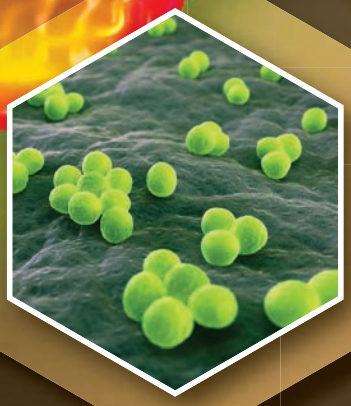
DEPARTMENT OF DEFENSE
BLAST INJURY RESEARCH PROGRAM
COORDINATING OFFICE

MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

2016 STATE-OF-THE-SCIENCE MEETING REPORT PROCEEDINGS, KEY FINDINGS, AND EXPERT PANEL RECOMMENDATIONS



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Previous State-of-the-Science Meetings

2009
International State-of-the-Science Meeting on Non-Impact, Blast-Induced Mild Traumatic Brain Injury, May 12–14, 2009, in Herndon, Va.
https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/meeting_proceedings/2009_SoS_Meeting_Proceedings.pdf

2010
International State-of-the-Science Meeting on Blast Injury Dosimetry, June 8–10, 2010, in Chantilly, Va.
https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/meeting_proceedings/2010_SoS_Meeting_Proceedings.pdf

2011
International State-of-the-Science Meeting on Blast-Induced Tinnitus, November 15–17, 2011, in Chantilly, Va.
https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/meeting_proceedings/2011_SoS_Meeting_Proceedings.pdf

2014
International State-of-the-Science Meeting on the Biomedical Basis for Mild Traumatic Brain Injury (mTBI) Environmental Sensor Threshold Values, November 4–6, 2014, in McLean, Va.
https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/meeting_proceedings/2014_SoS_Meeting_Proceedings.pdf

2015
International State-of-the-Science Meeting, Does Repeated Blast-Related Trauma Contribute to the Development of Chronic Traumatic Encephalopathy (CTE)? November 3–5, 2015, in McLean, Va.
https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/meeting_proceedings/2015_SoS_Meeting_Proceedings.pdf

Preface

On behalf of the Department of Defense (DoD) Executive Agent (EA) for Medical Research for Prevention, Mitigation, and Treatment of Blast Injury, I wish to commend and thank the meeting Planning Committee, meeting presenters, Expert Panel members, and attendees of the 2016 International State-of-the-Science Meeting, as well as the broader efforts of those contributing related science. Thanks to these efforts, we continue to advance our knowledge of the consequences of blast injury and foster new and better strategies to prevent, screen, diagnose, and treat affected service members and civilians.

The theme of this year's meeting was "Minimizing The Impact of Wound Infections Following Blast-Related Injuries." As is the custom, the meeting was devoted to critically assessing the state of the science, identifying important knowledge gaps, and focusing on future DoD research and policy opportunities. Meeting participants included representatives from the DoD, other federal agencies, academia, industry, and foreign allies. Topics addressed in these proceedings include research findings, identification of knowledge gaps, and recommendations for the future. Contributors to this collaborative work were a diverse group of subject matter experts, scientists, health professionals, and program directors.

The origins of the DoD Blast Injury Research State-of-the-Science Meetings date back to the early years of Operations Iraqi Freedom and Enduring Freedom (OIF/OEF) and the rise in blast injuries due to improvised explosive devices (IEDs). In 2006, the U.S. Congress responded by directing establishment of a DoD EA for blast injury research (Public Law 109-163, Sec. 256). In 2007, DoD Directive 6025.21E designated the Secretary of the Army as the EA (see Appendix G). Subsequently, the DoD Blast Injury Research Program Coordinating Office (PCO) was established within the U.S. Army Medical Research and Materiel Command to assist the EA. Today, the ongoing vision of the PCO is "a fully coordinated DoD Blast Injury Research Program that delivers timely and effective blast injury prevention, mitigation, and treatment strategies for Service Members today and in the future."

Under PCO coordination, the first Blast Injury Research Program's State-of-the-Science Meeting was held in 2009. The 2016 meeting was the sixth in the series, and these meetings have become an essential tool for strategic identification of the scientific research gaps and policy improvement opportunities in fields related to the prevention, mitigation, and treatment of blast injury. The meetings support the congressionally directed EA responsibilities to identify blast injury knowledge gaps and to foster collaborative medical research to close those gaps. We believe that the result is and will continue to be research that supports the development and delivery of effective blast injury prevention, mitigation, and treatment strategies—strategies that we hope will benefit those who serve the nation in uniform as well as society as a whole.

The report that follows summarizes the proceedings of the 2016 International State-of-the-Science Meeting, lays out the key findings and Expert Panel recommendations, and begins to

synthesize productive future directions in related policy and research. I am confident that you will find these proceedings, key findings, and expert panel recommendations are thoughtful, thorough, informative, important, and impactful. Most importantly, the insights described are certain to help the DoD, the military health system, clinicians, and others who provide care and support for the men and women serving in harm's way.

Michael J. Leggieri, Jr.
Director
DoD Blast Injury Research Program Coordinating Office
U.S. Army Medical Research and Materiel Command

Executive Summary

Blast injury is the leading cause of morbidity and mortality resulting from war, and wound infections following these injuries are among the most important contributors to these outcomes.

The theme of the 2016 Blast Injury Research International State-of-the-Science Meeting was "Minimizing The Impact Of Wound Infections Following Blast-Related Injuries." The meeting was held from November 29 to December 2, 2016 at the RAND Corporation in Arlington, Va.

The objectives of the 2016 meeting were:

1. determining predictive risk factors for wound infections following blast-related injuries, including individual susceptibility and environmental contributions, from point of injury through continued hospital care
2. identifying candidate biomarkers that would enable rapid and accurate diagnosis, management, and prognosis of wound infections following blast-related injuries
3. examining prevention strategies, including vaccines, for mitigating wound infections following blast-related injuries
4. proposing strategies that would mitigate the impact of multidrug resistant, virulent, or opportunistic organisms on wound infections following blast-related injuries.

Over 120 scientists, clinicians, and military leaders from related fields provided scientific overviews, presentations, and posters describing new and emerging science. Before the meeting, a conference planning committee invited a panel of five leading scientists and clinicians in related fields to serve as an Expert Panel, lead working groups, and develop overall recommendations. Working groups developed responses to four questions designed in advance to address the objectives above. Responses to these questions, provided in the following Proceedings, were informed by participant presentations and the expert panelist–led working groups. The working groups identified and prioritized unresolved challenges and recommended short-, intermediate-, and long-term actions and directions.

Following the meeting, the expert panel developed the following DoD research and policy recommendations to mitigate the impact of wound infections following blast-related injuries.

Recommendation 1. Ensure that proactive plans, policies, procedures, and clinical practice are in place to support and sustain a "Learning Trauma Care System" that is consistent with a recent Institute of Medicine report.[1] One goal of this approach should be to seek to improve theater-specific understanding, prevention, and treatment of wound infections following blast injuries.

Recommendation 2. Coordinate—by DoD Directive and all other appropriate regulatory mechanisms—routine research organizational support for sustained wound infection

[1] National Academies of Sciences, Engineering, and Medicine, *A National Trauma Care System: Integrating Military and Civilian Trauma Systems to Achieve Zero Preventable Deaths After Injury*, Washington, D.C.: National Academies Press, 2016.

surveillance and analytic epidemiology in current and future theaters of operation. Initiate and sustain research upon entry to any theater of operations.

Recommendation 3. Develop a proactive, comprehensive research strategy relating to blast-related wound care, enhanced infection control, and optimal antimicrobial prevention and treatment strategies for coordinated implementation within current and future theaters of operation.

Recommendation 4. Increase DoD efforts to engage and facilitate Food and Drug Administration (FDA) involvement in meetings, strategies, and other efforts to ensure research and development of innovative, integrated therapies tackling the growing, global problem of antimicrobial drug resistance. Use FDA collaboration to facilitate industry partnerships relating to antimicrobial drug development.

Recommendation 5. Implement a system to measure, compare, benchmark and reward compliance with existing Joint Trauma System clinical practice guidelines pertaining to blast-related injury, such as improving compliance with the Joint Trauma System guidelines relating to infection prevention in combat-related injuries (Clinical Practice Guideline [CPG] ID: 24)[2] and care of patients at high risk for invasive fungal infection in war wounds (CPG: 28)[3].

Recommendation 6. Preserve, sustain, and improve the DoD Trauma Registry and related programs (e.g., Trauma Infectious Disease Outcome Study and the Military Orthopedic Trauma Registry) to improve care and advance military relevant research relating to wound infections after blast-related injury.

[2] Omar Saeed, David Tribble, Kimberlie Biever, Michael Kavanaugh, and Helen Crouch, "Infection Prevention in Combat-Related Injuries (CPG ID: 24)," *Joint Trauma Clinical Practice Guidelines*, August 8, 2016.
[3] Carlos J. Rodriguez, David R. Tribble, Clinton K. Murray, Elliot M. Jessie, and Mansoor Khan, "Invasive Fungal Infection in War Wounds (CPG: 28)," *Joint Trauma Clinical Practice Guidelines*, November 1, 2012, last updated August 4, 2016.

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Introduction and Where to Find Key Information

The focus of the 2016 International State-of-the-Science Meeting was "Minimizing the Impact of Wound Infections Following Blast-Related Injuries." Wound infection has long been and continues to be a significant source of morbidity and mortality in the modern era of military healthcare. An estimated one quarter of combat wounds become infected, which has a significant impact on patient outcomes and healthcare costs. Several studies have reported increasing rates of nosocomial infections as service members experience prolonged hospitalization and progress through higher echelons of care. Additionally, combat wound infections from drug-resistant or multidrug-resistant organisms have increased in military personnel that served in Iraq and Afghanistan.

The DoD Blast Injury Research PCO hosted the International State-of-the-Science Meeting on November 29 to December 1, 2016 at the RAND Corporation in Arlington, Virginia, to further explore prevention, diagnosis, and treatment of wound infections following blast-related injury. This meeting brought together subject matter experts from across the DoD, other federal agencies, academia, and industry to address meeting objectives (see Table 1) assessing the current state of the science in this important area.

To prepare for and inform experts participating in the 2016 International State-of-the-Science Meeting, the DoD Blast Injury Research PCO commissioned a review of recent research literature directed at minimizing the impact of wound infections following blast-related injuries. This resulting literature review addresses specific research questions addressing the above objectives.

The PCO convened a 29-member, interagency planning committee that included members from clinical and research programs from the Army, Navy, Air Force, DoD, National Institutes of Health (NIH), and Centers for Disease Control and Prevention (CDC) (see Appendix B for a full list of Planning Committee members). The role of the Planning Committee was to refine meeting objectives, guide the literature review and address findings with implications for the structure of the meeting, formulate meeting working group questions (see Working Group Summary section, page 43), and solicit meeting abstracts and select abstracts for meeting posters

Table 1. 2016 International State-of-the-Science Meeting

1. Determine predictive risk factors for wound infections following blast-related injuries, including individual susceptibility and environmental contributions, from point of injury through continued hospital care.
2. Identify candidate biomarkers that would enable rapid and accurate diagnosis, management, and prognosis of wound infections following blast-related injuries.
3. Examine prevention strategies, including vaccines, for mitigation of wound infections following blast-related injuries.
4. Propose strategies that would mitigate the impact of multidrug resistant, virulent, or opportunistic organisms on wound infections following blast-related injuries.

and presentations (see Topical Presentations, page 21, and Poster Abstracts, page 81). The Planning Committee also guided the selection of a five-member Expert Panel (see Appendix C for biographies of the Expert Panel members). Expert panelists were charged with chairing the working group sessions and prioritizing the major meeting findings and recommendations to advance the state-of-the-science on prevention, diagnosis, and treatment of wound infections following blast-related injury.

Over 120 participants from the DoD, the Department of Veterans Affairs, the NIH, academia, the civilian and military medical research community, and industry attended the meeting (see Appendix D for the participant list). The agenda (see Appendix E) consisted of an invited keynote presentation, plenary presentations relating key background science and policy, Planning Committee–selected scientific presentations, a poster session, concurrent working group sessions, and Expert Panel member briefings summarizing findings from their working group sessions. Following the meeting, an Expert Panel session reviewed meeting data and formulated recommendations. Selected presentations from the 2016 State-of-the-Science Meeting are available from the DoD Blast Injury Research PCO website at https://blastinjuryresearch.amedd.army.mil/index.cfm/sos/minimizing_impact_of_wound_infections.

This meeting proceedings summarizes background from the literature review (see Background Literature Review, page 17). The meeting proceedings cover three types of presentations. First, topical presentations set the stage for the meeting by providing important background science- and policy-related information. Second, research presentations described current and ongoing scientific investigations submitted for the meeting. Those scientific abstracts of sufficient quality that were submitted for the meeting but not selected for podium presentations were accepted as posters (see also Appendix H).

The meeting was a productive opportunity for the scientific community to engage in intensive, rigorous, face-to-face dialogue about the knowledge gaps and requirements for advancing the state of the science on wound infections following blast-related injuries. The consolidated outputs from the five working group sessions are presented in the Working Group Summary. Finally, outputs from the Expert Panel session are summarized in the State-of-the-Science Expert Panel Findings and Recommendations.

Literature Review Summary

Matt Aldag, Ph.D., discussed the findings and conclusions of the extensive literature review he led on wound infections following blast-related injury. The review was completed to facilitate the PCO Planning Committee preparation for the State-of-the-Science Meeting. The results of this review are available online in their entirety (at https://blastinjuryresearch.amedd.army.mil/assets/docs/sos/lit_reviews/
2016_SoS_literature_Review.pdf) and are summarized only briefly here.[4]

Wound infection following blast-related injuries continues to be a significant source of morbidity and mortality in the modern era of military healthcare. Approximately a quarter of combat wounds become infected, having significant impact on patient outcomes and healthcare costs. Several studies report increasing rates of nosocomial infections as patients experience prolonged hospitalization and progress through higher echelons of care. Additionally, combat wound infections due to drug-resistant or multidrug resistant organisms have increased in military personnel that served in Iraq and Afghanistan.

To inform the 2016 International State-of-the-Science Meeting, the DoD Blast Injury Research PCO requested a review of recent research literature directed at minimizing the impact of wound infections following blast-related injuries. This literature review addresses specific research questions about (1) predictive risk factors of wound infection following blast-related injuries; (2) identification of candidate biomarkers to advance wound infection diagnosis capabilities; and (3) emerging prevention and treatment strategies, including vaccines, in an era of antimicrobial resistance.

A. Risk Factors

Risk factors associated with combat wound infection include injury characteristics, such as mechanism of injury, severity of injury, and region of injury. Environmental characteristics and healthcare-associated exposures, such as blood transfusions, medical implants, and delayed antibiotic treatment, also contribute to increasing risk of infection. Improved approaches to diagnose and detect infection would promote better prediction of infection, earlier diagnosis, earlier treatment application, individually tailored treatments, and improved understanding of the epidemiology of wound infection.

B. Biomarkers

While CPGs guide detection and diagnosis of wound infection and provide recommendations for postinjury antimicrobials and antifungals, debridement, irrigation, surgical wound management, and facility infection control measures—from prehospital field care to regional Level IV hospitals—limited information is available about specific diagnostic capabilities across military treatment facilities. Development of novel objective biomarkers would enable faster and more precise wound infection diagnosis. National and international researchers from government,

[4] DoD Blast Injury Research PCO, "2016 International State-of-the-Science Meeting:
Minimizing the Impact of Wound Infections Following Blast-Related Injuries, Literature Review," Fort Detrick, Md., 2016.

private, and nonprofit organizations are seeking to develop novel infection biomarker approaches, including proteins and enzymes, proteomic analysis, metabolomics, next-generation sequencing, biofilm detection, electrochemical sensors, intelligent wound dressings, and digital microscopy.

C. Prevention and Treatment
In addition, these organizations are collaborating to develop new prevention and treatment approaches as alternatives to antimicrobials, including vaccines, passive immunological therapy, phage therapy, antimicrobial peptides, photodynamic therapy, quorum sensing, nanoparticles, iron chelators, lectin inhibitors, FimH inhibitors, lactoferrin, hypothiocyanite, bioengineered tissue, bacterial gene transfer, probiotics, and plant compounds.

D. Limitations
Providing healthcare in austere environments, increasing nosocomial transmission, and emerging drug-resistant infections present capability gaps in the mission to minimize wound infection following blast-related injury.

E. Research Needs
To bridge these gaps, experts and researchers have identified research needs in three areas. First, basic science studies are needed to better understand physiological processes (the pathophysiology of infection and immune response to infection, the association between biofilms—aggregated bacterial cells that are intrinsically resistant to antibiotics—and infection, and the mechanism of action for existing antibiotics and immunoprotection). Second, studies should focus on the military healthcare system, including continued epidemiological assessment of bacterial and fungal infection, assessment of the availability and use of diagnostic techniques for wound infection, and the delivery of antimicrobials following injury and subsequent infection rates. Third, studies should advance the development of novel products or methods for diagnosis, prevention, and treatment approaches, including biomarkers including biofilm detection methods, new vaccine candidates, and improved animal models that more accurately reflect clinical wound infection.

Keynote Address

John Holcomb, M.D., delivered the keynote address for the 2016 International State-of-the-Science Meeting. Holcomb is a combat-experienced trauma surgeon and has extensively published on combat surgical trauma. He pointed out that the phenomenon of war-related blast injury is anything but new and that (contrary to popular public perspectives) improvised explosive devices are not the leading cause of blast-related injury. After blast injury, the risk of infection is great, particularly after traumatic amputation. Furthermore, with higher rates of survival after battlefield injuries, the long-term outcomes of survivors are often complicated by wound infections and osteomyelitis. Unfortunately, the microbial wound infection diagnostics available to medical teams operating in theater has not kept pace with the significance of the problem.

Holcomb encouraged meeting participants to give special consideration to improving microbial diagnostics available to medical teams in theater. He pointed out that blood cultures and other biological samples are notoriously slow, lack adequate sensitivity, and are often inaccurate. Molecular methods, such as polymerase chain reaction (PCR), are much quicker and more sensitive, but their pathological significance is sometimes difficult to discern, and false positive results may be common. Holcomb also pointed out that standard antibiotic treatment for wound infections is often driven by custom rather than empirical studies, and that the appropriate course of treatment is likely to vary by the theater of operation, particularly local indigenous microbial flora.

Holcomb closed with a challenge: If we can use molecular diagnostics for blast-related wound infections to (1) reduce antibiotic use and duration, (2) improve accuracy of antibiotic decisions, and (3) reduce antibiotic resistance, it would improve patient outcomes, decrease costs, and “dramatically change both the military and civilian world.”

Topical Presentation Summaries

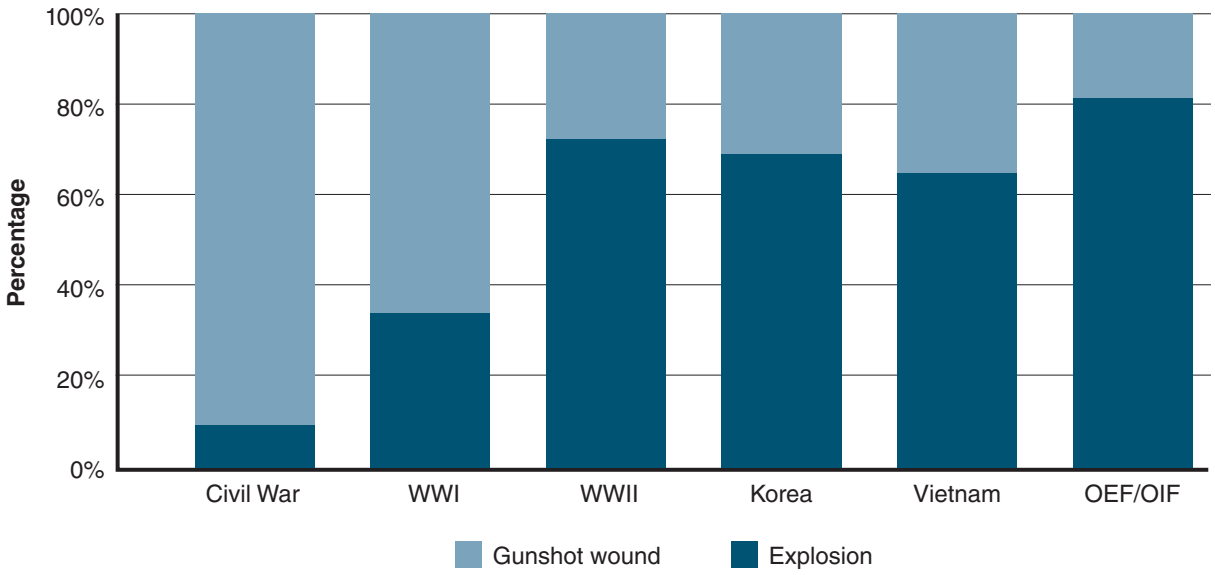
Following the keynote presentation, speakers from government and academia presented information on key topics, setting the stage for the meeting by identifying requirements, defining the scope of the problem, addressing policy issues, and describing the state of the science. The topic presentations discussed DoD perspectives, the activities of federal research partners, and assessments from academic research and industry.

Kirby Gross, M.D. (Colonel, Medical Corps, U.S. Army) addressed "Battlefield Blast-Related Wound Epidemiology and Clinical Management." Gross has deployed seven times to various theaters of operation, and these experiences have shaped his emphasis on early decisions in traumatic injury management.

Gross noted that 80 percent of the wounds in OIF/OEF have been from explosions (see Figure 1), as compared to only 10 percent of Civil War wounds (although the accuracy of this figure is open to question).[5]

Among the challenges related to determining the frequency of combat-related blast injury include variations in sample source (e.g., combat versus other types of units, different echelons of care). Virtually every traumatic injury mechanism (penetrating, blunt, thermal, crush) may be involved in blast injuries, making these injuries clinically complex and difficult to categorize.

Figure 1. Primary Mechanisms of Injury in U.S. Wars



[5] Sidney B. Brevard, Howard Champion, and Dan Katz, "Chapter 2, Weapons Effects," in *Combat Casualty Care Lessons Learned from OEF and OIF*, eds. Eric Savitsky, Brian Eastridge, Dan Katz, and Richelle Cooper, Fort Detrick, Md.: Borden Institute, 2012, p. 43.

As seen in Table 2, blast injuries may be primary (blast and accompanying blast overpressure), secondary (fragments of the exploding weapon or other flying objects), tertiary (when blast propels the body or when structures collapse on victims), quaternary (burns, inhalation injury, toxic exposures, or illnesses), or quinary (injuries from explosive additives, such as radiation).

Morbidity and mortality after blast injury is mainly a function of distance from the explosion. The impact of the blast wave decreases rapidly with distance from the explosion (see Figure 2).

However, secondary blast injury effects are typically the major cause of morbidity, leading to a myriad of complications, including polytrauma (e.g., traumatic brain injury; abdomen, thorax, spine, and genitorectal injuries; fractured pelvis and extremities; severe soft tissue injuries) that often results in massive bleeding, requiring transfusions and elevating risk of wound infection.

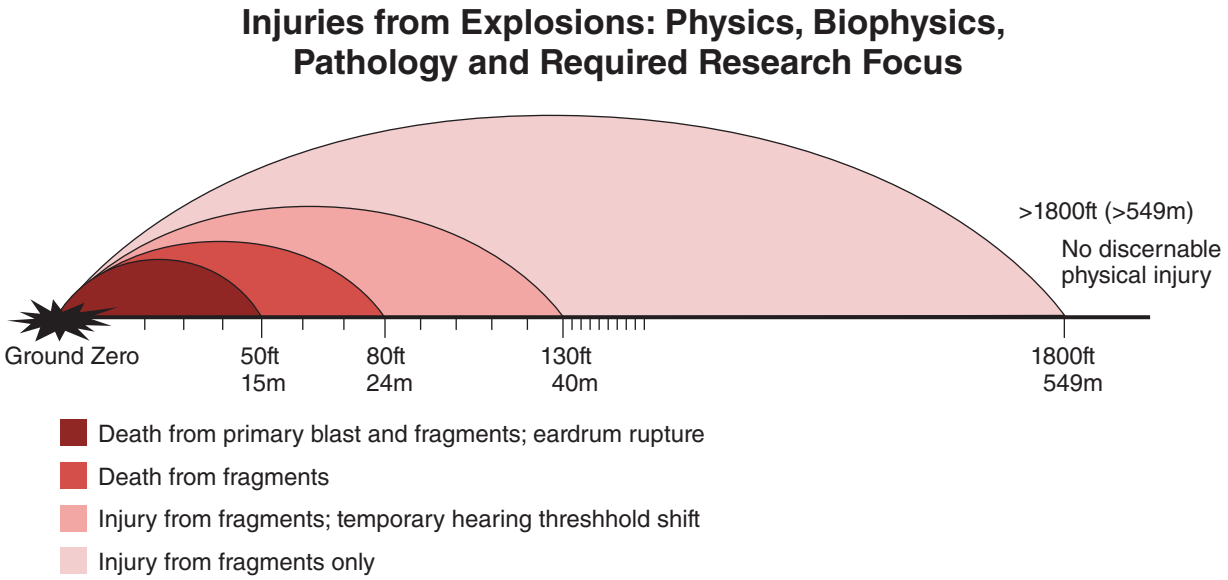
Finally, Gross emphasized the importance of efforts to improve the consistent delivery of medical care to the blast injury survivors and reviewed data from of a study of adherence to the Joint Trauma System Tactical Combat Casualty Care Clinical Practice Guidelines. Adherence to

Table 2. DoD Nomenclature for Blast Injury Categories After Explosions

Category	Definition	Typical Injuries
Primary	- Produced by contact of blast shockwave with body - Stress and shear waves occur in tissues - Waves reinforced and reflected at tissue density interfaces - Gas-filled organs (lungs, ears, etc.) at particular risk	Tympanic membrane rupture; Blast lung; Eye injuries; Concussion
Secondary	- Ballistic wounds produced by primary fragments (pieces of exploding weapon) and secondary fragments (environmental fragments, like glass) - Threat of fragment injury extends further than that from blast wave	Penetrating injuries; traumatic amputations; lacerations; concussion
Tertiary	- Blast wave propels individuals onto surfaces/objects or objects onto individuals causing whole body translocation - Crush injuries caused by structural damage and building collapse	Blunt injuries; crush syndrome; compartment syndrome; concussion
Quaternary	Other explosion-related injuries, illnesses, or disease	Burns; toxic gas and other inhalation injury; injury from environmental contamination
Quinary	Injuries resulting from specific additives, such as bacteria and radiation (“dirty bombs”)	

SOURCE: H.R. Champion, J.B. Holcomb, and L.A. Young, “Injuries from Explosions: Physics, Biophysics, Pathology, and Required Research Focus,” *Journal of Trauma and Acute Care Surgery*, Vol. 66, No. 5, 2009, pp. 1468–1477.

Figure 2. Morbidity and Mortality as a Function of Distance from Open-Space Detonation of a 155-mm Shell



Morbidity and mortality as a function of distance from open-space detonation of a 155-mm (220 lb, ~100 kg) shell. Distances are in proportion from ground zero to 130 feet (40 m). The interval between 130 feet (40 m) and 1,800 feet (549 m), however, is too large to allow proportional spacing.

HR Champion, JB Holcomb and LA Young, *Injuries from Explosions: Physics, Biophysics, Pathology and Required Research Focus*. J Trauma. 2009;66:1468-1477.

guidelines varied greatly across surgeons, ranging from 48 percent to 90 percent across 13 key standard guideline practices. Surgeons' adherence to individual practices (e.g., initial pain score, intra-abdominal antibiotics, deep-vein thrombosis prophylaxis) ranged from 40 percent (intra-abdominal antibiotics) to 100 percent (avoiding penicillin use as a soft-tissue antibiotic).

Matthew Bradley, M.D. (Lieutenant Commander, Medical Corps, U.S. Navy) discussed treatment challenges associated with the treatment of blast wounds. He reviewed data showing that of 1,544 extremity amputations during OIF/OEF, approximately 25 percent were bilateral lower extremity amputations (BLA) and asserted that an understanding of wound healing in these patients may improve diagnostics and therapeutics. Bradley reviewed traumatic amputation patients for whom the difference between a good outcome and a bad outcome—such as critical colonization ($\geq 10^5$ colony forming microbial units per gram of tissue tested), wound dehiscence, heterotopic ossification, or all three—was difficult to predict through visual clinical examination alone. His research evaluated whether inflammatory response might help identify traumatic amputation patients at greatest risk for these consequences. Bradley found

that about 80 percent of BLA patients were critically colonized, compared to about 20 percent of combat-related extremity wound patients overall. The most common bacterial isolate in the BLA group was *Enterococcus*, followed by *Acinetobacter baumanii*; in the control group, *Acinetobacter baumanii* was the most common bacterial wound isolate. The BLA group also had a distinct cytokine response pattern compared to control patients with other combat-related extremity wounds, a finding that held for testing of both serum and wound exudates. BLA patients who were critically colonized, those with wound dehiscence, and those with heterotopic ossification manifested comparatively distinct local and systemic cytokine responses; those with wound dehiscence showed a predominant local cytokine response and those with heterotopic ossification showed a more predominant systemic cytokine response. Researchers hypothesized that combat-related extremity wounds might mount a distinct local inflammatory response if associated with additional distant trauma and found that patients with multiple extremity wounds did have a distinct systemic cytokine response. The key finding was that the deregulated response in BLA patients was associated with wound dehiscence, additional distant trauma, and the development of heterotopic ossification. Bradley reported that his team is translating these findings into potential clinical practice improvements using an advanced bioinformatics tool to generate clinical decision models.

Paige Waterman, M.D. (Lieutenant Colonel, Medical Corps, U.S. Army) discussed the role of antibiotics in treating blast-related wound infections, focusing on the problem of antibiotic resistance. Waterman noted that the problem of antibiotic resistance was first discovered in Japan in the 1960s and has since grown into what is now widely viewed as a global problem with health policy implications. For example, in 2014, a White House[6] and subsequent National Action Plan[7] addressed the issue (as seen in Table 3), and the 2016 O'Neill Report[8] recommended a number of steps, including increasing awareness, improving sanitation to prevent spread of potentially resistant organisms, reducing agricultural use of antibiotics, global surveillance efforts, identifying and adopting of rapid diagnostics to reduce antibiotic use, developing therapeutic alternatives, and creating economic incentives for reduced antibiotic use.

Waterman noted that the issue of antimicrobial resistant organisms has direct relevance to the management of combat-related wound infections. Wounded soldiers are sometimes held in

Table 3. Combating Antibiotic Resistant Bacteria (CARB) National Action Plan Objectives

1. Promote the judicious use of antibiotics in health care and agriculture.
2. Strengthen surveillance efforts and data collection.
3. Further the development of new diagnostics.
4. Support research efforts for the development of new therapeutics.
5. Improve international collaboration on efforts to prevent and control the spread of antibiotic resistance.

[6] Office of the Press Secretary, White House, "Executive Order—Combating Antibiotic-Resistant Bacteria," Washington, D.C., September 18, 2014.
[7] White House, "National Action Plan for Combating Antibiotic-Resistant Bacteria," Washington, D.C., March 2015.
[8] J. O'Neill, "Tackling Drug-Resistant Infections Globally: Final Report and Recommendations: the Review on Antimicrobial Resistance, Review on Antimicrobial Resistance," May 2016.

theater alongside individuals with locally unique bacterial flora before being transported to other locations. These organisms develop resistance to antibiotics over the course of treatment and are then transferred from patients to clinical provider staff, propagating the spread of unique and resistant organisms across the military health system and elsewhere. Waterman said that to address this problem, the Office of the Under Secretary of Defense for Personnel and Readiness is currently formulating a DoD Instruction for CARB. Potential policy gaps the DoD Instruction might address include stewardship, enhanced surveillance for multidrug-resistant organisms, reporting requirements, development and evaluation of diagnostics, and experimental therapeutics activities. Potential DoD activities involved may include the Center for Infectious Disease Research, the Global Emerging Infectious Diseases Section, the Navy Marine Corps Epidemiologic Data Center, and the Army Pharmacovigilance Center.

Waterman closed with a review of the spread of multidrug resistant *Acinetobacter baumannii* from 2003 to 2011. She said that whole genome sequencing was used to elucidate the evolution of the six main strains of multidrug resistant organisms, and similar methods may be used for early detection of antibiotic-resistant bacteria in the future.

Terry Rauch, Ph.D. offered a "high-level review" of research policy as it relates to blast-related wound infection. Rauch, the director of Research and Development Policy and Oversight in the Office of the Assistant Secretary of Defense for Health Affairs, emphasized that while improved health, better care, and lower costs are important, the top DoD priority is military readiness. To this end, policy-relevant strategic guidance comes from many directions and places. Rauch cited the 2015 Precision Medicine Initiative, which provided $215 million to the 2016 budget, as an example of funding that might be leveraged for wound infection research. He observed that the recently passed fiscal year (FY) 2017 National Defense Authorization Act prescribed sweeping changes for DoD health care, including medical research, but the complete implications of this legislation are still unclear. The 2015 White House National Strategy for CARB emphasized collaboration across the FDA, U.S. Department of Agriculture, CDC, NIH, DoD, and Environmental Protection Agency and targeting current research resources to focus on high-priority antibiotic resistance issues. Rauch mentioned that one of the meeting's goals was to ensure that by 2020, data sets on antibiotic resistance generated through federally funded research, including genomic and proteomic data sets, will be publicly available through searchable online databases that protect personally identifiable information.

Rauch then reviewed the DoD strategic policy research drivers, such as the 2015 National Military Strategy, the 2015 Joint Concept for Health Services, and the robust military medical lessons learned literature. He suggested these sources offer valuable info on wound diagnosis and treatment. He also cited a Secretary of Defense memorandum with key guidance on resource allocation for military health system mission-essential research, including support for the CARB National Action Plan, infectious disease surveillance in support of the 2013 National Biosurveillance Science and Technology Roadmap,[9] and combat casualty care research.

[9] Office of the Assistant Secretary of Defense, "Memorandum for Director, Defense Health Agency, Fiscal Year 2017 Defense Health Program Interim Guidance for Research and Development Planning Activities," Washington, D.C., September 23, 2016.

MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

Colonel Michael Kozar reviewed the Military Infectious Disease Research Program (JPC-2) under U.S. Army Medical Research and Materiel Command. He directs the program, which focuses on appropriate infectious disease prevention, diagnosis, and treatment for the warfighter. The activity operates worldwide research laboratories in the United States, Peru, Kenya, Georgia, Egypt, Thailand, Singapore and Cambodia, some of which have operated for over 50 years. The focus is on naturally occurring threats, excluding Ebola, which is part of the chemical-biological portfolio. Most of the JPC-2 research funding addresses malaria drugs, malaria vaccines, dengue fever, Zika, and other vector-borne diseases. Within their bacterial diseases portfolio are a number of preclinical and early clinical emphases, advanced development work on rapid quantitative diagnostics for multidrug resistant organisms (MDRO), and fielded products (advances in antibiotic prescribing practices and FDA-approved single-site trial of Arbekacin, an antibiotic for MDRO). They are also engaged in vector research (e.g., transmission of Zika virus), field expedient mosquito repellants (e.g., DEET-laced camouflage makeup), and various Army and Defense Health Program (DHP) Task Areas (see Figure 3).

Figure 3. Army and DHP Task Areas

Army

Parasitic Diseases Research
- Anti-Parasitic Drug Development
- Malaria Vaccine Research

Viral Diseases
- Flavivirus Vaccine Research
- Lethal Virus (Hantann, Puumala) Countermeasures

Bacterial Diseases
- Prevention of Diarrheal Diseases
- Rickettsial Diseases

Vector Control
- Identification and Control of Insect Vectors of Infectious Diseases
- Rickettsial Diseases

Diagnostic Systems
- Diagnostic Systems for Infectious Diseases(far forward, rapid & easy to use point-of-care tests)

- Portfolio Managed by IIPT
- Intramural (WRAIR, NMRC, USAMRID) Awards

DHP

Viral Diseases
- Acute Respiratory Diseases/Emerging Infectious Diseases

Bacterial Diseases
- Antimicrobial Countermeasures
- Wound Infection Prevention and Management (applied & translational product development)

Diagnostic Development
- Diagnostic Systems for Infectious Diseases (integrated platform with multiple ID panels at role 3 and higher)

DHP Named Programs
- Military HIV Research*
- Combatting Antimicrobial Resistance*
- Deployed Warfighter*

- Portfolio Managed by IIPT
- Performers: Intramural (all DoD labs) and Extramural Awards/Grants

*Named Programs

Presentation figures and tables are attributable to the presenter unless otherwise attributed.

MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

Combat wound infection is a particularly important focus for the military because NIH does not work in this area. Here, JPC-2 goals and objectives relate to prevention, diagnosis and treatment and are summarized as (1) developing tools for early detection, identification, and characterization of drug-resistant organisms that cause wound infections; (2) discovering, characterizing, and developing host immune response and pathogen biomarkers associated with infection to inform clinical wound-management decisions; and (3) developing novel therapeutics and delivery technologies against wound infection pathogens and biofilm processes. They often focus on high-risk/high–pay off treatments or nontraditional treatments—areas that the private sector would not usually consider profitable enough to pursue.

Total JPC-2 funding from FY 2011 to FY 2015 was $126.8 million ($60.8 million core bacterial diseases funding, $59.1 million in Congressional Special Interest funding, and $6.9 million Small Business Innovation Research), with funding peaks in 2011 and 2015. Current research investments related to blast injury related infection are scattered from Technology Readiness Levels Two through Six and address rapid biomarker detection of infected wounds; optimization of clinical management of infected wounds, including MDROs in combat wounds; strategies to prevent, inhibit, and disperse biofilms; therapeutics for MDROs; and novel antibiotic delivery systems for field use. Kozar closed with more detailed descriptions of several key projects of relevance to blast-related wound infections. The first involved development of a rapid microbiological diagnostics platform for MDRO quantitative identification and resistance phenotyping to guide antibiotic selection in wounded warriors and veterans. This fully automated platform can identify bacteria within 90 minutes directly from a blood culture bottle and determines antibiotic sensitivities within seven hours. The current plan is to validate assays for wound swab samples. A second project was a biologically active antimicrobial human skin substitute for treating combat wounds infected with multidrug resistant (MDR) wound pathogens. When grafted, this skin substitute is bioengineered to provide sustained expression of human host defense peptides to expedite skin wound regeneration. A third effort involved testing of recombinant interleukin-12, a broad-spectrum biologic for the treatment of battle and traumatic wound infections. This is a phase two clinical trial that could lead to the production of an anti-infectious and healing accelerator to prevent amputations and repetitive surgeries in wounded military personnel. The fourth project tested bacteriophages as an alternative or adjunctive therapeutic for prevention and treatment of wound infections. The Naval Medical Research Center's Biological Defense Research Directorate and Infectious Diseases Directorate has developed libraries of naturally occurring phages, including phages against S. aureus, A. baumannii, and P. aeruginosa. This appears to be very promising work, with studies to date having demonstrated the efficacy of phage cocktails from these libraries in mouse wound infection models and an anecdotal report of efficacy in a patient treated under an FDA Emergency Use Authorization.

Michael Pucci, Ph.D. executive director of Early Drug Discovery at Spero Therapeutics in Cambridge, Massachusetts, provided an industry perspective of anti-infective drug development. Pucci said that in general industry no longer anticipates significant economic returns on antibacterial agents compared with other drugs. The drug review process is long, antibiotic use is relatively short in duration, and the expected onset of resistance has resulted in

diminishing returns. In addition, he said, genomic research has not yielded the dramatic results that some expected, and the bar is high for developing drugs—regulations are increasingly strict, compounds must reach bacteria but penetrating membranes is difficult, clinical trials are prohibitively expensive, and antibiotic resistance can often derail even the most promising agents. Only about 12 percent of antibiotics make it through the full development process, and these drugs have to turn enough profit to support the development of the drugs that succeed as well as those that fail. The fact that some of these drugs are curative might seem attractive to investors, but ironically it is not.

NIH funding for studies of antimicrobial drug resistance has been reasonably steady over time with more funding available in recent years, and the United States remains the leading funder in the world in this area. The FDA Generating Antibiotic Incentives Now (GAIN) Act is recent legislation that extends by five years the exclusivity period during which certain antibiotics (those that treat serious or life-threatening infections) can be sold without generic competition. This has created some hope for profit, and industry likes the idea of marketing exclusivity and associated potential for fast-track FDA approval processes.

Pucci concluded with the observation that antibiotic development has lacked innovation in recent years and, oddly, most of the antibiotics used today were discovered soon after antibiotics arrived on the clinical scene. The last major antibiotic development push was in the 1980s. However, partnerships involving government, academia, and industry can help to advance research in this increasingly important area.

Emerging Science Presentation Summaries

David Tribble, M.D., DrPH provided an overview of blast wound infection epidemiology and microbiology based upon the Trauma Infectious Diseases Outcomes Study (TIDOS). Combat-related infectious complications have changed over the last century. In the preantibiotic era, open wounds would be infected with anaerobes and streptococci in the first week, pyogenic streptococci in the second, and streptococci and staphylococci in the third. Since the introduction of penicillin and other antibiotics, surgical techniques have improved decreasing anaerobic soft tissue infections (i.e., Clostridial infection). There has also been increased recognition of hospital-acquired infections. Unfortunately, this has been accompanied by increasing rates of antibiotic resistance and greater prevalence of nosocomial pathogens, such as methicillin-resistant *Staphylococcus aureus* infection (MRSA), *E. coli*, *Acinetobacter spp.*, and *Enterobacter spp*.

Today, one-third of soldiers with combat-related injuries will develop infections. Table 4 lists the most common infections associated with blast trauma and the associated organisms.

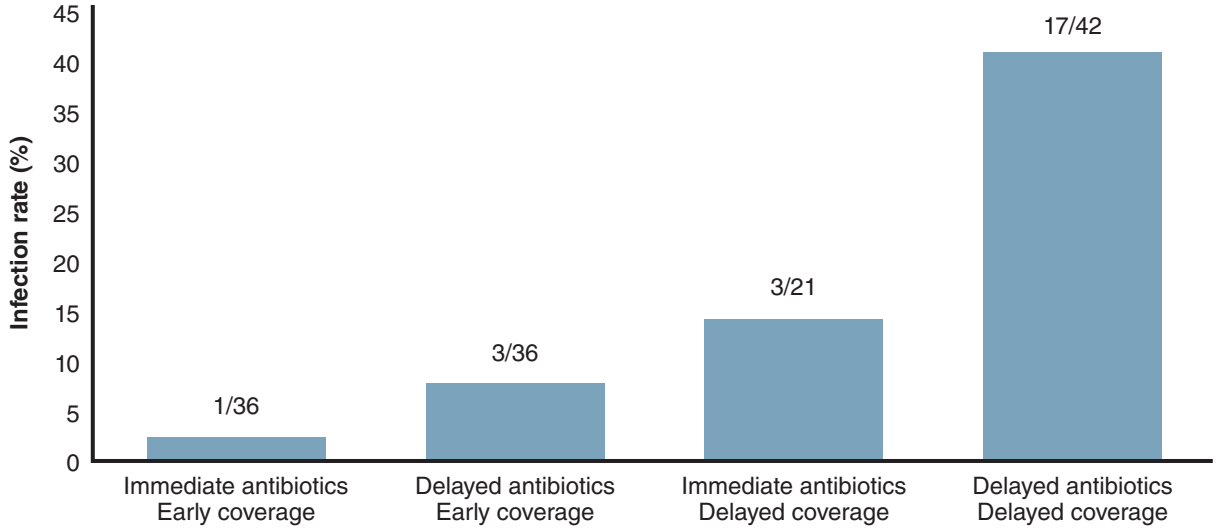
Risk factors include amputation, bloodtransfusion, intensive care unit admission, mechanical ventilation, and mechanism of injury. To combat these issues, and others, several prevention and management techniques are utilized. Tribble described the importance of wound coverage and immediate antibiotics to reduce the risk of osteomyelitis in open fractures (Figure 4). Second he summarized extremity wound microbiology. In OIF/OEF, polymicrobial and multidrug resistant infections were very common, often the result of inoculation with environmental and biological debris. As would be expected, causative agents differed depending upon the theater of injury.

Table 4. Blast Trauma Infection Syndromes

Infection Syndrome	% of Blast Trauma Patients	Median Time to Diagnosis Postinjury (Interquartile Range)	Top Three Organisms	% MDR	% Poly
Skin and soft-tissue infection (SSTI)	18.0	8 (4–16)	*E. faecium, P. aeruginosa, E. coli*	36.5	56.9
Osteomyelitis	3.5	16.5 (8–26.5)	ACB complex, *P. aeruginosa, E. coli*	44.2	54.8
Bloodstream infections	6.7	7 (4–14)	Coagulase-negative *staphylococci, P. aeruginosa,* ACB complex	29.3	32.3
Pneumonia	7.9	5 (3–8)	*P. aeruginosa,* ACB complex, *C. albicans*	33.9	54.5
Intra-abdominal infections	1.0	16 (10–21)	*P. aeruginosa, K. pneumoniae, C. albicans*	24.1	41.4

OIF infections were commonly multidrug-resistant *Acinetobacter baumannii-calcoaceticus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. Flora samples in OEF found invasive molds, and starting in 2009, a transition from *Acinetobacter spp*. to *E. coli*, extended spectrum beta-lactamase–producing bacteria, and multidrug-resistant gram-negative bacilli. Unusually, recurrent osteomyelitis was associated with *S. aureus* as the causative agent. A systematic assessment of microbiological factors and their impact on health risk, incorporating clinical microbiology, and surveillance of long-term outcomes are needed to improve prevention and treatment (see Figure 4).

Figure 4. Timing of Antibiotics and Wound Coverage Prevent Infection



Lack WD et al., J Orthop Trauma, 2015

A common theme running through the panels was the importance of building upon earlier research. This includes a blueprint for in-theater research, interdisciplinary information sharing, and real-time adaptation of data collection modules. Injury and better classification of injury and interventions are needed in the DoD Trauma Registry and Military Orthopedic Trauma Registry. The data need greater breadth and granularity, such as tracking injury severity levels. The experts similarly argued for improving cataloguing of sterile isolates, using serum and tissue repositories, and partnering with the civilian sector. All of these concepts would be strengthened if they are implemented before the next conflict.

Dr. Laveta Stewart, Ph.D., M.Sc., MPH spoke further on the use of TIDOS to calculate the rate of infection at the patient and wound level. TIDOS can be used to map wound and infection location, complexity, and severity. Within each patient classification, subjects with blast injuries had more infectious outcomes. More than 80 percent of subjects with one or more extremity wounds had a blast injury. Among the blast trauma patients, 23 percent experienced an extremity wound infection at a rate statistically higher than non-blast injured

patients. Categorizing patients by their most severe extremity injury highlighted that highest rates of extremity wound infection among patients with traumatic limb amputations (54 percent), compared to patients with open fractures of long bones (15 percent), and other patients with neither amputation or open fractures (3 percent) While subjects with blast-related injuries disproportionately have more infection, the representation of bone involvement is similar between blast and non-blast injured patients.

Katrin Mende, Ph.D. utilized the TIDOS to study the microbiology of complex combat-related extremity wounds. 95 percent of patients with infected wounds had organisms recovered, frequently multiple bacteria, molds, and yeasts. About 61 percent of the confirmed infections were polymicrobial, with 60 percent bacteria only and 30 percent a combination of bacteria and molds. Future analyses will determine the incidence of MDROs in combat extremity wounds and assess the impact on clinical outcomes.

Dr. Felipe Lisboa, M.D. presented on whether persistent critical colonization may be associated with different host response and healing outcomes. Lisboa and colleagues asked if successful healing of critically colonized wounds closed with critical colonization was associated with a distinct gene expression and cytokine response. In OIF/OEF, characterisation of explosive wounds have ranged from soft tissue injuries to complete traumatic amputations. High levels of bacterial colonization may interfere in normal wound healing. Critical colonization is defined as culture growth of at least 10^5 colony forming units by gram of tissue or microliter of wound effluent sample. More than 35 percent of patients may develop critical colonization in one or more of their wounds.

Lisboa's study tested 116 wounds from 73 patients' tissue biopsy and wound effluent samples by quantiative bacteriology. They confirmed that criticial colonization was associated with wound failure. Acinetobacter was the mostly commonly identified bacteria. Successful healing was associated with an increase of vascular growth factor beyond the initial three debriedments and a decrease in epethilial growth factor. All criticially colonized wounds also had increased gene expression of IL-1ß, IL-1ra, MCP-1, MIP-1α, and MIP-1ß as well as decreased CCL5/RANTES. Association with specific cytokine and gene response may be integrated into future clinical decision support tools.

New Antibiotic Therapeutics

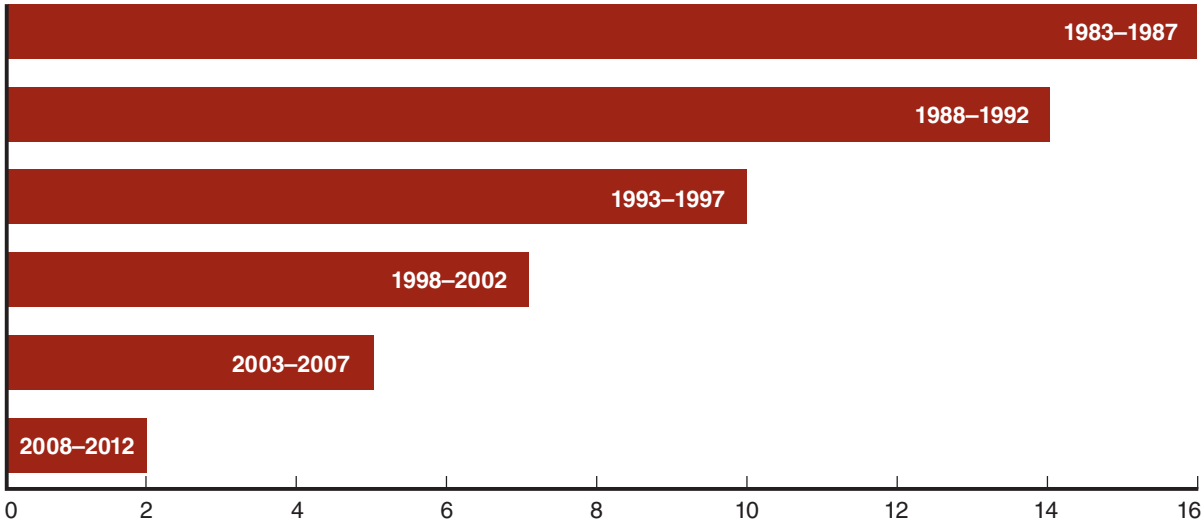
LTC Stuart Tyner, Ph.D. presented an overview of the current state of progress toward new antibacterial therapeutics for traumatic wound infections. To combat antibiotic-resistant bacteria, there are four streams of research into new treatments: small molecules, immunotherapy, phage therapeutics, and other novel therapeutics. Small molecules development includes antibiotic adjuncts that enhance the efficacy of current antibiotics or resensitize bacteria that have become resistant. Immunotherapy utilizes broad spectrum antibiotics directed at multiple virulence targets and epitopes. This multivalent approach targets different aspects of pathogenesis at multiple stages of a bacterial life cycle to prevent the development of drug resistence.

MAJ Chad Black, DVM, Ph.D. expanded on Walter Reed Army Institute of Research (WRAIR) research on small molecules. As displayed in Figure 5, antibiotic development has decreased from 16 agents in 1983 to two in 2012. WRAIR's experimental therapeutics division is primarily focused on developing the next generation of marlaria prophylaxis. Using the same antiparasitic paradigm and a five-year timeframe, WRAIR uses a gated-tier drug discovery approach:

1. targeted product profile developed with DoD infectious disease physicians
2. overall testing strategy created to find desired products
3. relevant assays identified to accomplish mission.

Meanwhile, the team assesses biological activity, physicochemical properties, and ADMET (drug absorption, distribution, metabolism, excretion, and toxicity).

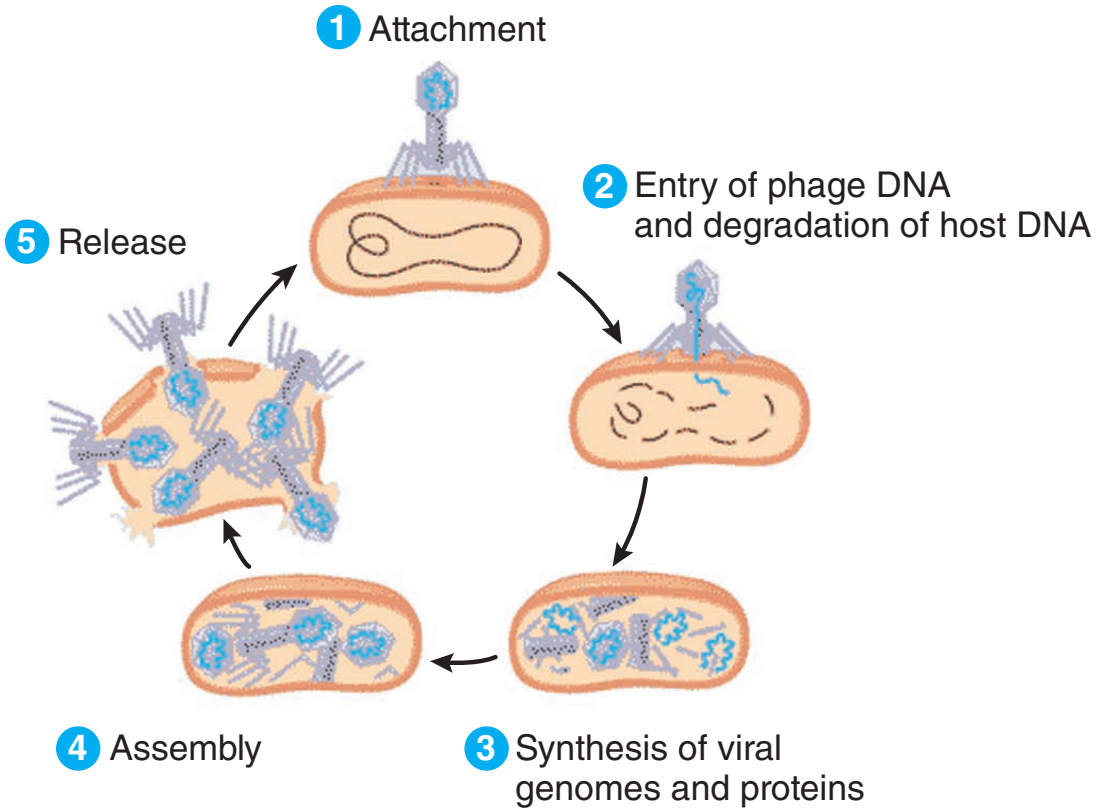
Figure 5. Antibiotic Development is Dwindling



Dr. Anna Jacobs, Ph.D. reviewed WRAIR's pre-clinical assessments of bacteriophage treatments for MDR bacterial wound infections. The majority of these infections are caused by the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*) pathogens and have been found to be highly resistant to standard of care antibiotics, such as imipenem and colistin. Based on a report by Pew Charitable Trust,[10] 37 antibiotics are in the development pipeline, but none of them are applicable to complex wound infections.

Bacteriophages are viruses that exclusively infect bacteria. Lytic phages specifically infect, replicate inside, and then lyse and destroy the host bacteria (see Figure 6). Phage therapy was popular in the United States until the antibiotic era and is still used in parts of Eastern Europe. Phages can penetrate mature biofilms and cause bacterial cell lysis without damaging the host microbiome. They can be used to treat an active infection or for prophylaxis.

Figure 6. Lifecycle of a Bacteriophage



[10] K. Talkington, C. Shore, and P. Kothari, "A Scientific Roadmap for Antibiotic Discovery," PEW Charitable Trusts, 2016.

Jacobs' team sought to develop personalized therapeutic cocktails and developed a library-to-cocktail approach to be delivered by systemic and topical applications. Future issues include administering phages, phage doses, phage activity against biofilms, and phages as adjunct therapy to antibiotics.

Dr. Magda Barbu, Ph.D offered a private-sector perspective on phage therapeutics using synthetic biology. Host range, biofilms, and resistance are three of the major challenges facing phage therapy (see Table 5). Synthetic genomics is overcoming the need for large phage cocktails with host range expansion.

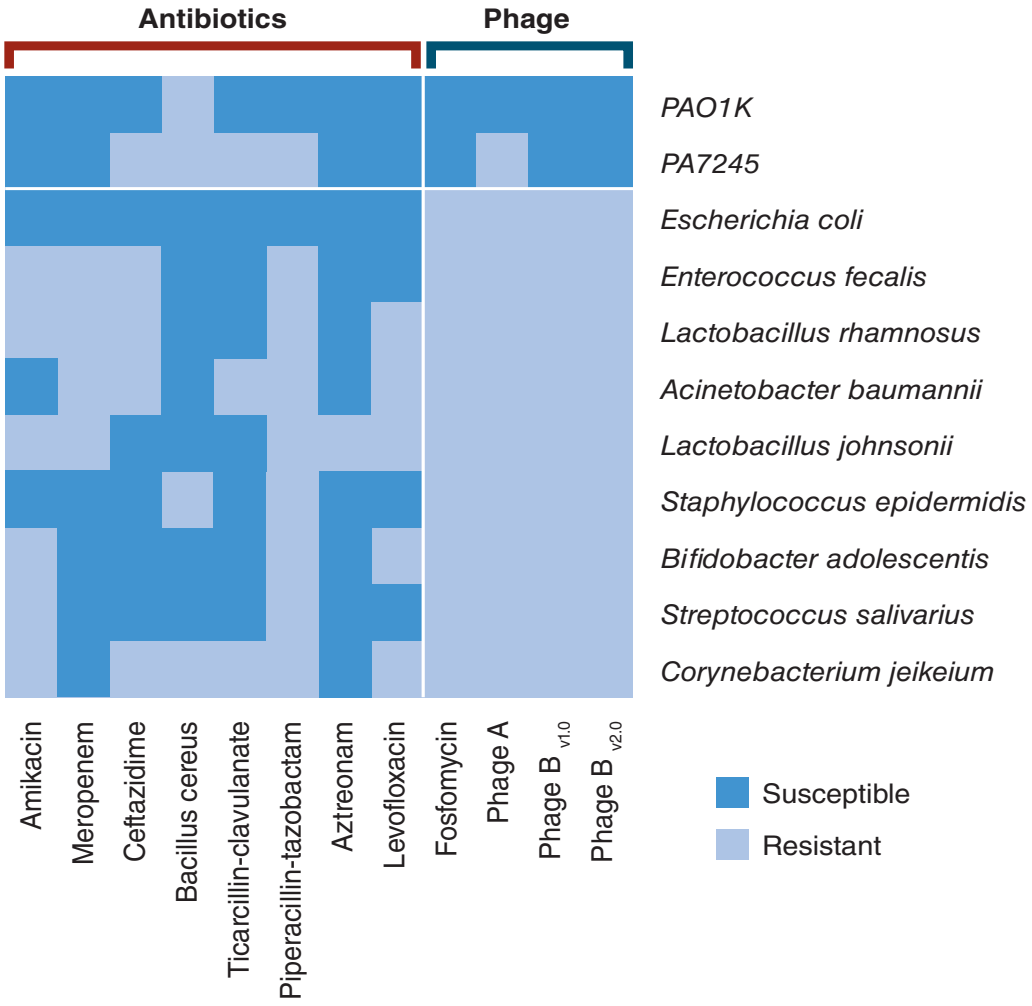
Biofilm-related infections are difficult to treat. For example, *P. aeruginosa* is associated with chronic otitis, chronic wound infections, and cystic fibrosis–associated infections. Synthetic Genomics is engineering multiple biofilm disruption agents in clinical isolates of *P. aeruginosa*. Testing a range of antibiotics and phage therapies against several bacteria revealed that: (1) antibiotics also eliminate commensal flora, (2) phages do not contribute to the emergence of antibiotic resistance or affect the microbiome, and (3) parental and engineered phage infect only target bacteria (see blue squares in Figure 7).

Monique van Hoek, Ph.D. described bioprospecting for antimicrobials and wound healing peptides. Cationic antmicrobial peptides (CAMPs) are a large and diverse "prescreened" library of antimicrobials. CAMPs are an ancient defense mechanism against infections that contribute to innate imunity with broad spectrum antimicrobial effectiveness. So far, more than 200 peptides have been screened against a panel of pathogens, including multidrug-resistant strains. Experiments with DRGN-1, a synthetic peptide inspired by American alligators' microbiology, determined that it has antibiofilm effects against both *Pseudomonas* and *Staphylococcus*, has host-directed effects for wound healing in vivo, and showed no cytotoxic effect at concentrations up to 100 micrograms per milliliter. Van Hoek's analysis of extreme species, like the American alligator, yielded multiple peptides that are effective against one or more bacteria, including several that are effective against antibiotic-resistant streams and/or demonstrate potent wound healing properties.

Table 5. Advantages and Disadvantages of Phage Therapy

Pros	Cons
Generally regarded as safe	Very limited host specificity
Abundant in nature (10^{31} virions)	Complex cocktails are needed to cover one species and prevent emergence of resistance
Highly specific	Immune clearance or neutralization
Self amplifying and self limiting	Only a subset can penetrate biofilms
80–90% efficacy in unregulated trials	Licensing and regulatory challenges
Approved as a food additive in the United States—EcoShield™ (for red meat) and ListShield™tates (for ready-to-eat foods)	Difficult to obtain intellectual property rights

Figure 7. Phage Versus Antibiotic Resistance Among Common Pathogens: Engineered Phage Demonstrated Target-Specific Killing *In Vitro*



MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

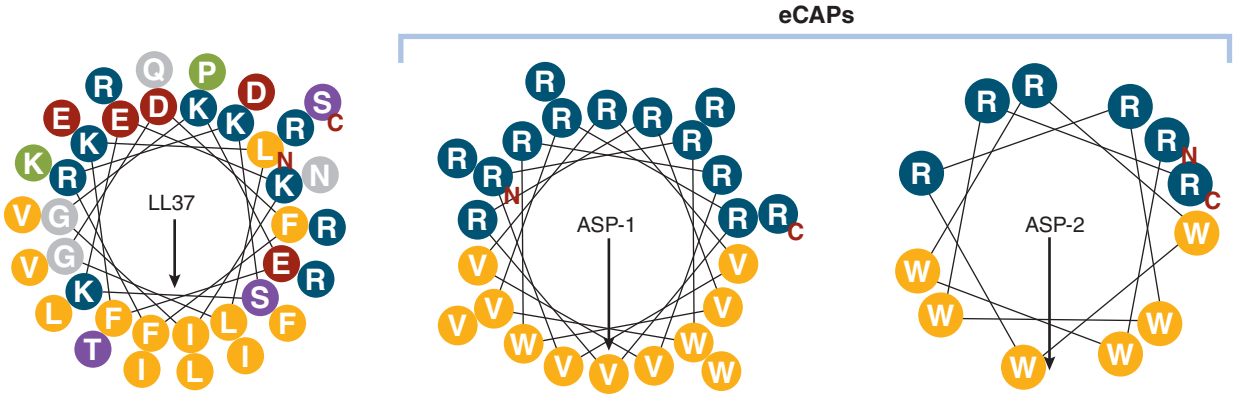
Jennifer Neff, Ph.D. shared another study of a novel peptide-based treatment with a focus on current treatment resistance. In addition to adaptive resistance mechanisms that bacteria develop to specific antibiotics, it is important to consider innate resistance associated with dormant bacteria and biofilm formation. Most conventional antibiotics were developed against targets in metabolically active bacteria and have poor activity against dormant bacteria in biofilms. Peptides have a lower propensity to invoke resistance than other conventional antimicrobial agents. CAMPs are ubiquitous and structurally diverse effector molecules that represent the first line of defense against microbial pathogens. Antimicrobial peptides generally recognize microbial organisms by electrostatic interactions with highly electronegative bacterial surface lipids. These cationic, amphipathic molecules typically consist of 12 to 50 amino acids, are prokaryotes to humans, and have shown antibacterial, antifungal, antiviral, and antiparasitic activity. Despite their flexibility, antimicrobial peptide activity is limited to a narrow range of tissues and conditions, is susceptible to proteolytic degradation and protein binding, and loses activity in high-salt conditions and in the presence of divalent cation.

Engineered Cationic Amphiphathic Peptides (eCAPs) are modeled after the membrane active, lentivirus lytic peptide (see Figure 8). Helical wheel analysis for peptides ASP-1 and ASP-2 were compared to the natural antimicrobial peptide, LL-37. Many antimicrobial peptides display an amphipathic conformation where they have positively charged and hydrophobic groups segregated onto opposite faces of an alpha helix, beta-sheet, or other tertiary structure. These features help in bacteria membrane binding and destabilization. Figure 8 shows how these features have been exaggerated in the two eCAPs. ASP-1 and ASP-2 were found to be effective against metabolically active and dormant bacteria, and had a lower propesnity to invoke resistance than other conventional antimicrobial agents. In a mouse model, it eradicated P. aeruginosa septicemia. It was also effective against ESKAPE pathogens in planktonic form, making it another candidate as a biofilm disruption agent.

By reducing bacteria contamination and the inflammation associated with that, an eCAP based treatment has potential to reduce risks for burn wound conversion. ASP-1 has an excellent

Figure 8. Example of Engineered Cationic Amphiphathic Peptides

- Nature inspired, sequence optimized



solubility and stability profile, and both ASP-1 and ASP-2 were active over a broad pH range and displayed broad spectrum activity against diverse gram-positive and gram-negative bacteria in preformed biofilms. Topical formulations can be placed in gel, cream, or dressing formation.

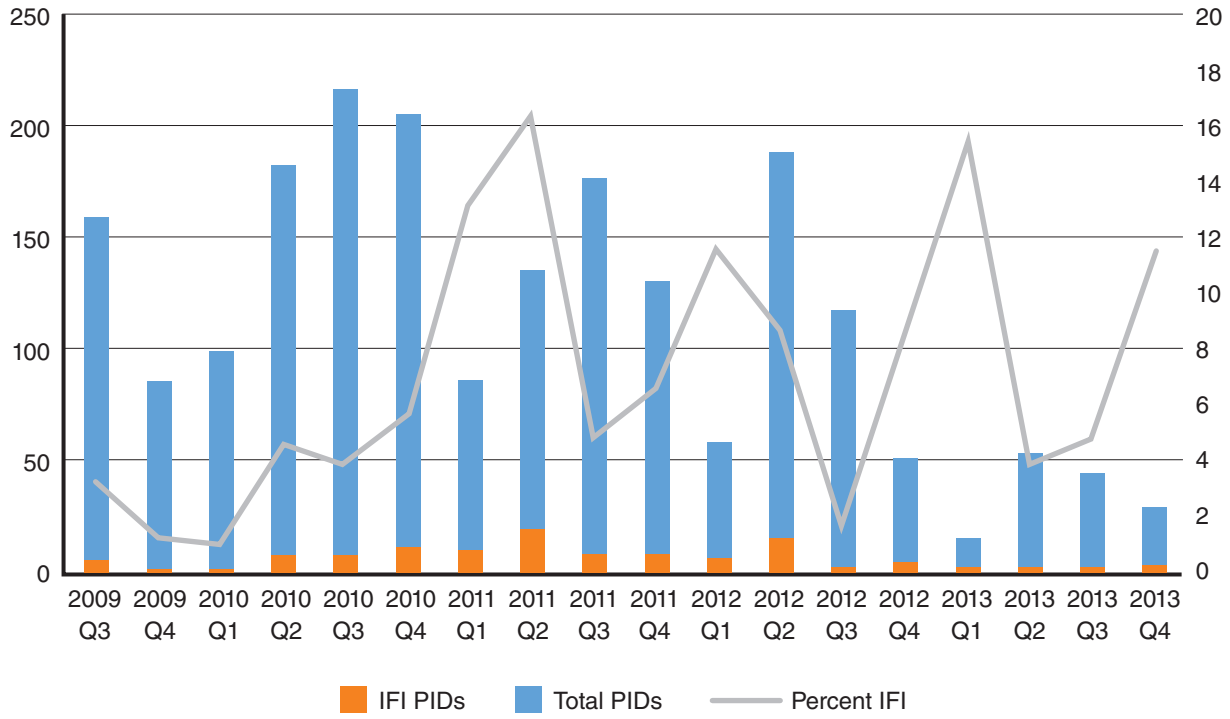
Daniel Kadouri, Ph.D. introduced predatory prokaryotes as "live antibiotics" to control infection. Predatory bacteria can attack human pathogens, including multidrug-resistant pathogens associated with wounds and burns. Predatory bacteria have the capacity to attack clinical isolates of a variety of ß-lactamase-producing, multidrug-resistant gram-negative bacteria. Predatory bacteria maintain their ability to prey on multidrug-resistant bacteria regardless of their antimicrobial resistance. They are nontoxic to human cell lines and do not impact cell migration and wound healing in vitro and ex vivo. Prokaryotes like *Bdellovibrio* have been recovered from biofilms, soils, and the rhizosphere. *Bdellovibrio* was able to prey, attack, and reduce 117 of the 135 examined bacteria in both single and multispecies culture suspension and biofilms. A reduction in biofilm biomass was observed as early as three hours after exposure to the predator.

Mark Smelzer, M.D. introduced the concept that *S. aureus* limits biofilm formation: i.e. formation, virulence in a sepsis model including in secondary infections, cytotoxicty for osteoblasts and osteoclasts, and reactive bone formation and cortical bone destruction. A chitosan paste was suggested as one method for drug delivery, as it offers completed wound coverage and is biocompatible, biodegradable, adhesive, and injectable.

Anuradha Ganesan, M.D., MPH described the Uniformed Services University's research on invasive fungal wound infections using TIDOS. Between 2009 and 2011, 6.8 percent of all admissions in the contiguous United States were complicated by an invasive fungal infection. There was significant morbidity, including high-level amputations. TIDOS was utilized to identify patients with laboratory evidence of fungal infection (i.e., histopathology or culture). Before OIF and OEF, fungal SSTIs were rare in military treatment facilities and were primarily observed in immunocompromised individuals. By the end of 2010, nearly 3.5 percent of all admissions to Landstuhl Regional Medical Center were fungal SSTIs, as charted in Figure 9. Mucor, aspergillus, fusarium, and other molds were commonly isolated from tissue specimens. On average, it took seven days to diagnose a fungal infection. Early aggressive debridement, coupled with antifungal chemotherapy, remains the optimal approach to clinical management. The role of molecular diagnostics in more timely and accurate diagnosis is currently being evaluated along with analyses to assess optimal clinical practice.

Robert Daum, M.D. modeled the role of the gut in MRSA infections. Most pathogens that cause severe infection follwing injury (e.g., MRSA, *Pseudomonas*, *Acinetobacter*) can be traced to the gut as their primary source of colonization. The gut shifts from harboring a "microbiome" to a "pathobiome" within hours following severe injury. The microbiome collapses in both composition, diversity, and abundance within six hours of a sudden physiologic insult by an unknown mechanism. Daum and his colleagues' hypothesis of surgical site infection posits that bloodstream leukocytes work like trojan horses for the metastasis of *Staphylococcus aureu*s. During times of physiological stress, MRSA colonizing the intestine is taken up by the intestinal neutrophil, where it survives in a dormant state. The neutrophil then homes to a

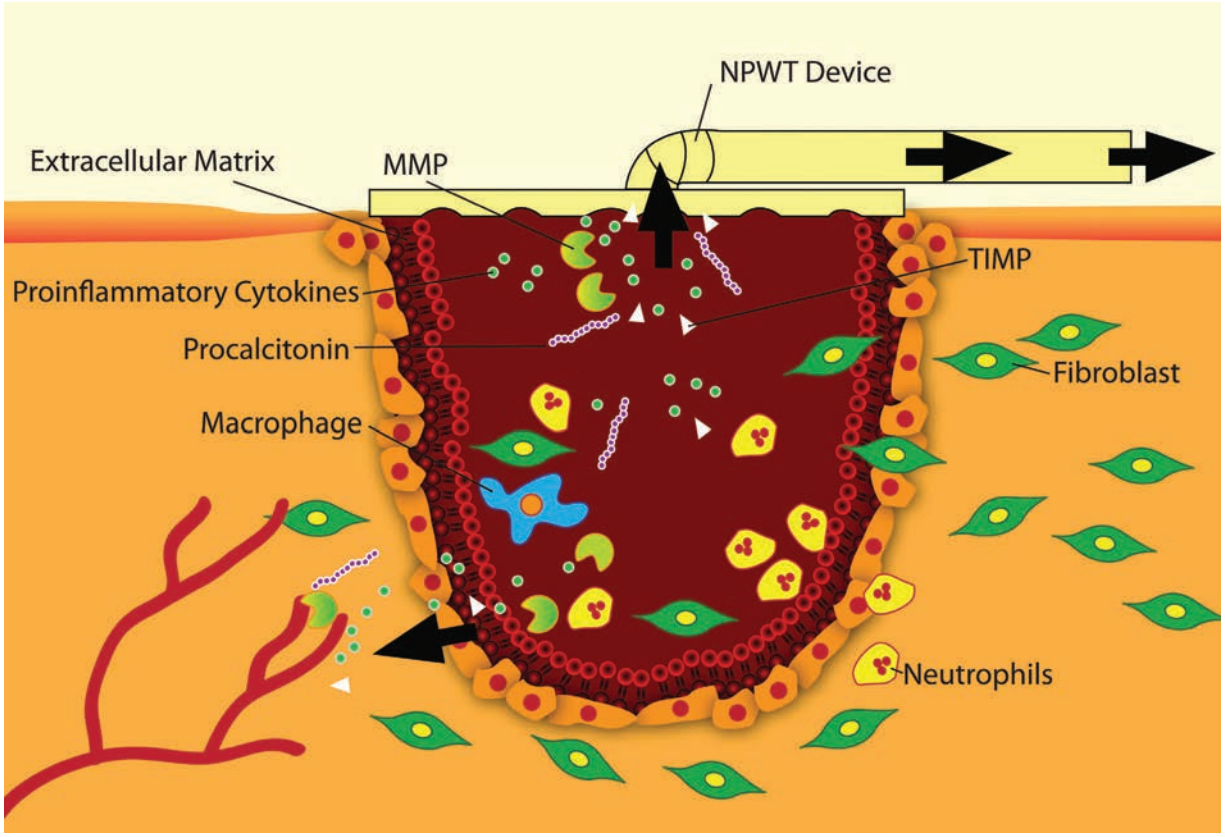
Figure 9. Landstuhl Regional Medical Center Trauma Admissions from Afghanistan Theater of Operations by Calendar Quarter



distant site of tissue injury and inflammation and releases its microbial payload, resulting in a metastatic surgical site infection. Daum's study in murine models found that MRSA successfully colonized the intestine and remained present for up to seven days following initial inoculation. An increasing amount of surgical trauma was also associated with increased abundance of MRSA per milligram of tissue. Wound ischemia and injury increased the severity of the infection.

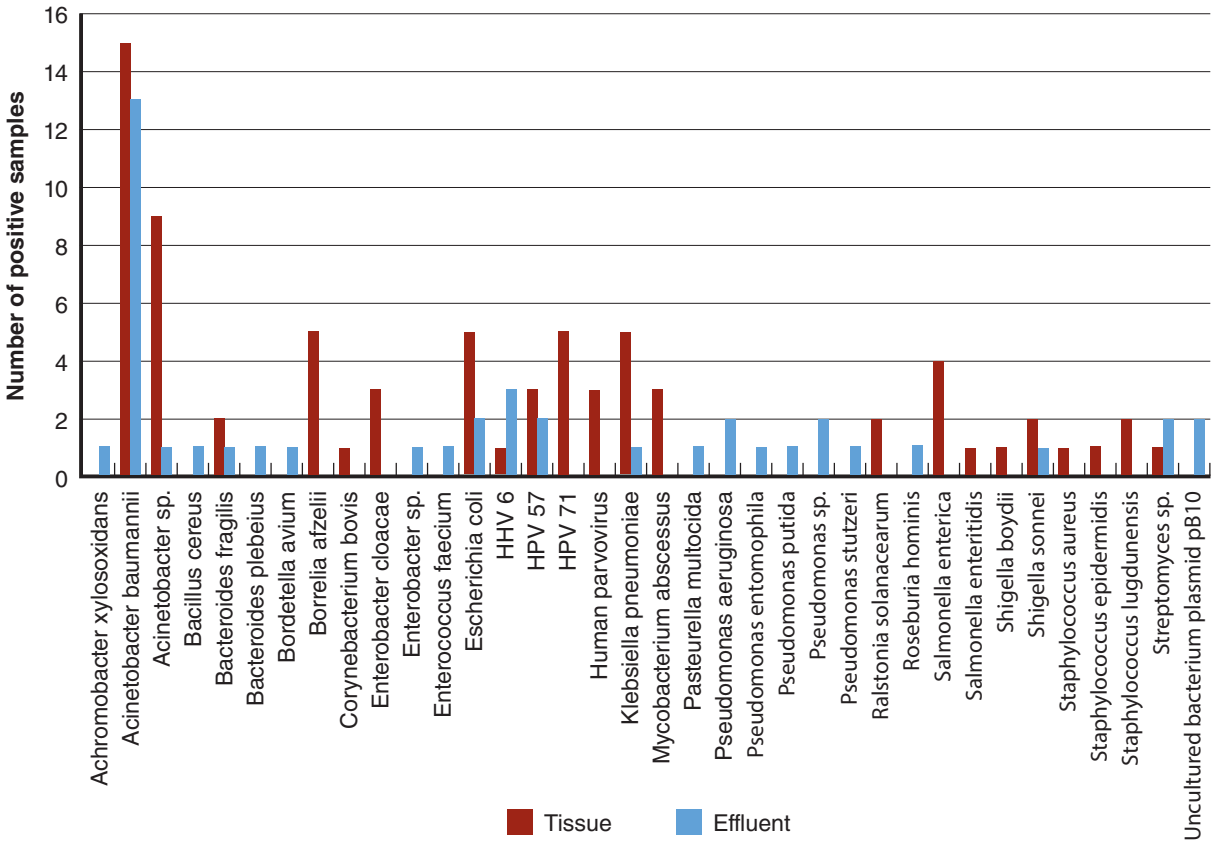
Nicholas Be, Ph.D. described genomics-based microbial detection in combat injuries. Complications related to infection occur even in culture-negative wounds with limited apparent pathology. Figure 10 depicts the microenvironment of a typical wound. An integrated study of the microbial profile in combat-injured service members can be used to predict outcomes and guide care. It is difficult to obtain reliable gene identification via unbiased desktop sequencing. High-performance computing, like the the Lawrence Livermore Microbial Detection Array, enables matching of DNA signatures with more than 12,000 of microbial species (see Figure 11). The Lawrence Livermore Microbial Detection Array detects a range of microbes not observed by quantitative bacteriology. Metagenome sequencing permits examination of the complete bioburden, which may track with clinical outcome. Bioburdens for individual patients can be tracked in individual patients over time. One can then examine the relative abundance of functional resistance capabilities within the metagenome. In the future, targeted sequencing may inform antimicrobial resistance detection.

Figure 10. Microenvironment of the Wound



Hahm G, Glaser JJ, Elster EA. Plast Reconstr Surg. 2011.

Figure 11. Lawrence Livermore Microbial Detection Array Detects a Range of Microbes Not Observed by Quantitative Biology



Working Group Summary

On the second and third days of the meeting, participants divided into five working groups, with an expert panelist chairing each working group (see Appendix D for Expert Panel biographies). Each separate working group discussed and addressed four questions (listed in Table 6 and before each response summary below) with the aid of a group discussion facilitator and note-taker.

Table 6. Working Group Questions

Question 1	How can our understanding of risk factors of wound infections, bacterial or fungal, following blast-related injuries, be applied to advance prediction, prevention, detection, and treatment strategies?
Question 2	What candidate biomarkers, from either host or pathogen, can potentially enable rapid and accurate diagnosis, management, and prognosis of wound infections and biofilm formation following blast-related injuries?
Question 3	What prevention strategies, to include the use of vaccines, can be employed to reduce the incidence of wound infections across the continuum of care (point of injury to U.S. military hospital setting) following blast-related injuries?
Question 4	What strategies hold the most promise for the treatment of wound infections associated with blast-related injuries and what are the challenges in fielding these?

Working group responses were based on the expertise of their members; findings from the literature review; and information from the 2016 meeting keynote, literature review, topical presentations, and emerging scientific presentations. Timeframe descriptors (see Table 7) were assigned to capture working group forecasts regarding when various policy, practice and research directions could be addressed. Working group responses are summarized by question below. The general consensus among the working groups and expert panelists was that there were many more questions than evidence-based answers in the area of wound infections after blast injury.

Table 7. Recommendation Timeframe Descriptors

Descriptor	Timeline	Example
Short-term	Actionable now or within 12 months	- Lessons learned from recent war experience that suggests the need for policy or practice changes - Existing data leading to potential policy, practice or research priority alterations
Intermediate-term	One to five years	- Pivotal research study that may drive a policy, practice, or readiness
Long-term	Six or more years	- Program of research or long term follow-up study of downstream policy, practice or readiness consequence

Question 1. How can our understanding of risk factors of wound infections, bacterial or fungal, following blast-related injuries, be applied to advance prediction, prevention, detection, and treatment strategies?

Simple visual appearance of the wound has long been the primary method used to assess the risk or likelihood of wound infection. However, the method is often unreliable and otherwise limited. Therefore, efforts to develop improved and more reliable risk assessment methods are essential (intermediate term). First, however, a standard nomenclature with clearly operationalized definitions is needed and should be developed in collaboration with the Joint Trauma Analysis and Prevention of Injury in Combat (JTAPIC) Program (short term). This nomenclature would be a foundational step toward a uniform and readily interpretable scientific, clinical, and policy literature on risk factors.

Second, research is needed to more fully understand unique aspects of wound microbiology and infections following blast versus other types of injuries (intermediate term). Efforts to characterize how geographic area of responsibility within the theater of operation, unique patterns of antimicrobial resistance, the impact of local flora on infection patterns, and injury susceptibility patterns are important. Local flora, geography, environmental factors, and patterns of antimicrobial resistance are constantly evolving, and therefore, such studies should be planned in advance, initiated on arrival to any new theater of operations, and sustained (intermediate term).

Data from civilian health care settings are likely to offer limited generalizability to military medical operations in deployed settings, making advance planning and research institutional review board approval for ecologically valid research studies essential (short term). To ensure infrastructure for planned studies in theater, the DoD Trauma Registry must be sustained, including the Trauma Infectious Disease Outcome Study and the Military Orthopedic Trauma Registry. These longitudinal data platforms should also be used to empirically validate and improve existing Joint Trauma System (JTS) CPGs to better prevent and treat wound infections after blast injuries and maximize associated outcomes (intermediate term). These longitudinal data platforms should also support research to assess the unintended consequences of antimicrobial therapies, such as the emergence of antimicrobial resistant organisms.

Third, the work groups suggested that future research should also investigate the unique ways that blast injury affects body and wound physiology, host microbiome, and associated risk of wound infection and treatment response (long term).

Question 2. What candidate biomarkers, from either host or pathogen, can potentially enable rapid and accurate diagnosis, management, and prognosis of wound infections and biofilm formation following blast-related injuries?

First, working groups suggested in response to this and all of the other questions relating to wound infection after blast injury that prompt policy steps are needed to address recurrent regulatory barriers to the initiation and completion of clinical investigations in the theater of

operations (short term). This is particularly important for enabling data collection on event-related biomarkers and analysis and knowledge that is generalizable to the battlefield context and to specific theaters of operation. Second, and related to the first, working groups felt strongly that a point-of-care diagnostic biomarker system should be developed. Third, baseline biomarker collections (effluent, serum, and tissue) should be integrated into the existing repository in a manner that can facilitate linkage to clinical outcomes (intermediate term) and enable a systems-biology approach analysis (intermediate term to long term). Fourth, a proactively planned research agenda is needed to ascertain the reliability of various biomarkers for clinical prediction and prognostication (intermediate term). Of particular interest to some was the extent to which the presence of biofilms can be used to aid treatment selection (intermediate term). Fifth, working groups identified long-term research interests. These included (1) prospective evaluation and monitoring of host biome in relationship to onset, course, and treatment of blast-related wound infections and (2) machine learning approaches to linking biomarker and host biome profiles for clinical prediction and treatment selection.

Question 3. What prevention strategies, to include the use of vaccines, can be employed to reduce the incidence of wound infections across the continuum of care (point of injury to U.S. military hospital setting) following blast-related injuries?

First, working groups indicated that compliance with preventive JTS clinical practice guidelines should be mandatory, and that senior military leaders should emphasize clinical practice guideline performance reporting that measures, compares, and rewards best guideline practices (short term). Particular attention is needed to proper adherence to enhanced infection control practices and related performance reporting (short term). Second, research is needed to examine and improve infection control practices (short term to intermediate term). Third, research should prospectively examine the relationship of specific antimicrobial drugs to subsequent development of antibiotic-resistant organisms, delayed wound healing, and other clinically important outcomes (intermediate term). Fourth, other wound infection prevention strategies suggested for future research include the use of advanced wound care strategies—e.g., negative pressure wound therapy, phage lysin, dressings, wound irrigation, topical and nonantibiotic antimicrobials, and microbicides (intermediate term). Fifth, the preventive implications of diet, nutrition, supplements, stress, and medications on immune fitness are largely unknown, and working groups felt this required systematic research examination (long term). Finally, working groups noted that the preventive role of passive immunotherapy/vaccines is at best a very long-term research interest, but perhaps worthy as an aspirational objective.

Question 4. What strategies hold the most promise for the treatment of wound infections associated with blast-related injuries and what are the challenges in fielding these?

First, working groups indicated the need to institute policies and processes that will ensure an expert trauma care workforce, including expertise in infectious disease aspects of blast-related would care and ongoing team training (short term). Second, as in prevention, compliance with preventive JTS clinical practice guidelines should be mandatory, and senior military leaders should emphasize comparative guideline performance reporting and best practice

incentives (short term). Third, an essential research direction, ideally accomplished with industry and FDA input, is the investigation of various combination therapies composed of new antimicrobial agents relying on different antimicrobial mechanisms (intermediate term to long term). Fourth, policies and organizational processes should help to engage, involve, and partner with the FDA and other regulatory agencies in these research efforts. This may foster mechanisms to fast track development and ultimate use for promising drugs and devices as supported by scientific studies (intermediate term). Fifth, policy efforts are needed to ensure that field clinical investigations can be and are proactively planned, rapidly implemented, and supported to regular completion (intermediate term). This can and should be facilitated through encouragement of and mechanisms for constructive non-DoD research partnerships and progressive clinical research funding. Partnerships and funding will advance promising concepts and drive timely adoption of new clinical practices as supported by the evidence (short term to intermediate term). Sixth, working groups indicated that the ultimate goal should be data-driven surgical (e.g., debridement, irrigation, fracture fixation), procedural, and antimicrobial treatment approaches (intermediate term to long term). To that end, ongoing efforts are needed to integrate intramural research across research program areas through the exercise of the executive coordinating function (short term, intermediate term, and long term).

Summary 2016 State-of-the-Science Expert Panel Recommendations

The Secretary of Defense and senior military leaders should:

Recommendation 1. Ensure that proactive plans, policies, procedures, and clinical practice are in place to support and sustain a "Learning Trauma Care System" that is consistent with a recent Institute of Medicine report. One goal should be to seek to improve theater-specific understanding, prevention, and treatment of wound infections following blast injuries. In support of this objective, which is consistent with a recent Institute of Medicine report, policies, procedures and organizational coordination are needed to permit, encourage and ensure real time, prospective data collection relevant to wound infection reduction strategies and allow the assessment of relevant clinical practice guideline compliance. These efforts must routinely begin on entry to new theaters of operation.

Recommendation 2. Coordinate—by DoD Directive and all other appropriate regulatory mechanisms—routine research organizational support for sustained wound infection surveillance and analytic epidemiology in current and future theaters of operation. Initiate and sustain research upon entry to any theater of operation. Organizational research support that ensures theater-wide research investigation into wound infections, related therapeutics, and antimicrobial resistance is necessary for achieving the Institute of Medicine's Learning Trauma Care System concept. Current policy often discourages, delays, or disincentivizes research investigation in theater. This is essential both to force health protection and to effective military medical care, given the limited generalizability of civilian trauma and infectious disease studies to the frontline environment.

Recommendation 3. Develop a proactive, comprehensive research strategy relating to blast-related wound care, enhanced infection control, and optimal antimicrobial prevention and treatment strategies for coordinated implementation within current and future theaters of operation.

Recommendation 4. Increase DoD efforts to engage and facilitate Food and Drug Administration (FDA) involvement in meetings, strategies, and other efforts to ensure research and development of innovative, integrated therapies tackling the growing, global problem of antimicrobial drug resistance. Use FDA collaboration to facilitate industry partnerships relating to antimicrobial drug development. Ironically, a frequently realized goal of antibiotic treatment is short-term treatment resulting in cure (in contrast to treatments for many chronic diseases), making it challenging for antibiotic manufacturers to recover drug development costs. DoD needs these partnerships, but it can be difficult to engage industry in this area; the FDA is actively involved with addressing this problem.

Recommendation 5. Implement a system to measure, compare, benchmark and reward compliance with existing Joint Trauma System clinical practice guidelines pertaining to blast-related injury, such as improving compliance with the Joint Trauma System guidelines relating to infection prevention in combat-related injuries (CPG ID: 24) and care of patients at high risk

for invasive fungal infections in war wounds (CPG: 28). Compliance with JTS clinical practice guidelines should be essential. Senior DoD leaders must emphasize clinical practice guideline performance reporting.

Recommendation 6. Preserve, sustain, and improve the DoD Trauma Registry and related programs (e.g., Trauma Infectious Diseases Outcome Study and the Military Orthopedic Trauma Registry) to improve care and advance military relevant research relating to wound infections after blast-related injury. The resulting data may be used to validate and improve clinical practice guidelines and support needed research.

Appendix A. Acronyms

ADMET	drug absorption, distribution, metabolism, excretion, and toxicity
AMR	antimicrobial resistance
BLA	bilateral lower extremity amputations
CAMPs	Cationic antmicrobial peptides
CARB	Combating Antibiotic Resistant Bacteria
CDC	Centers for Disease Control and Prevention
CPG	Clinical Practice Guidelines
DHP	Defense Health Program
DNA	Deoxyribonucleic acid
DoD	Department of Defense
EA	Executive Agent for Medical Research for Prevention, Mitigation and Treatment of Blast Injury
eCAPs	Engineered Cationic Amphiphathic Peptides
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, Klebsiella pneumoniae, *Acinetobacter baumannii*, Pseudomonas aeruginosa, and *Enterobacter* species pathogens
FDA	Food and Drug Administration
FY	fiscal year
IED	improvised explosive device
JPC-2	Joint Program Committee-2
JTAPIC	Joint Trauma Analysis and Prevention of Injury in Combat

JTS	Joint Trauma System
MDR	multidrug resistant
MDRO	multidrug resistant organisms
MRSA	methicillin-resistant Staphylococcus aureus infection
NIH	National Institutes of Health
NSAID	nonsteroidal antiinflammatory drug
OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom
PCR	polymerase chain reaction
PCO	DoD Blast Injury Research Program Coordinating Office
SSTI	Skin and soft-tissue infection
TIDOS	Trauma Infectious Diseases Outcomes Study
TBI	Traumatic brain injury
USAMRMC	U.S. Army Medical Research and Materiel Command
WRAIR	Walter Reed Army Institute of Research

Appendix B. Planning Committee

This meeting was made possible thanks to the guidance, planning, and insights of the members of the 2016 SoS Planning Committee:

Mr. Michael J. Leggieri, Jr.
Planning Committee Chair
DoD Blast Injury Research Program Coordinating Office

Dr. Raj Gupta
Planning Committee Co-Chair
DoD Blast Injury Research Program Coordinating Office

Dr. Rudy Alarcon
National Institutes of Health, National Institute of Allergy and Infectious Disease

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Dr. Michael Bell
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CAPT Timothy Burgess
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Joint Trauma Analysis and Prevention of Injuries in Combat

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Defense Advanced Research Projects Agency

COL Matthew Hepburn
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Dr. Kai Leung
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U.S. Army Medical Research and Materiel Command, Military Infectious Disease Research Program/Joint Program Committee 2

COL Clinton Murray
U.S. Army Surgeon General Consultant on Infectious Disease

Dr. Savita Nigam
U.S. Army Medical Research and Materiel Command Combat Casualty Care Research Program/Joint Program Committee 6

Dr. James B. Petro
Office of the Assistant Secretary of Defense Research and Engineering

MINIMIZING THE IMPACT OF WOUND INFECTIONS FOLLOWING BLAST-RELATED INJURIES

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U.S. Army Medical Research and Materiel Command, Office of the Principal Assistant for Research and Technology

MAJ Matt Scherer
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Dr. Richard Shoge
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COL Michael Zapor
Walter Reed Army Institute of Research

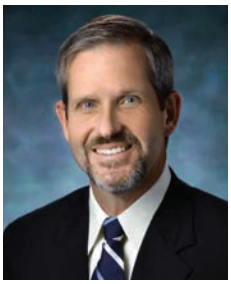
Dr. James Zheng
Program Executive Office Soldier

Appendix C. Expert Panel

An Expert Panel of five subject matter experts representing policy makers, clinicians, and scientists, will help lead and focus discussions during the plenary sessions. The Expert Panel members will also chair working group sessions, in which participants will address the four meeting questions. (Working group assignments can be found in the "Working Group" section of this booklet).

Dr. Ryan Fagan

Ryan Fagan, MD, MPH&TM is a Commander in the U.S. Public Health Service, trained in adult infectious diseases at Tulane University, and works as a medical officer at the Division of Healthcare Quality Promotion (DHQP) at CDC, Atlanta. Dr. Fagan's current responsibilities include response to U.S. outbreaks of healthcare-associated infections and leading CDC's infection control support activities for U.S. Ebola Assessment Hospitals. Dr. Fagan has been with the CDC since 2006 and his prior assignments include serving as: a CDC Epidemic Intelligence Service Officer with the Alaska Section of Epidemiology; the agency botulism subject matter expert; the DHQP lead for surveillance of surgical site infections in the U.S.; and the team lead of numerous field investigations including foodborne illnesses, rabies exposure, pandemic H1N1 influenza, fungal meningitis due to contaminated steroid injections, and healthcare infection control for Ebola in the U.S. and Liberia.



Dr. James Ficke

U.S. Army Col. James R. Ficke, M.D., a nationally renowned expert on the treatment of complex foot and ankle injuries and amputee care, is Professor and Chair of the Department of Orthopaedic Surgery at The Johns Hopkins University School of Medicine, and orthopaedist-in-chief of The Johns Hopkins Hospital.

A 1983 West Point graduate, Ficke, is the former chairman of the Department of Orthopaedics and Rehabilitation at the San Antonio Military Medical Center at Fort Sam Houston in Texas. He also served the U.S. Army Surgeon General as the senior advisor on policy and personnel for orthopaedics and extremity injuries for 7 years. Part of his responsibility was development of systems designed to foster exceptional outcomes and physical performance for wounded Warriors and their families across the country. Leading Army Orthopaedics and providing strategic direction for the six residencies across the nation, Ficke has helped train a substantial number of the Army's current orthopaedic professionals.

In addition to the awards he has received for his skills as a surgeon, educator, and military leader, he also has received some two dozen military decorations and awards, including the Bronze Star and Meritorious Service Medals. During Ficke's deployment as deputy commander of clinical services at the 228th Combat Support Hospital in Mosul, Iraq, from 2004 to 2005, he was the senior orthopaedic surgeon, treating more than 600 U.S. soldiers and Iraqi patients for

war injuries. His service there and elsewhere subsequently earned him the Society of Military Orthopaedic Surgeons' prestigious 2010 COL Brian Allgood Memorial Leadership Award, as well as The Surgeon General's 2010 Major General Lewis Aspey Mologne Award for excellence in military academics, education and clinical care.

Ficke received his medical degree from Uniformed Services University, completed a transitional internship at Madigan Army Medical Center in Tacoma, Wash., and finished his residency in orthopaedic surgery at Tripler Army Medical Center in Honolulu. He has completed an AO fellowship in trauma in Munich Germany, and a foot and ankle fellowship with James Brodsky, M.D., in Dallas, Texas. He is Chair, American Orthopaedic Association Leadership/ Fellowship Committee, and the AAOS Extremity War Injuries Project Team.

Colonel Kirby Gross

Colonel Gross is the incoming Director of the Army Trauma Training Department at Ryder Trauma Center in Miami, FL. His most recent duty stations include Division Chief Defense Medical Readiness Training Institute (DMRTI) and Director of the Joint Trauma System (JTS) at the U.S. Army Institute of Surgical Research in San Antonio, Texas. He had also previously served as the Trauma Consultant to the Office of the Surgeon General

Colonel Gross led Forward Surgical Teams and was an integral part of the 86th Combat Support Hospital. He has served as Chief of Surgery for the 10th Combat Support Hospital Ibn Sina in Baghdad, the Deployed Director of the Joint Trauma System on two occasions, attending surgeon at William Beaumont Army Medical Center, and Chief of Trauma and Critical Care at Walter Reed National Military Medical Center. In recognition of his service, Gross has received the Bronze Star, the Defense Meritorious Service, and the Joint Service Commendation Medals.

In addition to his work as a leader and surgeon, Colonel Gross is a distinguished educator. He had served as the steward of over 30 continuously updated clinical practice guidelines in trauma care and trauma systems. He teaches medics, established care providers, and surgical trainees about trauma care, trauma systems, and combat casualty care training. His expertise has been sought to develop and evaluate curricula. Additionally, Colonel Gross is responsible for creating incredible continuing education opportunities. For instance, Colonel Gross' leadership of the 5th Annual Joint Theater Trauma System Operation Enduring Freedom Trauma Conference brought together 150 medical professionals from around the world.

Dr. Thomas Patterson

Dr. Thomas F. Patterson received his Bachelor of Arts degree from Baylor University, in Waco, Texas and his medical degree from the University of Texas Medical School at Houston, Texas. He completed his internship and residency at Vanderbilt University Medical School, in Nashville, Tennessee and at Yale-New Haven Hospital, and a fellowship in infectious diseases at Yale University School of Medicine, New Haven, Connecticut,

where he also served as an Assistant Professor of Medicine. Dr Patterson currently is a Professor of Medicine and Chief, Division of Infectious Diseases at the University of Texas Health Science Center in San Antonio, Texas. He is also Director of the San Antonio Center for Medical Mycology. He has extensive experience in opportunistic fungal infections. His clinical and research interests focus on the diagnosis and treatment of fungal diseases, particularly in immunocompromised hosts. He has been involved in developing new antifungal drugs and in clinical trials of new antifungal compounds. Dr Patterson has published and lectured extensively on fungal infections. He is a past member of the ICAAC and IDSA Program Committees and is co-chair of the IDSA Aspergillus Guidelines committee. He is a Fellow of the American College of Physicians and IDSA and Past-President of the Texas Infectious Disease Society.

Dr. David Tribble

*Current Position***:** Science Director, Infectious Diseases Clinical Research Program, Preventive Medicine & Biostatistics Department, Uniformed Services University of the Health Sciences

Dr. Tribble completed his medical training at the University of Arkansas for Medical Sciences followed by Internal Medicine residency at the Naval Hospital Portsmouth, Infectious Diseases fellowship at the National Naval Medical Center (NNMC), and a Doctorate in Public Health at the Uniformed Services University of the Health Sciences (USU). After assignments at the Naval Medical Research Unit No. 3 in Cairo, Egypt, NNMC Infectious Diseases Division, and the Enteric Diseases Department, Naval Medical Research Center, Dr. Tribble joined the faculty at USU to establish the Infectious Disease Clinical Research Program (IDCRP), a Department of Defense and National Institute of Allergy and Infectious Disease (NIAID)/National Institutes of Health (NIH) collaborative research program. As IDCRP Science Director, Dr. Tribble provides senior leadership for strategic planning and clinical research. Additionally, Dr. Tribble is the Principal Investigator for the DoD-VA Trauma Infectious Disease Outcomes Study (TIDOS). Dr. Tribble's areas of research focus include trauma-related infectious complications, SSTI epidemiology and *S. aureus* infection prevention strategies, deployment/travel-associated infection epidemiology and clinical management, and bacterial diarrheal disease vaccine development.

Appendix D. Meeting Partcipants

Dr. Theresa Abraham
Strategic Marketing Innovations

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Dr. Angel Davey
DoD Congressionally Directed Medical Research Program

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MAJ Samandra Demons
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Dr. John DesJardins
Clemson University

Dr. Travis Dittmer
U.S. Army Medical Research and Materiel Command

Dr. Erik Edwards
Battelle Memorial Institute

Dr. Ronald Egres
DuPont Protection Solutions

Dr. Mark Ehrensberger
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Dr. Charles Engel
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Kerecis

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Beth Israel Deaconess Medical Center

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University of Texas Health Sciences Center at San Antonio

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Assistant Secretary of Defense for Research and Engineering, Office of the Secretary of Defense

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Lt Col Avraham Yitzhak
Israel Defense Forces

COL Michael Zapor
Walter Reed Army Institute of Research

Dr. Daniel Zurawski
Walter Reed Army Institute of Research

Appendix E. Meeting Agenda

Tuesday, 29 November

Time	Schedule	Presenter
8:00	Registration opens	
8:30	***Welcome & Meeting Overview***	Mr. Michael Leggieri
9:00	Keynote Address	Dr. John Holcomb
9:20	***Defining the Problem***	Dr. Charles Engel
9:25	Battlefield Blast-Related Wound Epidemiology and Clinical Management	COL Kirby Gross
9:45	Challenges in the Treatment of Blast Wounds: A Multidisciplinary Overview	LCDR Matthew Bradley LTC Paige Waterman
10:05	Q&A for All Speakers	
10:25	**AM BREAK**	
10:55	***Defining the Problem (continued)***	Dr. Charles Engel
11:00	Wound Infection Challenges and Policy: The DoD Perspective	Dr. Terry Rauch
11:20	Overview of DoD-Sponsored Research on Wound Infection	COL Michael Kozar
11:40	Industry Considerations for Anti-Infective Drug Development	Dr. Michael Pucci
12:00	Q&A for All Speakers	
12:20	**LUNCH & POSTER SET-UP**	
2:00	***Current State of the Science & What's Next***	Dr. Charles Engel
2:20	Literature Review Report: Findings and Conclusions	Dr. Matt Aldag
2:40	Wound Infection Epidemiology and Bacteriology	Dr. David Tribble
3:00	Q&A for All Speakers	
3:20	**PM BREAK**	
3:40	***Scientific Session 1***	Dr. Charles Engel
3:45	Assessment and Classification of Combat-Related Polytraumatic Extremity Wounds and Infectious Outcomes: Trauma Infectious Disease Outcomes Study 2009-2012	Dr. Laveta Stewart
4:05	Microbiology of Complex Combat-Related Extremity Wounds: Trauma Infectious Disease Outcomes Study 2009-2012	Dr. Katrin Mende
4:25	Persistent Critical Colonization May Be Associated With Different Host Response And Healing Outcomes In Combat-Related Extremity Wounds	Dr. Felipe Lisboa
4:45	***Closing Remarks & Adjourn***	Mr. Michael Leggieri

Wednesday, 30 November

Time	Schedule	Presenter
8:00	Registration opens	
8:30	***Welcome and Introduction to Scientific Session 2***	Mr. Michael Leggieri
8:35	Progress Toward New Antibacterial Therapeutics for Military Traumatic Wound Infections	LTC Stuart Tyner
8:55	Combating Antibiotic Resistant Bacteria (CARB) Program Leads to Novel Small Molecule Drug Screening Effort	Dr. Schroeder Noble
9:15	Pipeline Development for Pre-Clinical Assessment of Bacteriophage Treatment for MDR Wound Infections	Dr. Anna Jacobs
9:35	Engineered Bacteriophage Therapeutics Against Multidrug-Resistant Pathogens	Dr. Elena Barbu
9:55	**AM BREAK**	
10:15	***Scientific Session 3***	Dr. Charles Engel
10:20	Komodo Dragon-Inspired Synthetic Peptide DRGN-1Promotes Clearance and Healing of Polymicrobial-Biofilm Infected Wound	Dr. Monique Van Hoek
10:40	A Novel Peptide Based Antimicrobial Wound Treatment is Effective Against Biofilms of Multi-Drug Resistant Wound Pathogens	Dr. Jennifer Neff
11:00	Developing Predatory Bacteria as a 'Live Antibiotic' to Control Infection	Dr. Daniel Kadouri
11:20	Enhancing the Prevention and Treatment of Orthopaedic Infections Associated with Traumatic Injury	Dr. Mark Smeltzer
11:40	**LUNCH & POSTER SESSION I**	
1:10	***Scientific Session 4***	Dr. Charles Engel
1:15	Combat-Related Invasive Fungal Wound Infections among U.S. Blast Casualties	Dr. Anuradha Ganesan
1:35	Can Methicillin Resistant Staphylococcus Aureus Silently Travel From the Gut to the Wound and Cause Postoperative Infection? Modeling the "Trojan Horse Hypothesis"	Dr. Robert Daum
1:55	Association of Genomics-Based Microbial Detection with Wound Outcome in Combat Injuries	Dr. Nicholas Be
2:15	***Roles and Responsibilities of Working Groups***	Dr. Charles Engel
2:30	Working Groups*	Chaired by Expert Panelists
5:00	***Adjourn Directly from Working Groups***	
	*Breaks are determined within each Working Group	

Thursday, 1 December

Time	Schedule	Presenter
8:00	Registration	
8:30	Working Groups*	Chaired by Expert Panelists
11:30	**LUNCH & POSTER SESSION II**	
1:00	Working Groups*	Chaired by Expert Panelists
2:00	Working Groups Report Out	Expert Panelists
4:00	***Closing Remarks & Adjourn***	Mr. Michael Leggieri
	*Breaks are determined within each Working Group	

Appendix F. Welcome Letter

Dear Colleague:

As Director of the Department of Defense (DoD) Blast Injury Research Program Coordinating Office and on behalf of the Executive Agent (EA) for the DoD Blast Injury Research Program, I'd like to welcome you to the 2016 International State-of-the-Science (SoS) Meeting. The essential theme and focus of the 2016 SoS meeting is "Minimizing the Impact of Wound Infections Following Blast-Related Injuries." Wound infection continues to be a significant source of morbidity and mortality in the modern era of military healthcare. Approximately one quarter of combat wounds become infected, which has a significant impact on patient outcomes and healthcare costs.

We are very excited and pleased to address this core scientific aspect of blast injury. Indeed, over the next few days, you will play a pivotal role in the future of our research program in this topic area. Accordingly, we are extremely grateful for your participation and invaluable assistance during the conference. The objectives of this meeting are to:

1. Determine predictive risk factors for wound infections following blast-related injuries, including individual susceptibility and environmental contributions, from point of injury through continued hospital care.
2. Identify candidate biomarkers that would enable rapid and accurate diagnosis, management, and prognosis of wound infections following blast-related injuries.
3. Examine prevention strategies, including vaccines, for mitigation of wound infections following blast-related injuries.
4. Propose strategies that would mitigate the impact of multi-drug resistant, virulent, or opportunistic organisms on wound infections following blast-related injuries.

First established in 2009, the SoS meeting series is an essential tool for strategic identification of the scientific research gaps in fields related to the prevention, mitigation, and treatment of blast injury. These meetings support the EA's congressionally directed responsibilities to identify blast injury knowledge gaps and to foster collaborative medical research that will close the gaps and support the development and delivery of effective blast injury prevention, mitigation, and treatment strategies for our nation's Service Members.

This year's conference will address four specific questions, formulated and recommended by our interdisciplinary and multi-agency conference Planning Committee:

1. How can our understanding of risk factors of wound infections, bacterial or fungal, following blast-related injuries, be applied to advance prediction, prevention, detection, and treatment strategies?
2. What candidate biomarkers, from either host or pathogen, can potentially enable rapid and accurate diagnosis, management, and prognosis of wound infections and biofilm

formation following blast-related injuries?

3. What prevention strategies, to include the use of vaccines, can be employed to reduce the incidence of wound infections across the continuum of care (point of injury to U.S. military hospital setting) following blast-related injuries?
4. What strategies hold the most promise for the treatment of wound infections associated with blast-related injuries and what are the challenges in fielding these?

We are keenly aware that as a participating subject matter expert, you are volunteering your valuable time to help us gain the knowledge necessary to shape medical research in an effort to deliver timely and effective prevention, mitigation, and treatment strategies for our Service Members. Our expectations are high: We must maximize readiness and protect the men and women of our Armed Forces. These are sacred missions, and we have a responsibility to use the resources at our discretion to pursue the most prudent and promising research toward realizing these goals. Hence, we are placing great emphasis on expert collaboration and communication, strongly encouraging and expecting all in attendance to engage with each other and to share your diverse expertise in an attempt to strategically fill identified knowledge gaps.

The EA is devoted to ensuring that the eventual fruits of your efforts will help protect, save, and improve the lives of those who volunteer to serve and defend our nation. When the meeting ends, the work will continue, and we will continue to need your help and the help of other experts like you. By working with us, you are making a profound difference to protect our Service Members.

Michael J. Leggieri, Jr.
Director, DoD Blast Injury Research Program
Coordinating Office

Appendix G. DoD Directive 6025.21E

Department of Defense

DIRECTIVE

NUMBER 6025.21E
July 5, 2006

USD(AT&L)

SUBJECT: Medical Research for Prevention, Mitigation, and Treatment of Blast Injuries

References: (a) Section 256 of Public Law 109-163, "National Defense Authorization Act for Fiscal Year 2006"[1]
(b) DoD Directive 5101.1, "DoD Executive Agent," September 3, 2002
(c) DoD Directive 5134.3, "Director of Defense Research and Engineering (DDR&E),"November 3, 2003
(d) DoD Directive 5025.1, "DoD Directives System," March 2005
(e) through (g), see Enclosure 1

1. PURPOSE

This Directive:

1.1. Implements Reference (a) by establishing policy and assigning responsibilities governing coordination and management of medical research efforts and DoD programs related to prevention, mitigation, and treatment of blast injuries.

1.2. Designates the Secretary of the Army, in compliance with Reference (a) and consistent with Reference (b), as the DoD Executive Agent (DoD EA) for Medical Research for Prevention, Mitigation, and Treatment of Blast Injuries according to Reference (b).

1.3. Establishes the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee. The ASBREM Committee serves to facilitate coordination and prevent unnecessary duplication of effort within DoD biomedical research and development and associated enabling research areas, to include serving as the forum for implementation of subsections (d) and (g) of Reference (a).

[1] Federal legislative information is available through the Library of Congress THOMAS site, http://thomas.loc.gov.

2. APPLICABILITY

This Directive applies to:

2.1. The Office of the Secretary of Defense, the Military Departments, the Chairman of the Joint Chiefs of Staff, the Combatant Commands, the Office of the Inspector General of the Department of Defense, the Defense Agencies, the DoD Field Activities, and all other organizational entities in the Department of Defense (hereafter collectively referred to as the "DoD Components").

2.2. Medical and associated enabling research supported by any DoD Component for prevention, mitigation, and treatment of blast injuries.

3. DEFINITIONS

As used in this Directive, the following terms are defined as follows:

3.1. Blast Injury. Injury that occurs as the result of the detonation of high explosives, including vehicle-borne and person-borne explosive devices, rocket-propelled grenades, and improvised explosive devices. The blast injury taxonomy is provided at Enclosure 2.

3.2. Research. Any systematic investigation, including research, development, testing, and evaluation (RDT&E), designed to develop or contribute to general knowledge.

4. POLICY

It is DoD policy that:

4.1. DoD research related to blast injury prevention, mitigation, and treatment will be coordinated and managed by a DoD EA to meet the requirements, objectives, and standards of the DoD Military Health System as identified by the Under Secretary of Defense for Personnel and Readiness (USD(P&R)) and the unique combat casualty care requirements of the DoD Components.

4.2. Relevant research shall take maximum advantage of the scientific and technical capabilities of industry, academia, DoD Components, and other Federal Agencies.

4.3. The ASBREM Committee will be the venue for joint and cross-Service coordination specified by Reference (a).

4.4. DoD Components will gather and share medical information related to the efficacy of personal protective equipment and of vehicular equipment designed to protect against blast injury.

5. RESPONSIBILITIES AND FUNCTIONS

5.1. The Director of Defense Research and Engineering (DDR&E), under the Under Secretary of Defense for Acquisition, Technology and Logistics, according to DoD Directive 5134.3 (Reference (c)), shall:

5.1.1. Plan, program, and execute the functions and reports mandated for the DDR&E by Reference (a).

5.1.2. Have the authority to publish DoD Issuances consistent with Reference (d) for implementation of this Directive.

5.1.3. Establish, as needed, procedures to ensure that new technology developed under this Directive is effectively transitioned and integrated into systems and subsystems and transferred to and firmly under the control of the DoD Components.

5.1.4. Chair the ASBREM Committee to coordinate DoD biomedical research (see Enclosure 3 for additional detail), and employ that entity to facilitate the DoD EA's coordination and oversight of blast-injury research as specified in Reference (a).

5.1.5. Serve as the final approving authority for DoD blast-injury research programs.

5.1.6. Oversee the functions of the DoD EA and conduct/report on related periodic assessments (per Reference (a)).

5.2. The Assistant Secretary of Defense for Health Affairs (ASD(HA)), under the USD(P&R), shall:

5.2.1. Assist the DDR&E, the DoD EA, and the Director, Joint Improvised Explosive Devices Defeat Organization (JIEDDO), with identification of related operational and research needs, assessment of relevant research efforts, and coordination of planning to resolve capability gaps through focused research efforts.

5.2.2. Be the approving authority for Military Health System prevention and treatment standards developed and proposed by the DoD EA.

5.2.3. Appoint appropriate representatives to related coordinating boards or committees established by the DoD EA.

5.2.4. Ensure that the information systems capabilities of the Military Health System support the DoD EA and the functions specified by this Directive.

5.2.5. Serve as Co-chair of the ASBREM Committee. (See Enclosure 3 for additional detail.)

5.3. The Secretary of the Army is hereby designated as the DoD EA for Medical Research for Prevention, Mitigation, and Treatment of Blast Injuries, consistent with Reference (a), to coordinate and manage relevant DoD research efforts and programs, and in that role shall:

5.3.1. Give full consideration to the Research and Engineering (R&E) needs of the DoD Components and the Director, JIEDDO, addressing those needs/requirements by:

5.3.1.1. Maintaining a DoD technology base for medical research related to blast injuries and based on the DDR&E-approved program for the DoD Components.

5.3.1.2. Performing programming and budgeting actions for all blast-injury research to maintain the R&E programs based on DDR&E-approved priorities of the DoD Components.

5.3.1.3. Programming and budgeting for blast-injury research based on analysis and prioritization of needs of the DoD Components, consistent with paragraph 5.1 of this Directive.

5.3.1.4. Executing the approved DoD blast-injury research program consistent with DoD guidance and availability of annual congressional appropriations.

5.3.2. Provide medical recommendations with regard to blast-injury prevention, mitigation, and treatment standards to be approved by the ASD(HA).

5.3.3. Coordinate DoD blast-injury-research issues with the staffs of the DDR&E, the ASD(HA), and the Director, JIEDDO.

5.3.4. Support the development, maintenance, and usage of a joint database for collection, analysis, and sharing of information gathered or developed by the DoD Components related to the efficacy of theater personal protective equipment (including body armor, helmets, and eyewear) and vehicular equipment designed to protect against blast injury.

5.3.5. Appoint a medical general or flag officer representative to the ASBREM Committee.

5.3.6. Ensure that information is shared as broadly as possible except where limited by law, policy, or security classification and that data assets produced as a result of the assigned responsibilities are visible, accessible, and understandable to the rest of the Department as appropriate and in accordance with Reference (e).

5.4. The Secretaries of the Navy and the Air Force shall:

5.4.1. Forward their respective approved blast-injury medical R&E requirements to the DoD EA for consideration and integration.

5.4.2. Appoint medical general or flag officer representatives to the ASBREM Committee and appoint representatives to any other coordination, oversight, or assessment board established by DDR&E or the DoD EA.

5.4.3. Coordinate with other DoD Components on the assignment of Joint Technical Staff Officers to Army medical research entities, research and acquisition organizations, or installations for coordination of research programming and execution needs pertaining to their Component.

5.4.4. Provide an appropriate system for identification, verification, prioritization, and headquarters-level approval of their respective blast-injury R&E requirements before submission to the DoD EA.

5.5. The President of the Uniformed Services University of the Health Sciences (USUHS), under the ASD(HA) and USD(P&R), shall:

5.5.1. Ensure that education relating to blast-injury prevention, mitigation, and treatment is included in the USUHS medical and continuing education curriculum and programs.

5.5.2. Appoint a representative to any coordination, oversight, or assessment board established by DDR&E or the DoD EA.

5.6. The Chairman of the Joint Chiefs of Staff shall:

5.6.1. Coordinate input to the DoD EA and ensure integration of the requirements processes of the Joint Capabilities Integration and Development System[2] with the processes employed under this Directive.

5.6.2. Appoint a relevant senior representative to the ASBREM Committee.

5.6.3. Appoint representatives to organizational entities of the ASBREM Committee and to any other coordination, oversight, or assessment board established by DDR&E or the DoD EA.

5.7. The Commander, U.S. Special Operations Command shall establish procedures and processes for coordination of relevant Defense Major Force Program 11 activities with those planned, programmed, and executed by the DoD EA and shall also:

5.7.1. Forward that command's approved blast-injury R&E requirements for consideration and integration to the DoD EA.

5.7.2. Appoint representatives to organizational entities of the ASBREM Committee, as appropriate, and to any other coordination, oversight, or assessment board established by DDR&E or the DoD EA.

[2] CJCSI 3170.01E, "Joint Capabilities Integration and Development System," May 11, 2005, is available at http://www.dtic.mil/cjcs_directives/cjcs/instructions.htm.

5.7.3. Coordinate with the command on the assignment of Joint Technical Staff Officers to Army medical research entities, research and acquisition organizations, or installations for coordination of research programming and execution needs.

5.7.4. Provide an appropriate system for identification, verification, and headquarters-level approval of that command's blast-injury R&E requirements before submission to the DoD EA.

5.8. The Director, JIEDDO, consistent with Reference (f), shall:

5.8.1. Support development, maintenance, and usage of a joint database for collection, analysis, and sharing of information gathered or developed by DoD Components related to the efficacy of theater personal protective equipment (e.g., body armor, helmets, and eyewear) and vehicular equipment designed to protect against blast-injury.

5.8.2. Appoint representatives to organizational entities of the ASBREM Committee, as appropriate, and to any other coordination, oversight, or assessment board established by DDR&E or the DoD EA.

5.8.3. Assist the DoD EA, the DDR&E, and the ASD(HA) with identification of related operational and research needs, assessment of relevant research efforts, and coordination of planning to resolve capability gaps through focused research efforts.

6. AUTHORITY

The DoD EA identified by this Directive is hereby delegated authority to do the following:

6.1. Obtain reports and information, consistent with the policies and criteria of DoD Directive 8910.1 (Reference (g)), as necessary, to carry out assigned responsibilities and functions.

6.2. Communicate directly with the Heads of the DoD Components, as necessary, to carry out assigned functions, including the transmission of requests for advice and assistance. Communications to the Military Departments shall be transmitted through the Secretaries of the Military Departments, their designees, or as otherwise provided in law or directed by the Secretary of Defense in other DoD issuances. Communications to the Commanders of the Combatant Commands shall normally be transmitted through the Chairman of the Joint Chiefs of Staff.

6.3. Communicate with other Federal Agencies, representatives of the Legislative Branch, members of the public, and representatives of foreign governments, as appropriate, in carrying out assigned responsibilities and functions. Communications with representatives of the Legislative Branch shall be coordinated with the Assistant Secretary of Defense for Legislative Affairs and the Under Secretary of Defense (Comptroller)/Chief Financial Officer, as appropriate, and be consistent with the DoD Legislative Program.

7. EFFECTIVE DATE

This Directive is effective immediately.

Gordon England

Enclosures – 3
E1. References, continued
E2. Taxonomy of Injuries from Explosive Devices
E3. ASBREM Committee

E1. ENCLOSURE 1

REFERENCES, continued

(e) DoD Directive 8320.2, "Data Sharing in a Net-Centric Department of Defense," December 2, 2004
(f) DoD Directive 2000.19E, "Joint Improved Explosive Device Defeat Organization (JIEDDO)," February 14, 2006
(g) DoD Directive 8910.1, "Management and Control of Information Requirements," June 11, 1993

E2. ENCLOSURE 2

TAXONOMY OF INJURIES FROM EXPLOSIVE DEVICES

E2.1.1. Primary. Blast overpressure injury resulting in direct tissue damage from the shock wave coupling into the body.

E2.1.2. Secondary. Injury produced by primary fragments originating from the exploding device (preformed and natural (unformed) casing fragments, and other projectiles deliberately introduced into the device to enhance the fragment threat); and secondary fragments, which are projectiles from the environment (debris, vehicular metal, etc.).

E2.1.3. Tertiary. Displacement of the body or part of body by the blast overpressure causing acceleration/deceleration to the body or its parts, which may subsequently strike hard objects causing typical blunt injury (translational injury), avulsion (separation) of limbs, stripping of soft tissues, skin speckling with explosive product residue and building structural collapse with crush and blunt injuries, and crush syndrome development.

E2.1.4. Quaternary. Other “explosive products” effects – heat (radiant and convective), and toxic, toxidromes from fuel, metals, etc. – causing burn and inhalation injury.

E2.1.5. Quinary. Clinical consequences of “post detonation environmental contaminants” including bacteria (deliberate and commensal, with or without sepsis), radiation (dirty bombs), tissue reactions to fuel, metals, etc.

E3. ENCLOSURE 3

ASBREM COMMITTEE

E3.1. ORGANIZATION AND MANAGEMENT

The ASBREM Committee shall:

E3.1.1. Consist of general and flag officer and Senior Executive representatives of relevant DoD Components.

E3.1.1.1. Standing members include relevant senior officials of the DoD Components. At a minimum, the DDR&E, the ASD(HA), and representatives of the DoD Components' Acquisition Executives.

E3.1.1.2. The standing membership may be expanded by invitation of the Chair when issues require senior-level coordination outside the scope of the principal members. Such invited members will include a medical flag officer from the Joint Staff, a designee of the DoD EA specified by this Directive, the Director, JIEDDO, the Director of the Combating Terrorism Technology Support Office, and others as appropriate.

E3.1.2. Be chaired by the DDR&E or Senior Executive designee and co-chaired by the ASD(HA) or Senior Executive designee.

E3.1.3. Convene at the discretion of the Chair and Co-chair.

E3.1.4. Invite the attendance of observers from DoD boards, committees or offices, or from other Federal Agencies with interests in the deliberations of the ASBREM Committee.

E3.1.5. Establish subcommittees, Joint Technology Coordinating Groups, and other entities, as required, to facilitate and execute committee business.

E3.2. FUNCTIONS

The ASBREM Committee shall:

E3.2.1. Review medical RDT&E program plans and accomplishments for quality, relevance, and responsiveness to military operational needs, the needs of the Military Health System, and the goals of Force Health Protection.

E3.2.2. Review program plans and budgets in support of the various guidance documents relevant to National Security and to the missions and functions of the Department of Defense.

E3.2.3. Provide coordination, recommendations, and support to DoD EA(s) and other DoD officials as requested, directed, or otherwise appropriate.

Appendix H. Poster Presentations

The Virulence and Lethality of Acinetobacter baumannii *is Dependent on the Degree of Injury and Ischemia of the Wound: Insights into the Mechanism of Wound Infection*

Presenter: John C. Alverdy, University of Chicago

John C. Alverdy, MD FACS, Irma D. Fleming, MD, Monika A. Krezalek, MD, Natalia Belogortseva, PhD, Alexander Zaborin, PhD, Jennifer Defazio, MD, Luis A. Actis, PhD, Olga Zaborina, PhD

Background: The lethality *Acinetobacter baumannii* among military personnel remains significant and what effect, if any, traumatic tissues have on the virulence and lethality of this pathogen are unknown.

Methods: We developed a novel murine model of *A. baumannii* traumatic wound infection to study the effect of the wound environment on *A. baumannii* virulence. Mice underwent rectus muscle crush injury combined with ischemia created by epigastric vessel ligation followed by *A. baumannii* inoculation. Reiterative experiments were performed: 1) in mice without either ischemia or injury, 2) using a derivative mutant of *A baumannii* deficient in the production of its siderophore, acinetobactin, and 3) in mice whose wounds were supplemented with iron as a strategy to suppress *A. baumannii* virulence *in vivo*. All mice were euthanized 7 days later and the rectus muscle analyzed for clinical signs of wound infection, HIF1α accumulation, bacterial abundance and *A baumannii* phenotype expression. To determine the variation in in *vivo* virulence of *A. baumannii* within the various wound environments, isolates were injected into the moth worm *Galleria mellonella* and lethality determined.

Results: Results indicated that tissue injury is necessary to cause *A. baumannii* wound infection. The combination of rectus muscle injury with ischemia resulted in 100% incidence of clinical wound infection and the highest degree of HIF1α accumulation, whereas injury alone or ischemia alone resulted in a 20% and 0% incidence of infection, respectively. *A. baumannii* isolated from injured/ischemic muscle with clinical infection displayed a rough morphotype that induced a higher degree of virulence as judged by *G. mellonella* assays. A smooth morphotype was observed in injured muscle only without clinical infection and showed attenuated killing against *G. mellonella* compared to the rough morphotype. Iron supplementation of the wounds decreased HIF1α production, suppressed *A. baumannii* virulence and prevented wound infection. Experiments performed with the *A. baumannii* mutant deficient in acinetobactin did NOT produce infection associated with lower levels of HIF1α production.

Conclusions: *A. baumannii* wound infections require its siderophore acinetobactin and are dependent on tissue trauma. The process of infection involves HIF1α activation and can be abrogated by local iron, which affects both virulence and HIF1 α expression.

Potentiators Enhance Antimicrobial Activity to Overcome Bacterial Resistance

Presenter: Steven Arcidiacono, U.S. Army Natick Soldier Research, Development & Engineering Center

The prevalence of antibiotic resistant microorganisms highlights the need for new therapeutic strategies. The goal of this effort was to identify potentiators that enhance antimicrobial activity and provide efficacy against a range of resistant organisms. Activity was determined by a kinetic microplate assay to monitor cell optical density in the presence of the plant antimicrobial berberine plus each potentiator candidate; growth inhibition of was indicative of activity. A checkerboard assay was used to screen potentiators in combination with varying ratios and concentrations of berberine against clinical surrogates and was optimized through a series of formulation iterations. Berberine alone had no activity at the concentrations tested; however, in the presence of several potentiators, berberine exhibited broad spectrum activity against all surrogate organisms. Results against surrogates were validated when broad spectrum activity was demonstrated against multiple strains of bacterial (Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Acinetobacter baumannii) and fungal (Candida albicans) clinical isolates. These results demonstrate the efficacy of potentiator formulations using berberine as the proof-of-concept antimicrobial compound. In addition, potentiators combined with antibiotics overcame resistance of Methicillin Resistant S. aureus (MRSA), Vancomycin Resistant S. aureus (VRSA), and Vancomycin Resistant Enterococcus (VRE). Formulations of the select potentiators identified here, in combination with other antimicrobials including traditional antibiotics, may represent additional opportunities for overcoming antimicrobial resistance of clinical and biothreat organisms.

Establishment of a New Drug Discovery Algorithm and New Drug Pipeline for Discovery of Novel Antibacterials

Presenter: MAJ Chad Black, U.S. Army Natick Soldier Research, Development & Engineering Center

Lisa Read[1], Charles E. Bane[2], Mara Kreishman-Deitrick[2], Mark R. Hickman[2], Chad C. Black[2], Emil P. Lesho[3], R. Scott Miller[4], Robert M. Paris[2], Philip L. Smith[5], Paige E. Waterman[6] and Richard J. Sciotti[2]*
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[6]Armed Forces Health Surveillance Branch, Global Emerging Infections Surveillance, Washington, D.C.

Background: The Presidential Initiative to Combat Antibiotic Resistant Bacteria (CARB) was established to identify novel antibacterial agents for use against multi-drug resistant bacteria of military and public health importance. Due to its historic achievements in drug discovery, the Experimental Therapeutics Branch (ET) of the Walter Reed Army Institute of Research was charged to lead the effort to discover novel small molecule antibacterials for U.S. military forces, with the near-term objective of identifying a novel candidate antibacterial for human clinical testing within 5 years.

Methods: We adapted a gated-tier, testing paradigm to identify novel small molecules and profile identified chemical series that exhibit activity against multi-drug resistant bacteria that are relevant to military medicine. The strategy (already applied to our malaria and leishmania programs) uses a resource-sparing testing hierarchy whereby higher throughput/lower cost assays are used to identify hits and profile potential chemical series. Compounds that pass initial cutoff criteria are advanced to more costly and clinically relevant *in vivo* models. After a chemical series (structurally related compounds) is identified, the series is profiled against lead quality attributes to identify potential liabilities. Lead optimization of the chemical series begins with and evaluation of chemical tractability (ease of synthesis) followed by analysis of structural alerts to potential toxicity issues, metabolic liabilities and structural elements known to bind to specific biological targets that will influence the testing strategy. The analysis informs drug design strategy that uses multi-parameter optimization of biological activity, physicochemical properties and ADMET (absorption, distribution, metabolism, excretion and toxicity) to select for analogs that have improved drug-like characteristics.

Results/Conclusion: Leveraging ET's experience and expertise in antiparasitic drug discovery and development, we have established a diverse antibacterial pipeline that features internal projects directed toward novel chemical matter as well as projects with external collaborators in industry, academics and other government agencies. This diversity provides the maximum chance of successfully identifying a novel antibacterial clinical candidate within the ambitious timeline laid out by the CARB strategy, while ensuring optimal use of government and collaborators' resources.

Acute Injury, NSAID Use and Necrotizing Infections

Presenter: Dr. Amy Bryant, Boise Veterans Affairs Medical Center

Bacterial infections following traumatic battlefield injuries remain a significant cause of morbidity and mortality among our military personnel. In addition acute, non-penetrating muscle strain injuries are exceedingly common among those on active duty, those in training and in veterans in hospitals and nursing homes. NSAIDs are cornerstones of pain management in these settings, however, recent evidence suggests that NSAID use after acute muscle strain delays muscle regeneration and decreases muscle strength after repair. Further, clinical evidence and some experimental studies, including our own, suggest that NSAID use increases the risk of secondary necrotizing bacterial infection, especially that due to group *A. streptococcus* (GAS). These cryptogenic infections begin deep in the soft tissues and without an overt wound or cutaneous signs of infection, physicians often miss the diagnosis. Instead, patients are given NSAIDs for pain and are sent home, only to return 24-48 hours later in shock and multi-organ failure. Mortality can reach 85% and survivors often undergo multiple limb amputations, or are left with significant neurological impairments, necessitating prolonged hospitalization and rehabilitation.

Using a unique murine model of this infection, our proteomics analysis revealed that NSAID use after acute strain injury down-regulated proteins that inhibit programmed cell death (apoptosis). Such down-regulation was associated with increased caspase activity in muscles. In vitro, NSAIDs affected proliferating, but not differentiated, muscle progenitors (MPs) and which was associated with increased binding of GAS and a concomitant increase in MP expression of vimentin – a key GAS ligand. By down-regulating pro-survival proteins, we hypothesize that NSAIDs expand the nidus of injury and delay regeneration, thereby leaving muscles susceptible to GAS infection. Our ongoing work seeks to validate this cause-effect relationship in human tissues. Results may provide new information on the physiological role of anti-apoptosis mechanisms in muscle regeneration and the pathophysiological effects of NSAIDs in this process. New knowledge gained may shift the current paradigm of pain management in numerous clinical settings of importance to America's military and veteran populations and may offer new targets to reduce the risk of necrotizing infection after acute soft tissue injury.

Preliminary SEM and EDS Analysis of Novel Surface Modification After 1000 Cycles of Wear Testing

Presenter: Dr. John DesJardins, Clemson UniversityBoise Veterans Affairs Medical Center

Sarah M. Helms[1], Golnaz Najaf Tomaraei[2], Marian S. Kennedy, Ph.D.[2], John D. DesJardins, Ph.D.[2]
[1]Clemson University Department of Bioengineering, [2]Clemson University Department of Materials Science and Engineering

Infection is a concern for any open surgical wound and of particular concern for wounds exposed to debris, as is often the case in military trauma. Systemic antibiotic treatment can be used to combat this infection problem, however it is often ineffective and can lead to antibiotic resistant bacteria. Utilizing a surface modification technology developed by ENBIO in Dublin, Ireland, we have proposed a method in which to modify the surface of commonly used fracture fixation implants such as dynamic compression plates (DCPs) that will result in the localized inhibition of bacterial adherence to the implant. This treatment, consisting of a combination of alumina, polytetrafluoroethylene (PTFE), chitosan, and the antibiotic vancomycin, is proposed to resist bacterial adherence to the implant and minimize infection at the site. Surface characterization of the different combinations of dopants is being analyzed, with focus on wettability, wear resistance, and surface roughness of the modified surfaces. It has been found that the modified surfaces exhibit varying degrees of roughness, however all rougher than the standard fracture fixation implant, and have been shown to have improved wear resistance to traditional titanium and stainless steel metals, which are common DCP materials. After performing a 24 hour viable cell count test on all combinations of dopants inoculated with a bacterial concentration of 1E+03, it was found that the samples modified with vancomycin showed no signs of bacterial growth, however all other sample dopant combinations grew 1E+06 to 1E+08 cells, including blank stainless steel and titanium samples used as controls. In vitro studies are being performed to confirm the proper combination of dopants to minimize bacterial adhesion, followed by a pilot and full in vivo rabbit models to test the efficacy of the treatment in a more realistic and dynamic environment.

Acute Care Cover for Severely Injured Limbs (ACCSIL) Protective Oxygenating Wrap for Enhanced Recovery (Power)

Presenter: Dr. Erik Edwards, Battelle Memorial Institute

Battelle is designing a device under the Acute Care Cover for Severely Injured Limbs (ACCSIL) program to assist military personnel providing point of injury care to servicepersons that have experienced traumatic limb injuries. The device, called the Protective Oxygenating Wrap for Enhanced Recovery (POWER) pack will utilize externally supplied oxygen and the delivery of antimicrobial or biostatic materials to preserve injured tissue. This will ultimately improve the overall, post-injury quality of life of servicepersons.

It is envisioned that tissue will be preserved using a two component approach. One component, a bioactive coating, will minimize infection, help achieve hemostasis, provide pain relief and supply oxygen to the wound. A second, conformal cover component, will contain the wound and the bioactive coating protecting the limb from further injury. Additionally, the conformal cover will supply medication at regular intervals to the wound site. Finally, the POWER pack is being designed to provide the above listed key functions for 72 hours post-injury, so that injured individuals can be taken to a definitive medical care facility in a broad range of operational scenarios.

The objectives of the program are to develop functional prototypes through a series of interviews and discussions with individuals that could potentially interact with the POWER pack device, improve the design through customer and user feasibility feedback, and demonstrate the tissue preservation and antimicrobial performance of the prototypes.

Interviews with potential device users are being conducted through a series of contextual inquiries and formative usability studies to develop use cases, user needs, concepts and prototypes that will be iteratively refined through the interview and discussion process. In parallel with the interviews, technical development activities will be used to identify potential chemistries, materials, and technologies that can meet user needs and provide key functions of the device.

Two rounds of contextual inquiries have been conducted with individuals who could potentially interact with the POWER pack device. These interviews have investigated a series of different form factors, embodiments and features that could be useful for preserving tissue after the time of injury. These contextual inquiries have provided key insights into the needs of potential users and are being used to guide the prototype development activities of the program.

Cathodic Voltage Controlled Electrical Stimulation for Prevention and Eradication of Acinetobacter baumannii *Implant Associated Infections*

Presenter: Dr. Mark Ehrensberger, University at Buffalo

Introduction: Infection subsequent to orthopedic intervention is a primary complication associated with blast-related injuries. Bacterial biofilms on fracture fixation hardware and the surrounding tissue can further compound the situation. Cathodic voltage-controlled electrical stimulation (CVCES) applied directly to titanium (Ti) implants has recently been shown to eradicate methicillin-resistant Staphylococcus aureus implant-associated infections (IAIs) in an in-vivo model. This study evaluated the effectiveness of CVCES to prevent and eradicate Gram-negative Acinetobacter baumannii (A. baumannii) IAIs.

Methods: A clinical isolate of A. baumannii (Ab307) was utilized for all tests. In-vitro studies utilized a 3-electrode system, within a custom chamber designed to simulate soft tissue coverage of an orthopedic implant, to apply CVCES to Ti coupons. In prevention tests, Ti coupons were placed in fresh Ab307 cultures while receiving -1.8V for 2,4,or 8 hrs. In eradication tests, Ab307 biofilms were preformed on Ti coupons and then received stimulation of -1.8V for 1 hr. In both sets of tests, after stimulation the Ti and media was harvested and dilution plated to enumerate colony-forming units (CFU). In-vivo eradication studies utilized an IACUC approved rat prosthetic infection model. Briefly, a Ti rod was implanted through the humeral head of Long-Evans rats and inoculated to establish a local infection. On post-op day 6 an implantable 3-electrode csystem delivered -1.8V to the Ti for 1 hr. Subsequently the Ti and bone tissue were collected for enumeration of CFU. Control experiments (no stimulation) were performed for all tests. Six samples were conducted for each in-vitro test group and eight samples were conducted for each in-vivo test group. Student t-tests compared the CVCES and control groups (significant if $p<0.05$).

Results: CVCES prevented in-vitro coupon-associated CFU and planktonic CFU from reaching detectable levels in a time-dependent manner. Remarkably, no CFU were detectable at 8 hours. CVCES treatment significantly reduced the in-vitro coupon-associated CFU by over 99.9% and coupon-associated CFU by over 99.99%. CVCES treatment significantly reduced the in-vivo implant-associated CFU by over 91% and bone-associated CFU by over 88%.

Significance: CVCES of Ti implants is an effective antimicrobial strategy and may represent a paradigm shift in the prevention and eradication of infections associated with blast-related injuries.

An Overview of Improvised Explosive Devices and Other Blast Related Injuries

Presenter: CAPT Mark Fleming, United States Navy

A lasting legacy of war is knowledge of how wounds heal and how we can help them to do so. This hard-won knowledge is often forgotten between conflicts. This fact is critical, because the wartime lessons and advances in healthcare are applicable to the wounds of peacetime as well. Additionally, much of the dogma that is so common in healthcare have been dispelled by the lessons learned during conflict. An example is the use of a tourniquet. Prior to 2005 the use of tourniquets for hemorrhage control was considered Heresy. However, several peer reviewed reports suggested that 1/3 of combat fatalities were a result of compressible extremity hemorrhage. Therefore, the development of a practicable field tourniquet, its universal distribution, individual training in its application and a paradigm shift away from considering the tourniquet as a tool of last resort has greatly facilitated the survival of critically injured patients.

Since the global war on terror commenced in 2001 over 1600 U.S. service members sustained nearly 2300 combat related extremity amputations and thousands more sustained severe injuries secondary to IEDs and other explosive ordinances. Almost 50% of amputees sustaining a combat related amputation between 2010 and 2014 sustained multiple amputations. As Combat operations lasted well over a decade and the types of wounds that were seen during the early parts of the wars were drastically different than those seen during the later parts of the conflicts. The Injury patterns evolved with changes in tactical operations, particularly as troops transitioned from mounted to dismounted. Early in the conflict the injuries were often isolated system or single extremity injuries as a result of gun shots or during mounted operations. During the latter parts of the conflict the injury patterns were characterized by multiple injuries and amputations usually secondary to IEDs. Multiple extremity injuries and amputations combined with a constellation of pelvic, abdominal, and urogenital wounding secondary to an IED blast represented a frequently encountered treatment dilemma. Treatment strategies were aimed at preserving viable tissue while minimizing complications and maximizing return of function.

The aim of this report will be to describe the mechanisms of blast related injuries, the initial management, the complications (including infectious) associated with blast related injuries and the initial outcomes of treatment.

Blast Injury Infection Prevention Through Enhanced Local Delivery with Biomaterials

Presenter: Dr. Warren Haggard, University of Memphis

Blast injuries create complex wounds with multiple contaminating materials and pathogens. Infection prevention treatments need to have flexibility for wound type, geometry and size, treatment approach, contaminating pathogen, antimicrobial selection and dosage, ease of use, compatibility, and removal. Our research group has pursued multiple investigations for treating complex extremity wounds that are commonly found with blast-related injuries. Those investigations have created an improved understanding of antimicrobial concentration, activity, elution, and diffusion within the wound. Three investigated biomaterial technologies for the mitigation of wound infections were evaluated and one of those technologies, the chitosan sponge, was developed and commercialized. All the investigated technologies have the ability for clinician selection of the antimicrobial or antimicrobials to allow for treatment flexibility and a continuum of care. Past and current infection prevention research outcomes of the three investigated technologies will be discussed to examine enhanced local delivery for mitigating wound infections with an emphasis on in-vitro testing and pre-clinical model results. The advantages and disadvantages of these technologies will be highlighted. A few examples of clinical use of the commercialized chitosan sponge as a wound dressing in civilian cases will also be discussed to illustrate the sponge's use in complex wound environments. The next steps for enhancing the infection prevention outcomes with improved biomaterial technologies will be reviewed.

Rapid Determination of Infection Etiology and Antibiotic Susceptibility

Presenter: Dr. Jane Hill, Dartmouth College

Bacterial colonization and infection of wounds contributes to substantial patient morbidity and mortality. Current gold standard methods to determine infection etiology and antibiotic susceptibility require culturing the organism, a slow process that can take more than 48 hours. Emerging nucleic amplification technologies, while substantially faster than culture methods, are not able to distinguish between bacterial phenotypes, nor differences between infection, colonization, and dead bacteria. Here, we present an approach that captures bacterial metabolism biomarker suites ("fingerprints") of infection using system that can be adapted to real-time analysis. I present two case studies that reflect our progress to-date.

The first case study demonstrates that multidrug-resistant Gram-negative bacteria can be distinguished from their susceptible siblings using a suite of volatile metabolites. For example, *in vitro* studies of over 100 heterogeneous clinical isolates show that only 20 molecules are needed to discriminate carbapenem-resistant from susceptible bacteria. And, in a mouse model using isogenic pairs of *Staphylococcus aureus* differing only in their ability to withstand methicillin assault, a suite of volatile metabolites were able to discriminate between phenotypes without antibiotic addition.

The second case study demonstrates application of the volatile metabolite approach for the determination of infection etiology in an animal model (mouse) of infection as well as human infection, including differentiation from colonization. In a study of six bacterial pathogens in an acute infection scenario in a mouse model, volatile molecules were used to discriminate infection etiology. Translating this approach to a human system, less than 20 molecules from the headspace of over 130 lavage fluids samples from polymicrobial systems (including colonization and infection examples) to identify the dominant organism in the context of heterogeneous co-morbidities. Gross infection was distinguished from colonization and genera level discrimination, including infections with *Pseudomonas aeruginosa* and *S. aureus*. Pilot data from human infection lavage samples indicates that 12 volatile molecules are needed for discrimination between infections caused by MRSA versus MSSA.

The volatile molecule metabolic fingerprint approach is one which has the potential to allow for the rapid determination of infection etiology and drug resistance phenotype. The approach can also be deployed to evaluate the efficacy of new therapeutics.

Promoting Tissue Regeneration with Low Level Energy Therapy to Minimize Infection Risk

Presenter: Gregory Nichols, Homeland Defense and Security Analysis Center

Kunal Mitra, PhD, Professor of Biomedical Engineering, Florida Institute of Technology, Melbourne, Florida
Gregory Nichols, MPH, CPH, Science and Technology Advisor, Homeland Defense and Security Information Analysis Center

Blast injuries, are among the most common injuries found in veterans of the recent conflicts in Iraq and Afghanistan. Additionally, they are among the most extensive to treat in terms of complex tissue damage; chronic pain endured; and long-term rehabilitation needed. In addition, since these wounds can take a long time to heal, tissues are exposed to sources of infection during the healing process. Healing the wound faster and facilitating a rapid immune response would help minimize the potential for serious infection to develop.

Low level energy therapy (LLET) has been explored as a treatment for a number of injury models. Light is an important factor in both the normal functioning of organisms as well as in the correction of dysfunction. This method of treatment involves light with intensities too low to cause significant heating. The observed biological changes, including increases in mitochondrial respiration rate, suppression of inflammatory cytokine response, and cell proliferation, are hypothesized to be due to the interaction of a photon of light with a photoreceptor. Additionally, LLET has been shown to attract neutrophils to infection sites and also promote the absorption of antibiotics by infected tissues. Low level light therapy is attractive as a safe, non-invasive therapy that could be administered to an acutely injured warfighter to stabilize his injury, accelerate wound healing, and minimize infection.

Research regarding how LLET can be utilized in clinical settings have been inconsistent and certain parameters, such as wavelength, irradiance and pulse structure, have never been standardized. Furthermore, little research, if any, has been done regarding the use of LLET in field conditions. Our proposed research will investigate the most efficient sources for LLET and will measure effectiveness of tissue regeneration and assess whether or not LLET is viable for directly reducing microbial growth as well. The ultimate goal of this research is to design a compact mobile delivery system, incorporating the optimal low level energy therapy or combination of therapies that can be to be used in forward deployed areas to promote regeneration of tissue and reduce infection following a traumatic injury.

Posttraumatic Blood Brain-Barrier Defects and Changes in Cytochrome P450 Superfamily: A Concern After Blast Polytrauma

Presenter: Dr. Marten Risling, Karolinska Institute

Alpha-toxin is a major cytotoxic agent released by *Staphylococcus aureus*. Alpha-toxin has been shown to play a role in pathogenesis of disease, since knockout strains show reductions in invasiveness and virulence. Recently, studies indicate that alpha-toxin can induce apoptosis in certain human immune cells. In previous studies we have shown that the alpha-toxin can enter the brain and spinal cord in areas lacking blood brain-barrier (BBB) function. In the normal intact brain, the BBB is absent is restricted areas such as the area postrema in the brain stem, the superficial part of the olfactory bulb and the first segment of the optic nerve, just behind the eye. In addition, we have shown that alpha-toxin can enter the central nervous system in areas affected by traumatic injuries, due to a breakdown of the BBB. This indicates the possibility that circulating alpha-toxin from wounds infected by *Staphylococcus aureus* could be a concern after blast polytrauma and could exert effects in the central nervous system through passage across a defective BBB.

The cytochrome p450 (CYP) is a superfamily of genes encoding for genes involved in metabolism of compounds such as hormones, toxins and pharmaceutical drugs, including antibiotics. It is known that Interaction of drugs sometimes can be due to a competition when more than one compound is metabolized by the same CPY enzyme. Using gene-expression arrays we have studied changes in the expression of members of the CYP superfamily 3 experimental models for Blast TBI. It was observed that there were significant changes in CYP expression after secondary blast (fragment penetration) but not primary or tertiary blast. For example, the CYP 1B1 showed a 20-fold increase in expression. This increase was verified by in situ hybridization and immunohistochemistry. CYP 1B1 is involved in metabolism of both hormones and the pro-inflammatory prostaglandins. Several other members of the CYP family showed an increased expression, but at more modest levels (1-2 fold change). It should be noted that this injury zone also has a defect in the BBB. Therefore, it should not be excluded that such dynamic changes in the CYP superfamily in the injured area of the brain might influence the metabolism of bacterial toxins or antibiotics in the injured brain.

PUL-042 – Novel, Broad Spectrum, Immune Stimulant to Prevent Infection and Mortality From a Broad Range of Respiratory Infections

Presenter: Dr. Brenton Scott, Pulmotect, Inc.

A broad array of medical countermeasures (MCMs) for infections have been proposed. Military and civilian populations are at added risk against conflict and terrorism, requiring improved mitigation and treatment options. Opportunistic infections and multi-drug resistance infections are increasing, presenting unique challenges for blast victims. These threats can be contagious respiratory illnesses that lead to hospitalization or death. While vaccines offer pre-exposure protection, they are limited to specific strains and are difficult to manufacture and distribute quickly, offering a limited response.

When the respiratory tract is exposed to virulent pathogens, a microscopic arms race ensues. Time for treatment is critical. Pulmotect is developing a clinical stage drug, PUL-042, for prevention and treatment of infection by inhaled pathogens that is complimentary to vaccine and anti-viral approaches that has immediate activity boosting the host's immune system to fight off a broad range of infections.

PUL-042 is a novel, first-in-class immunomodulatory agent consisting of two stable synthetic molecules that can be mass-produced. It exploits a phenomenon of stimulated antimicrobial resistance in respiratory epithelia by locally activating the lung's innate immune defenses. The host-based resistance mechanism is initiated in seconds and is "pathogen agnostic."

In vitro and lethal challenge models were used to demonstrate the breadth and strength of PUL-042. It maintains its activity with multiple doses and protects for up to 4 weeks. In addition, PUL-042 synergizes with antivirals to enhance efficacy and expand treatment windows.

PUL-042 is a clinical stage drug with two active INDs and two Phase I clinical trials characterizing safety/tolerability. It is currently being developed for immunocompromised cancer patients at risk of pneumonia in collaboration with researchers at MD Anderson Cancer Center. Based on efficacy against a wide range of pathogens, four other indications have been identified: 1) Combining with antivirals/antibiotics to improve treatment regimens 2) Preventing asthma exacerbations by fighting off viral infections, 3) Addressing patients with chronic airway diseases, such as COPD and cystic fibrosis, and 4) Use as a broad spectrum defense against emerging, biodefense, and opportunistic pathogens from major events.

Novel Diagnosis, Treatment, and Prevention Strategies of Infections Following Blast Injury

Presenter: Dr. Mark Shirtliff, University of Maryland–Baltimore

The long-term survival of wounded warriors following traumatic blast injuries is fraught with complications, including chronic biofilm infections with the resulting high rates of morbidity and mortality. A biofilm can be defined as a microbial community that shows a unique phenotype and dramatically higher tolerance to antimicrobial agents than their planktonic counterparts. This biofilm phenotype results from a unique 'omic (transcriptomic, proteomic, and immunoproteomic) expression of antigens that can be exploited by clinicians for opportunities to diagnose, treat, or even prevent these infections following blast injury.

We have identified antigens that are expressed when microbes are in a biofilm mode of growth (including *Staphylococcus aureus* and *Pseudomonas aeruginosa*) in musculoskeletal infections, infected burns, and chronic wounds. By measuring the host antibody response to particular biofilm antigens, these notoriously difficult-to-culture infections can be quickly (<5 min.), inexpensively (<$20), and accurately diagnosed. Clinical studies have shown both a sensitivity and specificity of 100% when compared to polymerase chain reaction diagnostics. In addition, when unique biofilm and planktonic antigens are delivered as a pre-challenge vaccination or passive immunization with antibodies against these antigens biofilm infections are prevented in animal models of sepsis, osteomyelitis, and prosthetic implant infection. Even in cases of post-infection administration, passive immunization was able to eliminate infection dissemination and mortality. These novel strategies represent a fundamental leap in diagnosing, treating, and preventing the infectious complications following blast injuries, thereby preventing morbidity and mortality in wounded warriors.

Omega3 Rich Fish Skin as Infection-Prevention Strategy after Blast Injuries: An In Vitro Bacterial Barrier Study

Presenter: Hilmar Kjartansson, Kerecis

Skuli Magnusson BSc[1,2], Baldur Tumi Baldursson MD PhD[1,3], Hilmar Kjartansson MD[1,3], Gudmundur Fertram Sigurjonsson MSc Eng[1]
[1]Kerecis
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Introduction: Use of improvised explosive devices in modern warfare has increased the frequency of blast injuries. Victims of explosions often suffer from multiple traumatic injuries with high risk of wound infection. The most frequently multidrug-resistant identified drug resistant strains of bacteria is *Staphylococcus aureus*. Current pre-hospital treatments for blast-related injury involve simple non bioactive dressings to limit secondary wound contamination. No disease transmission risk exists between codfish and humans, allowing for total preservation of the natural elements of the fish skin. Mammalian derived tissues however require treatment with harsh detergents due to disease transmission. Randomized and double blind clinical trial have shown that cod fish skin promotes faster healing in acute wounds compared to a mammalian derived product. Fish skin is adapted to constant thread of invading pathogens in the aquatic environment. Kerecis™ Omega3 is FDA cleared acellular fish skin that possesses multiple natural biomechanical properties that facilitate tissue protection and regeneration.

Objective: The objective of this study was to assess the ability of cod fish skin to act as a barrier to bacterial invasion.

Method: Biomaterials (1.5 cm x 1.5 cm) were placed between a two chamber apparatus. Broth with log 4.0 colony-forming units (CFU)/ml of *Staphylococcus aureus* (ATCC 25923) was injected into the upper chamber and sterile broth into the lower chamber. Kept at 37°C until breached by *S. aureus*, calculated from *S. aureus* growth curve.

Results: The fish skin is more effective barrier to *S. aureus* compared to Puraply™ (Organogenesis) type I porcine collagen matrix, Epifix® (Mimedx) human amniotic membrane allograft and Endoform (Hollister) dermal template dressing. Spiking the Omega3 content of the fish skin further augments its barrier properties.

Conclusion: The Kerecis Omega3 fish skin technology is naturally more effective bacterial barrier compared to mammalian tissues. Kerecis Omega3 provides a new and more advanced treatment option for blast-related injuries. Serving both as tissue-preservation and infection-prevention strategy.

Navy Medical Research for the Management of Wound Infections: Basic Science and Novel Antimicrobial Therapeutics

Presenter: CDR Michael Stockelman, Naval Medical Research Center

Multidrug resistant bacterial infections of combat extremity wounds have become a severe challenge affecting both quality of life and capacity to return to duty for wounded warriors. Management of these infections places a significant burden on the Military Health System. As antibiotic resistance continues to limit treatment options, new preventive and therapeutic approaches are needed. The Wound Infections Department at the Naval Medical Research Center and its collaborators have an active portfolio of projects to respond to this need, both with the development and testing of novel therapeutic approaches, and with the investigation of basic mechanisms of the infection process.

We have several new therapeutics at various stages of technological maturity, as well as in vitro and in vivo systems for testing them. Phage therapeutics using cocktails of naturally occurring phage types is a promising approach on the cusp of testing in clinical trials. Phage libraries are in development for all the ESKAPE pathogens significant for their frequent multidrug resistant phenotypes. Photodynamic therapy, in which functionalized nanoparticles are activated by visible light to induce antimicrobial activity, are being tested preclinically in vivo. Products of beneficial bacteria, and other natural antimicrobial products, are in early stages of testing. We are looking at applications of our most advanced strategies, such as integration into deployable wound dressings.

In addition to product-oriented research, we are investigating basic processes within the wound infection. Most prominently, we are studying the immune response to skin and soft tissue infections. By studying what kinds of responses correlate with the best clinical outcomes, and by testing immune responses for their functional effects on pathogens, we can identify new vaccine candidates and targets for therapeutic intervention.

These efforts are closely coordinated with the programs of other Navy laboratories and with the work of our Army counterparts to sustain a robust pipeline for future solutions to the challenge of antibiotic resistance in combat wound infections.

Pathogen-Specific Biomarker for Diagnosis and Management of Wound Infections

Presenter: Dr. Michael Super, Wyss Institute, Harvard University

Current diagnostics of sepsis using blood cultures and molecular diagnostic tests fail to detect wound and bloodstream infections in most infected patients, whereas the inflammatory biomarkers of infection that have a higher sensitivity of detection, lack specificity in distinguishing infection from trauma-related inflammation. Therefore we have leveraged a broad-spectrum pathogen binding opsonin (FcMBL) and developed a rapid test to directly diagnose the presence of infection in the blood to triage patients and guide antibiotic therapy. The FcMBL ELLecSA (Enzyme-Linked Immuno-Sorbent Assay) is able to detect PAMPs (pathogen associated molecular patterns) present on, or released by, 85% of clinical isolates representing 73 of 86 different pathogen species, including the most common causes of sepsis and wound infections. The PAMPs assay rapidly (81%), specificity (>89%), and diagnostic accuracy (0·87). It also distinguished infection from trauma-related inflammation in patient cohorts with a higher specificity than the clinical sepsis biomarker, C-reactive Protein. The FcMBL ELLecSA-based PAMPs assay offers a rapid, simple, sensitive and specific method for diagnosing infections, even when blood cultures are negative and antibiotic therapy has been initiated, that is amenable to development as a point of care device. (Ref Cartwright et al eBiomedicine 2016)

Bacterial Colonization Modulates Host Inflammatory Responses in Dehisced Wound

Presenter: Dr. Meenu Upadhyay, Uniformed Services University Health Sciences

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Background: Wound dehiscence is a common problem in the treatment of combat-related trauma and often associated with microbial bioburden. According to our previous finding microbial factors can modulate inflammatory signals that impede normal healing.

Methods: We identified 4 patients (total 8 wounds) in a group of 75 enrolled in a IRB approved protocol with at least two critically colonized (CC, 105 cfu / g of tissue) wounds from each patients in the same study subject. Each one of these patients had one wound that healed successfully and the other dehisced. Gene expression profile of healed and dehisced wound were compared in presence of critical colonization by qPCR.

Results: We evaluated critically colonized dehisced and healed wounds in this study and found most of the dehisced wounds were colonized with Acinetobacter species (105 cfu / g of tissue). We found statistically significant difference in the local expression of LPS binding protein (LBP) p< 0.05) in healed wound compared to dehisced. Furthermore, local tissue gene expression of MCP-1 and CSF-2 were also exhibit differential expression (p 0.05) in healed and dehisced wound.

Conclusions: The variable expression of pro-inflammatory responses we detected in dehisced and healed wound may play an important role in the interaction of immune cells at the site of infection and affect phagocytosis.

Novel Strategies to Prevent and Treat Recalcitrant Biofilm Wound Infections

PresenteR: Dr. J. Scott VanEpps, University of Michigan

Blast injuries commonly result in wounds which are susceptible to infection. These infections can be difficult to treat due to the development of bacterial biofilms. Biofilms are communities of bacteria encapsulated in a polymeric matrix that confer significant protection from both topical and systemic antibiotics as well as host immune response. This motivates the development of new technologies and strategies to prevent and treat these recalcitrant infections. Here we present two novel strategies to improve outcomes from biofilm infections. First, we describe the development of zinc oxide nanoparticles (ZnO-NPs) with potent antibacterial properties against multidrug resistant organisms including methicillin resistant *Staphylococcus aureus* (MRSA). These particles can be engineered with specific shape and/or surface chemistry to tune their antimicrobial effects. Aqueous dispersions of these ZnO-NPs are bactericidal with almost 4 log reduction in colonies in just thirty minutes. When applied as a coating to surfaces using a layer-by-layer (LBL) technique, this material reduces biofilm development by *S. aureus* by more than 95%. The LBL technique can be applied to many different materials post fabrication including dressing materials and implanted medical devices. It represents a promising technology to prevent biofilm formation at vulnerable sites such as blast wounds. Second, when a biofilm does form it is recalcitrant to standard antibiotic therapy and in the case of blast wounds may require multiple debridement procedures to obtain source control. We demonstrate that modest elevations in temperature (i.e., 45° C) may be a useful adjunct for treating biofilm infections in situ. We show that changes in temperature result in irreversible softening of the viscous and elastic moduli of Staphylococcal biofilms. More importantly, increasing temperatures augment conventional antibiotic killing of common biofilm forming pathogens including *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, and *Klebsiella pneumoniae*. Together, this suggests that applying a modest amount of heat to a biofilm may increases its susceptibility to antibiotic treatment. The goal is for in situ biofilm treatment to reduce surgical debridement of wounds. Biofilms have multiple protective features to ensure survival. Only through multiple adjuvant preventative and treatment strategies can we expect to improve outcomes of these infections.

Narrow Spectrum Therapeutics for the Treatment of Acinetobacter baumannii *Infections*

Presenter: Dr. Daniel Zurawski, Walter Reed Army Institute of Research

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The Wounded Warrior is susceptible to bacterial infections after blast and other battlefield injuries. These bacteria are often extensively drug-resistant (XDR), making most antibiotic regimens ineffective, which then leads to numerous debridements, wash outs, amputations, amputation revisions, sepsis, and other associated morbidity. While four new drugs have been FDA-approved for gram positive bacterial infections to include methicillin-resistant *Staphylococcus aureus* (MRSA), gram negative bacteria still pose a grave threat to soldiers as the drug pipeline is limited. Of these, *Acinetobacter baumannii* posits a difficult challenge to both researchers and caregivers because of a plastic genome that can rapidly acquire resistance and because of an innate ability to persist inside and outside of the human body.

Our team took two approaches to combat *A. baumannii*. First, we developed monoclonal antibodies (mAb) against surface targets that are required for bacterial pathogenesis, which serves as a proof-of-concept study for an anti-virulence therapeutic. Second, we partnered with Spero Therapeutics, Inc. to utilize their "potentiator" molecule SPR741, which on its own does not kill bacteria, but instead, disrupts the bacterial membrane increasing the efficacy of antibiotics. This combination approach also limits resistance. *A. baumannii* is particularly vulnerable to SPR741 making both approaches a narrow spectrum attack. For the mAb study, we targeted the Type Six Secretion System (T6SS) that is responsible for secreting toxins into host cells. Our first step showed that Hcp, the T6SS needle, is required for virulence. Then, we generated mAbs against Hcp, and showed a specific mAb could protect mice from XDR-*A. baumannii* infection in pulmonary and wound models when injected prophylactically.

For the potentiator approach, we compared a mixture of SPR741 and rifampin to both compounds alone and an untreated, negative control in our murine pulmonary model of A. baumannii infection. Rifampin alone at a 10 mg/kg/BID dosing regimen provided some efficacy, 50% animal survival after a weeklong infection. However, when rifampin was combined with SPR741 at 60 mg/kg/BID, we achieved 95% survival over the same time course. This was in contrast to SPR741 alone that only provided 20% animal survival or no treatment, which provided 0% survival. Both studies also showed concurrent drops in bacterial burden and are promising starts that warrant further development.

Appendix I. Keynote Speaker

John B. Holcomb, MD, FACS

John Bradley Holcomb received his M.D. from the University of Arkansas Medical School in 1985. Dr. Holcomb entered the U.S. Army in 1985, and completed his general surgery training in 1991. Dr. Holcomb then deployed with the Joint Special Operations Command for the next decade. From 2002 to 2008, COL Holcomb was the Commander of the U.S. Army Institute of Surgical Research and Trauma Consultant for the Army Surgeon General. Over the years he deployed in multiple real world operations. He is a three time recipient of the Army's Greatest Invention award. COL Holcomb retired from active duty in 2008 and received the Lifetime Achievement Award in Trauma Resuscitation Science from the American Heart Association, the United States Special Operations Command Medal, and the Service award from the American College of Surgery. He has been a member of the Committee on Tactical Combat Casualty Care since 2001. In 2008, Dr. Holcomb joined the University of Texas Health Science Center at Houston as a Professor of Surgery. He was named the Director of the Center for Translational Injury Research and Vice Chair of the Department of Surgery. Dr. Holcomb is actively involved in clinical medicine, education, research, entrepreneurship and is a founder of a small health IT company. He reviews papers for more than 25 journals, has published more than 400 peer-reviewed articles and serves on multiple boards. Dr. Holcomb and his wife, Dr. Kelly Wirfel, have been married since 1998 and have 2 children.

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