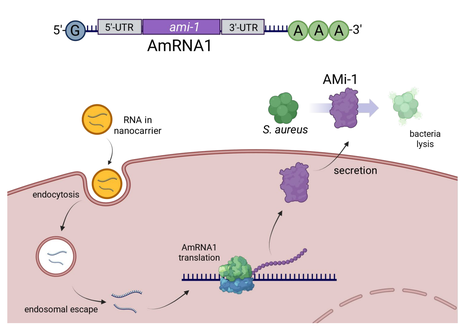
Engineering Antimicrobial RNA Therapeutics to Combat *Staphylococcus aureus* in Human Cell Models of Combat Wound Infections

Mackenzie Ryan PhD[1,2,*], Zachary Rivas PhD[1,2*], G. Corley Bernstein PhD[1,2], Lucas Brown PhD[1,2], LT Sara Tuck PhD[1,3], Frédéric Poly PhD[3], Evelina Angov PhD[4,#], Yoann Le Breton PhD[1,3,#]

Walter Reed Army Institute of Research
Defense Health • Global Health

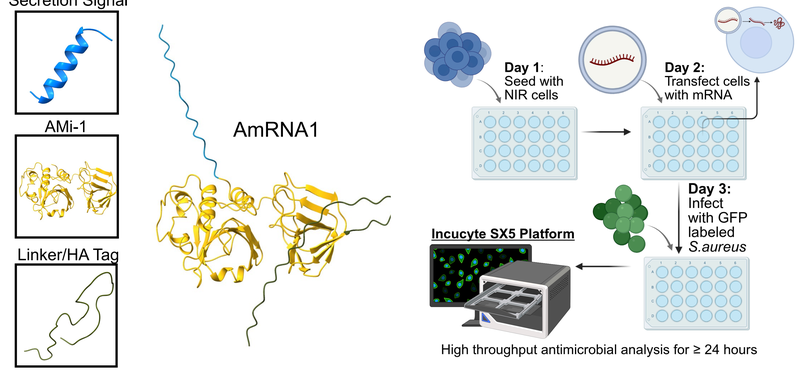
INTRODUCTION

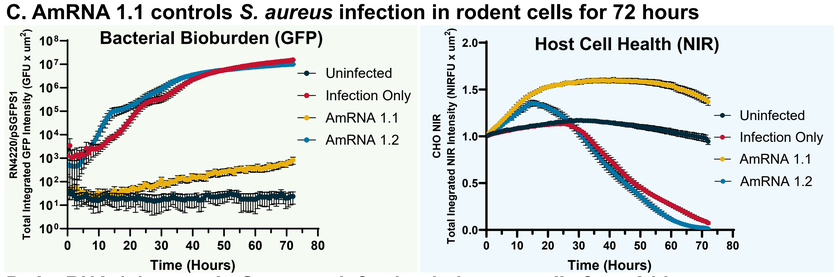
Introduction: The rise of multidrug-resistant (MDR) pathogens during recent global conflicts highlights the urgent need for innovative, novel antimicrobial development. To address this issue, we have established an R&D effort between Navy and Army labs to develop an antimicrobial mRNA-based approach against MDR pathogens responsible for combat wound infections: AmRNA (Antimicrobial RNA). An advantage of this approach is the modular, "building block"-like framework of the AmRNA constructs, which facilitates rapid design and optimization of new candidates.



Here, we present our efforts to generate AmRNA products optimized for human cell types associated with wound infection. We engineered modular AmRNA constructs with interchangeable non-coding sequence elements, enabling rapid iteration to the mRNA backbone to optimize antimicrobial activity against *Staphylococcus aureus*, a major MDR wound pathogen.

BACKGROUND





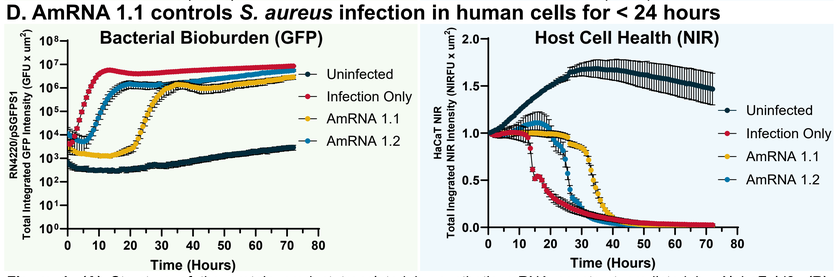


Figure 1. (A) Structure of the protein product translated by synthetic mRNA construct predicted by AlphaFold3. **(B)** Schematic of high throughput *in vitro* cell infection study workflow using Incucyte SX5 Live Cell imaging system. **(C)** AmRNA 1.1 controls *S. aureus* infection and protects rodent host cell health for over 72 hours in Chinese Hamster Ovary (CHO) cells, purchased from ATCC. **(D)** AmRNA 1.1 controls *S. aureus* infection for ~20 hours in a human keratinocyte (HaCaT) cell line, purchased from AddexBio.

AmRNA OPTIMIZATION IN HUMAN CELL LINES

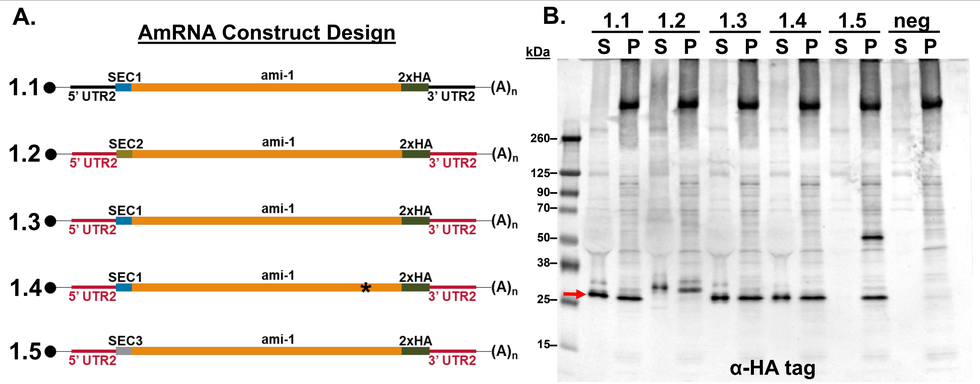
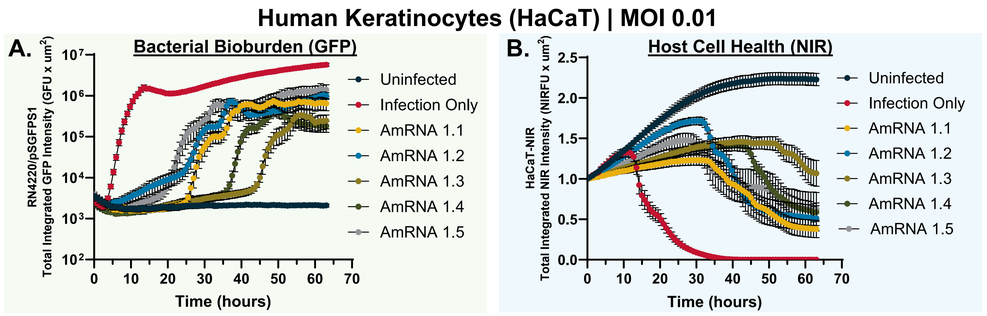
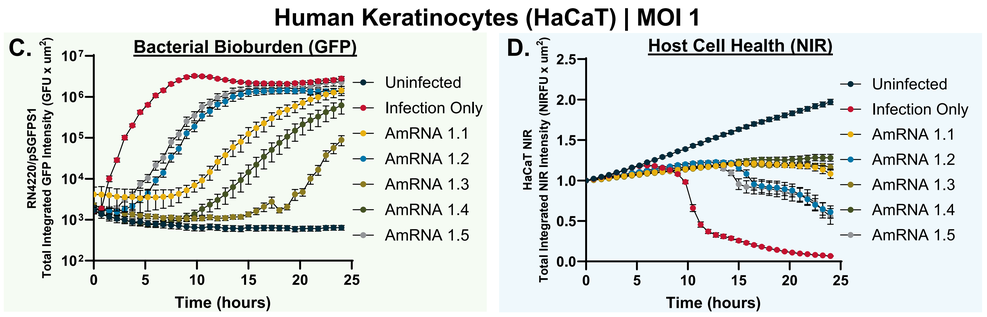


Figure 2. Optimized AmRNA 1 for improved expression and activity in human cell types. **(A)** AmRNA construct design with prototype 5'- and 3'-untranslated regions and secretion signals for enhanced expression in mammalian cells. **(B)** Western blot analysis of *in vitro* AMi-1 protein expression at by AmRNA 1.1-1.5 24 hours post transfection (~29kD, red arrow).

ENHANCED ACTIVITY IN HUMAN vs. RODENT CELL LINES





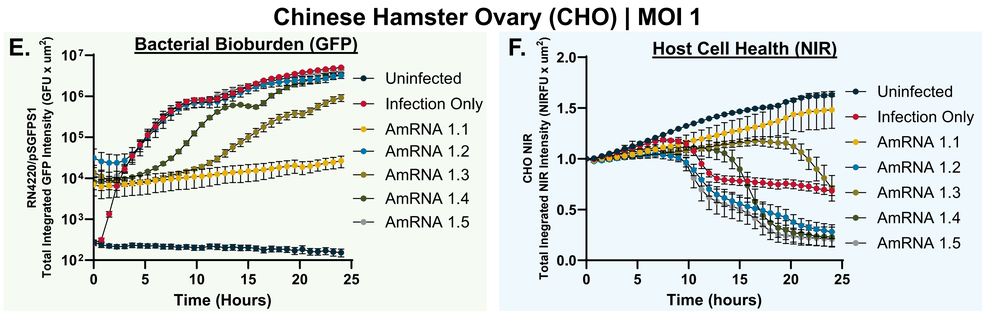
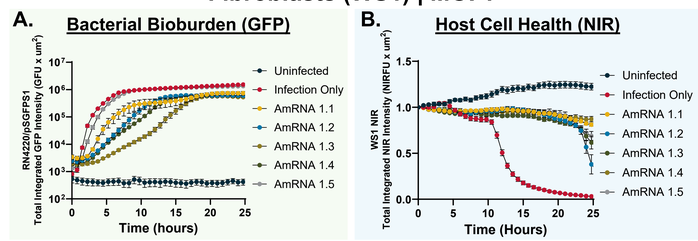
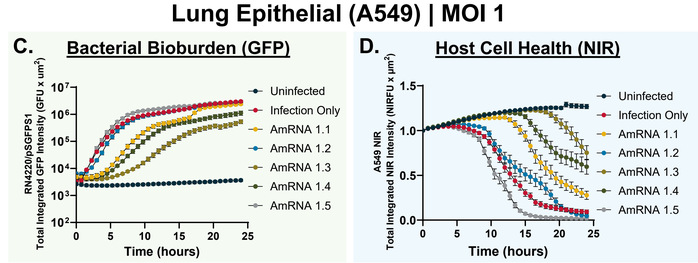


Figure 3: AmRNA 1.1-1.5 efficacy against *S. aureus* evaluated in human and rodent cell types. **(A-D)** AmRNA 1.3 demonstrates significantly improved control of bacterial bioburden (GFP, green box) and protection of host cells (NIR, blue box) compared to AmRNA 1.1 in human cells **(E,F)** AmRNA 1.3 did not improve antimicrobial efficacy compared to AmRNA 1.1 the CHO cell line, however, host cell protection by AmRNA 1.3 and AmRNA 1.4 was comparable to AmRNA 1.1.

AmRNA EVALUATION IN SKIN AND LUNG CELLS





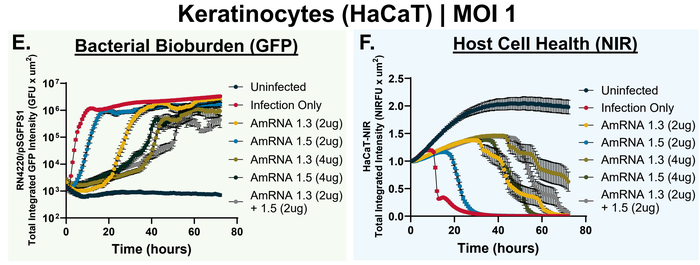


Figure 4: AmRNA 1.1-1.5 antimicrobial efficacy against *S. aureus* in skin and lung cell types associated with combat wound infections. **(A,B)** AmRNA 1.3 maintains enhanced antimicrobial efficacy and protection of host cell health when expressed in a human skin fibroblast line (WS1). **(C,D)** AmRNA 1.3 maintains enhanced antimicrobial efficacy and protection of host cell health when expressed in a lung epithelial cell line (A549). **(E,F)** Treatment with both AmRNA 1.3 (extracellular and intracellular) and AmRNA 1.5 (intracellular only) results in ~10-hour delay in bacterial growth.

CONCLUSIONS

- The modular, "building block" like nature of the NMRC/WRAIR AmRNA production pipeline facilitates the rapid design and optimization of new candidates with enhanced antimicrobial activity.
- Through the iterative process of interchanging the non-coding elements of the AmRNA construct, we identified AmRNA 1.3 as an optimized construct with increased antimicrobial efficacy against *S. aureus* in several human cell types associated with wound infections, improving control of bacterial bioburden by ~20 hours.
- These results demonstrate the power of AmRNA as a customizable, scalable platform with the potential to deliver "fire-and-forget" antimicrobial products at the point of injury in both austere military and civilian settings and highlight the potential of AmRNA therapeutics of as an innovative treatment for MDR wound pathogens.

PARTNERS/COLLABORATORS

Funding:

IP: Joint filing by NMRC/WRAIR (inventors: YLB and EA)
US19/742,500; PCT/US26/36792; 64/042,035

Affiliations: [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, Bethesda, MD, USA; [3] Military Relevant Bacterial Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA; [4] Biologics Research and Development Branch, Walter Reed Army Institute of Research, Silver Spring, MD, USA

2. This work was funded through MID MI240169 and MI260057, and DTRA CB11469.