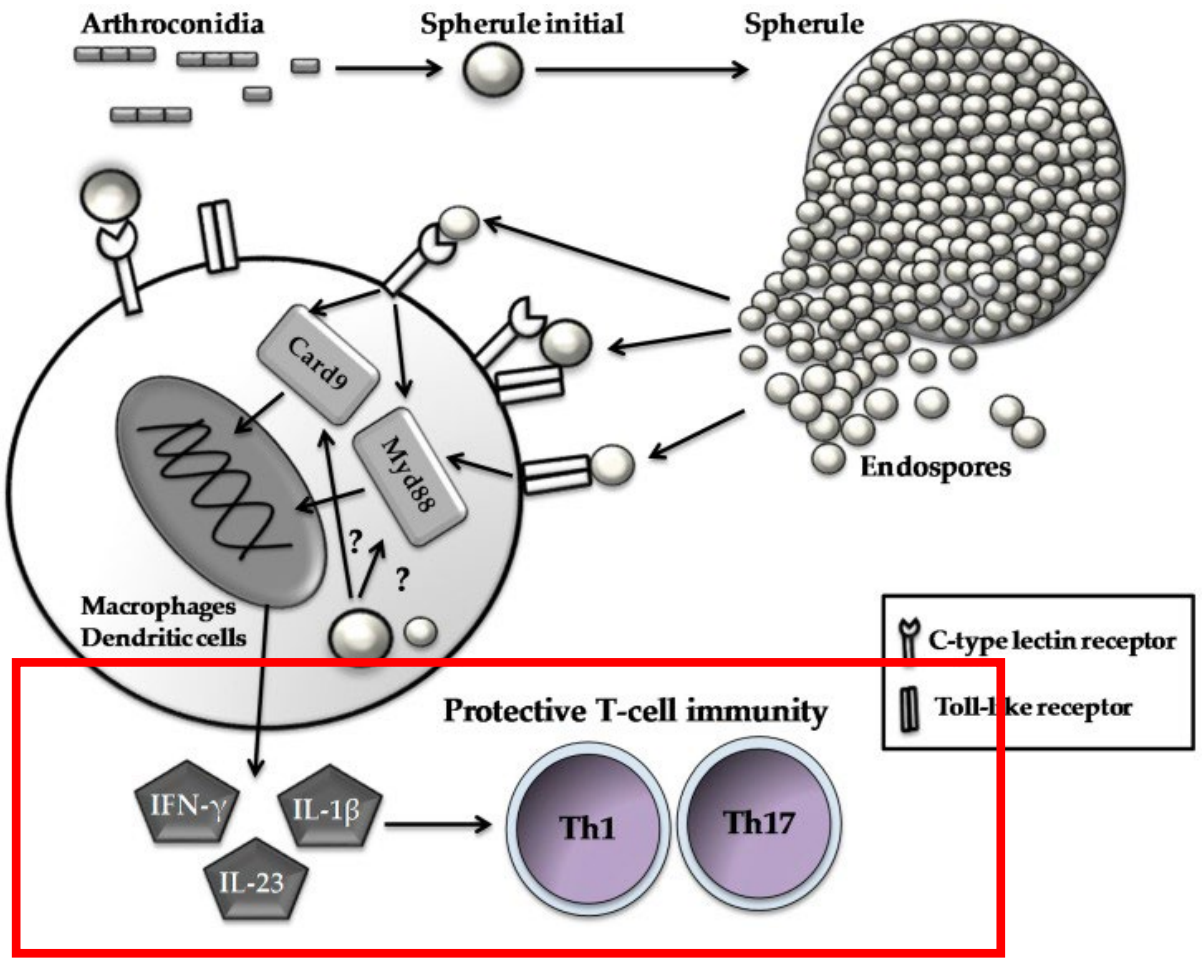
- Rapid design and development
- Can be designed to precisely focus the immune responses only against those antigens needed for protection
- Enables rapid discovery of new immunogens
- Highly amenable to multi-antigen delivery
- High safety, low reactogenicity




***DNA and RNA vaccines induce robust T cell responses***

- Induces robust T cell responses believed to be important for an effective Valley Fever vaccine
  - CD4+ T cell responses (Th1, Th17)
  - CD8+ T cell responses
- Plasmids (genetic adjuvants) can be co-delivered to modulate and enhance the type of response induced
- Can be delivered to induce mucosal immune responses in the lung

Nucleic Acid Vaccines are very effective for inducing the types of responses likely need for protection from Valley Fever

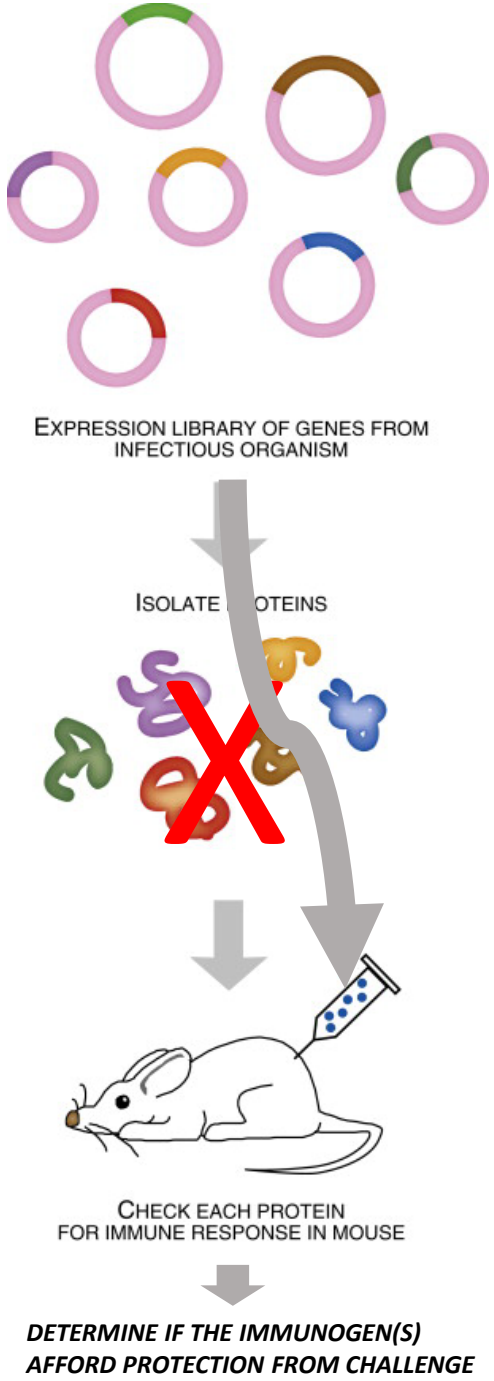



***These are precisely the responses DNA and RNA vaccines are especially good at inducing***

Castro-Lopez N, Hung CY. Immune Response to Coccidioidomycosis and the Development of a Vaccine. Microorganisms. 2017 Mar 16;5(1):13. doi: 10.3390/microorganisms5010013. PMID: 28300772; PMCID: PMC5374390.

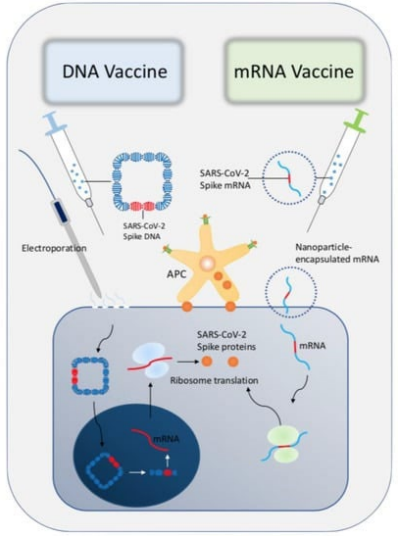
# Nucleic Acid vaccines enable rapid discovery of protective immunogens

- Known immunogens
- Virulence factors as candidate immunogens



# Differences between DNA and RNA vaccines

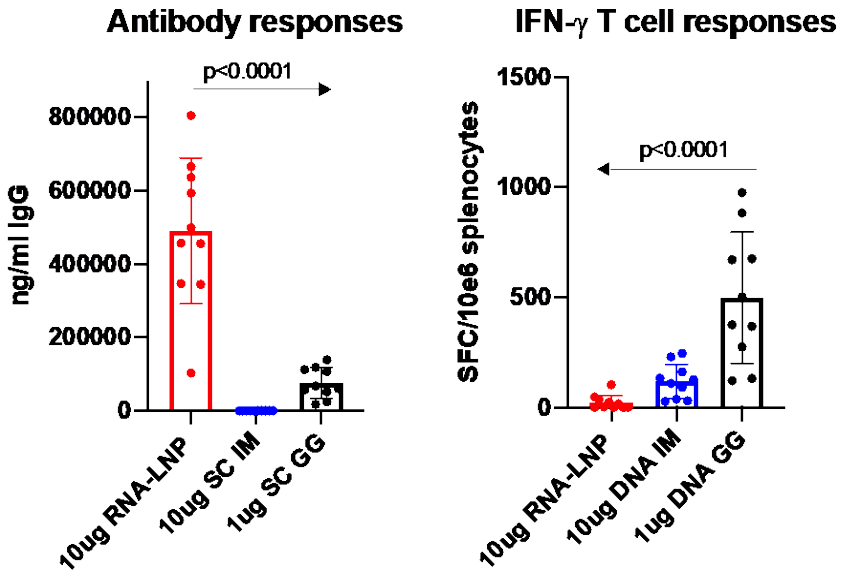
1. **Immunogenicity: RNA is better**
   - DNA needs to be delivered into the nucleus. RNA only needs to be delivered into the cell
   - RNA has self-adjuvanting properties, DNA vaccines alone are poorly immunogenic




2. **Stability: DNA is better**
   - DNA is stable at room temp for years
   - mRNA requires ultra-cold storage

| Feature | DNA | RNA |
|---|---|---|
| Immunogenicity | ++ | +++++ |
| Stability | +++++ | ++ |

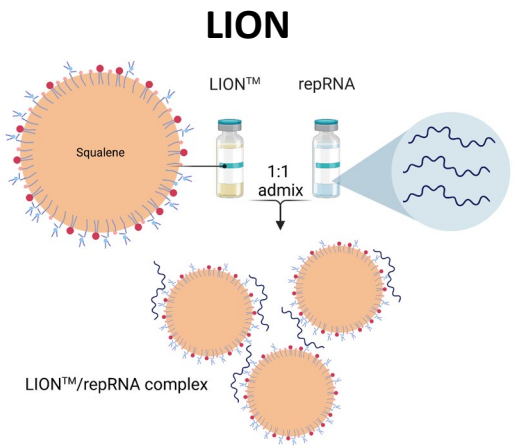
**3. DNA and RNA vaccines can induce distinct immune responses**

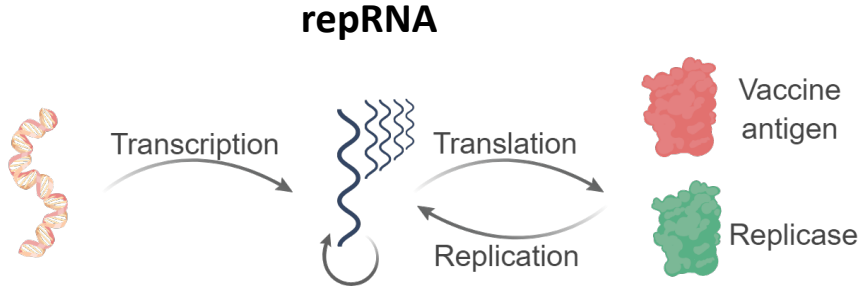



***Can we leverage DNA and RNA vaccines in combination to induce a broad range of immune responses to different immunogens?***

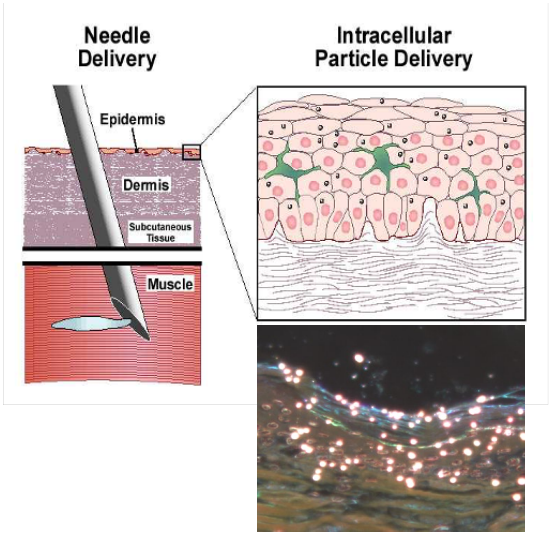
# Our Nucleic Acid Vaccine Platforms

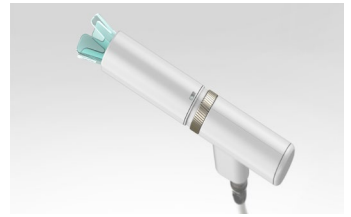
## Self-amplifying RNA vaccine (repRNA/LION) delivered by a nanolipid carrier

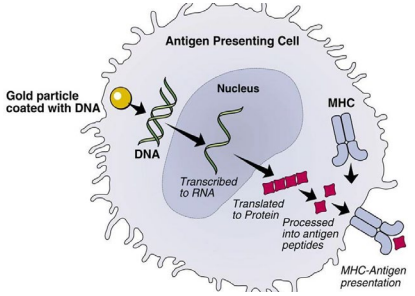


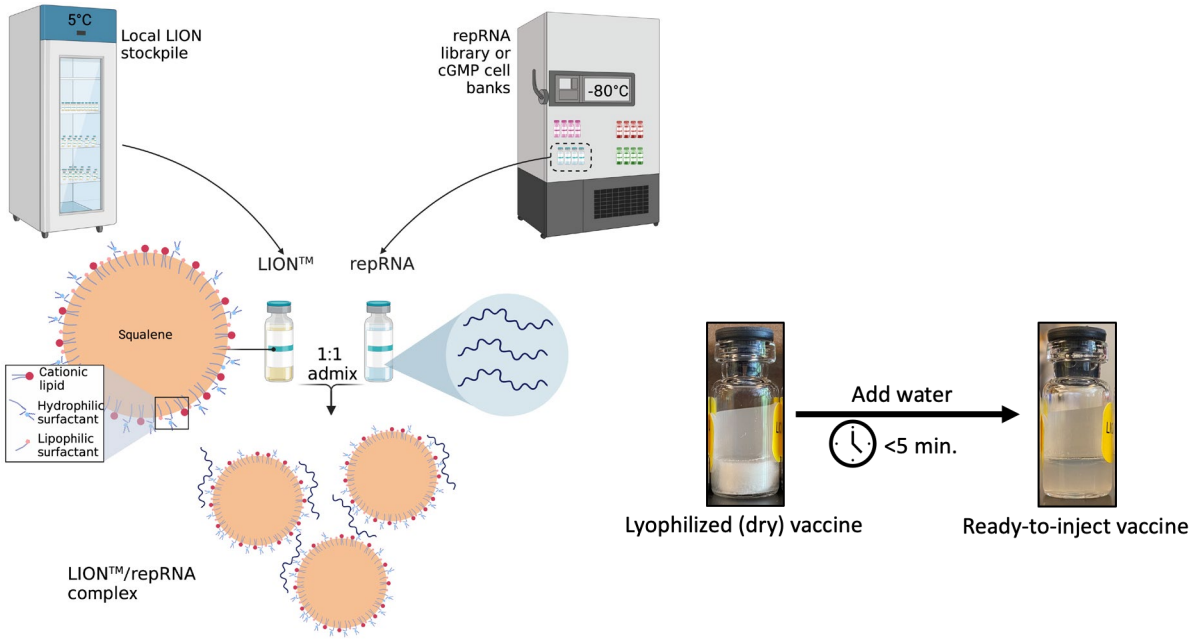

## repRNA and adjuvanted DNA vaccines delivered into skin by needle-free gene gun

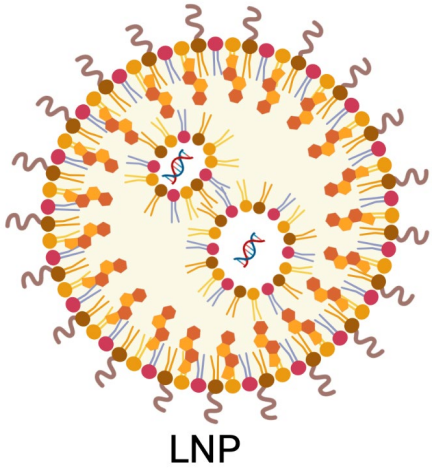




# LION™ is distinct from LNP

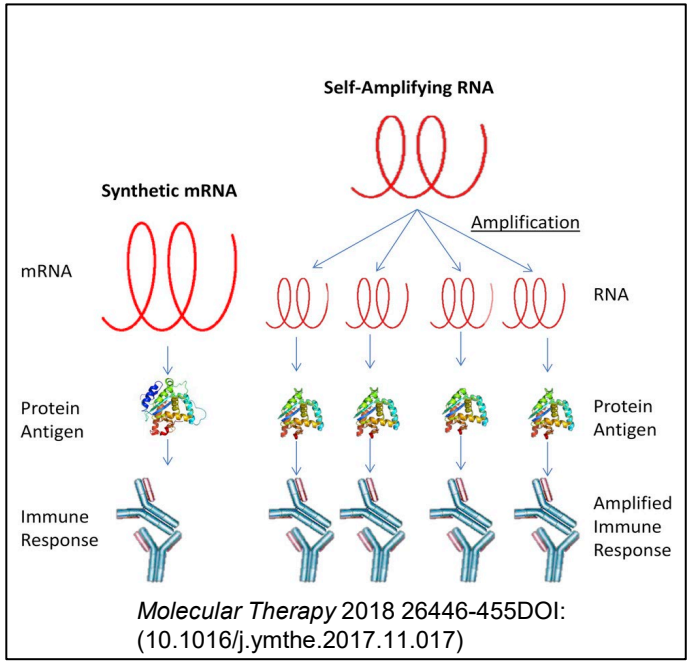



- ✓ Formulation is stable at 5C >1 year and can be stockpiled
- ✓ "Plug-and-play" functionality
- ✓ Local retention at injection site → enhanced safety

- X Must be stored frozen at ultracold temperatures
- X Formulation "married" to RNA limits use flexibility
- X Systemic distribution → safety concerns

# RepRNA is distinct from mRNA

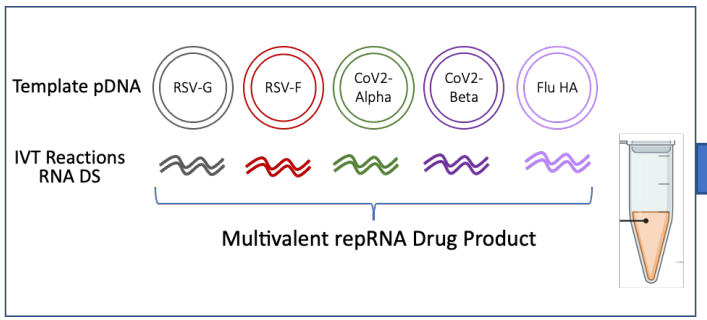


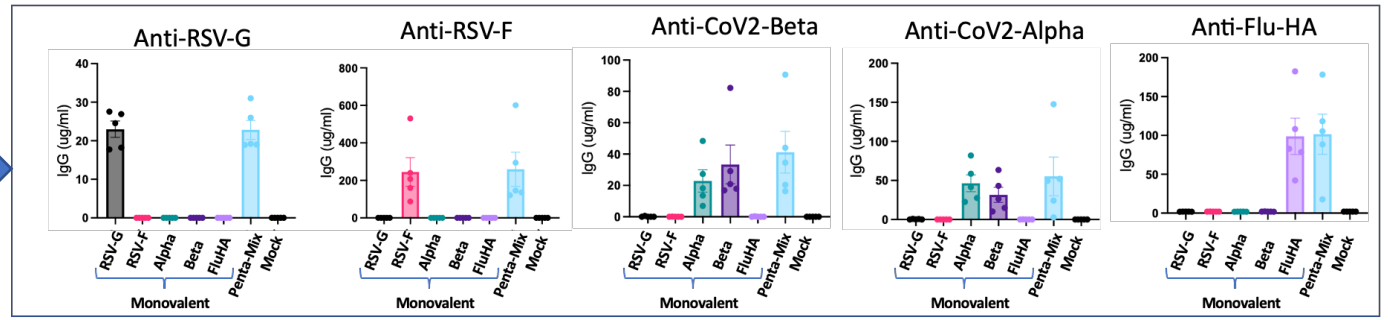

*Molecular Therapy* 2018 26446-455DOI: (10.1016/j.ymthe.2017.11.017)

- ✓ Expresses more protein > amplified immune responses
- ✓ Self-adjuvanting -induces higher Type I IFNs > more robust Th1 T cell responses
- ✓ Stronger immune responses at lower doses (5 μg) and lower reactogenicity at high doses (100 μg) > Wide dosing window enables co-delivery of multiple antigens, each at their optimum dose

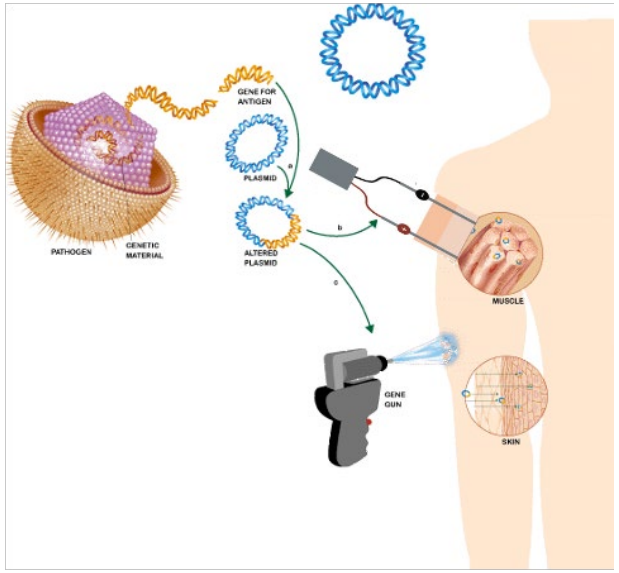
***An effective Valley Fever vaccine may need to be be multi-valent to target the pathogen at different stages of its life cycle***

POC: LION/repRNA can deliver multiple RNAs without compromising in vivo immunogenicity of each antigen

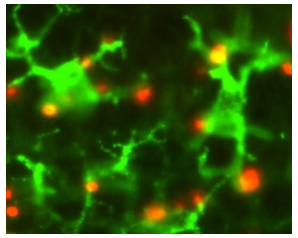


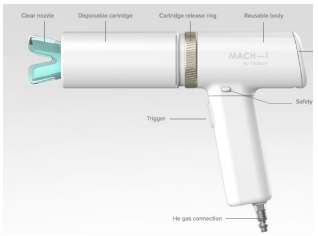



# Gene gun delivery is distinct from electroporation delivery

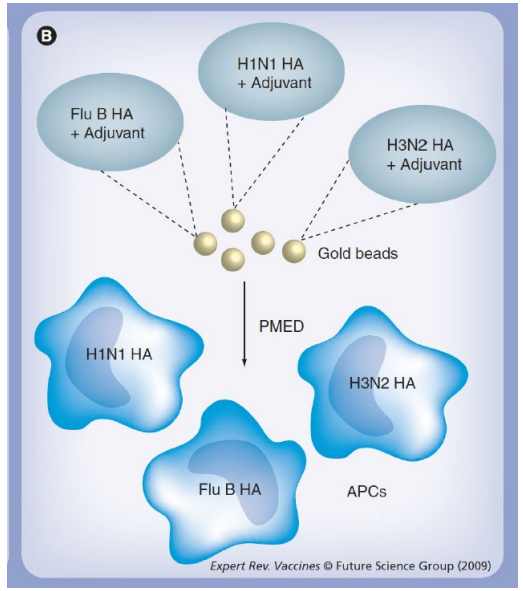



- ✓ Pain-free
- ✓ Direct intracellular delivery into the highly immunocompetent skin

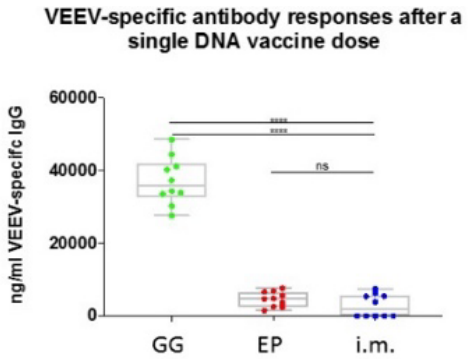


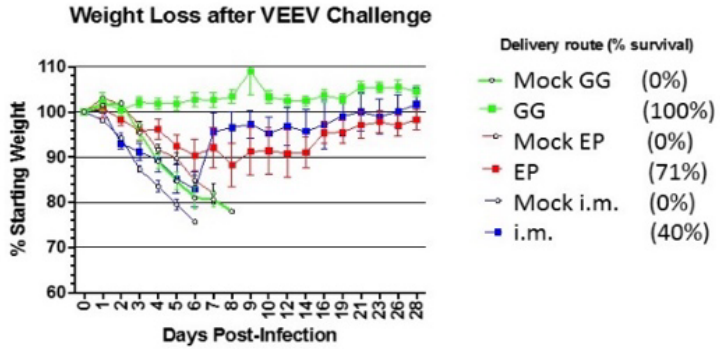


- ✓ Delivers multiple DNA vaccines without compromising in vivo immunogenicity of each antigen



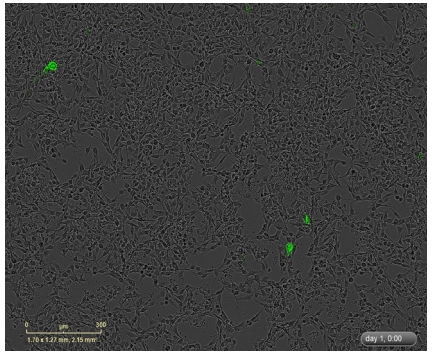

- ✓ Induces stronger immune responses with lower doses of DNA



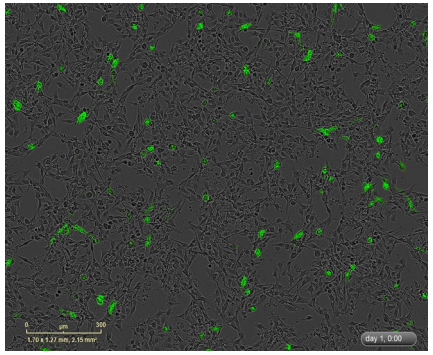



# Gene gun delivery is distinct from other methods of nucleic acid delivery

✓ Can deliver either DNA or RNA

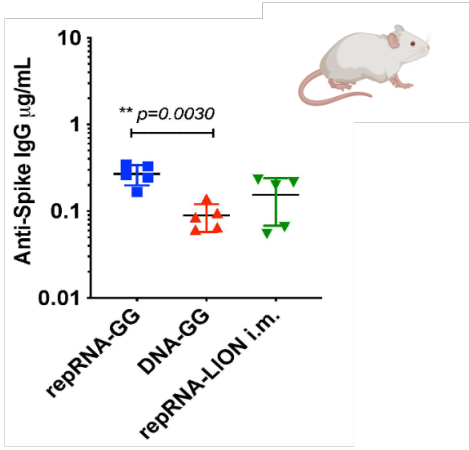


**DNA**

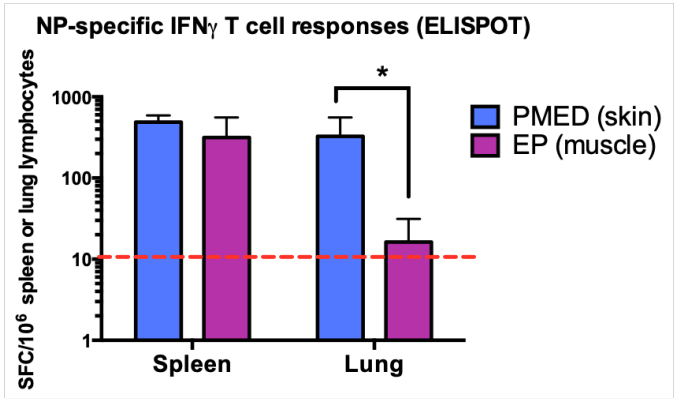


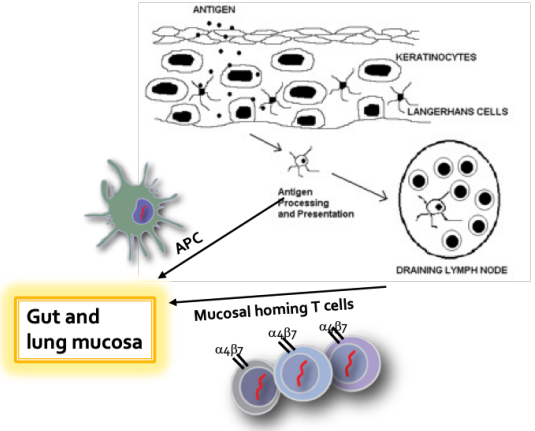
**RNA**

SARS-CoV-2 gene gun delivered RNA and DNA vaccines



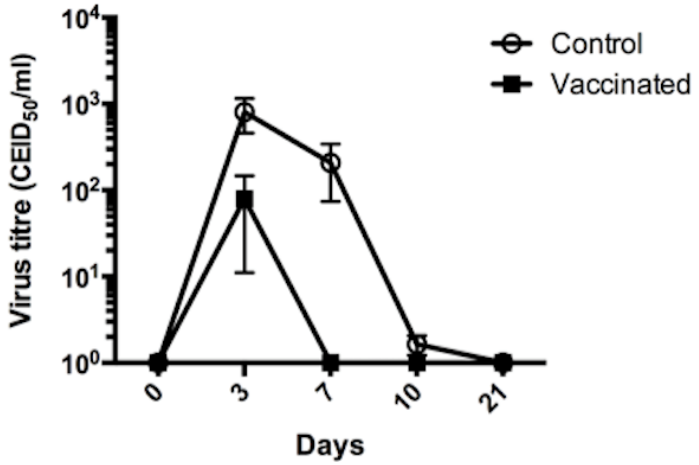

✓ Epidermal delivery induces mucosal responses

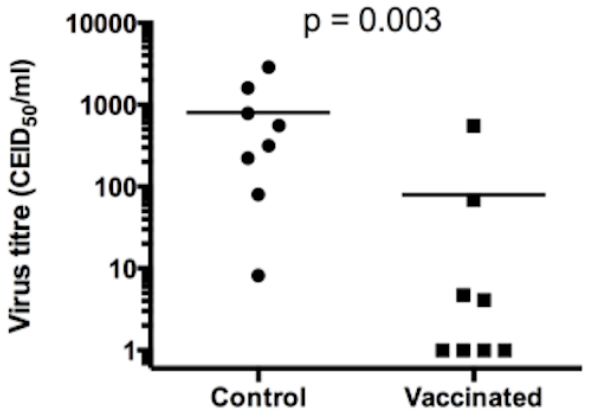


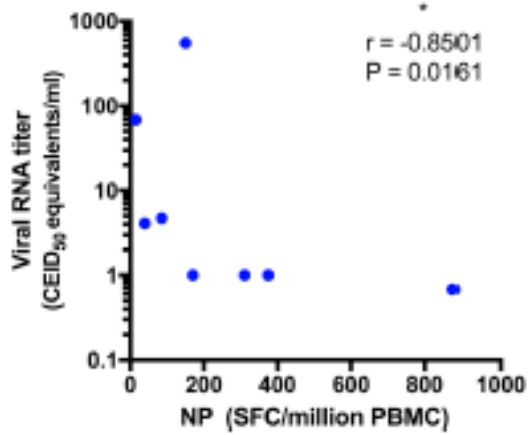
# Gene gun delivered DNA vaccine induced mucosal protection from a respiratory challenge with influenza in nonhuman primates

Influenza DNA vaccine induced mucosal protection from heterologous challenge in nonhuman primates (Koday et al 2017 PloS One)



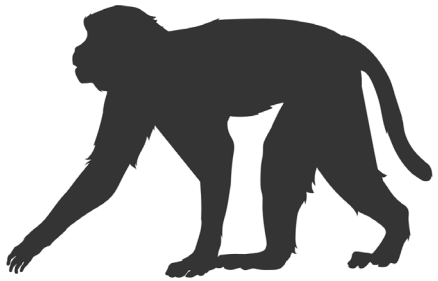




**Protection correlated with NP-specific T cell responses in the lung**

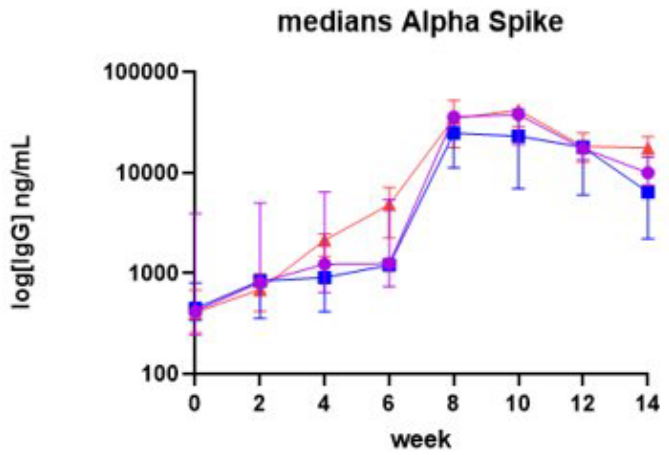


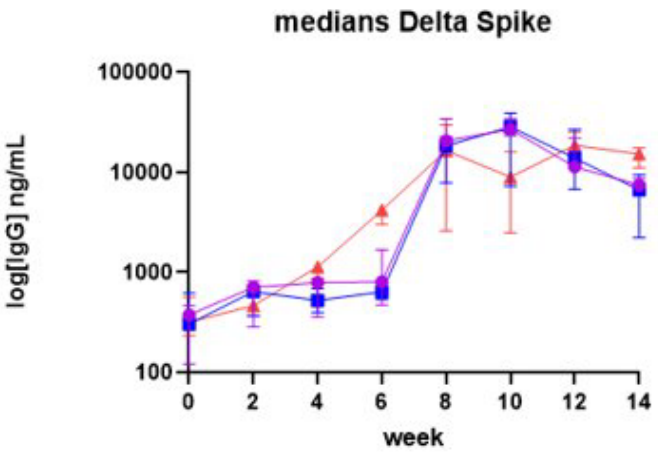

***A gene gun delivered Valley Fever vaccine that induces mucosal immune responses at the site of exposure could provide superior protection***

# Comparison of Gene gun delivery of DNA or repRNA to LION/repRNA in in nonhuman primates

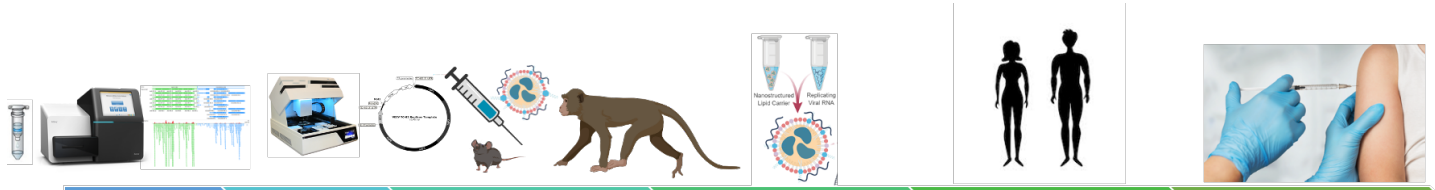


| Experimental groups | n | Vaccination | Delivery |
|---|---|---|---|
| 1 | 5 | repRNA | gene gun |
| 2 | 5 | DNA | gene gun |
| 3 | 4 | repRNA/ LION | IM |



# repRNA/LION and gene gun have proven to be safe and very effective for inducting protective levels of immunity in humans





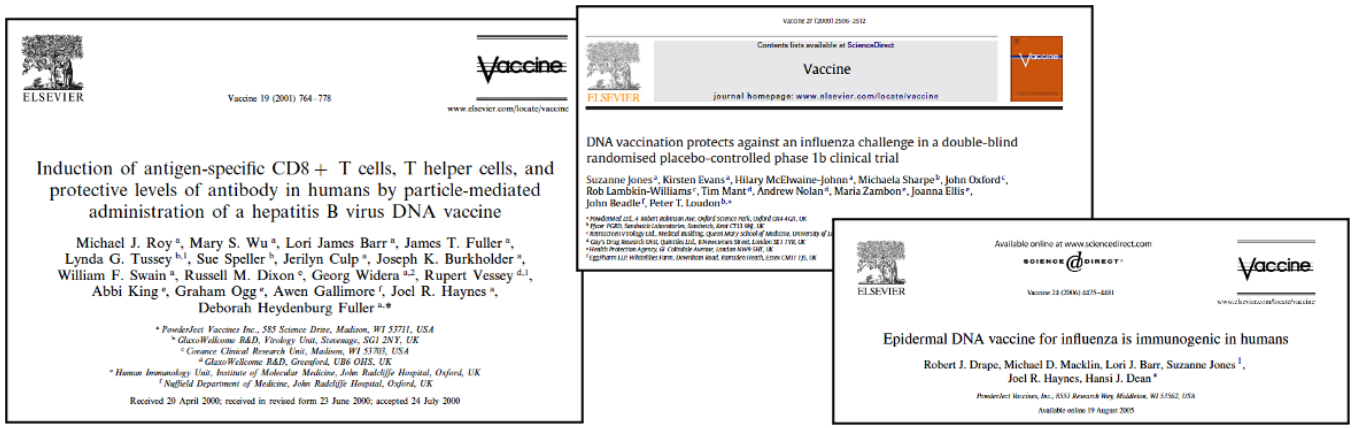





**repRNA/LION**

**FIRST** self-amplifying RNA vaccine licensed for human use
**FAST** We designed, produced and started testing a candidate replicating RNA vaccine within 7 days after the sequences of SARS-CoV-2 were published
**ADAPTABLE** We are currently testing LION/repRNA COVID-19 vaccines to protect against emerging variants that are more resistant to current vaccines

**DNA/gene gun**



Induction of antigen-specific CD8 + T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine

DNA vaccination protects against an influenza challenge in a double-blind randomised placebo-controlled phase 1b clinical trial

Epidermal DNA vaccine for influenza is immunogenic in humans

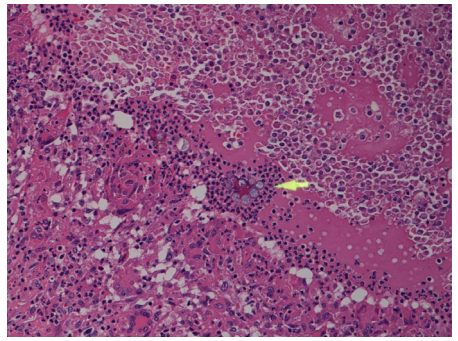
**PAIN FREE, EFFECTIVE AT LOW DOSES:** Induced Th1 T cell responses and protective levels of antibody using 1000-fold lower doses of DNA than electroporation

# Development of a nonhuman primate model for Valley Fever

# WaNPRC's Pigtail macaques (*M. nemestrina*) could play a key role in the development of an effective vaccine for Valley Fever

***PTMs are naturally vulnerable to Valley Fever and if infected, exhibit similar clinical disease as humans***

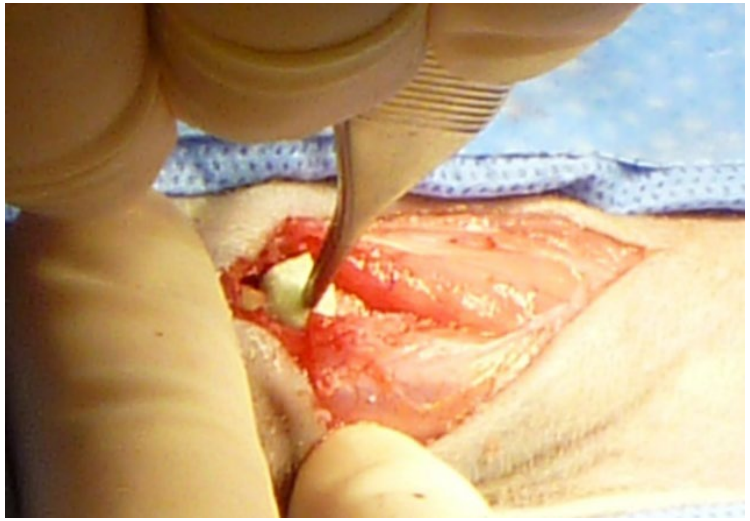
- ✓ Primary route of infection is inhalation
- ✓ The same organs are affected (i.e. lungs, lymph node, liver, kidney, bone, skin etc)
- ✓ Diverse clinical presentation (average 7-28 days after exposure)
- ✓ Lethargy
- ✓ Coughing
- ✓ Shortness of breath
- ✓ Fever
- ✓ Inappetence and/or weight loss
- ✓ Joint pain or lameness
- ✓ Skin rash or nodules
- ✓ Neurological symptoms
- ✓ Affected animals are treated early with anti-fungals and treatment is usually curative



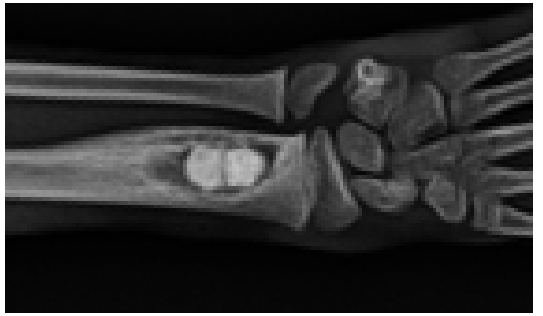

WANPRC
Washington National Primate Research Center
UNIVERSITY of WASHINGTON

# Fluconazole-impregnated beads surgically implanted

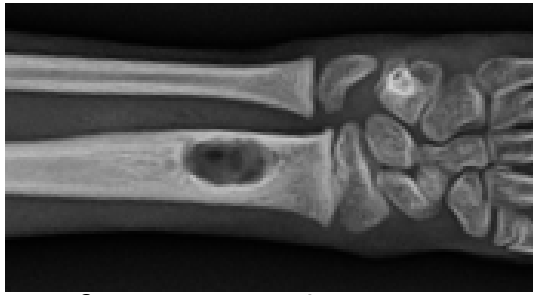
***Because systemic treatment was not effective, surgery was performed to debride infected material and insert fluconazole-impregnated methylmethacrylate beads***

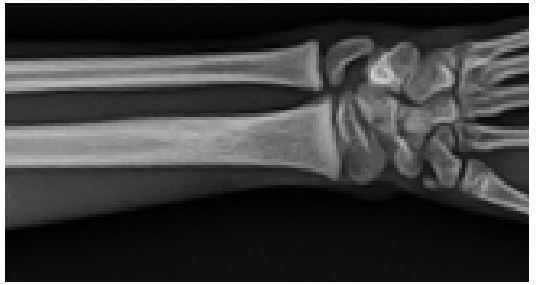
***Four months after implantation beads were removed, and bone healed completely.***



After implant surgery



After removal surgery



8 months after removal

Hotchkiss CE, Jeffery DA, Vogel KW. Use of Fluconazole-impregnated Beads to Treat Osteomyelitis Caused by *Coccidioides* in a Pigtailed Macaque (*Macaca nemestrina*). Comp Med. 2022;72(4):273-279. PMID: 35835541; PMCID: PMC9413521.

# We are developing a controlled experimental Pigtail macaque model of coccidioidomycosis

- Will provide highly relevant preclinical model closely modeling humans to test new vaccines and therapeutics

- Will also help us to answer some important questions:

  - ***What are the earliest immune responses to infection?***

    This will inform vaccine design

  - ***What are the mechanisms driving different outcomes in pathogenesis?***

    This will help us to refine our vaccine to focus on immune responses contributing to improved outcomes.

# Summary

**Nucleic Acid vaccines offer many features that align with what may be needed for an effective VF Vaccine**

- ✓ Th1/T17 T cell responses, mucosal immunity
- ✓ Enables multi-antigen vaccine composition
- ✓ Easy to manufacture and scale up, needle-free/self administration
- ✓ Strong track record of safety & efficacy in humans

**Nucleic Acid vaccines offer a valuable tool for immunogen discovery**

- ✓ Only the genetic sequence is needed– enables rapid immunogen discovery
- ✓ ***CAVEAT:*** ***Viral antigens expressed by NA vaccines closely mimic viral infections >> Fungal antigens may present a new challenge***

**Nonhuman primates are a promising model to support development of a vaccine for Valley Fever and improve our understanding of the mechanisms of disease and protection**

- ✓ Closest model to humans will enable the study of stages of infection not possible in humans.
- ✓ Gain a better understanding of how Cocci causes disease and immune mechanisms of protection

# Acknowledgments


**Fuller lab (UW)**
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Kris Aalto
Dennis McCabe

**Northern Arizona University**
Bridget Barker
Erik Settles
Paul Keim

**Washington National Primate Research Center**
Charlotte Hotchkiss
Richard Grant
Veterinary staff

**HDT Bio Corp**
Jesse Erasmus
Amit Khandhar
Malcolm Duthie
Darrick Carter
Steven Reed
Peter Berglund
Steve Reed

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