

**DEFENSE HEALTH AGENCY**  
**20.2 Small Business Innovation Research (SBIR) Program**  
**Proposal Submission Instructions**

The Defense Health Agency (DHA) SBIR Program seeks small businesses with strong research and development capabilities to pursue and commercialize medical technologies.

Broad Agency Announcement (BAA), topic, and general questions regarding the SBIR Program should be addressed according to the DoD SBIR Program BAA. For technical questions about a topic during the pre-release period, contact the Topic Author(s) listed for each topic in the BAA. To obtain answers to technical questions during the formal BAA period, visit <https://www.dodsbirsttr.mil/submissions/login>

Specific questions pertaining to the DHA SBIR Program should be submitted to the DHA SBIR Program Management Office (PMO) at:

E-mail - [usarmy.detrick.medcom-usamrmc.mbx.dhpsbir@mail.mil](mailto:usarmy.detrick.medcom-usamrmc.mbx.dhpsbir@mail.mil)

Phone - (301) 619-7296

**PHASE I PROPOSAL SUBMISSION**

Follow the instructions in the DoD SBIR Program BAA for program requirements and online proposal submission instructions.

DHA SBIR Phase I Proposals have four Volumes: Proposal Cover Sheets, Technical Volume, Cost Volume and Company Commercialization Report. **Please note that the DHA SBIR will not be accepting a Volume Five (Supporting Documents) as noted at the DoD SBIR website.** The Technical Volume has a 20-page limit including: table of contents, pages intentionally left blank, references, letters of support, appendices, technical portions of subcontract documents (e.g., statements of work and resumes) and any other attachments. Do not duplicate the electronically generated Cover Sheets or put information normally associated with the Technical Volume in other sections of the proposal as these will count toward the 20-page limit.

Only the electronically generated Cover Sheets, Cost Volume and Company Commercialization Report (CCR) are excluded from the 20-page limit. The CCR is generated by the proposal submission website, based on information provided by small businesses through the Company Commercialization Report tool. Technical Volumes that exceed the 20-page limit will be reviewed only to the last word on the 20<sup>th</sup> page. Information beyond the 20<sup>th</sup> page will not be reviewed or considered in evaluating the offeror's proposal. To the extent that mandatory technical content is not contained in the first 20 pages of the proposal, the evaluator may deem the proposal as non-responsive and score it accordingly.

Companies submitting a Phase I proposal under this BAA must complete the Cost Volume using the on-line form, within a total cost not to exceed \$250,000 over a period of up to six months.

The DHA SBIR Program will evaluate and select Phase I proposals using the evaluation criteria in Section 6.0 of the DoD SBIR Program BAA. Due to limited funding, the DHA SBIR Program reserves the right to limit awards under any topic and only proposals considered to be of superior quality will be funded.

Proposals not conforming to the terms of this BAA, and unsolicited proposals, will not be considered. Awards are subject to the availability of funding and successful completion of contract negotiations.

## **RESEARCH INVOLVING ANIMAL OR HUMAN SUBJECTS**

**The DHA SBIR Program discourages offerors from proposing to conduct human subject or animal research during Phase I due to the significant lead time required to prepare regulatory documentation and secure approval, which will significantly delay the performance of the Phase I award.**

The offeror is expressly forbidden to use or subcontract for the use of laboratory animals in any manner without the express written approval of the US Army Medical Research and Development Command's (USAMRDC) Animal Care and Use Review Office (ACURO). Written authorization to begin research under the applicable protocol(s) proposed for this award will be issued in the form of an approval letter from the USAMRDC ACURO to the recipient. Furthermore, modifications to already approved protocols require approval by ACURO prior to implementation.

Research under this award involving the use of human subjects, to include the use of human anatomical substances or human data, shall not begin until the USAMRDC's Office of Research Protections (ORP) provides authorization that the research protocol may proceed. Written approval to begin research protocol will be issued from the USAMRDC ORP, under separate notification to the recipient. Written approval from the USAMRDC ORP is also required for any sub-recipient that will use funds from this award to conduct research involving human subjects.

Research involving human subjects shall be conducted in accordance with the protocol submitted to and approved by the USAMRDC ORP. Non-compliance with any provision may result in withholding of funds and or termination of the award.

## **PHASE II PROPOSAL SUBMISSION**

Phase II is the demonstration of the technology found feasible in Phase I. All DHA SBIR Phase I awardees from this BAA will be allowed to submit a Phase II proposal for evaluation and possible selection. The details on the due date, content, and submission requirements of the Phase II proposal will be provided by the DHA SBIR PMO. Submission instructions are typically sent toward the end of month five of the phase I contract. The awardees will receive a Phase II window notification via email with details on when, how and where to submit their Phase II proposal.

Small businesses submitting a Phase II Proposal must use the DoD SBIR electronic proposal submission system (<https://www.dodsbirsttr.mil/submissions/login>). This site contains step-by-step instructions for the preparation and submission of the Proposal Cover Sheets, the Company Commercialization Report, the Cost Volume, and how to upload the Technical Volume. For general inquiries or problems with proposal electronic submission, contact the DoD SBIR/STTR Help Desk (1-703-214-1333) or Help Desk email at [DoDSBIRSupport@reisystems.com](mailto:DoDSBIRSupport@reisystems.com).

The DHA SBIR Program will evaluate and select Phase II proposals using the evaluation criteria in Section 8.0 of the DoD SBIR Program BAA. Due to limited funding, the DHA SBIR Program reserves the right to limit awards under any topic and only proposals considered to be of superior quality will be funded.

Small businesses submitting a proposal are required to develop and submit a Commercialization Strategy (please refer to DoD Instructions, section 7.4) describing feasible approaches for transitioning and/or

commercializing the developed technology in their Phase II proposal. This plan should be included in the Technical Volume.

The Cost Volume must contain a budget for the entire 24-month Phase II period not to exceed the maximum dollar amount of \$1,100,000. These costs must be submitted using the Cost Volume format (accessible electronically on the DoD submission site), and may be presented side-by-side on a single Cost Volume Sheet.

DHA SBIR Phase II Proposals have four Volumes: Proposal Cover Sheets, Technical Volume, Cost Volume and Company Commercialization Report. The Technical Volume has a 40-page limit including: table of contents, pages intentionally left blank, references, letters of support, appendices, technical portions of subcontract documents (e.g., statements of work and resumes) and any attachments. Do not include blank pages, duplicate the electronically generated Cover Sheets or put information normally associated with the Technical Volume in other sections of the proposal as these will count toward the 40-page limit.

Technical Volumes that exceed the 40-page limit will be reviewed only to the last word on the 40<sup>th</sup> page. Information beyond the 40<sup>th</sup> page will not be reviewed or considered in evaluating the offeror's proposal. To the extent that mandatory technical content is not contained in the first 40 pages of the proposal, the evaluator may deem the proposal as non-responsive and score it accordingly.

### **PHASE II ENHANCEMENTS**

The DHA SBIR Program has a Phase II Enhancement Program which provides matching SBIR funds to expand an existing Phase II contract that attracts investment funds from a DoD Acquisition Program, a non-SBIR government program or eligible private sector investments. Phase II Enhancements allow for an existing DHA SBIR Phase II contract to be extended for up to one year per Phase II Enhancement application, and perform additional research and development. Phase II Enhancement matching funds will be provided on a dollar-for-dollar basis up to a maximum \$550,000 of SBIR funds. All Phase II Enhancement awards are subject to acceptance, review, and selection of candidate projects, are subject to availability of funding, and successful negotiation and award of a Phase II Enhancement contract modification.

### **TECHNICAL AND BUSINESS ASSISTANCE (TAB A)**

The DHA SBIR Program does not participate in the Technical and Business Assistance (formally the Discretionary Technical Assistance Program). Contractors should not submit proposals that include Technical and Business Assistance.

The DHA SBIR Program has a Technical Assistance Advocate (TAA) who provides technical and commercialization assistance to small businesses that have Phase I and Phase II projects.

**PROTEST PROCEDURES**

Please refer to the DoD Program Announcement for procedures to protest an Announcement. As further prescribed in FAR 33.106(b), FAR 52.233-3, Protests after Award should be submitted to:

Ms. Micaela Bowers  
SBIR/STTR Contracting Officer  
U.S. Army Medical Research Acquisition Activity  
Phone: (301)-619-2173  
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## **DEFENSE HEALTH AGENCY SBIR 20.2 Phase I Topic Index**

- DHA202-001 Companion Diagnostic Platform for Rapid Assessment of Bacteriophage Susceptibility in Antibiotic-Resistant Bacterial Pathogens
- DHA202-002 A Multiplexed, Functional Assay to Determine the Bactericidal Activity of Antibodies Against Multiple Enteric Bacterial Pathogens
- DHA202-003 Development of Human Monoclonal Antibody Therapeutic against *Klebsiella pneumoniae* Infection
- DHA202-004 On-Site Creation of Dialysate Fluid

DHA202-001 TITLE: Companion Diagnostic Platform for Rapid Assessment of Bacteriophage Susceptibility in Antibiotic-Resistant Bacterial Pathogens

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Develop state-of-the-art diagnostic technology for rapid detection of antimicrobial susceptibility in pathogens from infected wounds that can be used to guide clinical decisions for use of non-traditional antimicrobials.

DESCRIPTION: U.S. military service members who are medically evacuated from theatre due to combat-related injuries have sustained high impact insults such as explosions, gunshot wounds and motor vehicle accidents, leading to significant skin and soft tissue injuries that may be frequently contaminated. A large proportion of these service members are at increased risk for infectious complications of their traumatic injuries, and the most common infections involve skin and soft tissue, wound infections, and osteomyelitis and sepsis if not treated in a timely manner. *Acinetobacter baumannii* has been identified as one of the most frequently associated organisms with skin and soft tissue infections among wounded warriors, occurring in 35% of wound infections. Within this 35%, up to 90% of the culture isolates were assessed to be antimicrobial resistant (AMR) [1]. Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a well-recognized cause of skin and soft tissue infections (SSTI) in US military hospitals with a reported prevalence of 68% to 70% in selected military hospital emergency rooms [2]. High rates of MRSA skin and soft tissue infections have been observed among soldiers in training [3]. In addition to skin and soft tissue infection, MRSA is the most frequently isolated organism late in infection in traumatically injured service members [2]. Late infection in this population often results in limb salvaging amputation. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are responsible for significant morbidity and mortality among both civilian and military populations, often colonizing mucosal surfaces, wounds, and foreign devices such as catheters and endotracheal tubes with biofilms that are highly resistant to antibiotic penetration and clearance by the immune system. In civilian and veteran populations these same types of infections frequently occur in individuals that have skin and soft tissue and prosthetic joint infections [4]. In a patient infected with multi-drug resistant organisms, the treatment choices often become limited due to waning approvals of new antibiotics. Frequently, these patients are hospitalized for prolonged periods of time and subsequently experience multiple episodes of hospital readmissions related to infectious complications of their wound or orthopedic implants. In addition to increased patient morbidity, provision of medical care for service members with infected traumatic wounds can be very costly and lead to intense resource utilization.

Furthermore, the prolonged systemic administration of broad spectrum antibiotics to soldiers and sailors escalates the risk of selecting for bacterial organisms with increased antibiotic resistance profiles. The dwindling arsenal of antibiotics active against multidrug-resistant organisms urgently necessitates novel therapeutics such as phage to decrease the rates of mortality and morbidity associated with MDR infections and maintain current standards of medical practice in the future. However, the clinical utility of novel antimicrobials, such as bacteriophage [5], will be limited by the inability to monitor the bacterial susceptibility in real time to guide clinical decisions and therapeutic use. Current antibiotic susceptibility testing systems are closed both from hardware and reagents to the software analytic pieces that prevent evaluation of alternative antimicrobials such as phage. This topic seeks an open standards system (easily expandable or modified for evaluation of different antimicrobial types) that can monitor individual bacterial susceptibility to bacteriophage and eventually other non-traditional antimicrobial agents to support preclinical development and evaluation of therapeutic candidates and for future use as a companion diagnostic in the clinics to guide treatment decisions.

PHASE I: Selected performer determines the feasibility of the concept by developing a prototype diagnostic susceptibility-based assay that has the potential to meet the broad needs discussed in this topic description. Currently there are no FDA-cleared, field-capable assays that can be used to rapidly identify the most common bacterial pathogens causing wound and sepsis infections as described in references 1-6 (to include but not limited to the ESKAPE group of pathogens: Enterococcus spp., Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp, and Escherichia coli), as well as an ability to determine the respective susceptibility of the detected pathogen to bacteriophage. Development of an assay for that can rapidly determine the susceptibility of AMR bacteria to bacteriophage is therefore a high priority for development and eventual clinical use of these promising therapeutics. Assay run time should be congruent with or more rapid (less than or equal to overnight culture; 16-18h) than current automated antibiotic susceptibility testing systems in order to provide results within a clinically relevant timeframe to guide therapeutic use.

PHASE II: Based on the results from Phase I, the selected performer provides up to 3 initial lots of at least 50 prototype assays (tests or plates) each to the COR. These initial lots will be evaluated for sensitivity and specificity using a diversity set of bacterial strains and cognate bacteriophage for evaluation in vitro. Can coordinate with WRAIR/NMRC for materials and assistance with preclinical evaluation if needed. Feedback regarding the sensitivity/specificity of each lot of prototype assays will be provided to the performer. This data will then be used to optimize each subsequent lot of assays. The goal in Phase II is the development of a prototype assay that provides 85% sensitivity and 85% specificity when compared to phage plaque assays. Once sensitivity and specificity requirements have been met in preclinical tests, the selected performer will confirm the performance characteristics of the assay (sensitivity, specificity, positive and negative predictive value, accuracy and reliability) using preclinical or clinical specimens. The elected performer will require a Federal-Wide Assurance of Compliance before government funds can be provided for any effort that requires human testing or uses of clinical samples. The selected performer will also conduct stability testing of the prototype device in Phase II. Stability testing will follow both real-time and accelerated (attempt to force the product to fail under a broad range of temperature and humidity conditions and extremes) testing in accordance with FDA requirements. The data package plan required for application to the U.S. Food and Drug Administration will be prepared at the end of phase II.

PHASE III: During this phase the performance of the assay should be evaluated in field studies or clinical trials that will conclusively demonstrate that the assay meets the requirements of this topic. The performer may coordinate with WRAIR/NMRC for this objective. Military applications: AMR bacterial infections occur worldwide. The diagnosis of these wound infections and sepsis cases are often delayed, because the currently available tests, mostly reliant on bacterial culture or high-complexity nucleic acid amplification, are not field-capable, not rapid, and can vary considerably among different laboratories even when using the same procedure or method. With the availability of an easy and rapid assay developed under this topic, wounded and ill soldiers can be treated with more effective antimicrobials such as bacteriophage, alongside traditional antibiotics, in a timely manner in any military medical organization (such as a Battalion Aid Station, a Combat Support Hospital, Forward operation base, or a fixed medical facility). The performer should coordinate with WRAIR/NMRC to establish a National Stock Number (NSN) for potential inclusion in into appropriate "Sets, Kits and Outfits" that are used by deployed medical forces. Civilian applications: MDR bacterial infections occur in communities and hospitals, in wounds, skin and soft tissue infections, pneumonia, and blood stream infections. We envision that the performer that develops the rapid diagnostic assay and will be able to sell and/or market this assay to a variety of civilian medical organizations, and that this market will be adequate to sustain the continued production of this device.

REFERENCES:

1. Davis, K.A., et al., Multidrug-resistant *Acinetobacter* extremity infections in soldiers. *Emerg Infect Dis*, 2005. 11(8): p. 1218-24.
2. Morrison-Rodriguez, S.M., et al., Community-associated methicillin-resistant *Staphylococcus aureus* infections at an Army training installation. *Epidemiol Infect*, 2010. 138(5): p. 721-9.
3. Burns, T.C., et al., Microbiology and injury characteristics in severe open tibia fractures from combat. *J Trauma Acute Care Surg*, 2012. 72(4): p. 1062-7.
4. Hospenthal, D.R., et al., Guidelines for the prevention of infections associated with combat-related injuries: 2011 update: endorsed by the Infectious Diseases Society of America and the Surgical Infection Society. *J Trauma*, 2011. 71(2 Suppl 2): p. S210-34.
5. Kutter, E., et al., Phage therapy in clinical practice: treatment of human infections. *Curr Pharm Biotechnol*, 2010. 11(1): p. 69-86.

**KEYWORDS:** Wound Infections, ESKAPE, AMR, MDR, Diagnostic, Bacteriophage, Phage therapy, antimicrobial, susceptibility testing

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DHA202-002 TITLE: A Multiplexed, Functional Assay to Determine the Bactericidal Activity of Antibodies Against Multiple Enteric Bacterial Pathogens

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Develop a qualified, multiplexed, functional assay that can be used to evaluate bactericidal activity of antibodies against an array of *Shigella* enabling high-throughput sample analysis.

DESCRIPTION: Bacterial pathogens that cause diarrhea are a significant threat to the warfighter on a global scale and consistently rank at the top of the list of infectious agents for which the Army requires countermeasures. Infection with these pathogens leads to a reduction in warfighter readiness, morale, lost duty days. *Shigella* is a major cause of diarrhea in children and adults in low- to middle-income countries (LMICs), and among travelers and US Service Members (1). *Shigella* infection can lead to persistent diarrhea ( $\geq 14$  days) in travelers to endemic areas, and can also have long-term health impacts including irritable bowel syndrome and reactive arthritis (2). The high incidence of infection, the rise of antibiotic resistance, the long duration of illness and the potential long-term side effects make prevention of *Shigella* infection a high priority for US Service Members deployed to endemic areas.

One of the major technical issues facing the field when developing prophylactic or therapeutic products is the broad species diversity (3) and the serotype specific immune responses that are generated after infection (4). The focus of the *Shigella* research has been on the development of countermeasures that are capable of protecting against multiple serotypes of *Shigella*. These countermeasures most often target four *Shigella* species, *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6 and *S. sonnei*. A countermeasure that was capable of protecting against these serotypes would significantly reduce global disease burden. The need for effective prophylactic and therapeutic products to combat *Shigella* also requires immunological methods to evaluate these products and their efficacy. Many of the current *Shigella*-specific immunological assays are only quantitative, and do not offer any qualitative information about the immune response being investigated. Functional immunological assays to assess immune responses to *Shigella* do exist (5), but these are typically specific for only a single serotype. Evaluating responses to multiple serotypes in a single-plex assay is time consuming and also requires greater quantities of serum and mucosal samples, many of which are limited.

An assay to evaluate the shigellacidal activity of antibodies is imperative for the vaccine development field, but the new push for non-vaccine countermeasures to combat *Shigella* will also require robust functional assays. Both vaccine and non-vaccine countermeasures will need reliable, validated assays to show product efficacy, and this will include qualitative analysis of immune responses and immunoprophylactic products. Any successful countermeasure product will need to protect against or treat infection with multiple *Shigella* serotypes to be highly efficacious, so the development of multiplexed immunoassays will save time, supplies, and biological sample volumes. The development and validation of a multiplexed, *Shigella*-specific, functional assay will support the rapid development and evaluation of efficacious prophylactic and therapeutic countermeasures to prevent and treat *Shigella* infections. The ultimate problem to be solved, and the central focus of this topic, is the development of an assay platform to measure functional antibody activity and immune responses to *Shigella* and other enteric bacterial pathogens.

PHASE I: Phase I will focus on assay conceptualization including assay parameters, internal controls, and data analysis package. A major component of phase I will be concept design of the multiplex assay format to include discrete readouts for each bacterial serotype to be analyzed (e.g. fluorescence, antibiotic resistance cassettes). The concept design will also require that assay qualification parameters are defined including: a) bio-specimen types (e.g. blood, sera, fecal), bio-specimen volume required (e.g. finger-stick,

500 ul sera derived from venipuncture; b) generation of positive and negative controls (e.g. monoclonal antibodies, pooled sera). Specifically, the awardee will have performed the assay in a research laboratory setting and demonstrated that it can be repeated by additional users. In order to demonstrate the feasibility of multiplexing, a minimum of two *Shigella* serotypes will be evaluated using the proof-of-concept prototype assay and a pilot panel of control samples and monoclonal antibodies that target *Shigella* serotypes in the assay.

**PHASE II:** Phase II will focus on finalizing and refining the optimal multiplex assay approach from Phase I. The workflow from Phase I should be refined to expand on the proof-of-concept into a product that enables high-throughput screening of serum or other clinical samples against multiple *Shigella* targets. The assay will be performed in a research laboratory setting to demonstrate the feasibility of multiplexing using a minimum of four *Shigella* serotypes. In addition to execution of the assay, a qualification of the inter- and intra-assay reproducibility in-house should be performed. This qualification will include metrics of assay precision, repeatability and reproducibility, with estimates of uncertainty around these metrics. The assay should be specific for at least four *Shigella* targets, but the platform should also be flexible and allow expansion to other enteric bacterial pathogens such as ETEC, Cholera, *Campylobacter* or *Salmonella*. A detailed plan for assay qualification should be developed across multiple laboratory sites. This phase should also demonstrate evidence of commercial viability of the product.

**PHASE III:** The expected Phase II end-state is a qualified, easy to use, multiplexed, functional assay kit which can be used on a relatively low volume of biological sample of varying types and evaluates bactericidal activity to at least five *Shigella* serotypes simultaneously. This assay platform should also be under development for expansion to measure responses to other bacterial enteric pathogens beyond *Shigella*. This assay kit represents a method to evaluate functional antibody responses targeting *Shigella*. The development of effective anti-*Shigella* countermeasures relies heavily on the identification of products that are effective at functionally inhibiting bacterial infection. The assay kit described here is unique to anything currently in development, as it is multiplexed to include many cynically relevant strains of *Shigella* and it measures functional activity of antibodies. This end-product has the potential to be used by research laboratories to examine the potency of enteric countermeasures. These countermeasures may include hyper-immune bovine colostrum products, monoclonal antibodies, and passive vaccine strategies; all of which are aimed at preventing or treating disease caused by *Shigella*. A validated functional assay would help to harmonize immunological assessment of *Shigella*-specific countermeasures globally, which will allow for accurate comparisons between products and speed the production of efficacious prophylactics. This product would also likely be used in the immunological analysis of controlled human infection models (CHIMs) for *Shigella* to understand the development of serotype specific immunity, and facilitate development and evaluation of pan-*Shigella* countermeasures. A potential method of transition for this product will be through the Army futures command following the decision gate process. This product may also be attractive to private industry, as this multiplexed assay kit is ideally suited to evaluate immunoprophylactic products as well as commercial vaccines. Assays that evaluate functional antibody activity are essential for vaccine licensure in many current vaccines including seasonal influenza and meningococcal polysaccharide vaccines. Civilian commercialization of this product is likely to include GLP production and GMP manufacture and distribution.

#### REFERENCES:

1. Porter CK, Olson S, Hall A, & Riddle MS (2017) Travelers' Diarrhea: An Update on the Incidence, Etiology, and Risk in Military Deployments and Similar Travel Populations. *Mil Med* 182(S2):4-10.
2. Connor BA & Riddle MS (2013) Post-infectious sequelae of travelers' diarrhea. *J Travel Med* 20(5):303-312.
3. Anderson M, Sansonetti PJ, & Marteyn BS (2016) *Shigella* Diversity and Changing Landscape: Insights for the Twenty-First Century. *Front Cell Infect Microbiol* 6:45.

4. Formal SB, et al. (1991) Effect of prior infection with virulent *Shigella flexneri* 2a on the resistance of monkeys to subsequent infection with *Shigella sonnei*. *J Infect Dis* 164(3):533-537.
5. Nahm MH, et al. (2018) Development, interlaboratory evaluations, and application of a simple, high-throughput *Shigella* serum bactericidal assay. *mSphere* 3(3).

KEYWORDS: *Shigella*, Bacterial Diarrhea, Diagnostic Assay, Immunoassay, Multiplex, Validation, Antibody

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DHA202-003      TITLE: Development of Human Monoclonal Antibody Therapeutic against *Klebsiella pneumoniae* Infection

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Develop human monoclonal antibodies against *K. pneumoniae* for therapeutic use. Candidate antibodies will be tested for binding to the surface of the target bacterium and evaluated for efficacy as a prophylactic and therapeutic with and without standard of care antibiotics in relevant animal models and downstream human clinical trials. Infection rates occur in 20-35% of combat-associated traumatic injuries; *K. pneumoniae* has been responsible for 8-10% of these infections resulting loss of life, limb, and delayed or prohibited return to duty at an estimated cost of \$1M-\$2M per injured military member. A monoclonal antibody therapeutic is a promising solution to prevent these infections, deaths, amputations, and to enhance return to duty.

DESCRIPTION: U.S. military members medically evacuated from theater because of combat injuries sustain high impact insults such as explosions, gunshot wounds and motor vehicle accidents, leading to significant injuries that are frequently contaminated. Without timely treatment, injuries are at increased risk for infectious complications, especially skin and soft tissue, wound, osteomyelitis and sepsis<sup>1</sup>. *K. pneumoniae* poses a serious threat and will be a threat in future conflicts because:

- 1) *K. pneumoniae* has grown significantly resistant to antibiotics, and there are now multidrug-resistant (MDR), extensively drug-resistant (XDR) and even pandrug-resistant (PDR) strains leaving clinicians in the military health system (MHS) with few or no treatment options.
- 2) Although antibiotic discovery has caught up with drug-resistant Gram-positive pathogens, such as *S. aureus*, the same is not true for drug-resistant Gram-negatives. Specifically, the recently approved antibiotic, ceftolozane-tazobactam, provides coverage against *P. aeruginosa* infections, but is not effective against *K. pneumoniae*. Similarly, although ceftazidime-avibactam is effective against most serine carbapenemase-producing bacteria, but not many *K. pneumoniae* isolates.
- 3) Irrespective, monotherapy is subject to resistance.

Therefore, because of the looming threat of drug resistance and a paucity of effective antibiotics, wound infections caused by *K. pneumoniae* will not be resolved by traditional antibiotics, and investment in alternative strategies is paramount. Monoclonal antibody therapy is a non-traditional, antibacterial approach, which works on its own or as an adjunct to antibiotics, both prophylactically or as treatment, to resolve infection. In the 19th century, serum was successfully used to treat bacterial infections<sup>2</sup>. Now, with 21st century technology, generation of human monoclonal antibodies (Hu-mAb) is a viable and attractive antibacterial strategy that can be somewhat fast-tracked through clinical trials given the inherent lack of toxicity and stability issues, which often accompany other traditional antibacterial approaches. Other advantages of Hu-mAb therapy are: (1) longevity, as this product is not cleared by the immune system as fast as mAbs from other animal sources; (2) confers inherent pathogen specificity without disrupting the microbiome; (3) potentiates rapid and sustained killing via multiple mechanisms including: direct killing, anti-virulence, neutralization, complement deposition, and opsonization by phagocytes<sup>2</sup>. Furthermore, mAbs with Fc domains that bind to the host phagocyte receptor FcγRII result in downstream suppression of inflammation and sepsis caused by Gram-negative bacteria<sup>3</sup>. Killing bacteria by multiple mechanisms limits toxic shock seen in sepsis and limits emerging resistance.

Recently, companies have successfully developed Hu-mAb to treat bacterial infections. The FDA approved two Hu-mAb products: Bezlotoxumab for *Clostridium difficile* infection and Raxibacumab for *Bacillus anthracis* infection<sup>4</sup>. There are six additional Hu-mAb antibacterial solutions in the development pipeline.

Preferred Features of monoclonal antibody deliverable:

- human or humanized antibodies will be given highest priority
- if non-human antibodies will be made, a plan for humanization must be included in Phase III

PHASE I: Selected performer determines the feasibility of the concept by identifying at least 100 unique mAbs that bind to at least 5 unique targets on the native, bacterial surface or secreted factors of a clinically-relevant strain of *K. pneumoniae* by ELISA, fluorescent microscopy, or other like methods. 50% of mAbs must bind to biofilm-grown bacteria or supernatants of biofilm-grown bacteria and 50% must bind planktonically grown bacteria or secreted factors. Half of each group of mAbs must bind in the presence of capsule. Further, these 100 mAbs may not bind either the capsule or lipopolysaccharide (LPS). Selected performer will coordinate with WRAIR/NMRC for required bacterial strains to help facilitate assay results, and any work by WRAIR/NMRC with respect to this deliverable will be done at no cost. Deliverable 1: The selected performer will provide the COR with 100 unique mAb sequences (to a minimum of 5 unique bacterial proteins) and mAbs.

PHASE II: Selected performer will epitope map mAbs and establish broad reactivity (80% or greater reactivity) of mAbs against a diverse set of at least 100 clinically-relevant *K. pneumoniae* strains. Performer will determine mAb function by using secondary screens to include, at minimum: anti-growth, anti-biofilm, anti-virulence, complement, and opsonizing activity against the bacteria of interest. Performer will determine identity of bacterial targets of mAbs with activity in any assay listed above. The results of the secondary screen must yield at least 10 antibodies (to at least five unique bacterial targets) that bind to the surface of the bacterium or to secreted bacterial factors and have some antibacterial or enhanced immunologic function, such as increased bacterial killing via complement or opsonophagocytosis. Ultimately, candidates need to be narrowed to at least 10 mAb that reduce bacterial numbers or show in a tissue culture assay that bacteria can no longer kill or intoxicate host cells. Finally, this set of antibodies will be tested in an in vivo efficacy model to identify the best single or combination of antibodies for Phase III. WRAIR/NMRC could assist with this work, and this work would be at no cost. Deliverable 2: Performer will deliver results of in vitro assays to COR.

PHASE III: Positive Phase II results infers that the product will move forward with a series of preclinical experiments to support Deliverable 4: an IND and clinical trial for a Hu-mAb product against *K. pneumoniae*. This phase will encompass both small and large animal models such as mouse, rabbit, mini-pig and/or pig, for survival, sepsis and SSTI/wound infections. These should be in addition to the animal model done in Phase II to address both safety and efficacy. The models should consider endpoints such as: survival, bacterial burden, and time to wound closure, which reflects the requirements for the U.S. Food and Drug Administration (FDA) with regard to a product for ABSSSI. Promising antibodies will be combined into a defined mix or cocktail and in vivo efficacy experiments repeated. Performer will investigate efficacy of the mixture alone and in combination with antibiotics to evaluate synergy in an appropriate animal model. This phase will also include a formal clinical indication for the cocktail, which would be SSTI, ABSSSI and/or other relevant clinical indication. Additionally, the selected performer will establish an escalating toxicity model to establish a therapeutic window for the FDA. Finally, the performer will address the serum longevity of the final product(s) in a representative animal model of infection. All experiments should be completed GLP-like/GMP-like as best as possible. Funding for this effort could come from the Joint Warfighter Medical Research Program (JWMP) or from awards in Congressionally Directed Medical Research Program (CDMRP), Additionally, CARB-X a spin off programs of the Biomedical Advanced Research and Development Authority (BARDA) is an additional potential funding source.

The Government customer would use this product a number of ways to include prophylactic therapy or treatment along with the standard of care for wound infections. The market value of a product would be

estimated around \$100M-\$150M as there are about 50,000 of these infections worldwide and current pricing for novel antibiotics is at least \$3000 a dose<sup>4</sup>. Once developed and demonstrated, the technology can be used both commercially in civilian or military settings. The selected performer shall make this product available to potential military and non-military users throughout the world.

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**KEYWORDS:** Wound Infections, ESKAPE, AMR, monoclonal antibodies, prolonged field care, bacteria, pathogens, antibacterial treatments

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DHA202-004 TITLE: On-Site Creation of Dialysate Fluid

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)  
TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: The objective of this Small Business Innovation Research topic is to develop a technology that can create dialysate fluid on-site using potable water, non-potable water, and salt water without a source of electrical power that weighs less than 1lb. and is FDA approved for its intended purpose.

DESCRIPTION: It is anticipated that future battlefield environments will have prolonged care scenarios in which critically injured patients will not be evacuated out of theater for extended periods of time, up to and beyond 72 hours. Based on these evacuation times, it is anticipated that patients will arrive at Field Hospitals in critical condition, leading to increased rates of acute kidney injury (AKI) ~19-40% of patients arriving to the Field Hospital, similar to those seen in civilian hospital emergency rooms<sup>1-2</sup>.

Current technologies that provide support for AKI require large amounts of dialysate fluid to function, ~75L of fluid per patient per day. Shipping this amount of fluid into the battlefield environment is not logistically feasible in future battlefields that will be conducted using Multi-Domain Operations (MDO).

Therefore, the DoD is seeking new and innovative technologies that are able to create dialysate fluid on the battlefield that don't require power, are lightweight, and are rugged enough to withstand military environments. The technology should be able to create the dialysate fluid from any source of available water including, but not limited to potable, non-potable, and salt water.

PHASE I: The contractor should provide basic proof of concept that their selected technology has the ability to create dialysate fluid for use in extracorporeal life support of the kidneys. The basic principle that is demonstrated should be expandable for use with different water types with no or minimal modifications to the process. The technology should function without electrical power. Design drawings for the fully functional prototype device should be completed by the end of this phase.

PHASE II: The contractor should develop and demonstrate their technology showing that it can create dialysate fluid for use in extracorporeal life support of the kidneys. The technology should function without electrical power and be able to use any source of available water to create the dialysate fluid, including, but not limited to potable water, non-potable water, and salt water. The contractor should then validate that the created dialysate fluid meets US standards for dialysate<sup>3</sup>. Finally, the contractor should conduct a Pre-Submission meeting with the FDA to validate their regulatory strategy and testing of the technology aligns with FDA requirements. Deliverables include 5 prototypes, validation test reports, the contractor's proposed regulatory strategy, FDA pre-submission meeting minutes providing feedback on the contractor's proposed regulatory strategy, and a technology commercialization strategy.

PHASE III: The contractor should refine and implement their regulatory strategy for obtaining FDA approval of their technology for use as dialysate fluid based off of their initial FDA feedback. This phase should culminate in submission to the FDA of the developed technology for approval. In conjunction with FDA submission, the contractor should develop scaled up manufacturing of the technology that follows FDA quality regulations. Work may result in technology transition to an Acquisition Program managed by the Warfighter Expeditionary Medicine and Treatment (WEMT) Project Management Office (PMO) and/or commercialization of this technology capability. Contractor shall seek additional funding from other government sources and/or private sector investors to develop or transition the prototype into a viable product for sale to the military and private sector markets. The ability to create dialysate fluid on the battlefield will remove the logistical constraint for providing kidney support to critically injured soldiers on the battlefield, allowing kidney support to be provided in theater. This type of technology

may also be of interest to large-scale dialysate manufacturing companies for further partnership and commercialization.

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KEYWORDS: Dialysate, Kidney, Extracorporeal, Life Support, Renal Replacement Therapy, Fluid

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