Intranasal Nanovaccines for Tuberculosis Prevention: Novel Strategies in Formulation and Delivery

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NASAL INNOVATION FORUM

Respiratory Mucosal Vaccination

Generation of memory and plasma IgA+ B cells

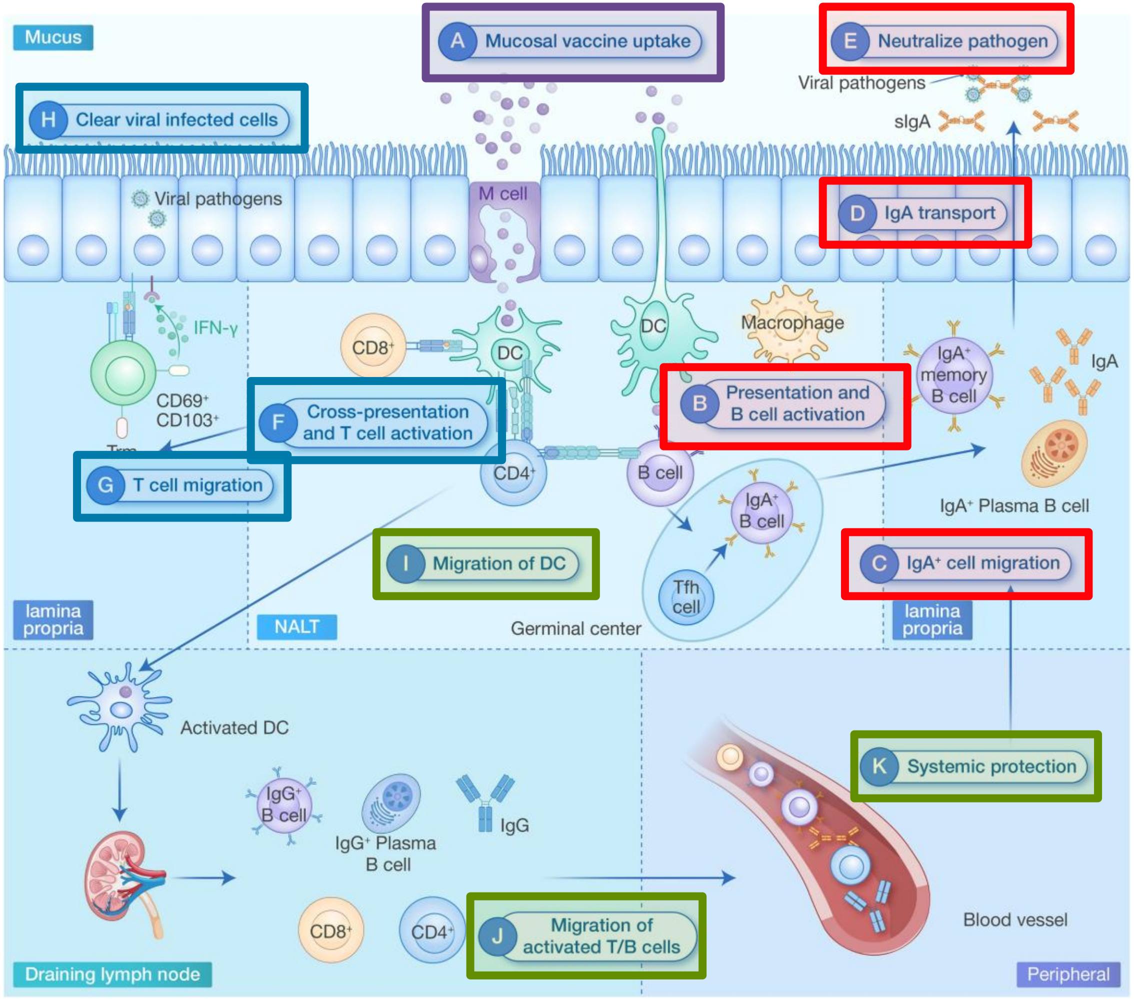
- *Secreted IgA in the mucus rapidly neutralize pathogens*

Generation of antigen-specific T cells

- *Mucosal tissue-resident T cells clear infected host cells*

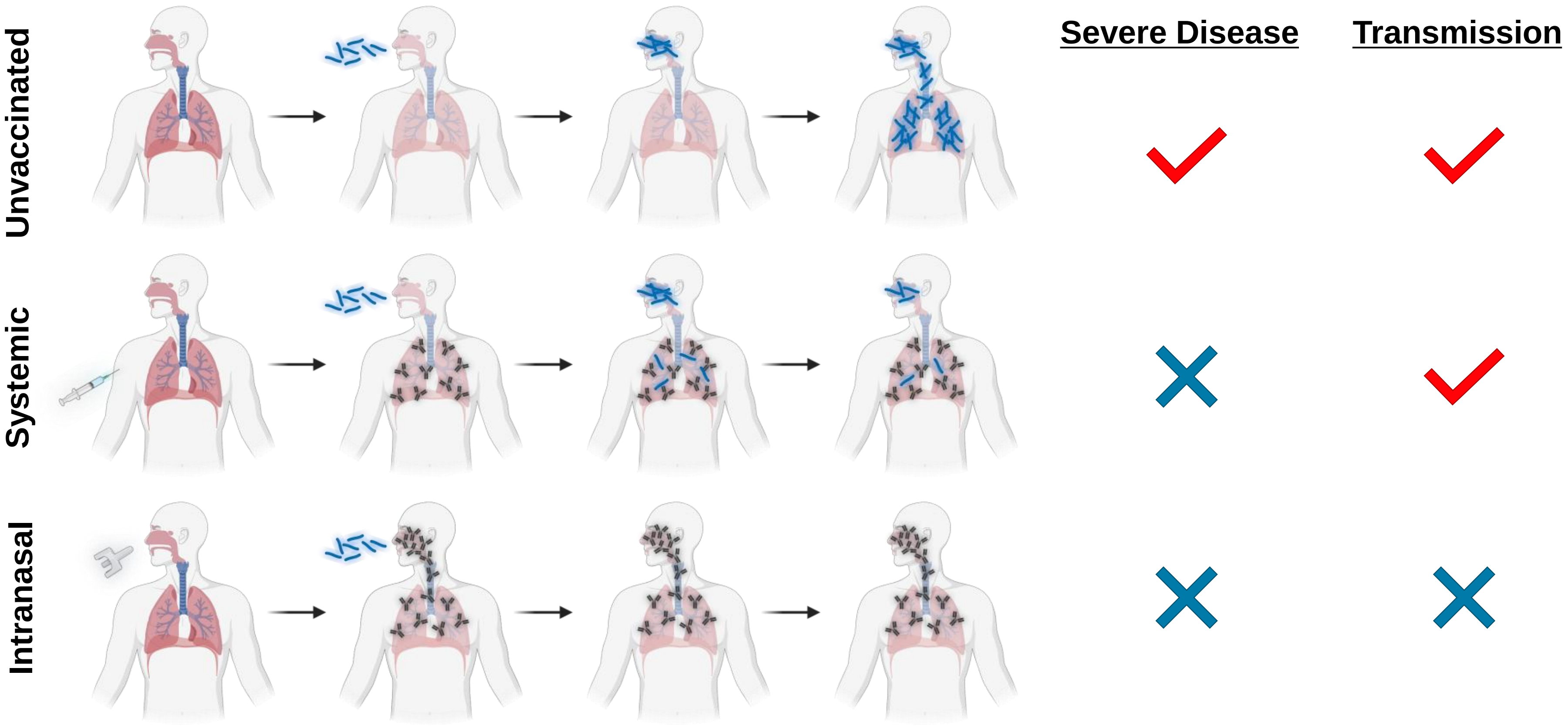
Stimulation of B and T cells in the draining lymph nodes

- *Produced IgG can enter the bloodstream, conveying systemic immunity*



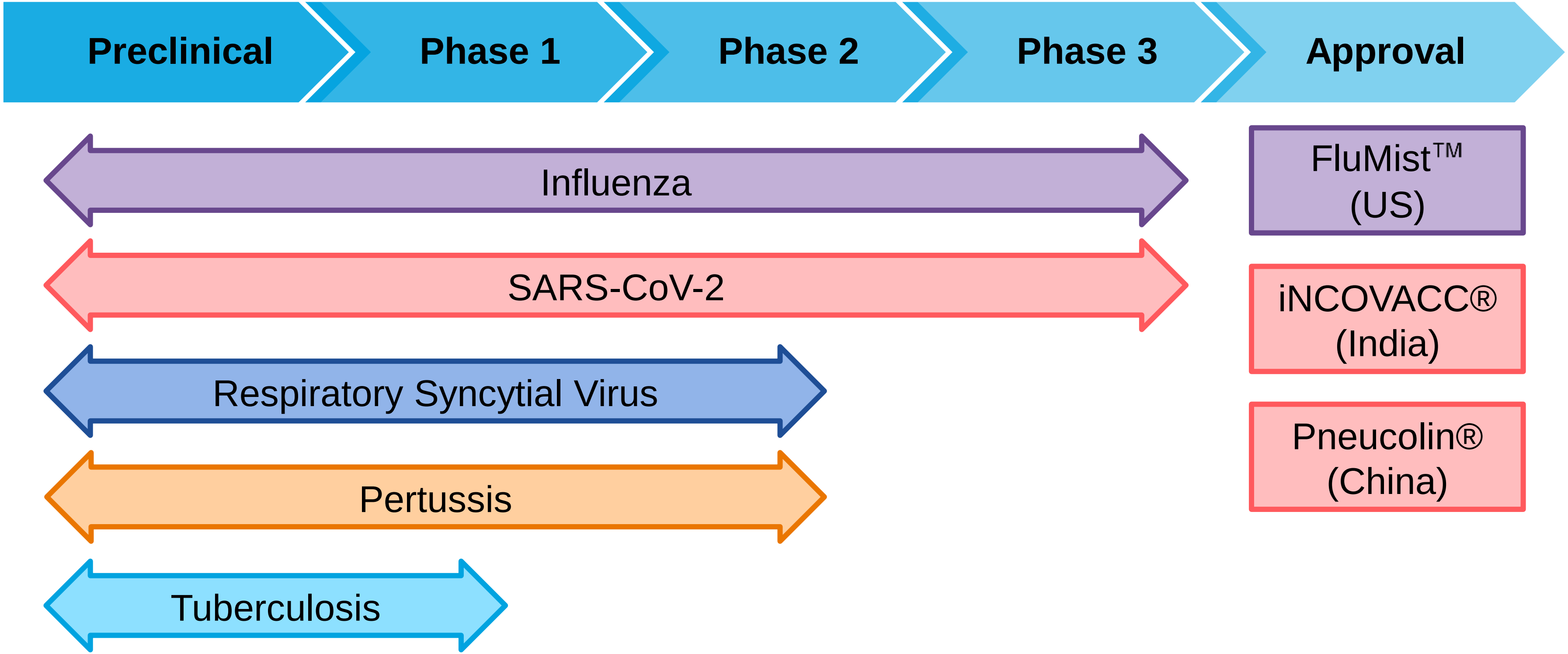
Lin et al. *hLife*. **2024.** Zhang et al. *Mol. Biomed.* **2025.**

Vaccination against Respiratory Infectious Disease



Adapted from Garcia-Sastre. *Curr. Res. Immunol.* **2022.** Figure made with BioRender.

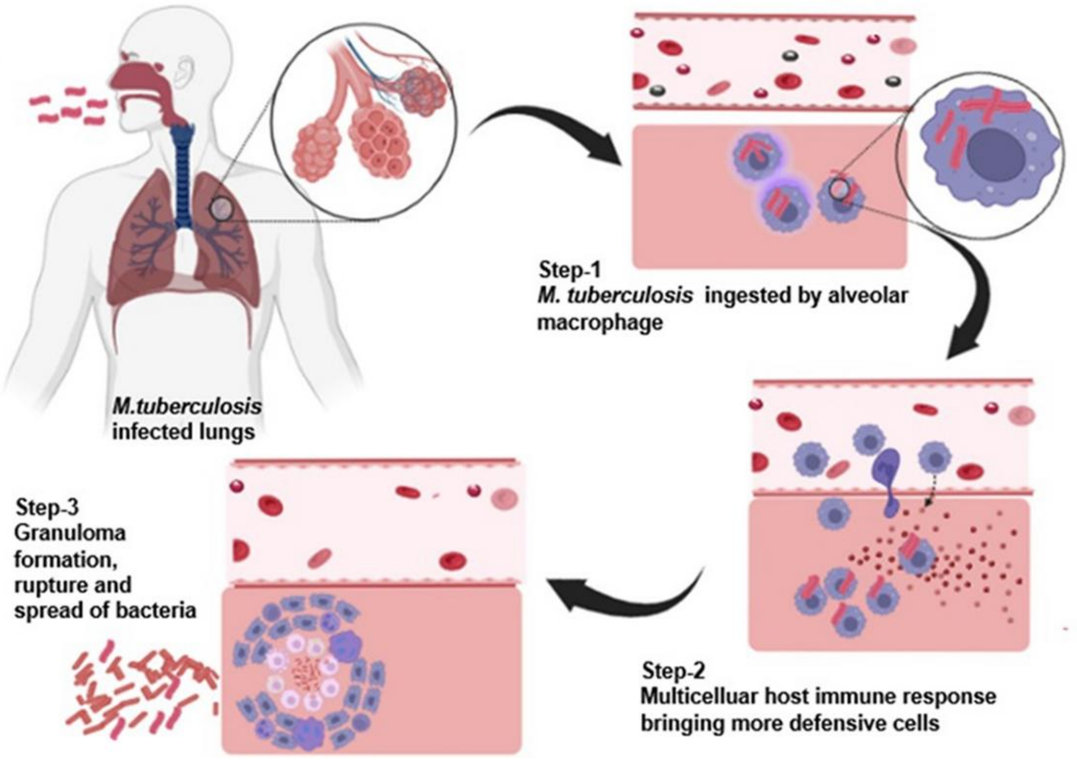
Intranasal Vaccine Landscape



Alu et al. *eBiomedicine*. **2022.** Bai et al. *ACS Nano*. **2024.**

Tuberculosis (TB)

- Results from infection with *Mycobacterium tuberculosis* (*Mtb*)
- World's leading cause of death from a single infectious disease
- 1.25 million deaths in 2023
- High prevalence in resource-limited countries



Nair et al. *J. Nanobiotechnol.* **2023.** Global tuberculosis report. WHO, **2024.**

Tuberculosis Vaccination

Bacille Calmette-Guérin (BCG) vaccine

- Only licensed and widely used vaccine for TB
- Global use, with limited use in US
- Protects children from disseminated TB and TB meningitis

Limitations to BCG vaccine

- Less effective in preventing pulmonary TB in adults

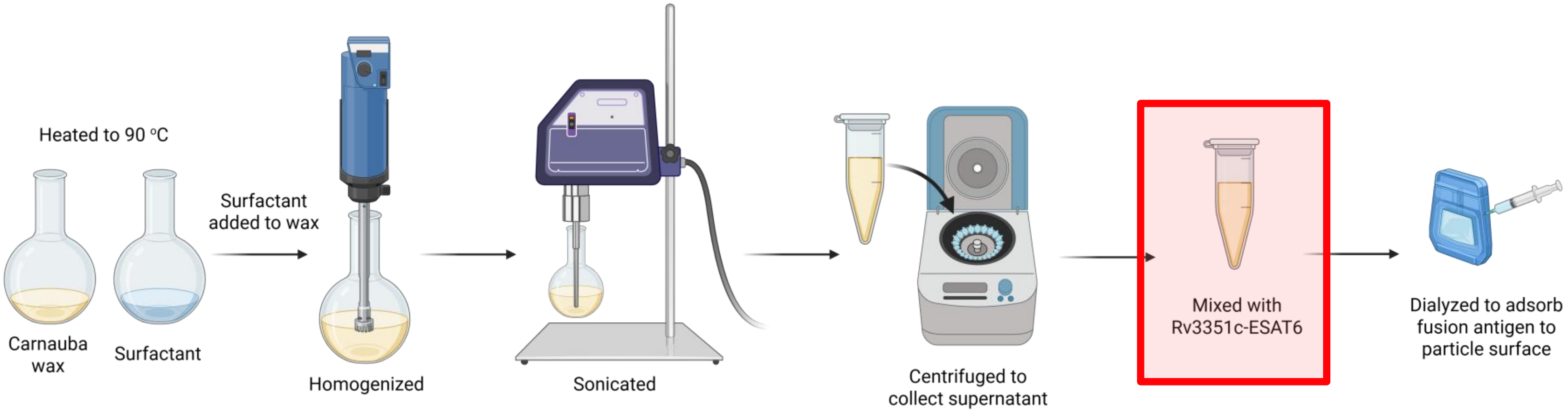
Program Goal: to combine the benefits of the BCG vaccine with a nanovaccine booster to enhance and extend immunity against *Mtb*

Nanovaccine Components

Mtb Antigens	Nanoparticle Vehicle	Adjuvants	Vaccine Matrix
Heparin-binding hemagglutinin adhesin (HBHA) *Extrapulmonary dissemination*	**Carnauba Wax** *Antigen on surface* *Faster release*	**CpG oligodeoxynucleotides (CpG 2395)** *TLR-9 stimulant*	**Dilute saline** *Control matrix*
Fusion antigen: **Rv3351c** *Lipid raft formation* **Early Secretory Antigenic Target 6 (ESAT6)** *Pore-forming activity*	**Poly(lactic-co-glycolic acid) (PLGA)** *Antigen on surface and embedded* *Slower release*	**Glucopyranosyl Lipid A (GLA-LS)** *TLR-4 agonist*	**Vaccine self-assembling immune matrix (VacSIM®)** *Forms hydrogel depot at site of administration*

Wax Nanoparticles

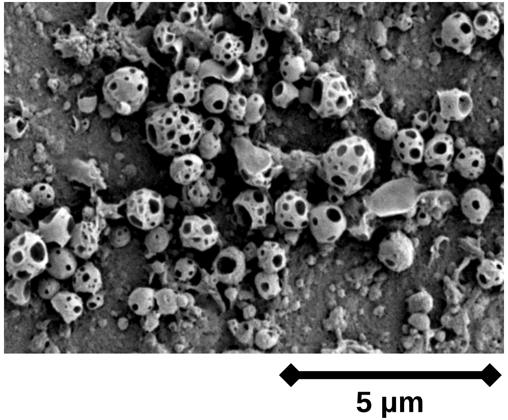
Nanoparticles prepared via hot homogenization technique with surfactant



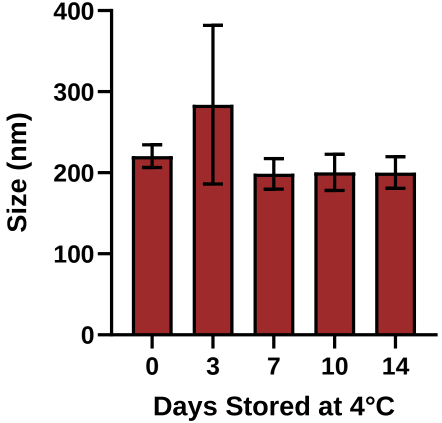
Schematic made with BioRender.

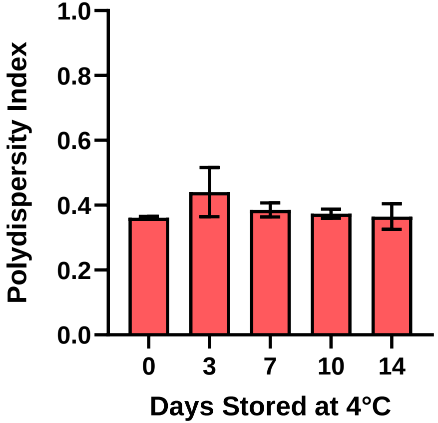
Wax Nanoparticles

Rv3351c-ESAT6



200-400 nm



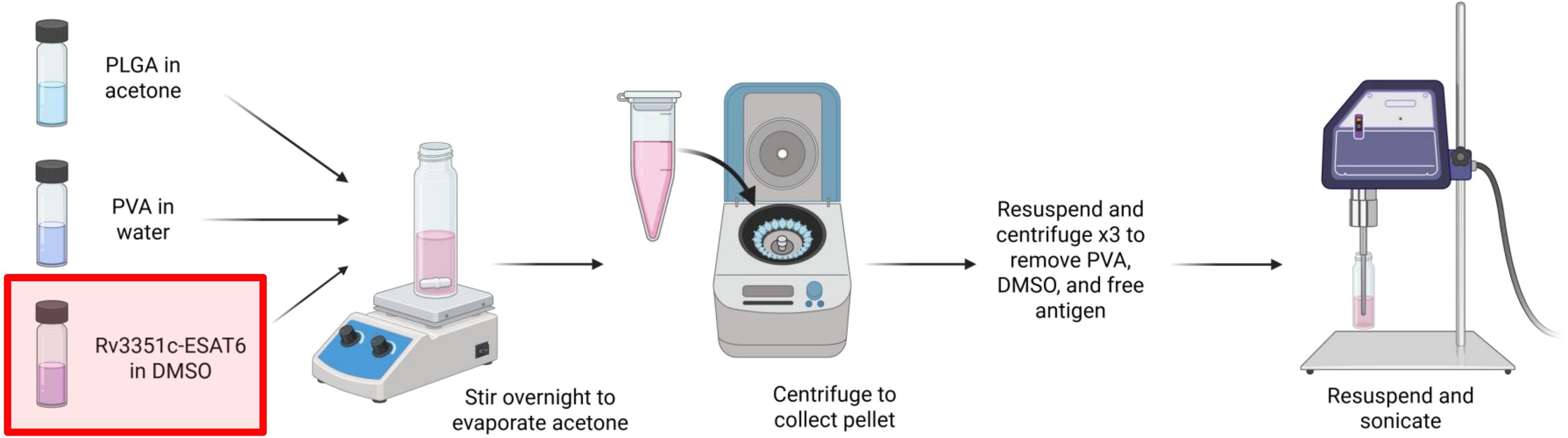


Particles maintained size and polydispersity after 14 days of storage at 4 ˚C

n ≥ 3

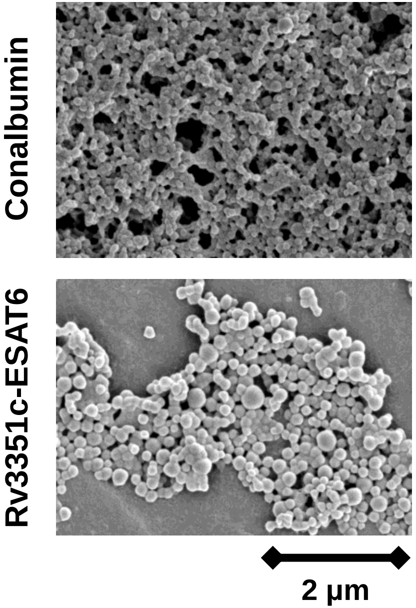
PLGA Nanoparticles

Nanoparticles prepared via solvent displacement technique

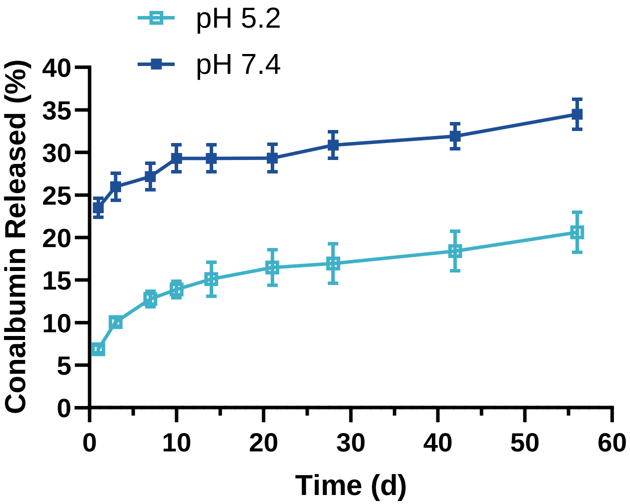


Schematic made with BioRender.

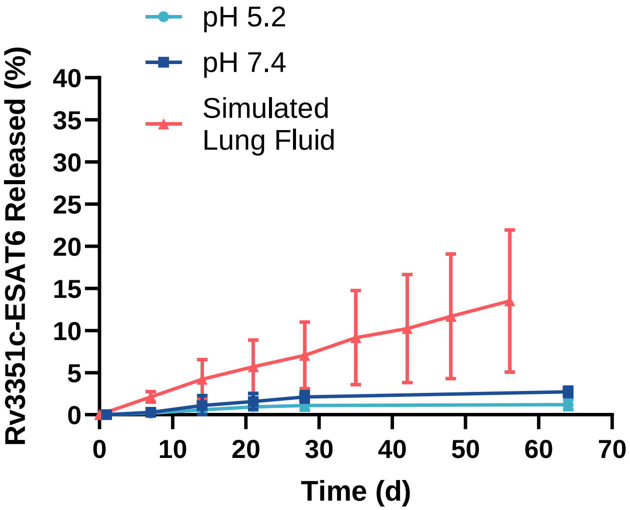
PLGA Nanoparticles



200-400 nm



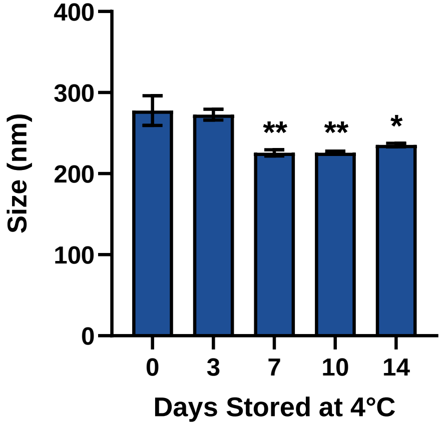
EE = 89.7 ± 0.6%
Release ≥ 8 weeks

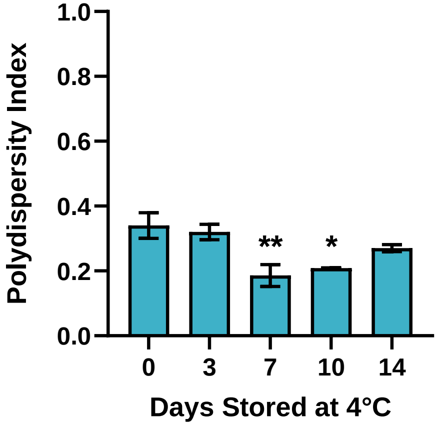


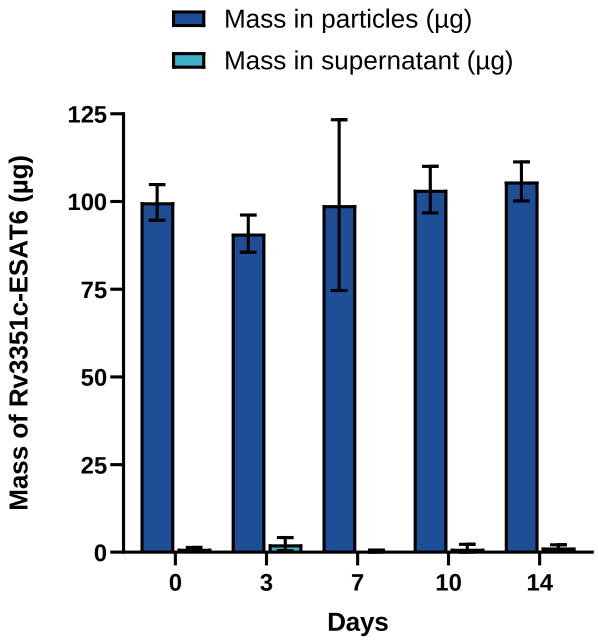
EE = 82.2 ± 3.5%
Slow release in vitro likely due to hydrophobicity

n ≥ 3

PLGA Nanoparticles







Particles slightly decreased in size and polydispersity over 14 d storage at 4 ˚C

>97% antigen retained in particles after 14 d storage at 4 ˚C

n ≥ 3

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Nanovaccine Candidates

Rv3351c-ESAT6 Nanocarrier × Adjuvant/ Matrix × Route of Administration = 20

Rv3351c-ESAT6 Nanocarrier
- Wax
- PLGA

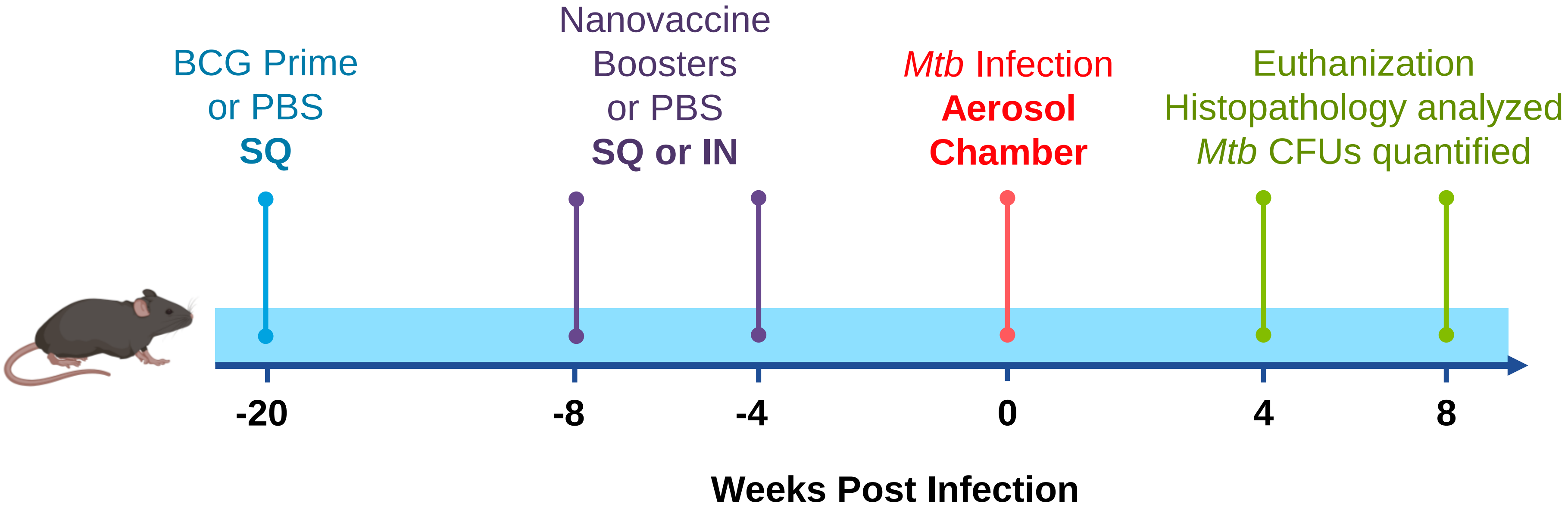
All vaccine formulations contained HBHA

Adjuvant/ Matrix
- VacSIM
- CpG
- GLA-LS
- VacSIM + CpG
- VacSIM + GLA-LS

Route of Administration
- Subcutaneous (SQ)
- Intranasal (IN)

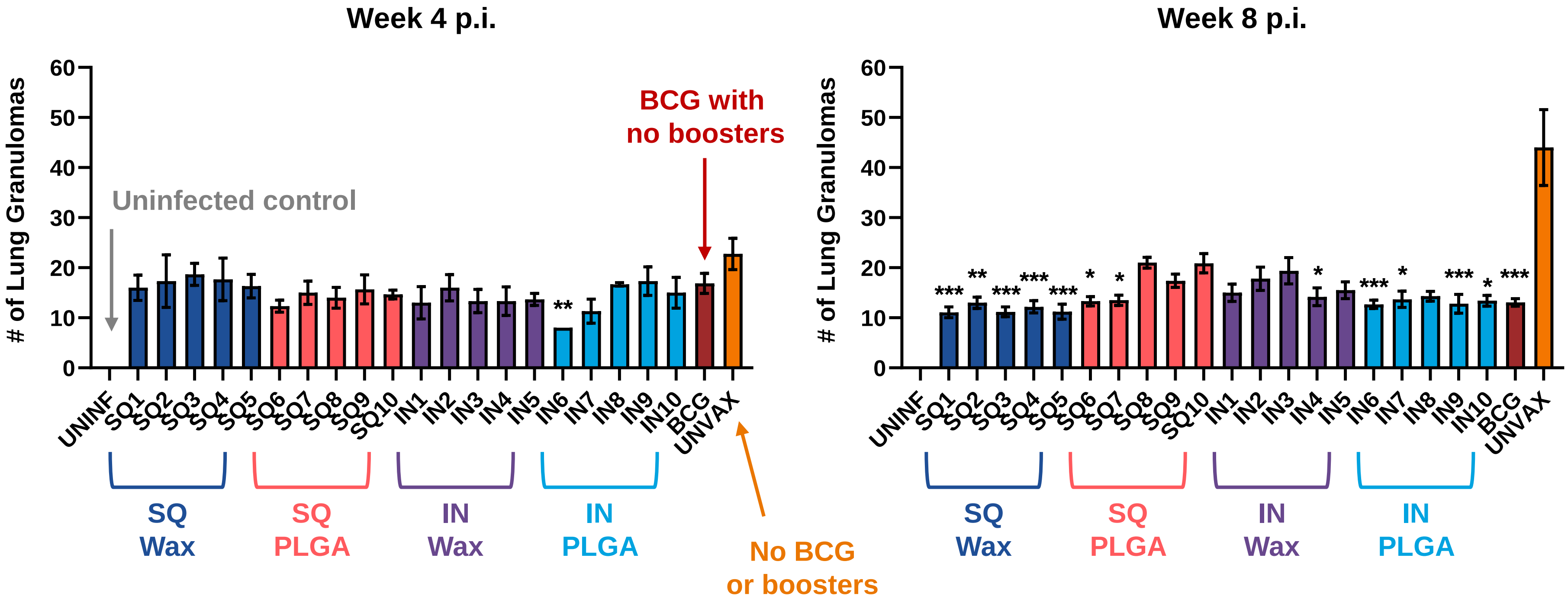
Controls:
1. Uninfected
2. BCG only
3. Unvaccinated

Preclinical Murine TB Challenge – Experiment Outline



Histopathology – Number of Granulomas

Kruskal-Wallis statistical test
All comparisons are to UNVAX
*p<0.05 **p<0.01 ***p<0.005

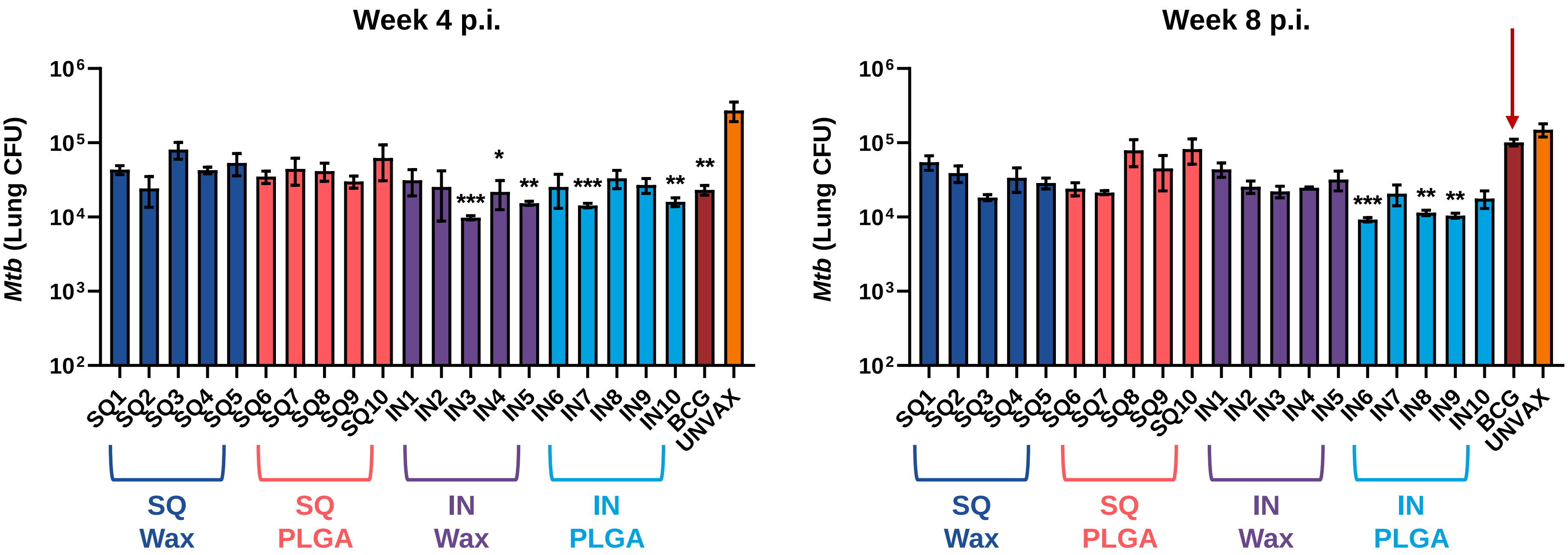


BCG + Booster protects similarly to BCG only in reducing granuloma formation

n ≥ 3

Bacterial Burden – Lungs

Kruskal-Wallis statistical test
All comparisons are to UNVAX
*p<0.05 **p<0.01 ***p<0.005



Intranasal PLGA formulations maintain reduced lung bacterial burden at 8 weeks

n ≥ 3

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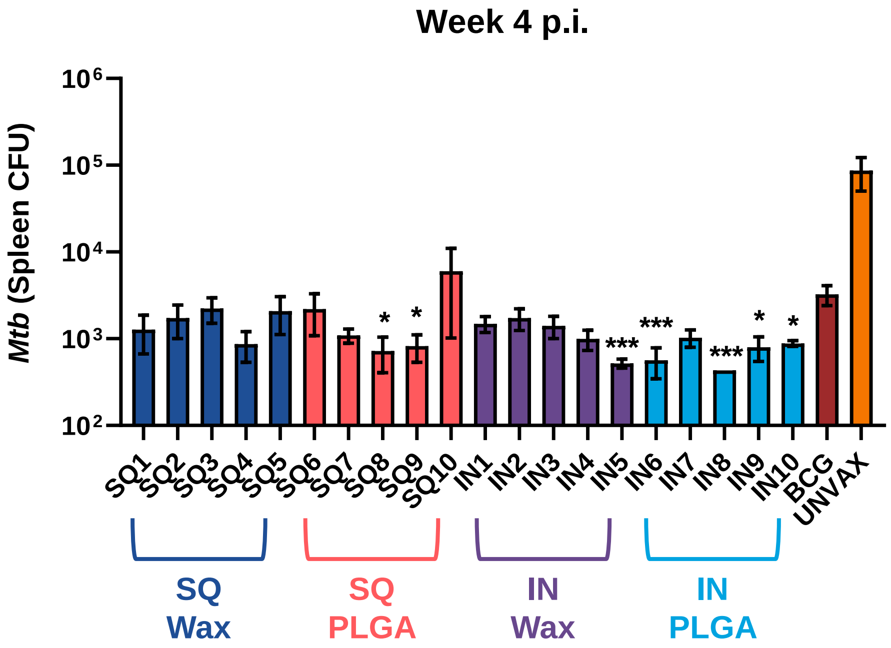
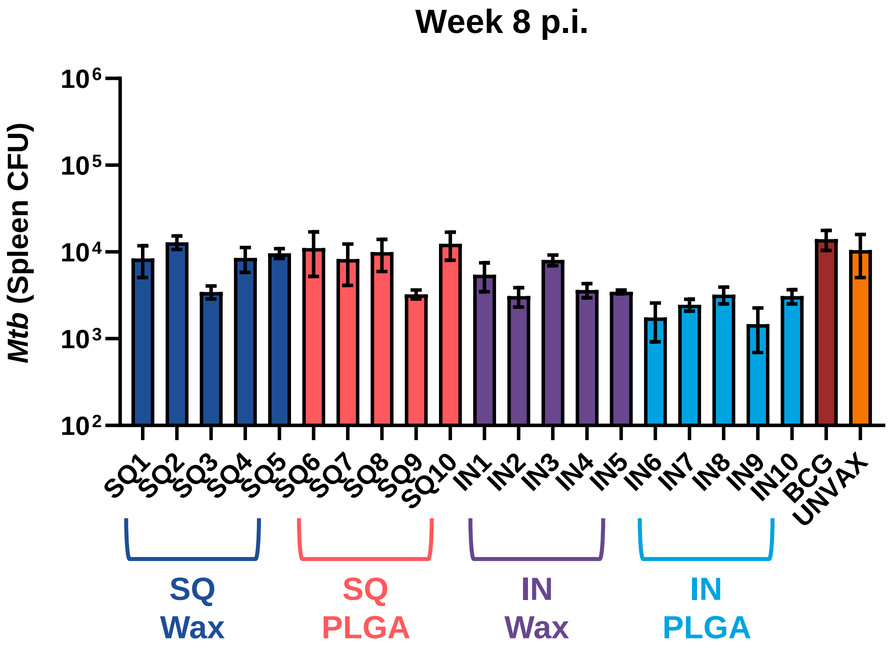
Bacterial Burden – Spleen

Kruskal-Wallis statistical test
All comparisons are to UNVAX
*p<0.05 **p<0.01 ***p<0.005

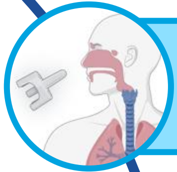




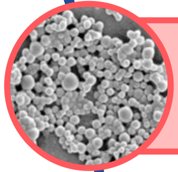
Intranasal PLGA formulations reduced spleen bacterial burden at 4 weeks

n ≥ 3

Conclusions and Next Steps

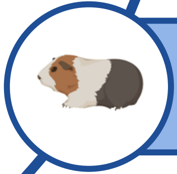


Intranasal vaccination generates mucosal immunity, reducing both the severity of infection and the likelihood of transmission.



An intranasal TB nanovaccine was successfully formulated to control the release of a novel fusion antigen.

Intranasal vaccines with extended antigen release outperformed those delivered subcutaneously or with rapid antigen release in mice.



The top intranasal PLGA formulations will be evaluated for stability and safety in mice as well as safety and efficacy in guinea pigs.

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For additional questions, please contact:
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