Abstract # MHSRS-26-2218

# Novel Human Cholera Toxin (CT) Vaccine to Prevent and Treat MRSA Staphylococcal Front-Line Combat Wound Infections

A non-toxic CTA2/B chimeric subunit platform inducing mucosal and tissue-resident immunity against *Staphylococcus aureus*

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PENTAMER BIO

**A needle-free, cold-chain-independent vaccine that clears the nasal reservoir driving ~80% of endogenous *S. aureus* surgical site infections — validated in large-animal One-Health challenge trials.**

## 01 UNMET NEED

CTA2/B ACTS HERE

**Nasal colonization**
the reservoir — carried, not caught

▼

**Wound contamination**
point of injury, austere field

▼

**Bacteremia · osteomyelitis**
bone and hardware involvement

▼

**Sepsis**
limb loss, mortality, evacuation burden

IM VACCINES ACT HERE

**Fig 1.** Endogenous seeding pathway. Intramuscular vaccination reaches only the systemic phase; CTA2/B targets the barrier where colonization and contamination occur.

- *S. aureus* remains a primary driver of wound sepsis and chronic osteomyelitis in combat-related trauma.
- Combat wounds are frequently polymicrobial with MRSA predominance; systemic antibiotics alone increasingly fail.
- Cold-chain logistics for antibiotics and conventional vaccines are unreliable in austere settings.
- No licensed *S. aureus* vaccine exists for any population, military or civilian.

| Prior approach | Immunity targeted | Outcome |
|---|---|---|
| Capsular polysaccharide, IM | Systemic IgG | Phase III failure |
| Single-antigen subunit, IM | Systemic IgG | Halted — no clinical benefit |
| Topical antibiotic decolonization | None — chemical clearance | Recolonization; resistance pressure |
| **CTA2/B chimera, IN / SQ / patch** | **Mucosal IgA + barrier T cells** | **This work** |

**~80%** of *S. aureus* SSIs originate from the patient's own nasal colonization[1]

### THE GAP

- Every prior *S. aureus* vaccine to reach Phase III has failed — all targeted systemic humoral immunity alone.
- IM vaccination generates circulating IgG but **does not establish mucosal IgA or tissue-resident memory T cells ($T_{RM}$)** at the nasal and skin interfaces.
- Protecting the warfighter requires immunity **at the barrier**, not only in the blood.

### WHAT PCC REQUIRES

| | |
|---|---|
| **Needle-free**<br>no sharps, no injector | **Thermostable**<br>no cold chain |
| **Self / buddy dose**<br>no clinician required | **Point of injury**<br>effective in the field |

## 02 PLATFORM & METHODS

- Recombinant subunit fusion of *S. aureus* adhesins **IsdA** and **ClfA** to non-toxic derivatives of cholera toxin, **CTA2/B**.
- CTA2/B is **dual-function**: mucosal adjuvant *and* delivery vehicle. The pentameric CTB ring binds GM1 ganglioside on epithelial and dendritic cells, driving uptake without CT enterotoxicity.
- Patented platform; bivalent (IsdA+ClfA-CTA2/B) and trivalent (**SA3-CTA2/B**) candidates.

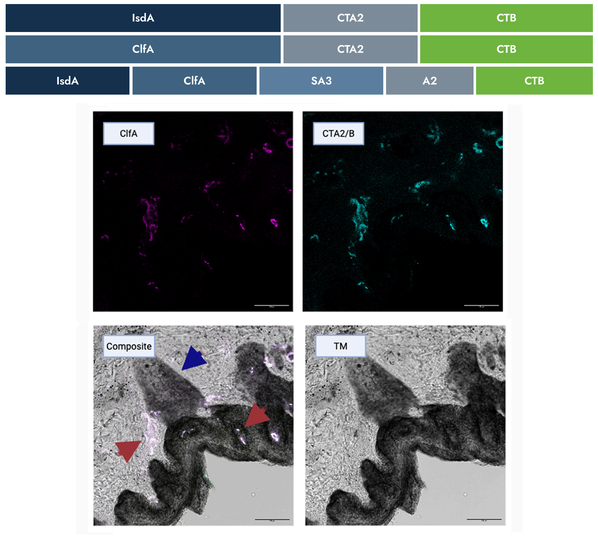


**Fig 2.** Domain maps of the bivalent and trivalent chimeras (top). Expression from pLR001/pLR003 yields the adhesin fused to CTA2, which assembles with five CTB subunits into the GM1-binding A2/$B_5$ pentamer (bottom; IsdA construct shown, ClfA equivalent).

### MANUFACTURE

*E. coli* aerobic culture ▸ Expression & folding ▸ Glycan affinity chromatography ▸ Analysis & concentration

### STUDY DESIGN

| Aim | Model | Route | Readouts |
|---|---|---|---|
| **Immunogenicity** | BALB/c mouse | IN · SQ · IM | Serum IgG, mucosal IgA, T-cell subsets |
| **$T_{RM}$ induction** | BALB/c mouse | IN vs. SQ vs. IM | $CD69^+$, $CD103^+$ tissue-resident memory |
| **One-Health efficacy** | Bovine challenge trial | SQ, 400–600 µg prime + boost | Antigen-specific IgG in milk and serum, SCC, shedding, temperature, milk yield |
| **Trivalent scaling** | BALB/c mouse | IN · SQ | Cross-reactivity vs. diverse human isolates incl. MRSA |

Comparators: adjuvant-only (CTA2/B) and naïve/PBS controls. Significance at $p < 0.05$; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

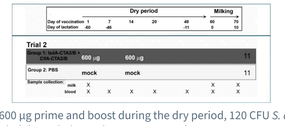


**Fig 3.** One-Health trial design: 600 µg prime and boost during the dry period, 120 CFU *S. aureus* intramammary challenge, milk and blood sampled through day 30 (n = 11 per group).

## 03 RESULTS

### MUCOSAL IMMUNITY, MOUSE

Intranasal IsdA-CTA2/B induced significantly higher antigen-specific **mucosal IgA** than adjuvant-only controls, alongside serum IgG, with a **Th2-type** profile — IL-4 production and IgG1 isotype switching[2]. Immune serum blocked *S. aureus* adherence to human epithelial cells.

**Antigen-specific IgG after SQ vaccination**

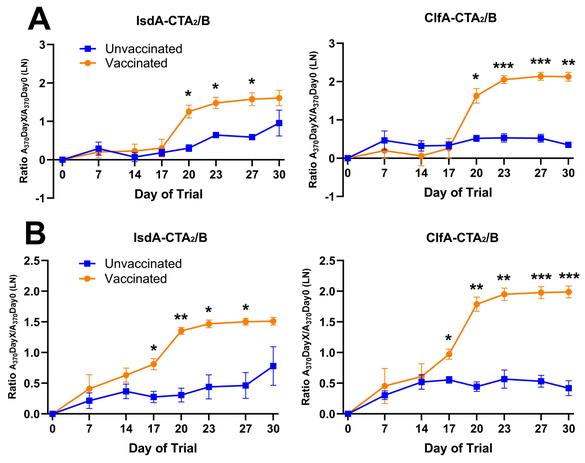


**Fig 4.** IsdA- and ClfA-specific IgG in milk **(A)** and serum **(B)** through challenge. Vaccinated animals diverge from unvaccinated controls from day 17–20 and hold a 2–4× ratio to day 30.

**Inflammatory cell count**

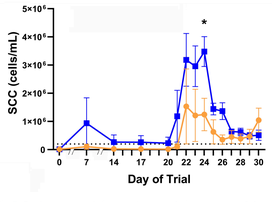


**Fig 5.** Post-challenge inflammatory cell influx peaked ~2.8× lower in vaccinated animals (day 24, *).

***S. aureus* shedding**

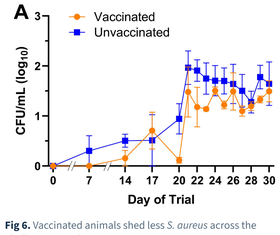


**Fig 6.** Vaccinated animals shed less *S. aureus* across the challenge period.

**Safety: no vaccine-attributable fever**

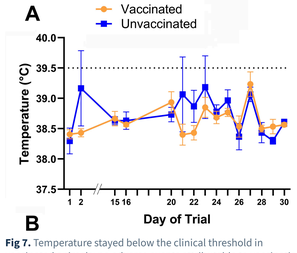


**Fig 7.** Temperature stayed below the clinical threshold in vaccinated animals; no adverse events attributable to vaccination.

### NO ENTEROTOXICITY

CTA2/B retains adjuvanticity without the ADP-ribosyltransferase activity of holotoxin CT.

### TRIVALENT SCALING

SA3-CTA2/B is being scaled to broaden coverage across diverse human isolates including MRSA. **In progress.**

## 04 WARFIGHTER APPLICATION

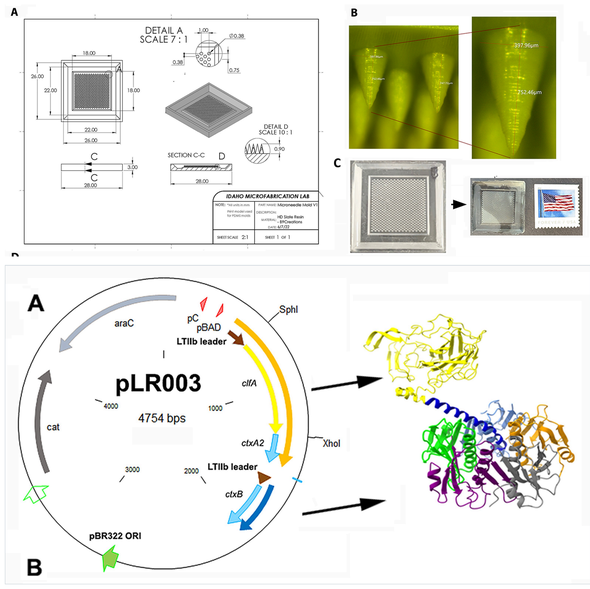


**Fig 8.** Needle-free format. *Left:* PDMS-moulded sucrose/CMC dissolvable microneedle array (750 × 400 µm needles) carrying ClfA-CTA2/B — postage-stamp footprint, no sharps, no cold chain. *Right:* chimera delivered into *ex vivo* skin, with ClfA and CTA2/B co-localizing in the dermal layer where antigen-presenting cells reside.

| | |
|---|---|
| PRE-DEPLOYMENT | Decolonize nasal carriage in units at high trauma risk |
| POINT OF INJURY · ROLE 1 | Intranasal or patch dose by medic, buddy, or self |
| PROLONGED CASUALTY CARE | Less reliance on cold-chain antibiotics through 72 h+ evacuation delays |
| ROLE 2 / 3 | Lower rates of wound sepsis, osteomyelitis, and MDR treatment failure |

## 05 CONCLUSIONS

- CTA2/B chimeras induce mucosal IgA and antigen-specific antibody at the barrier — immunity IM vaccination does not deliver.
- Large-animal One-Health challenge data support safety and translatability of the bivalent construct.
- A dissolvable microneedle patch makes the platform administrable at the point of injury without sharps or cold chain, and immunity is antigen-directed — the isolate's resistance profile is irrelevant to the mechanism.

## 06 NEXT STEPS

**VACCINATE** IN / patch ▸ **COLONIZE** nasal carriage ▸ **WOUND CHALLENGE** does the reservoir seed it?

- Run the sequential colonization-to-wound challenge above with a DoD partner — standard models inoculate exogenously and never test the endogenous premise. Lead candidates: rat open femur fracture with muscle crush and MRSA contamination; contaminated-suture incisional SSI.
- Complete trivalent SA3-CTA2/B breadth and $T_{RM}$ ($CD69^+/CD103^+$) route-comparison studies.
- Advance toward GMP manufacture and human clinical licensure.

### SEEKING PARTNERS

Seeking **government program sponsors and prime contract partners** to move this platform toward an IND: co-authored grant submissions (CDMRP, DTRA, JPC-2, SBIR/STTR Direct-to-Phase II), DoD-relevant traumatic wound challenge models, and OTA or Phase III contract vehicles for PCC delivery formats.

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**COI:** B. Allinson is an officer and equity holder of Pentamer Biologics LLC.

### DISCLAIMER

Views are those of Pentamer Biologics LLC and do not necessarily reflect the policy or position of the USDA, Innovate Alabama, Station 41, Southern Research, or the Department of Defense. The platform uses patented CTA2/B technology for preventative and therapeutic use. This research is preclinical; test articles (IsdA+ClfA-CTA2/B, SA3-CTA2/B) are non-GMP grade. Safety and efficacy were validated in bovine clinical trials; mouse-model studies are non-GLP and intended to de-risk the technology for human licensure.

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