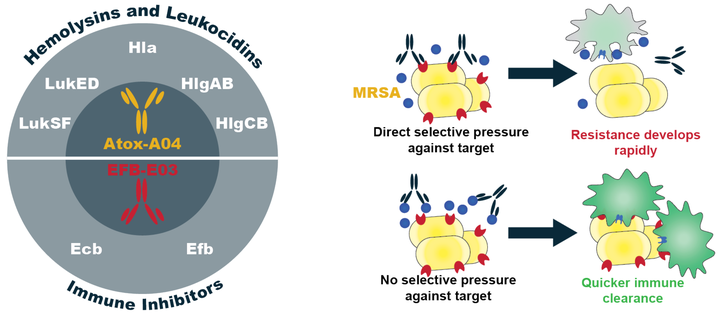
Evaluation of Field Deployable Broadly Neutralizing Monoclonal Antibodies to Counter MRSA Infections in Combat Wounds

Authors: Lucas Brown PhD[1,2], Mackenzie Ryan PhD[1,2], Jacob Glanville PhD[4], David Tsao MSc[4], Jerry Sadoff MD[4], Nicholas Bayless PhD[4], Michael Madejczyk PhD[5], Frédéric Poly PhD[3], LT Sara Tuck PhD[1,3], Zachary Rivas PhD[1,2,*], Yoann Le Breton PhD[1,3,*]

1. Combat Wound Infections Division, Naval Medical Research Command, Silver Spring, MD, USA 2. The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. Bethesda, MD, USA 3. Military Relevant Bacterial Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA 4. Centivax, San Francisco, CA, USA 5. Experimental Therapeutics Branch, Walter Reed Army Institute of Research, Silver Spring, MD, USA

Background and Aims



Multi-drug resistant (MDR) bacterial infections pose a potent problem for warfighter injuries during prolonged combat casualty care in battlefield environments. To alleviate the burden caused by these pathogens, we evaluated two humanized broadly neutralizing antibodies developed by Centivax (Atox-A04, EFB-E03) which **each target multiple secreted toxins** expressed by methicillin-resistant *Staphylococcus aureus* (MRSA). We hypothesize that, by **targeting secreted toxins instead of surface proteins**, we will minimize the emergence of resistance mechanisms while facilitating quicker clearance by the immune system. We further hypothesize that targeting multiple toxins with a single antibody will provide a **synergistic benefit to host survival and bacterial clearance**, as well as prove **effective against a wide diversity of strains**.

Aims

- Develop *in vitro* assays to screen Atox-A04 and EFB-E03 efficacy against a diverse panel of MRSA strains
- Demonstrate the feasibility of using broadly neutralizing antibodies as a prophylactic against wound infection in *in vivo* murine and porcine wound and sepsis models

EFB-E03 Opsonophagocytic Killing Assay

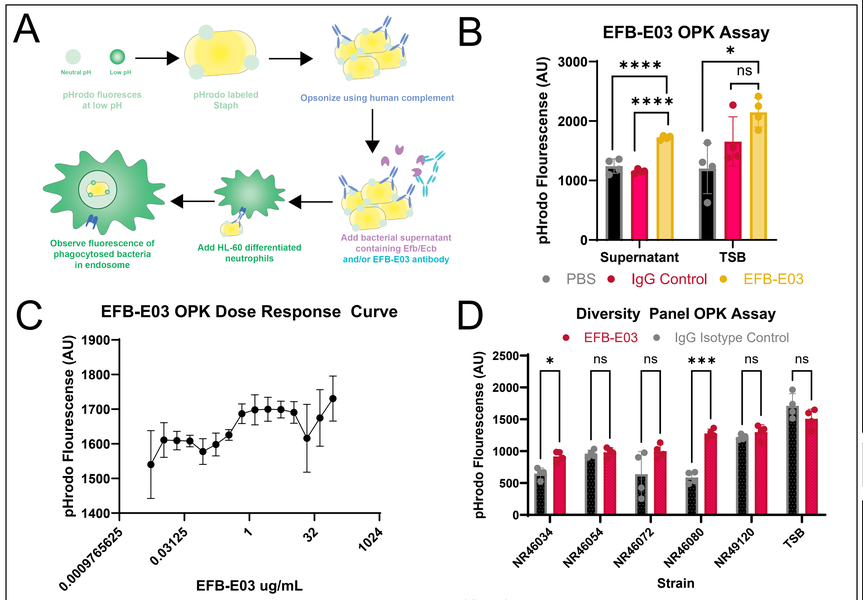


Figure 1: EFB-E03 improves opsonophagocytic killing (OPK) in neutrophil-like cells against a diverse panel of MRSA strains. **(A)** Diagram of the custom OPK assay using pHrodo-labeled MRSA as a reporter for phagocytosis by HL-60 cells differentiated towards a neutrophil-like state. **(B)** Addition of 1 µg/mL EFB-E03 significantly improved differentiated HL-60 phagocytic activity in the presence of supernatant from *S. aureus* JE2 bacteria. **(C)** Dose response curve of EFB-E03 OPK improvement using supernatant from *S. aureus* JE2 bacteria. **(D)** Initial screening of EFB-E03 antibody effectiveness against a panel of MRSA strains. Error bars represent the standard deviation of n=4 replicates. Significance is calculated using a one-way ANOVA **(B)** or Student's t-test with Welch's correction **(C, D)**: *P<0.05, **P<0.01, ***P< 0.001, ****P<0.0001.

Atox-A04 Cytoprotective Assay

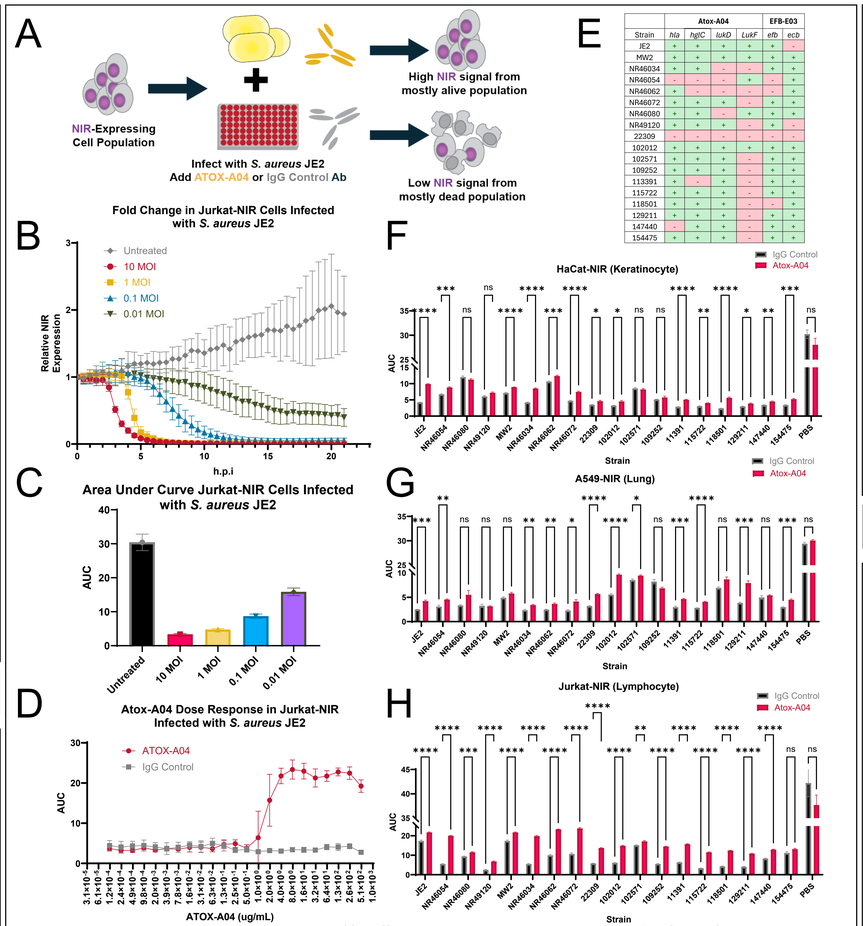
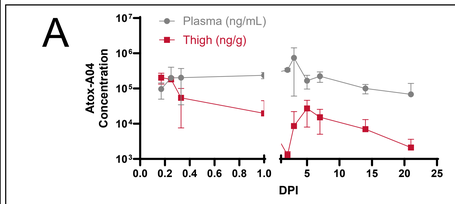


Figure 2: Atox-A04 demonstrates 81% efficacy against a diverse panel of MRSA strains in a cytoprotective assay. **(A)** Diagram of the cytoprotective assay using fluorescence as a proxy for cell health. **(B)** Determination of ideal *S. aureus* JE2 infection MOI. NIR signal for each well is normalized to the first reading. **(C)** Area under the curve of each experimental group in **(B)** using GraphPad Prism 11. **(D)** Dose response curve of Atox-A04 in Jurkat-NIR cells infected with *S. aureus* JE2 for 24 hours. EC50 was determined to be 8µg/mL. **(E)** Genetic screen of MRSA diversity panel for ATOX-A04 gene targets via colony PCR. **(F-G)** Screening of Atox-A04 cytoprotective efficacy against an 18-member panel of MRSA strains in keratinocytes **(F)** lung **(G)** and lymphocyte **(H)** cell lines using 8µg/mL of Atox-A04 or IgG isotype control antibody. All experiments were measured every 45 minutes for 24 hours. Error bars represent the standard deviation of n=8 replicates. Significance is calculated using Student's t-test with Welch's correction: *P<0.05, **P<0.01, ***P< 0.001, ****P<0.0001.

Intramuscular Injection Pharmacokinetics



Tissue	Plasma	Thigh
HL_Lambda_z(day)	8.22821	4.90768
Tmax(day)	3	0.083
Cmax(ng/mL)	743667	215000
AUClast(day*ng/mL)	3835349	265595
Cl_F_obs(mL/day)	0.01828	0.30295
AUCINF_pred(day*ng/mL)	4595190	281668
AUC_%Extrap_pred({%})	16.5356	5.70621
Cl_F_pred(mL/day)	0.0185	0.30177

Figure 3: Pharmacokinetic study of intramuscular injection of Atox-A04 antibody in mice. **(A)** Female 6-week old Balb/c were injected intramuscularly with 5mg/kg ATOX-A04 antibody and sacrificed at various time points. Thigh muscle and plasma were collected and analyzed via human IgG ELISA (n=3). Error Bars represent standard deviation. **(B)** Pharmacokinetic parameters extracted from data modeling.

Mouse Sepsis Model

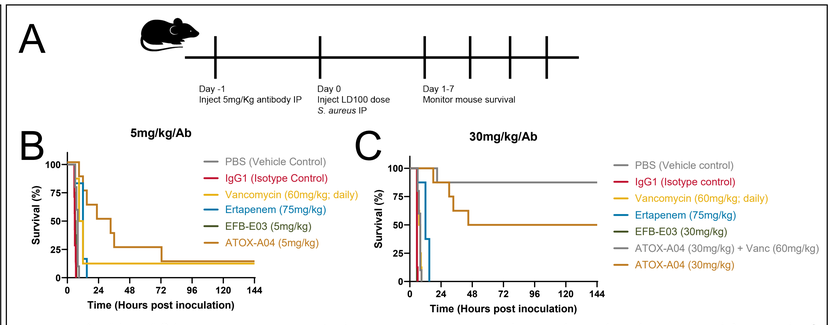


Figure 4: Atox-A04 improves survival alone and in combination with antibiotics in an IP model of mouse sepsis. **(A)** 6-week-old female Balb/c mice (n=8) were prophylactically injected in the IP cavity with Atox-A04, EFB-E03 or human IgG control 24 hours before being injected with a LD100 dose of *S. aureus* JE2. Mice were monitored for 7 days, and surviving mice were sacrificed at the end of the experiment. Survival curves for mice dosed with at 5mg/kg **(B)** or 30mg/kg **(C)** of antibody. While mice treated with EFB-E03 died within 24 hours along with the other control groups, mice treated with Atox-A04 survived significantly longer than untreated, EFB-E03 or vancomycin alone.

Mouse Dorsal Wound Infection Model

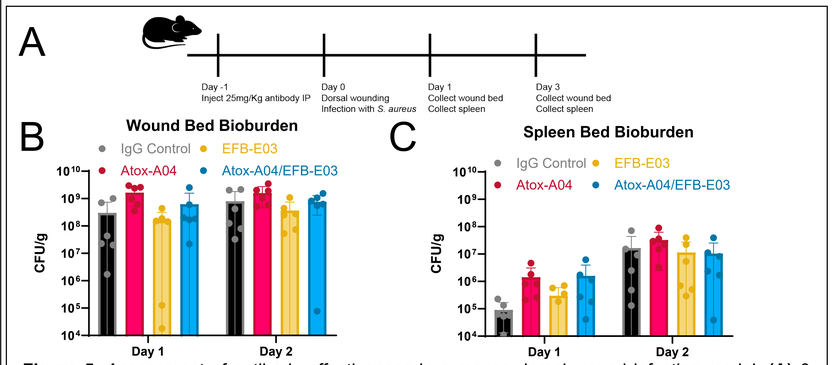


Figure 5: Assessment of antibody effectiveness in a mouse dorsal wound infection model. **(A)** 6-week-old female Balb/c mice (n=6) were injected in the IP cavity with 25mg/kg of Atox-A04, EFB-E-3, a cocktail of both, or human IgG control 24 hours before wounding and infection with JE2 MRSA. Wound beds **(B)** and spleens **(C)** were taken 24 and 72 hours after infection and analyzed for bacterial bioburden. CFUs were normalized to the mass of tissue homogenized. Error bars represent the standard deviation of 6 replicates. Significance is calculated using an ordinary one-way ANOVA test comparing the means of the three experimental groups to the IgG control group, with Dunnett's multiple comparisons test: *P<0.05, **P<0.01, ***P< 0.001, ****P<0.0001.

Conclusions and Next Steps

- We developed *in vitro* assays for assessing the efficacy of both Atox-A04 and Efb-E03 antibodies against a diverse panel of MRSA strains
- Atox-A04 showed effectiveness against 81% of strain-cell line combinations
- Atox-A04 increased the survival rate of mice in a sepsis model, but did not significantly reduce MRSA bioburden in a mouse dorsal wound model
- Both antibodies will be evaluated individually and combinatorially in a mouse pneumonia model and porcine dorsal wound model.

Partners/Collaborators

Defense Health Agency ®

Walter Reed Army Institute of Research
Defense Health • Global Health

centivax

Source of support: This work was supported by the MID Program (MI250071).
The animal study protocol was reviewed and approved by the NMRC IACUC in compliance with all applicable Federal regulations governing the protection of animals in research.