WELCOME

November 29, 2012

Dear Colleagues:

Welcome to the FDA White Oak campus and to this Public Workshop on *Burkholderia*: Exploring Current Issues and Identifying Regulatory Science Gaps. This workshop has been made possible through collaboration with the Defense Threat Reduction Agency; the Joint Science and Technology Office for Chemical and Biological Defense; the U.S. Strategic Command Center for Combating Weapons of Mass Destruction; the National Institute of Allergy and Infectious Diseases; the Centers for Disease Control and Prevention; the U.S. Army Medical Research Institute of Infectious Diseases; the Biomedical Advanced Research and Development Authority; and the Chemical Biological Medical Systems Joint Project Management Office.

Two years ago, the U.S. Public Health Emergency Medical Countermeasures Enterprise sponsored the 2010 HHS *Burkholderia* Workshop, which focused on developing consensus recommendations for the post-exposure prophylaxis and treatment for melioidosis and glanders. Since then, interest has increased and research efforts have expanded to understand the microbiology and applied aspects of animal models and clinical disease.

Over the next day and a half, we invite you to join us in this interagency and international effort to explore current information on melioidosis (caused by *Burkholderia pseudomallei*) and glanders (caused by *Burkholderia mallei*). The workshop goals are to identify solutions or future areas of research needed to further animal model development and to advance candidate medical countermeasures (MCMs) for approval, licensure, or clearance. You will hear from many of the scientists who are involved in critical areas of *Burkholderia* countermeasure development and research as well as from colleagues who are partnering with FDA in this important endeavor.

We would like to thank all of you who have made this event possible. Thanks go to our partner agencies for their support, and staff, who have worked tirelessly to plan the workshop. Special thanks go to the presenters, many of whom have traveled long distances to be here today. Last, but far from least, we want to take this opportunity to thank all of you for your enthusiasm and commitment to this critical national effort.

Sincerely,
The Program Committee

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AGENDA

THURSDAY, NOVEMBER 29, 2012

8:00 – 8:10 Welcome
Lisa Hensley, U.S. Food and Drug Administration

8:10 – 8:20 Introductory remarks
Tracy MacGill, U.S. Food and Drug Administration

8:20 – 9:00 SESSION 1: PUBLIC HEALTH EMERGENCIES: PREPAREDNESS FOR MELIOIDOSIS AND GLANDERS EVENTS

Moderator: *Mark Albrecht, Biomedical Advanced Research and Development Authority*

8:20 – 9:00 Report on 2010 HHS *Burkholderia* Clinical Guidance Workshop
Rebecca Lipsitz, Office of the Assistant Secretary for Preparedness and Response

9:00 – 10:15 SESSION 2: CURRENT KNOWLEDGE OF CLINICAL DISEASE IN HUMANS

Moderator: *David Blaney, Centers for Disease Control and Prevention*

9:00 – 9:20 Epidemiology, route of infections, clinical risk factors, and prevention
Direk Limmathurotsakul, Mahidol University, Bangkok, Thailand

9:20 – 9:40 Clinical manifestations, standard and acquired antimicrobial sensitivity and resistance, and therapy guidelines
David Dance, Mahosot Hospital, Vientiane, Laos

9:40 – 10:15 Glanders
David Dance, Mahosot Hospital, Vientiane, Laos

10:15 – 10:30 BREAK*

10:30 – 11:15 SESSION 3: DIAGNOSTICS

Moderator: *Alex Hoffmaster, Centers for Disease Control and Prevention*

10:30 – 10:45 Review of diagnostic tests for naturally-acquired melioidosis and accidental or deliberate *B. Pseudomallei* exposure
Sharon Peacock, University of Cambridge, United Kingdom

10:45 – 11:00 Melioidosis diagnostics at CDC: Currently, in the works, and in the future
Alex Hoffmaster, Centers for Disease Control and Prevention

11:00 – 11:15 Lateral flow assay
Dave AuCoin, University of Nevada School of Medicine

11:15 – 12:15 SESSION 4: GENOMICS AND STRAIN CHARACTERISTICS

Moderator: *Clint Florence, Defense Threat Reduction Agency*

11:15 – 11:35 Genomic analysis of differential virulence of *B. pseudomallei* in a BALB/c mouse model of acute melioidosis
Apichai Tuanyok, Northern Arizona University

11:35 – 11:55 Developing a reference panel of *Burkholderia pseudomallei* strains
Carl Gelhaus, Battelle Memorial Institute

11:55 – 12:15 Antibiotic resistance and its impact on strain selection
Herbert Schweizer, Colorado State University

12:15 – 13:00 LUNCH*

13:00 – 16:15 SESSION 5: ANIMAL MODELS

Moderator: *Mark Albrecht, Biomedical Advanced Research and Development Authority*

13:00 – 13:30 Comparative aspects of caprine melioidosis
Carl Soffler, Colorado State University

13:30 – 14:00 Rodent models of glanders and melioidosis
Dave Waag, United States Army Research Institute of Infectious Diseases

14:00 – 14:30 Characterization of *Burkholderia pseudomallei* NCTC 13392 for evaluation in a NHP model
Julia Vipond, HPA Microbiological Services, Salisbury, United Kingdom

14:30 – 14:45 BREAK*

14:45 – 15:15 Novel imaging techniques to monitor therapeutic treatments against *Burkholderia* respiratory infections
Alfredo Torres, University of Texas Medical Branch - Galveston

15:15 – 15:45 Autotransporter and two partner secretion pathway proteins and their roles in *B. pseudomallei* pathogenesis in a mouse model
Peggy Cotter, University of North Carolina School of Medicine

15:45 – 16:15 Kinetics of growth and dissemination of *Burkholderia mallei/pseudomallei* after aerosol infection
Jeff Hogan, University of Georgia College of Veterinary Medicine

16:15 – 16:45 Wrap-up discussion and adjournment
Pamela Chamberlain, U.S. Food and Drug Administration

FRIDAY, NOVEMBER 30, 2012

8:05 – 9:35 SESSION 6: CANDIDATE MEDICAL COUNTERMEASURES

Moderator: *Anthony Macaluso, Chemical Biological Medical Systems*

8:05 – 8:20 Antibacterial activity and in vivo efficacy of pyrimidoindoles versus *B. pseudomallei*
John Finn, Trius Therapeutics

8:20 – 8:35 Optimization of pre-IND leads with activity against *B. pseudomallei*
Rick Slayden, College of Veterinary Medicine, Colorado State University

8:35 – 8:50 Fully synthetic tetracyclines designed for biothreat and multidrug-resistant hospital pathogens
Joyce Sutcliffe, Tetraphase Pharmaceuticals

8:50 – 9:05 Vaccines against chronic melioidosis
Richard Titball, University of Exeter, Devon, United Kingdom

9:05 – 9:20 Virus-like particles as a novel platform for *Burkholderia* protective antigens
Joann Prior, Defence Science and Technology Laboratory, Wiltshire, United Kingdom

9:20 – 9:35 Melioidosis and glanders vaccine development
Eric Lafontaine, University of Georgia

9:35 – 9:45 BREAK*

9:45 – 10:45 SESSION 7: SCIENTIFIC AND REGULATORY CHALLENGES TO COUNTERMEASURE DEVELOPMENT

Moderator: *Rosemary Roberts, U.S. Food and Drug Administration*

9:45 – 10:05 New Drug Application (NDA) for an antimicrobial drug for a medical countermeasure indication
Elizabeth O'Shaughnessy, U.S. Food and Drug Administration

10:05 – 10:25 Review of clinical trials of antimicrobial therapy for melioidosis in relation to intervention development
Sharon Peacock, University of Cambridge, United Kingdom

10:25 – 10:45 Animal Rule
Rosemary Roberts, U.S. Food and Drug Administration

10:45 – 11:00 BREAK*

11:00 – 11:50 SESSION 8: NEXT STEPS

Moderators: *Lisa Hensley and Pamela Chamberlain, U.S. Food and Drug Administration*

This will be an interactive panel discussion in which the critical points and next steps will be captured in real-time on the screen. The panel of speakers will assist in leading the discussion to supplement audience participation.

11:50 – 12:00 Closing Remarks
Tracy MacGill, U.S. Food and Drug Administration

*Conference attendees and/or presenters are responsible for meals and/or light refreshments on their own and at their own cost. Government staff and/or Government contractors may not be involved in the provision or facilitation of food and/or light refreshments for conference attendees and/or presenters.

SPEAKER BIOGRAPHIES

Mark Albrecht, Ph.D., joined the Biomedical Advanced Research and Development Authority (BARDA) in March 2012. As a BARDA project officer, he provides program management oversight and technical support to the animal model development efforts of the Broad Spectrum Antimicrobials program. Dr. Albrecht received his B.A. in Biology from the Whitman College, Walla Walla, WA. He went onto the University of California, Riverside, where he received his Ph.D. in microbiology while studying the properties of sensitivity and resistance of Pseudomonas aeruginosa and Burkholderia cepacia to antimicrobial peptides and the role of alginate lyase during P. aeruginosa biofilm formation. Dr. Albrecht went on to serve two years as a Post-Doctoral Fellow at the Naval Medical Research Center (NMRC) charged with evaluating the therapeutic and post-exposure efficacy of two fully human monoclonal antibodies with specificity to Bacillus anthracis Protective Antigen and Lethal Factor. While at the NMRC he was promoted to Staff Scientist and later Senior Staff Scientist at the Biological Defense Research Directorate. In these roles he was responsible for continued evaluation of passive immune-therapy against anthrax, development of multi-valent/agent vaccination strategies against B. anthracis and Y. pestis based on recombinant proteins and DNA, support of the Navy's and DARPA's Seven-Day Burkholderia Biodefense Research Program, and maintained corporate research collaborations between industry and the Navy. Dr. Albrecht has 14 years of multidisciplinary expertise in project management, animal modeling, biodefense biology, and novel therapeutics and vaccine countermeasures against human bacterial pathogens. Dr. Albrecht has authored multiple peer-reviewed scientific articles and holds two patents on the use of antibodies to improve vaccine efficacy and one on DNA sequence optimization for eukaryotic expression.

David AuCoin, Ph.D., M.S., is a principal investigator within the Diagnostics Discovery Laboratory, which is a research group within the University of Nevada School of Medicine's Department of Microbiology and Immunology. Currently, his laboratory is focused on developing diagnostics and therapeutics for microbial infections. Attention has been focused on targeting capsules that surround the exterior of microbes. Often these structures make ideal targets for immunodiagnosis. Additionally, development of a novel strategy termed "In vivo Microbial Antigen Detection" (InMAD) has allowed for the identification of secreted/shed antigens that may be targets for diagnosis or immunotherapeutics. Following identification of secreted microbial antigens, his laboratory develops monoclonal antibodies (Mabs) targeting these antigens. The MAbs are then used to construct lateral flow immunoassays to rapidly diagnose diseases such as melioidosis, aspergillosis and candidiasis. The capsular polysaccharide (CPS) produced by B. pseudomallei was identified by InMAD as an encouraging diagnostic target. A monoclonal antibody has been produced that is reactive with CPS in patient samples. InBios International has incorporated the CPS specific mAb into a lateral flow immunoassay (LFI), which is currently undergoing pre-clinical testing in endemic areas of Thailand and Australia.

David Blaney, M.D., M.P.H., is a medical epidemiologist at the U.S. Centers for Disease Control and Prevention in Atlanta, Georgia. He has been working with the Bacterial Special Pathogens Branch at the Centers for Disease Control and Prevention since August of 2008. Since starting with the Branch, Burkholderia has been one of his pathogen focuses, primarily addressing biopreparedness activities, epidemiology, and clinical diagnosis and treatment. As a part of his work, he has been consulted on numerous occasions by physicians and state health departments for guidance on epidemiologic investigation and case management, and was a lead organizer for an HHS-sponsored meeting of subject matter experts to develop consensus guidelines for post-exposure prophylaxis and treatment in an intentional release situation. He has co-authored four manuscripts related to melioidosis and co-authored three book chapters on melioidosis; he has also performed peer review of numerous manuscripts on melioidosis.

Pamela Chamberlain, D.V.M., D.A.B.T., Ph.D., has extensive experience and training in private clinical practice, as a reviewer and food safety toxicologist at the FDA Center for Veterinary Medicine, Temporary Advisor to the Joint FAO/WHO Expert Committee on Food Additives for veterinary drugs, and Lead Delegate for the US Delegation to the Codex Committee on Residues of Veterinary Drugs in Foods. Dr. Chamberlain also has industry experience working at a contract research organization as a Study Director Manager specializing in preclinical safety toxicology studies, and as Associate director of Veterinary Services. Currently with FDA, Dr. Chamberlain is applying her diverse background, experience, and knowledge to help advance the important mission of the Office of Counterterrorism and Emerging Threats (OCET), and in addition is serving as the current Institutional Official for the FDA's White Oak campus animal program, and is a member of the Federal Veterinarian Workforce Talent Management Advisory Council.

Peggy Cotter, Ph.D., is a professor in the department of microbiology and immunology at the UNC School of Medicine. Her lab is currently characterizing the expression, maturation and cellular localization of the B. pseudomallei TPS and AT proteins and is investigating their potential roles in adherence, immunomodulation, and other aspects of B. pseudomallei

pathogenesis. She has discovered that the TPS proteins function as Contact-Dependent Growth Inhibition (CDI) systems and has recently published the first characterization of these systems in Burkholderia. Her characterization of putative AT proteins indicates that at least two are critical for virulence.

David Dance, M.B., Ch.B., M.Sc., has practiced as a medical microbiologist in the UK, Thailand and Laos for the past 30 years. He originally undertook specialist training with the UK Public Health Laboratory Service and then in 1986 helped set up clinical studies on melioidosis in Ubon Ratchathani, north east Thailand, which continue to this day. He returned to the UK in 1990, initially continuing research into Burkholderia pseudomallei, but in 1994 he moved to Plymouth where for 10 years he worked as Director of the Public Health Laboratory and as a Consultant Clinical Microbiologist. Following the establishment of the Health Protection Agency, he was appointed Regional Microbiologist for the south west of England, a post he held for 6 years. In these latter two roles, he was national lead for clinical and public health aspects of imported melioidosis and he became increasingly involved in planning for deliberate release of this and other bacteria, writing national guidelines and developing and delivering training programs. In 2010, he returned to clinical research in Southeast Asia and he is currently based in Mahosot Hospital, where the first case of melioidosis recognized in the Lao PDR was diagnosed in 2000 and where more than 500 cases of culture positive melioidosis have subsequently been seen.

John Finn, Ph.D., has been working in the field of industrial organic chemistry for thirty years, and in the area of antibacterial drug discovery since 1998. From December 1984 to January 1995, he served as the senior scientist of American Cyanamid Company. From January 1995 to December 1997, he served as associate director at Synaptic Pharmaceutical Corporation, a biopharmaceutical company. From January 1998 to March 2003, he served as the senior director of lead discovery and optimization at Cubist Pharmaceuticals, Inc., a biopharmaceutical company. From December 2003 to June 2004, he served as the vice president of drug discovery at Elitra Pharmaceuticals Inc., a biopharmaceutical company. He founded Rx3 Pharmaceuticals in 2004 with five other scientists. He led the company and served as the main synthetic and computational chemist until they attracted their Series A round and changed their name to Trius Therapeutics. At that point, he became CSO and helped in-license tedizolid, which he has taken from preclinical through to Phase 3 clinical studies. The research group he lead as CSO has had several successful structure-based drug design projects, including one targeting Gram-positive MetRS. Projects at Trius have included iterative structure-based drug design and collaborations with academic and government laboratories.

William Florence, Ph.D., attended Texas Tech University Health Sciences Center and obtained a Bachelor's of Science in Clinical Laboratory Science and obtained his Ph.D. in Microbiology/Immunology from Ohio State in 2007 studying MHC Class II antigen presentation pathway and its interaction with intracellular pathogens. Following a post-doctoral fellowship at Vanderbilt University Medical Center in the Department of Microbiology and Immunology to study the Immunology of Invariant Natural Kill T-cells (iNKTs), Dr. Florence joined the Defense Threat Reduction Agency as a Science and Technology Manager. There, he manages the Vaccines portfolio for the development of Vaccines directed against bio-warfare agents and a portfolio dedicated to the proof-of-concept research to develop an in vitro platform of human organ constructs that could accurately predict human safety, efficacy, and pharmacokinetics of candidate Medical Countermeasures (MCMs) (i.e., therapeutics and pharmaceuticals). It is envisioned that the platform could be utilized in the development of MCMs by generating data to support in vivo testing and evaluation plans for investigational products. A long-term goal of this research is to explore the potential for this technology to reduce the overall burden of in vivo testing in the development and management of products for human use.

H. Carl Gelhaus, Ph.D., has worked for 14 years in the field of Immunology, covering a diverse range of topics including 7 years on bioweapons and bioterror threats. At the United States Army Medical Research Institute for Infectious Disease (USAMRIID), Dr. Gelhaus performed research on the role of toll-like receptors (TLR) involved in the pathogenesis of diseases caused by gram-negative select agent bacteria. Namely, the roles of TLR1, 2, 4, 6, and 9 were investigated in Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis, and Yersinia pestis. This work was conducted both in vivo and in vitro, using mouse models, including bone marrow derived dendritic cell cultures. Dr. Gelhaus was able to demonstrate that that CpG oligodeoxynucleotides alleviated B. pseudomallei infections while exacerbating F. tularensis infections. Dr. Gelhaus also participated in research efforts involving the recombinant F1-V plague vaccine under development at USAMRIID and investigated the role of quorum sensing in Y. pestis pathogenesis. At Battelle, Dr. Gelhaus has been involved in the development of animal models associated assay of tularemia and melioidosis to support licensure of medical countermeasures through the US Food and Drug Administration Animal Rule. Dr. Gelhaus has begun to investigate the efficacy of vaccines and drugs using these models. Dr. Gelhaus has 6 years of experience handling bacterial select agents under BSL-3 and ABSL-3 conditions, including Bacillus anthracis, B. mallei, B. pseudomallei, F. tularensis, and Y. pestis. At

Battelle, Dr. Gelhaus is a Principal Research Scientist and serves as a Study Director for Good Laboratory Practices regulated studies.

Lisa Hensley, Ph.D., M.S.P.H., is Director of Regulatory Science, in the Office of Counterterrorism and Emerging Threats (OCET), Office of the Chief Scientist, at the Food and Drug Administration. Dr. Hensley works closely with medical counterterrorism scientific leads in FDA's medical product centers and other relevant offices to promote and coordinate the scientific research and regulatory science activities conducted under FDA's Medical Countermeasures initiative (MCMi). She also serves as the principal liaison on medical countermeasure regulatory science issues to FDA's federal partners in the Public Health Emergency Medical Countermeasures Enterprise. Dr. Hensley's extensive background in epidemiology, microbiology, immunology, and infectious disease makes her one of the premier scientists in the development and characterization of animal models and in the field of therapeutics and vaccine discovery. Before joining FDA in January 2012, Dr. Hensley served as Chief of Viral Therapeutics, Virology Division, with the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID). During her 14 years at USAMRIID, she focused on infectious disease research, including on the identification and development of medical products to counter chemical, biological, radiological, and nuclear (CBRN) agents and emerging pathogens. Much of her work addressed issues around development and characterization of animal models to test medical countermeasures. Dr. Hensley received her BS in natural science/public health and a MHS in immunology and infectious diseases through a joint program at The Johns Hopkins University and The Johns Hopkins school of Hygiene and Public Health. She received her MSPH and PhD from the Department of Epidemiology at the University of North Carolina, Chapel Hill. She has authored more than 95 peer review articles and contributed to numerous book chapters.

Alex Hoffmaster, Ph.D., performs reference work and research on detection, identification, and molecular characterization of B. anthracis, Brucella spp., Burkholderia pseudomallei, B. mallei, and Leptospira spp. He has co-authored over 50 research papers and book chapters in this field. His melioidosis reference laboratory performs agent identification and characterizes strains for the Laboratory Response Network by multi-locus sequence typing (MLST), multiple-locus variable-number tandem repeat analysis (MLVA), or whole genome sequencing. He also provides serology for melioidosis in the United States to aid in diagnosis and for monitoring exposed personnel. Current laboratory projects are focused on rapid antigen detection. He is also a member of the Department of Homeland Security sponsored Stakeholder's Panel on Agent Detection Assays (SPADA) which has developed strain panels for the evaluation and validation of bioagent detection assays including Burkholderia pseudomallei and B. mallei.

Robert Jeffrey Hogan, Ph.D., focuses on the interactions of the host and pathogen. By better understanding these processes, he aims to develop novel approaches to prevent the disease transmission and mortality associated with viral and bacterial infections. Vaccination represents one of the most important public health tools available. In this regard, he has been working together with Dr. Eric Lafontaine for the last 4 years to develop protein-based vaccines against the gram-negative bacteria Burkholderia mallei (Bm) and Burkholderia pseudomallei (Bp) using both mouse and equine models of infection. These bacteria are endemic to areas near the equator including Southeast Asia and Northern Australia and can be isolated from the soil (Bp) or from an equine reservoir (Bm). The most severe form of disease occurs during inhalation which results in pneumonia and subsequent bacteremia. His recent data suggests that several of the proposed vaccine antigens are highly immunogenic and can provide protection against lethal challenge.

Eric Lafontaine, Ph.D., has been studying bacterial pathogenesis with emphasis on the mechanisms used by Moraxella catarrhalis, Burkholderia pseudomallei, Burkholderia mallei and Francisella tularensis to interact with host mucosal surfaces. During this time, his lab has identified and characterized several adherence factors and autotransporter proteins expressed by these organisms. His lab is currently examining the vaccinogenic potential of these molecules using animal models including mice, chinchillas and horses. He has established strong collaborations with other investigators, produced several peer-reviewed publications, administered multiple extramurally funded research projects, and trained graduate students, post-doctoral researchers, undergraduate, as well as professional (medicine and veterinary medicine) students.

Direk Limmathurotsakul, M.D., Ph.D., is head of the microbiology department at Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, in which academic activities address the burden of tropical infectious diseases, particularly melioidosis. His research interests include epidemiological aspects, environmental studies, and development of diagnostic tests and treatment guidelines. During the last 7 years, he has been running clinical studies at Sappasithiprasong, Ubon Ratchathani, Thailand, with more than 400 melioidosis patients presenting each year. He is currently running: a RCT comparing between 12 and 20 week of oral eradicative treatment in melioidosis; a Wellcome trust-funded project "Determining routes of B. pseudomallei infection with development of evidence-based guidelines for the prevention of melioidosis"; and an NIH-funded project, "Regulation of host inflammatory responses and outcome in melioidosis by TLR5."

He was invited to present at the World Melioidosis Congress 2010, Townsville, Australia, and to present the work of a case-control study to determine the route of melioidosis infection at European Melioidosis Network 2012, Amsterdam, Netherlands.

Rebecca Lipsitz, Ph.D., is a policy analyst at Health and Human Services (HHS) within the Division of Medical Countermeasure and Strategy. She oversees efforts to develop medical countermeasure requirements for public health emergencies, working closely with colleagues throughout the agencies and departments in the Public Health Emergency Medical Countermeasure Enterprise (PHEMCE). She has experience leading inter-agency working groups and developing consensus policy recommendations on a wide range of medical countermeasure issues. Dr. Lipsitz has led numerous stakeholder activities to solicit input on medical countermeasure issues from internationally renowned subject matter experts. Prior to joining HHS, Dr. Lipsitz was an AAAS fellow at the National Institutes of Health (NIH) and HHS. At NIH, she helped to coordinate new strategic opportunities for concerted research under the auspices of the NIH Common Fund. Dr. Lipsitz received her AB from Barnard College and her PhD from the University of California, San Diego. In addition, Dr. Lipsitz completed post-doctoral training at the NIH, where she developed novel spectroscopic methods to study protein-protein interactions during apoptosis.

Anthony Macaluso, Ph.D., is the Chief Technical Officer for the Chemical Biological Medical Systems Joint Project Management Office (CBMS-JPMO) located in Frederick, Maryland. In this role, he provides direct input into identifying and shaping the goals of CBMS in the areas of product development, technology and regulatory affairs. He assists the JPM/DJPM with technical oversight and acquisition support with emphasis in the areas of science and technology availability, technology maturity, applicability of proposed products to pharmaceuticals/biologics advanced development and production leading to FDA licensed/approved products. Dr. Macaluso earned a Bachelor of Science degree from the Massachusetts Institute of Technology (MIT) and a Doctor of Philosophy from the University of Oregon's Institute of Molecular Biology. Prior to joining the private sector, he completed postdoctoral fellowships at MIT and the University of Michigan. While working in the private sector, Dr. Macaluso developed microbial strains and processes for the production of biological pesticides, biotin, detergent enzymes, amino acids, and animal vaccines. Upon leaving the private sector, he joined the Scientific Review Program at the National Institute of Allergy and Infectious Diseases (NIAID/NIH) and later moved to the Division of Microbiology and Infectious Diseases at NIAID/NIH. As a Project Officer at the Biomedical Advanced Research and Development Authority (BARDA), Dr. Macaluso managed a portfolio of contracts for the development of Rad/Nuc medical countermeasures. Dr. Macaluso participates on multiple committees as a Subject Matter Expert in the areas of Select Agent Regulations and Dual Use Research of Concern.

Tracy MacGill, Ph.D., is a Scientist in the Office of Counterterrorism and Emerging Threats (OCET), in the Office of the Chief Scientist (OCS), Office to the Commissioner (OC), FDA supporting the Regulatory Science component of the Medical Countermeasures Initiative. She came to OCET from the Office of Biodefense Research Affairs (OBRA), NIAID, NIH where she served as a Program Officer managing a portfolio focused on the development biodefense animal models to support product development. Previously, Dr. MacGill was a Microbiologist in the Office of Counter-Terrorism and Emergency Coordination (OCTEC), CDER, FDA. Dr. MacGill also served on active duty with the United States Army as a research microbiologist in the Department of Immunology at the Walter Reed Army Institute of Research (WRAIR) working with non-human primate malaria models. Following the anthrax mail attacks in fall, 2001, she was assigned to a counterterrorism augmentation team at the United States Army Research Institute of Infectious Diseases at Fort Detrick, MD. Dr. MacGill is now an officer in the Commissioned Corps of the United States Public Health Service and holds the rank of Commander. CDR MacGill earned a doctorate in Cellular and Molecular Biology from the University of Nevada, Reno

Elizabeth O'Shaughnessy, M.B., B.Ch., B.A.O., is a board certified infectious diseases physician working as a medical officer at the Food and Drug Administration. She has expertise in the primary review of efficacy and safety information for investigational new drugs (IND), new drug applications (NDA) and biologic applications (BLA) for treatment of anthrax, plague, and tularemia, and infections caused by Burkholderia species. She has experience in the review of new drug applications under the Animal Rule and in the review of complex clinical trial data, such as assessment of composite endpoints and surrogate endpoints in the evaluation of drug efficacy, as well as evaluation of complex safety data which are relevant to the design of studies of investigational treatments for infections caused by Burkholderia species.

Sharon Peacock, M.B., Ph.D., directed a program of clinical and basic research into melioidosis and Burkholderia pseudomallei at the Wellcome Unit based in the Faculty of Tropical Medicine, Mahidol University, Thailand between Jan 2003 and Oct 2009. She is now based in Cambridge, UK, but continues to play an active part in melioidosis research in the Wellcome Unit. She led the development of consensus guidelines for post-exposure prophylaxis (PEP) following accidental

laboratory exposure to B. pseudomallei, which have become international guidelines. She sat on the Steering Committee of the Health and Human Services working party on PEP and treatment following intentional release of B. pseudomallei. She belongs to a consortium formed to map the global presence of environmental B. pseudomallei, and is funded by the Wellcome Trust for studies defining the relative importance of different routes of human infection (inoculation, inhalation, or ingestion). These data will underpin a public health initiative on melioidosis prevention. She has published > 90 papers and book chapters in peer reviewed journals on the topic of melioidosis, out of a total of >220 publications.

Joann Prior, Ph.D., has extensive experience in developing pretreatments for potential biothreat agents. Her research is particularly focused on studying polysaccharides of these agents. She has published articles in this area of research: for example, research characterizing the lipopolysaccharide of Yersinia pestis (J. L. Prior, et al 2001a) and the genetic influences on its production (J.L.Prior et al 2001b); and research to establish the protective efficacy and mechanism of polysaccharide production in Francisella tularensis (J.L.Prior et al 2003 and R.M. Thomas, et al 2007) and examine the glycosylation processes in this spp. (R. M. Thomas, et al 2011a, Wolfgang Egge-Jacobsen, et al 2011b). She has also investigated the protective efficacy of polysaccharides from Burkholderia pseudomallei (M. Nelson et al 2004) and studied the system of glycosylation (A. Scott et al 2011) and polysaccharide production (J. Cuccui, et al 2012) in this organism.

Rosemary Roberts, M.D., is the Director of the Office of Counter-Terrorism and Emergency Coordination (OCTEC) since September 2004. OCTEC works with sponsors developing countermeasures against chemical, biological, radiological and nuclear (CBRN) threats prior to the pre-IND stage. In this setting, OCTEC works with the sponsor to be prepared for its pre-IND meeting with the regulatory division and helps sponsors and CDER's regulatory divisions in the interpretation and implementation of the "Animal Rule" and Emergency Use Authorization. In addition, OCTEC is the FDA point of contact for the Strategic National Stockpile (SNS) which is maintained by the CDC. OCTEC members participate in HHS led interagency working groups to discuss prioritization and requirements for medical countermeasures to be developed against CBRN threats. OCTEC is responsible for coordinating emergency preparedness and response for CDER. Prior to being a director, Dr. Roberts was the Deputy Director of the Office of the Counter-Terrorism and Pediatric Drug Development for three years. In this position, Dr. Roberts' major responsibility was the implementation of pediatric provisions of the FDA Modernization Act, and the Best Pharmaceuticals for Children Act. She served as Federal Advisor to the National Advisory Committee on Children and Terrorism. Dr. Roberts received her BS from the Ohio State University (OSU) in 1971 and her Doctor of Medicine from the OSU College of Medicine in 1974. She completed a pediatric residency at the Children's Hospital in Columbus, Ohio. Pediatric infectious disease training consisted of one year of clinical infectious disease training at the Oklahoma Children's Memorial Hospital in 1980 to 1981 and two years of post doctoral research at Georgetown University in Washington, DC from 1981 to 1983. Dr. Roberts is board certified in Pediatrics and Pediatric Infectious Diseases.

Herbert Schweizer, Ph.D., M.S., is currently a Professor of Microbiology and Associate Department Head of the Department of Microbiology, Immunology and Pathology at Colorado State University. He also serves as Co-Director of the Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research. He is a Fellow of the American Academy for Microbiology. His main research interests include understanding drug resistance mechanisms in Gram-negative bacteria, especially the function and regulation of multidrug efflux pumps, evaluation of new antibacterials and development of state-of-the-art genetic tools for pathogenic bacteria. He has published over 150 manuscripts and is recognized for his contributions in these areas of research. For the past six years studies in his laboratory were focused on understanding the biology, pathogenesis and drug resistance of various Burkholderia species, including B. pseudomallei, and B. mallei. His contributions to Burkholderia research are documented by 27 publications and invitations to present at and participate in national and international meetings, advisory panels and workshops. His research is funded by grants from NIH, DOD and the pharmaceutical industry. He serves on and chaired NIH and European Union study sections in the areas of drug discovery research.

Richard Slayden, Ph.D., has expertise in all stages of academic drug development ranging from target discovery and validation, preclinical drug optimization to advanced efficacy testing and development of drug formulations. He has been involved in M. tuberculosis research since 1993 and F. tularensis, B. pseudomallei and Y. pestis research since 2005. Uniquely, his work includes a wide variety of integrative research that allows for a complete investigation from basic biology to evaluation of efficacy in animal models of infection. Specifically, his research team has experience identifying clinically relevant drug targets, assessing protein function via gene dosage [knockout and knockdown mutants, and dominant negative and merodiploid strains], determining mode of action, and advanced lead compound and formulation development. Importantly, these strategies were developed to specifically manipulate essential molecular targets and exploit them for the development of novel broad-spectrum chemotherapies with potency against priority pathogens and medically important pathogens.

Carl Soffler, D.V.M., M.S., Ph.D., D.A.C.V.I.M., began his training in veterinary medicine at Cornell University and went on to complete a residency and master's program at Colorado State University, culminating in board certification in large animal internal medicine. Building on his clinical background he pursued a PhD program in the lab of Dr. Richard Bowen developing large animal models of Burkholderial infection. He recently completed his PhD which focused on aerosol and percutaneous infection models of caprine melioidosis and their comparison to naturally occurring human and caprine disease. The goal of this research is to develop an alternative model system for the comparative study of melioidosis pathogenesis, antimicrobial testing, and vaccine development. Additionally, he has also conducted preliminary research on aerosol and intratracheal models of glanders in horses and donkeys.

Joyce Sutcliffe, Ph.D., is Senior Vice President for Tetraphase Pharmaceuticals, Inc. Her responsibilities encompass managing the discovery biology (strategy for screening and preclinical development of new compounds) internally and with outside contractors. Working with other members of the senior management team, she is involved in strategy for clinical development, marketing assessments, budgeting, resource allocation, government contract writing and management. She has more than 30 years of experience in antibiotic research and development. Prior to joining Tetraphase, she was Vice President, Research, NanoBio Corporation where she led antimicrobial, antiviral and antifungal discovery activities. In addition, she was Chief Research Scientist for six years at Rib-X Pharmaceuticals and previously spent 16 years at Pfizer where she played key roles in early-stage antibiotic discovery and development through Phase 2 activities. She is currently the PI on a BARDA-funded contract to develop eravacycline, a novel broad-spectrum fluorocycline, for treatment of serious hospital infections and as an empiric agent for biothreat pathogens. Further, she is co-PI on a NIAID-funded project to advance TP-271, a novel tetracycline, through IND-enabling studies into phase 1 clinical studies towards the empiric treatment of moderate-to-serious pneumonia, pneumonic plaque, and other infections caused by aerosolization of biothreat pathogens. Dr. Sutcliffe received her Ph.D. in Microbiology from the University of Florida, Gainesville, followed by postdoctoral positions at the University of Massachusetts Medical School and a staff fellowship at the National Institutes of Health (NIH). Her expertise is antimicrobial resistance mechanisms, a challenge and driver for new antibiotics.

Richard Titball, Ph.D., D.Sc., moved to the School of Biosciences at the University of Exeter from the Defence Science and Technology Laboratory (DSTL) at Porton Down in 2007, where he had been a Senior Fellow. He has worked extensively on a range of bacterial pathogens, including Burkholderia pseudomallei, Francisella tularensis, Yersinia pestis, Campylobacter jejuni and Clostridium perfringens. His past work has provided new insight into the molecular architectures and mode of action of C. perfringes toxins and he led initiatives to sequence the first genomes of a range of candidate biowarfare agents. His work also resulted in the development of vaccines against plague and C. perfringes toxins which have been trialed in humans and animals respectively. His principal interests now lie with understanding the molecular basis of disease caused by B. pseudomallei and C. jejuni and the molecular mechanisms used by these pathogens to establish persistent disease and to persist in the environment. He also has a strong interest in the development of vaccines against B. pseudomallei and C. perfringens toxins. This work includes understanding the role that vaccines might have in controlling chronic and persistent disease and the use of novel vaccine delivery systems such as gold nanoparticles. His work is funded by the U.S. Defense Threat Reduction Agency, DSTL, BBSRC, Cariplo Foundation, Wellcome Trust and the NIH. He has published a total of 269 papers in peer-reviewed journals including Nature, Science and PNAS; of these, 53 are on Burkholderia.

Alfredo Torres, Ph.D., is a Professor at UTMB Health. He has extensive experience in the study of category B pathogens, such as pathogenic Escherichia coli, Shigella, Salmonella, Burkholderia mallei and B. pseudomallei, and has published more than 75 peer-review publications, 10 book chapters and 1 book in topics related to microbial pathogenesis, food safety, therapeutics, and vaccine development. His major interest includes the elucidation of the mechanisms used by pathogenic E. coli to adhere and colonize the intestinal epithelia and to characterize regulatory networks controlling their expression. His laboratory is also characterizing the pathogenic mechanisms of B. mallei and B. pseudomallei with the goal of developing suitable vaccines and therapeutics, and it has developed in vivo imaging techniques to study pathogenesis in small animal models of infection. He is Associate Editor of Frontiers in Cellular and Infection Microbiology and he is the founder and current coordinator of the Latin American Coalition for Escherichia coli Research.

Apichai Tuanyok, Ph.D., has a long-term research goal of better understanding the molecular and genetic basis of virulence mechanisms of Burkholderia pseudomallei and B. mallei. His studies include the identification and characterization of potential virulence genes that are responsible for differential virulence of B. pseudomallei and B. mallei in animal models. He has been working on B. pseudomallei, B. mallei, and their near-relative species since 2002 when he was a postdoc in Dr. Don Woods' Laboratory at the University of Calgary, in Alberta, Canada. He joined Northern Arizona University (NAU) in 2006 as a postdoctoral associate to work on the Pacific Southwest Regional Center of Excellence (PSWRCE)-funded Burkholderia projects. Since that time, he has helped build the Burkholderia research group at NAU and has started to build an

independent research program, including serving as the PI on studies funded by the PSWRCE, DHS, DTRA, and BARDA. Also, he has been instrumental in setting up a BSL3 facility at the Center for Microbial Genetics and Genomics (MGGen), as well as collecting/obtaining B. pseudomallei and B. mallei strains from around the world. He is now an Assistant Research Professor and Assistant Director of the MGGen Center at NAU and has published at least 27 peer-review papers on genomic analyses, animal models, immunology, and gene expression of B. pseudomallei and B. mallei. He currently supervises three post-doctoral fellows, three graduate students, and five undergraduate students.

Julia Vipond, Ph.D., received her doctorate in microbiology from the University of Glasgow and is currently a senior scientist at the Health Protection Agency (UK) with the responsibility as the operational lead in the area of Select Agent research. Dr. Vipond has 10 years of post-doctoral experience in the research arena, primarily concentrated on vaccine evaluation against bacterial pathogens; initially, Bordetella pertussis and Mycobacterial infections but more recently on medical countermeasures for a range of Select Agent pathogens including Y. Pestis, B. anthracis, C. Burnetii, monkeypox and Burkholderia species. Dr. Vipond was previously a project team leader for multiple projects involving aerosol-infection of small and large animal models and has vast experience as a study director on numerous model development studies as well as several vaccine assessment studies, all involving the use of aerosolized Select Agents. This work has involved coordinating a large number of personnel with a broad range of technical expertise and has resulted in the submission of data packages to the FDA for evaluation of safety and efficacy supporting product licensure under the FDA Animal Rule.

Dave Waag, Ph.D., is a microbiologist in the bacteriology division at USAMRIID in Frederick, MD. He has planned, conducted, supervised, coordinated, and evaluated research on the immunogenicity and efficacy of vaccines against Burkholderia mallei. He developed animal models used to evaluate the efficacy of putative B. mallei vaccines and therapeutics. He has also developed diagnostic reagents and techniques for the diagnosis of, and coordinated the USAMRIID research effort against, glanders.

SPEAKER ABSTRACTS

David AuCoin, Ph.D., M.S.

The goal of our research is to develop a point-of-care (POC) immunoassay that detects secreted bacterial antigens in patient samples for early diagnosis of melioidosis. A POC immunoassay could greatly impact patient outcome because a high percentage of melioidosis patients with acute septicemia die before culture results are available. Also, the antibiotics used for empirical treatment of septicemia are not effective for *B. pseudomallei*. In order to develop a POC diagnostic our first objective was to identify *B. pseudomallei* antigens that are secreted/shed during infection. We employed a novel approach termed In vivo Microbial Antigen Discovery (InMAD) to identify secreted/shed *B. pseudomallei* antigens. By using this method we have identified a candidate polysaccharide and numerous protein antigens that could be targeted for diagnosis. The *B. pseudomallei* capsular polysaccharide (CPS) was one antigen identified by InMAD and a CPS-specific mAb was produced. The mAb was able to directly detect antigen in urine from melioidosis patients by Western blot and antigen-capture ELISA. A prototype lateral flow immunoassay (LFI) was developed in collaboration with our private-sector partner InBios International (Seattle, WA). The LFI has a sensitivity of 0.2 ng/ml when purified CPS is used as an antigen source. The CDC is currently evaluating the effectiveness of the LFI for identification of *B. pseudomallei* colonies grown on solid agar. In addition, the LFI is being evaluated in a pre-clinical setting in the endemic areas of Thailand and Australia. Preliminary studies indicate that the LFI can detect CPS in a variety of patient samples including serum, urine, pus and respiratory secretions.

Peggy Cotter, Ph.D.

Burkholderia pseudomallei is a NIAID Category B priority pathogen. It causes the serious and often lethal human disease melioidosis and represents both a worldwide emerging infectious disease problem and a bioterrorism threat due to its extremely low infectious dose, the ability to initiate infection by an aerosol route, an intrinsic resistance to commonly used antibiotics, and the lack of a currently available vaccine. Little is known about the molecular bases of *B. pseudomallei* pathogenesis. Autotransporter (AT) and Two Partner Secretion (TPS) pathway proteins are large, surface-localized and secreted proteins that function as virulence factors in many Gram-negative bacterial pathogens. Some are effective as vaccine components. Available genome sequence information indicates that *B. pseudomallei* has the potential to produce at least ten AT proteins and three TPS pathway proteins. Our goals are to determine the roles of the *B. pseudomallei* AT and TPS pathway proteins in pathogenesis. We have constructed *B. pseudomallei* strains containing either disruption or deletion mutations in each of the genes encoding predicted AT proteins and compared them with wild type bacteria for their ability to adhere to and invade epithelial and macrophage-like cell lines *in vitro* and to form plaques, which requires that the bacteria spread from cell-to-cell without leaving the host cell intracellular environment. We also compared the mutants with wild type bacteria for their ability to cause disseminated infection in mice when delivered by the intranasal route. We found that two of the mutants were severely defective in their ability to invade mammalian cells and to disseminate from the respiratory tracts of mice to cause lethal infection. Our data suggest that the corresponding AT proteins function as invasins, making them good candidate vaccine components. We have also discovered that the *B. pseudomallei* TPS proteins function as Contact-Dependent Growth Inhibition (CDI) systems that are involved in interbacterial killing and are also required for biofilm formation. Our data suggest that these CDI systems contribute to the ability of *B. pseudomallei* to compete successfully for niche establishment in the rhizosphere and may therefore contribute to transmissibility of these bacteria.

David Dance, M.B., Ch.B., M.Sc.

First described in1812, some 100 years before melioidosis, human glanders amongst people in contact with infected horses has never been common. Since the decline in the importance of horses as a mode of transport and the success of efforts to control the disease amongst equines, it is now vanishingly rare. Over the past 50 years, descriptions of human glanders have largely been restricted to occasional laboratory-acquired cases. Naturally acquired infection is thought usually to have been acquired by contamination of wounds or mucous membranes or ingestion of infected water or meat, but infection by inhalation of the organism is also possible. Human-to-human transmission has occasionally been reported. Broadly speaking the clinical manifestations of glanders in humans are similar to those of melioidosis, although involvement of the nasal and oral mucosae and the lymphatics appear to be more frequent than in melioidosis, analogous to glanders in animals. Traditionally, by analogy with infection in animals, the disease was called 'glanders' when there was nasal and respiratory tract involvement and 'farcy' when the primary site of infection was the skin and local lymphatics. The disease may run an acute fulminating and rapidly fatal course or may be chronic, relapsing and occasionally asymptomatic. Disseminated nodules or abscesses may be found throughout the body but especially in the liver, spleen and lungs. The development of widespread cutaneous papules that evolve into ulcerating abscesses ("Hautrotz") is associated with a particularly poor prognosis. The host adapted pathogen *Burkholderia mallei* grows rather less luxuriantly than *Burkholderia pseudomallei* in culture and would present an even greater diagnostic challenge to modern clinical laboratories, whose staff are likely to be

unfamiliar with its features, than the latter. Since most human cases of glanders occurred in the pre-antibiotic era there are few hard data on which to base treatment recommendations. In the absence of antibiotic treatment there were several graphic and dramatic cases described in which the disease persisted for many years, necessitating as many as 40 operations to drain abscesses, causing untold misery and death in over 90% of reported cases. The antibiotic susceptibility of *B. mallei* is similar to that of *B. pseudomallei* with the exception that the former is susceptible to aminoglycosides *in vitro*, so it is anticipated that regimens that are effective in melioidosis would also be so in glanders. The only recently reported case recovered after treatment with imipenem followed by azithromycin and doxycycline.

John Finn, Ph.D.

The pyrimidoindoles are a new class of broad-spectrum antibacterial drugs that inhibit two novel bacterial targets: the GyrB and ParE subunits of DNA gyrase and topoisomerase IV. This class of compounds has excellent activity on all Category A and B bacterial biodefense pathogens and is especially potent on *Burkholderia pseudomallei*. This talk will highlight issues specific to *B. pseudomallei* including: the use of *B. thailandensis* as a surrogate pathogen in the initial antibacterial screen, *in vivo* testing and use of PK-PD in selecting a dosing strategy. We will also discuss thoughts on a development path for demonstrating effectiveness of this class of drugs in treating melioidosis.

H. Carl Gelhaus, Ph.D.

Burkholderia pseudomallei is the causative agent of melioidosis, a rare disease of biodefense concern with high mortality and extreme difficulty in treatment. No approved human vaccines are available that protect against *B. pseudomallei* infection, and with the current limitations of antibiotic treatment, the development of new preventative and therapeutic interventions is crucial. Although clinical trials could be used to test the efficacy of new medical countermeasures (MCMs), the high mortality rates associated with melioidosis raises ethical issues with experimental infections. The US Food and Drug Administration (FDA) has formulated a set of guidelines for the licensure of new MCMs to treat diseases in which it would be unethical to test the efficacy of these drugs in humans. The FDA "Animal Rule" 21 CFR 314 calls for consistent, well-characterized *B. pseudomallei* strains to be used as challenge material in animal models. In order to facilitate efficacy testing of new MCMs for melioidosis using animal models, we identified a panel of strains isolated from human cases that have a low passage history, are virulent in animal models, and are well-characterized phenotypically and genotypically. We reviewed published and unpublished literature on various *B. pseudomallei* strains to establish an objective method for selecting the strains with attention to five categories: animal infection models, genetic characterization, clinical and passage history, and availability of the strain to the research community. We selected strains, and produced master and working cell banks. Characterization included microbiological (e.g. colony morphology, Gram stain) and genetic (e.g. PCR, whole genome sequencing) methods. These strains are a high-quality resource for the development of melioidosis animal models.

Alex Hoffmaster, Ph.D.

Early detection of *Burkholderia pseudomallei* and diagnosis of melioidosis is important due to the specific antimicrobial therapy that it requires. Clinical suspicion and identification of this bacterium can be difficult in regions where the disease is not common such as the United States. The Zoonoses and Select Agent Laboratory (ZSAL) is the reference laboratory for melioidosis at the Centers for Disease Control and Prevention (CDC). In this role, ZSAL provides subject matter expert guidance and consulting to the Laboratory Response Network (LRN) on confirmatory identification of *B. pseudomallei* and diagnosis of melioidosis. ZSAL also provides additional testing and agent characterization that are not available within LRN reference laboratories. This presentation will provide an overview of current testing capability within the LRN/CDC and describe current work and future directions of melioidosis diagnostics at CDC.

Robert Jeffrey Hogan, Ph.D.

Burkholderia pseudomallei (*Bp*), the causative agent of meliodosis, is a gram-negative bacterium that can be isolated from the soil and wet areas in Southeastern Asia and Northern Australia. *Burkholderia mallei* (*Bm*), the causative agent of glanders, is a host-adapted clone of *Bp* thought to only persist in its equine hosts. *Bp* and *Bm* share characteristics including inherent resistance to antimicrobial therapy, invasion and replication in host cells, extremely high morbidity and mortality upon respiratory tract infection, and the ability to persist for months or years should the host survive the acute stage of disease. Unfortunately, there are no efficacious vaccines for these two pathogens and data regarding the initial stages of the infection and disease are limited. With this in mind, the kinetics of *Bp* and *Bm* growth and dissemination were examined in mice at early time points after aerosol infection. Both *Bp* and *Bm* replicated to high levels in the lung tissue and disseminated to the spleen. However, the control of bacterial replication, dissemination, bacterial burden in these tissues were different between the two isolates examined. Whereas *Bm* replicated without any apparent interference from the host immune system at all doses administered, low dose *Bp* infection showed a plateau of replication in the lungs. These and other differences were magnified with increasing time post-infection. Taken together, these data demonstrate that respiratory tract infection with

Bp or *Bm* leads to rapidly increasing bacterial burden in the lungs followed by dissemination to the spleen. However, these parameters are variable based upon the organism (*Bp* versus *Bm*) and dose of inoculum.

Eric Lafontaine, Ph.D.

Burkholderia pseudomallei (*Bp*) and *Burkholderia mallei* (*Bm*) are the causative agents of melioidosis and glanders, respectively. *Bp* is a bacterium recovered from water and wet soils in endemic areas (*e.g.* Southeast Asia, Northern Australia). *Bm* is a host-adapted clone of *Bp* which does not persist outside its equine host. Though glanders has been eradicated in North America and most European countries since the late 1930s, the disease is still endemic in many parts of the world including Asia, Africa, and the Middle East. Infection by these closely-related bacteria occurs via percutaneous inoculation or inhalation of contaminated aerosol particles, and the most common manifestation is severe pneumonia leading to highly fatal bacteremia. Glanders and melioidosis are difficult to diagnose and necessitate prolonged antibiotic therapy that has a low success rate due to the intrinsic resistance of *Bp* and *Bm* to most antimicrobials. In addition, *Bp* and *Bm* invade host cells and multiply intracellularly, which provides protection from antibiotics and the host humoral immune response. This ability to thrive intracellularly also allows the organisms to rapidly disseminate to organs during the course of infection and further complicates treatment. There is no vaccine to protect against these pathogens and there is concern regarding their use as biological warfare agents given that *Bm* has previously been utilized in this manner. For these reasons, *Bm* and *Bp* are classified as Select Agents by the CDC and the Federal Select Agent Program (FSAP).

To address this lack of vaccine and shortage of effective antimicrobials, we analyzed the genomic sequences of *Bp* and *Bm* strains in search of novel targets to develop anti-infective approaches. These analyses revealed that *Bp* and *Bm* share several adhesin and autotransporter gene products that are highly-conserved between species at the amino acid level. Published work by our group and others indicate that adhesins and autotransporters are excellent vaccine candidates. They are expressed on the bacterial surface and are readily accessible for recognition by the immune system. Many adhesins and autotransporters have been shown to be expressed during infection and to elicit protective immune responses when used as vaccine antigens. Additionally, adhesins and autotransporters play key roles in pathogenesis including host cell invasion, phospholipolysis, and colonization. Our objective is to test whether immunization with conserved adhesins and autotransporters will protect against *Bp* and *Bm* in aerosol models of infection.

Through funding from DTRA, we developed a murine aerosol model of infection for *Bm* and *Bp*, which allows for evaluating various therapeutic approaches (*e.g.* vaccines, antimicrobial compounds, MAbs). Through funding from NIH, we also developed an equine model of glanders, which allows for evaluating therapeutic approaches in the natural host for *Bm* and satisfy the FDA's two-animal rule (*i.e.* mice and horses). These models, and their use for melioidosis and glanders vaccine development, will be discussed.

Direk Limmathurotsakul, M.D., Ph.D.

Melioidosis is a fatal infectious disease caused by the Category B select agent and environmental saprophyte, *Burkholderia pseudomallei.* This disease has emerged over the past 25 years as an important cause of morbidity and mortality in Southeast Asia and northern Australia, and is also endemic in other tropical regions. All age groups can develop melioidosis, but incidence peaks between the ages of 40 and 60 years. Melioidosis is markedly seasonal in most settings with 75% of cases presenting during the rainy season. Predisposing conditions in adults include the presence of diabetes mellitus, chronic renal failure, immunosuppressive treatments, including steroids, thalassemia, chronic liver disease, chronic lung disease (including cystic fibrosis) and kava consumption, one or more of which are found in 60–90% of cases. The most frequent clinical picture is a septicaemic illness, often associated with bacterial dissemination to distant sites such that concomitant pneumonia and hepatic and splenic abscesses are common. Bacteraemia and pneumonia occur in 50% of cases, but not necessarily together.

A recent case-control study was conducted in northeast Thailand to provide evidence for inoculation, inhalation and ingestion as routes of infection, and develop preventive guidelines based on this evidence. Multivariable conditional logistic regression analysis based on 286 cases and 512 controls showed that activities associated with a risk of melioidosis included working in a rice field, other activities associated with exposure to soil or water, an open wound, eating food contaminated with soil or dust, drinking untreated water, outdoor exposure to rain, water inhalation, current smoking and steroid intake. *B. pseudomallei* was detected in water source(s) consumed by 7% of cases and 3% of controls, suggesting that consuming water containing *B. pseudomallei* was associated with melioidosis. First evidence-based guideline for the prevention of melioidosis from all three routes of infections was proposed.

Rebecca Lipsitz, Ph.D.

The Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) coordinates Federal efforts to enhance chemical, biological, radiological and nuclear threats (CBRN) and emerging infectious diseases (EID) preparedness from a medical countermeasure (MCM) perspective. In 2010, the PHEMCE convened the 2010 HHS *Burkholderia* Workshop which brought together international experts to generate expert consensus recommendations for melioidosis therapy and post-exposure prophylaxis (PEP). These recommendations will help the PHEMCE in its preparedness and response efforts as well as provide information that can assist clinicians in case management of infections. In addition to discussions on appropriate medical countermeasures for melioidosis infections, workshop participants provided recommendations on diagnostics and other aspects of patient management.

Elizabeth O'Shaughnessy, M.B., B.Ch., B.A.O.

Guidelines and general principles for a new drug application (NDA) for an antimicrobial drug related to a counterterrorism indication will be reviewed. Recommendations regarding the safety database for antimicrobial drugs studied under the "Animal Rule" will be reviewed with reference to new molecular entities and currently marketed antimicrobial drugs.

Sharon Peacock, M.B., Ph.D.

Session 3 lecture:

The recommended approaches to the diagnosis of naturally acquired melioidosis will be discussed, including the central importance of bacteriological culture and the role of molecular detection tests such as PCR. The available serological tests will be reviewed in relation to natural infection, including a discussion of their advantages and pitfalls. The performance of these tests will also be considered in the context of accidental or deliberate exposure to *B. pseudomallei*. The talk will end with a summary of the outstanding needs for diagnostic test development and evaluation.

Session 7 lecture:

The published clinical trials of antimicrobial therapy for melioidosis will be reviewed and critiqued, including a discussion of their limitations. Current recommendations will be described for the therapy of culture-proven melioidosis, and for post-exposure prophylaxis following accidental or deliberate exposure to *B. pseudomallei*. Knowledge gaps will be highlighted in relation to intervention development.

Joann Prior, Ph.D.

Currently there is no licensed vaccine available for the bacterial biothreat agents *Burkholderia pseudomallei* and *Burkholderia mallei,* causative of melioidosis and glanders, respectively. These two closely related bacteria produce a common capsule polysaccharide (CPS) which consists of an unbranched homopolymer of 1-3 linked 2-O acetyl-6-deoxy-β-D-manno-heptopyranose that has been identified as a major virulence determinant in both species. Loss of CPS expression in *B. pseudomallei* increases the MLD in mice from 70 cfu to greater than 10^6 cfu. The CPS of *B. pseudomallei* has protective capacity against pathogen challenge and the passive transfer of antibodies raised against CPS also induces protection in experimental models.

Although there are some exceptions, polysaccharides in general are T-cell independent antigens which are poor inducers of immunological memory. Conjugation of polysaccharides to protein carriers has previously been shown to be an efficient method of directing a more appropriate immune response which is T-cell dependent. Recombinant virus-like particles (rVLP) are non-infectious but highly immunogenic protein particles and are thus the vaccine vector of choice for less potent antigens. Tandem core™ has been developed by iQur as a highly immunogenic rVLP which is designed to carry two antigenic inserts simultaneously. Immunogenic peptide sequences and conjugated polysaccharide antigens are thus presented in a highly immunogenic context, stimulating strong and appropriate T- and B-cell responses.

This work aims to develop an efficacious vaccine against *B. pseudomallei* and *B. mallei* based on Tandem core™ technology combined with antigens from *Burkholderia*. Targeted linker peptide sequences will be cloned into the major insertion regions (MIRs) of Tandem core™. This will provide specific attachment sites for the conjugation of both natural CPS and synthetic mono- and oligo-saccharide antigens. Synthetic carbohydrate antigens will be prepared commensurate with scale-up of antigen production. They will be designed such that their structural similarity to CPS will be sufficient to generate a cross-reactive immune response to the natural antigen. The various rVLP will be evaluated for immunogenicity in mice and lead candidates will be examined for efficacy against aerosol challenge with both *B. pseudomallei* and *B. mallei*.

Herbert Schweizer, Ph.D., M.S.

Burkholderia pseudomallei infections are difficult to treat because of the bacterium's intrinsic antimicrobial resistance and propensity to acquire resistance. In contrast to other bacteria where mobile elements play major roles in resistance acquisition, all clinically significant resistance identified to date in *B. pseudomallei* is mediated by chromosomally encoded mechanisms. Of clinical consequence is resistance to ceftazidime, trimethoprim + sulfamethoxazole and doxycycline. Resistance to ceftazidime is mostly mediated by overexpression or mutation of the chromosomally-encoded PenA β-lactamase. Trimethoprim, trimethoprim + sulfamethoxazole and doxycycline resistance is mediated by overexpression of the BpeEF-OprC efflux pump. Expression of this efflux system also causes cross-resistance to chloramphenicol and fluoroquinolones. Another efflux pump, AmrAB-OprA, is the cause for the intrinsic high-level aminioglycoside and macrolide resistance observed in the majority of *B. pseudomallei* strains. This talk will illustrate how inclusion of defined mutants in tester panels assists with and adds value to assessment of *in vitro* efficacy of investigational drugs.

Richard Slayden, Ph.D.

We have an ongoing academic drug discovery program targeting the enoyl-ACP reductase enzymes involved in fatty acid biosynthesis. A typical observation in bacterial drug discovery is that often times there is limited correlation between *in vitro* potency of a drug candidate and the observed efficacy in infection models. The observed limited correlation between potency and efficacy may be attributed to a combination of factors including (1) a limited knowledge about the host-response to infection and dissemination, (2) lack of information about pathogens metabolism during infection and in in different tissues, (3) mechanism of target inhibition and (4) resistance mechanisms and tolerance. This is particularly true in the case of *B. pseudomallei* drug discovery. Despite these factors, lead enzyme inhibitors that target enoyl-ACP synthases have been designed and developed around several pharmacophores. Success with other bacteria pathogens including *M. tuberculosis* and *F. tularensis* have been extended to *Burkholderia* species where significant challenges are presented by the presence of both FabI (bpFabI1 & bpFabI2) and FabV (bpFabV) enoyl-ACP reductases and the intrinsic ability of *Burkholderia* to efflux drugs. Screening has identified several enoyl-ACP reductase inhibitors with MIC values less than 1 ug/ml on average against the efflux mutant strain *B. pseudomallei* Bp400, and MIC values only ~10 fold greater against the efflux competent strain *B. pseudomallei* 1026b. Lead compounds **PT52,** and **PT01** and **PT68** demonstrated efficacy in the burkholderia animal model of infection as determined by a reduction of bacterial load in the lungs and spleen. Notably, inhibition or reduction of dissemination to secondary sites of infection correlates well with observed efficacy. In addition to development of lead inhibitors and Pre-IND studies, there is particular interest in the identification of all the clinically relevant drug targets in *Burkholderia* species, understanding the host & pathogen mechanisms that are involved in resistance to treatment, and investigating the impact of genome organization and redundant coding on virulence and treatment.

Carl Soffler, D.V.M., M.S., Ph.D., D.A.C.V.I.M.

Melioidosis, the disease resulting from infection with *Burkholderia pseudomallei*, is a serious emerging infectious disease endemic to Southeast Asia and Northern Australasia and a leading infectious cause of death in the former. Additionally, *B. pseudomallei* has been designated a Category B Select Agent by the United States Centers for Disease Control and Prevention because of its potential use in bioterrorism, which has led to intensive research on inhalational models of murine melioidosis. Natural infection is believed to occur predominantly through percutaneous inoculation or inhalation in the rainy season in endemic areas, with infection also occurring to a lesser extent following oral exposure. However, the actual importance of each route of infection in natural disease is unknown.

Goat models provide the opportunity to study the importance of the route of infection and its effect on disease pathogenesis in a naturally affected species. Disease and outcome can be evaluated relative to natural presentations in both human and goat populations as they both exhibit a similar epidemiology/epizoology of melioidosis, which corresponds to comparable environmental exposure to *B. pseudomallei* within its endemic range. Furthermore, the larger body size of goats allows for human-relevant clinical monitoring as well as longer-term serial evaluation of disease progression and therapy in individual animals.

Using a caprine model system, we have investigated the pathogenesis of infection following intratracheal aerosol and percutaneous exposure to 10^4 delivered CFU of *B. pseudomallei*. Disease was observed in all animals following infection. Fever and hematologic changes were more severe in aerosol infected goats, but both groups tended to develop subacute to chronic active disease, with percutaneously infected goats showing regression of lesions at the later time points. Percutaneously infected goats generally exhibited more variable clinical signs, hematologic changes, and gross pathology, but often had more severe histologic lesions. Dissemination from the site of infection was more rapid in the percutaneously infected animals, with bacteria detectable in the lungs and spleen as early as Day 2 post-infection (PI) and gross abscessation evident in distant sites as early as Day 7 PI. Extrapulmonary dissemination after aerosol infection appeared to occur around

Day 7 with splenic or renal abscesses not grossly detectable until day 14. Lesion development was closely associated with a leukocytoclastic vasculitis observed in affected tissues in both aerosol and percutaneous infection. Pulmonary involvement was evident in all but one goat (Day 2 PI percutaneous) by culture or the presence of histologic lesions. The rapid dissemination of *B. pseudomallei* after percutaneous inoculation challenges the perception that inhalational melioidosis is more severe or will affect the lungs more frequently than percutaneous infection.

The findings presented here provide a clinical, radiographic, and pathologic description of the pathogenesis of subacute to chronic aerosol and percutaneous caprine melioidosis. However, acute presentations are possible in association with concurrent disease or debility, suggesting that the caprine model system may be amenable to the incorporation of risk factors to increase susceptibility/acute disease as typically seen in human melioidosis. It is hoped these models will help broaden the scope of melioidosis research to fill remaining voids, particularly in the areas immunology, vaccine development, and evaluation of novel antimicrobial therapeutics through the comparative study of disease.

Joyce Sutcliffe, Ph.D.

By extending the total synthetic methodology developed by Myers, *et al*., multiple novel antibiotic classes retaining the druggable tetracyclic core have been made. Many of these compounds are efficacious by both oral and intravenous (IV) routes. TP-434 (eravacycline) is a broad-spectrum antimicrobial with an *in vitro* spectrum of activity that includes all multidrug-resistant gram-negative and gram-positive aerobic and anaerobic bacteria except *Pseudomonas aeruginosa.* Eravacycline is in clinical development for treatment of serious infections in the hospital and is being evaluated as an empiric countermeasure for inhalational exposure to aersolized biothreat pathogens (BARDA Contract HHSO100201200002C). It has potent antibacterial activity ($MIC_{50/90}$ values) against 35 isolates of *Bacillus anthracis* (≤0.016/≤0.016 μg/ml), 34 isolates of *Yersinia pestis* (0.06/0.125 μg/ml), 32 isolates of *Francisella tularensis* (0.125/0.5 μg/ml), 30 isolates of *Burkholderia mallei* (0.016/0.25 μg/ml), and 35 isolates of *Burkholderia pseudomallei* (1/1 μg/ml). Thus, this compound has promising activity as an empiric countermeasure, incluing *Burkholderia* species. Eravacycline is protective in murine infection models challenged with key public health pathogens and is orally bioavailable in humans with a long half-life consistent with once-daily dosing. The IV formulation of eravacycline was efficacious in a recently completed Phase 2 clinical trial for the treatment of complicated intra-abdominal infections. Under the BARDA Contract, eravacycline will be assessed for its ability to treat plague, tularemia, and anthrax in small animals and non-human primates. In addition, BARDA funding will advance eravacycline through six Phase 1 studies and a Phase 2 study for the treatment of serious community-acquired bacterial pneumonia. TP-271 is another novel tetracycline being developed by Tetraphase Pharmaceuticals for the treatment of respiratory infections caused by biothreat or public health pathogens (NIAID contract HHSN272201100028C and NIAID grant 1R01AI093484-01). TP-271 is a potent broad spectrum antimicrobial *in vitro* and has demonstrated protection via both oral and IV routes in rodent models of *Streptococcus pneumoniae, Haemophilus influenzae*, or *Staphylococcus aureus* (including MRSA) infection. This compound has potent antibacterial activity ($MIC_{50/90}$ values) against 30 isolates of *B. anthracis* (≤0.008/≤0.008 μg/ml), 30 isolates of *Y. pestis* (0.12/0.25 μg/ml), 27 isolates of *F. tularensis* (0.25/0.5 μg/ml), 30 isolates of *B. mallei* (0.06/0.12 μg/ml), and 30 isolates of *B. pseudomallei* (1/4 μg/ml). The NIAID programs will support further efficacy testing of TP-271 against tularemia, plague and anthrax in murine and non-human primate models as well as determination of the safety and pharmacokinetics in animal species and humans by the IV and oral routes. Both novel tetracyclines have no known cross-resistance to other antibiotic classes and retain activity against known tetracycline resistance mechanisms. Thus, they would be a useful addition to the armamentarium for both empiric treatment of biothreat category A and B pathogens and public health pathogens.

Richard Titball, Ph.D., D.Sc.

Melioidosis, caused by *Burkholderia pseudomallei*, is often a chronic disease in humans and recurrent infections are often reported. These observations suggest that it is difficult to elicit sterile immunity. Similar findings have been reported from work where small animal models of disease have been used to evaluate candidate vaccines. All of the candidates tested to date can elicit sterile immunity against low challenge doses. However, animals challenged with higher doses develop chronic disease and eventually succumb to the infection. Recently we have been investigating the possibility that the pathogen is able to manipulate the response to infection, by producing a proteasome inhibitor. The ability of the pathogen to modulate the response to infection might make the development of an effective vaccine difficult and might indicate the need to use combination approaches to the prevention of disease.

Alfredo Torres, Ph.D.

Burkholderia mallei and *B. pseudomallei* are two intracellular pathogens causing glanders and melioidosis, respectively, and which are classified as Category B select agents, due to their biothreat potential and lack of effective therapeutic treatment.

We previously showed that intranasal treatment of mice with CpG oligodeoxynucleotides confers protection against acute meliodosis, due to the recruitment of inflammatory monocytes and neutrophils. Recently, we have monitored neutrophil recruitment to the lungs in response to CpG treatment using *in vivo* whole body imaging technique and demonstrate protection in the murine glanders model. We have observed that CpG administration reduced the robust production of chemokines and pro-inflammatory cytokines in lungs, which is a hallmark of the infection in non-treated animals. Lungs of infected control animals were infiltrated with neutrophils, as compared to CpG-treated animals. A stable luminescent reporter *B. mallei* strain was initially detected in the nose of infected animals and progressed to the lungs and spleen over the course of infection. CpG-treatment 48 h pre-infection resulted in increased recruitment of neutrophils (visualized by near infrared fluorescent imaging) to the lungs and reduction of bioluminescent bacteria, correlating with decrease bacterial burden in target organs and protection against acute murine glanders. In summary, we have developed optimized *in vivo* imaging methods to monitor disease progression, which is effective to evaluate efficacy of therapeutic treatment/vaccination during *Burkholderia* infections. Further, protection of CpG-treated animals was associated with recruitment of neutrophils prior to infection and demonstrated, for the first time, real time *in vivo* imaging and co-localization of neutrophils and bacteria in the lungs.

Apichai Tuanyok, Ph.D.

It has been well-recognized that various clinical manifestations are associated with melioidosis. Its pathogen, *Burkholderia pseudomallei,* is known as one of the most genetically diverse bacterial species. This is because it has an "open genome" that recombines at a high frequency. Genetic recombination is the major cause of gene gain and/or loss in *B. pseudomallei*. We believe that various clinical outcomes observed in melioidosis patients are controlled by genomic differences. Here, we used BALB/c mice as an animal model to study differential virulence of twenty genetically diverse *B. pseudomallei* strains which were collected from two different sources: human cases and soils, in Thailand and Australia. A single dose (500 – 3,000 CFU) of intraperitoneal (I.P.), intranasal (I.N.), or subcutaneous (S.C.) inoculation was used to assess the virulence in BALB/c mice. Interestingly, not all *B. pseudomallei* strains were able to cause acute melioidosis in BALB/c mice by these delivery routes. This suggests that *B. pseudomallei* strains have differential virulence in BALB/c mice, even though most of clinical strains used in this study were isolated from the fatal melioidosis cases. In addition, post-mortem examination and histopathology were performed to determine cause of death or changes produced by disease. The liver and spleen were the most affected organs following the I.P. inoculation, whereas the lungs and brain were mostly affected by I.N. inoculation. Comparative genomics has revealed a great diversity of their accessory genes e.g., genomic islands (GIs). This study has confirmed that differential virulence does exist naturally among strains of *B. pseudomallei.*

Julia Vipond, Ph.D.

Burkholderia pseudomallei and *B. mallei*, the causative agents of melioidosis and glanders respectively, are recognized as pathogens of public health consequence and also as potential biological warfare agents, especially in the aerosolized form. Due to limited proven pre- or post-exposure prophylaxis after inhalational exposure for use in humans, it is essential to have a robust animal model of these two diseases. A model system that represents human disease would enable the generation of data packages that could support the licensure of medical countermeasures against diseases caused by pathogens of high consequence, in this instance specifically *B. pseudomallei* and *B. mallei*. Work performed to evaluate the aerosolization of *B. pseudomallei* in mice using a well characterized strain and the resources required to prepare for GLP pivotal efficacy studies in non-human primates will be presented; with particular focus on *in vitro* strain characterization and telemetry infrastructure and validation.

David Waag, Ph.D.

Background. At USAMRIID, we have established rodent animal models that are used to test the efficacy of vaccine candidates and therapeutics. Animal models have been established for glanders and melioidosis in hamsters and mice infected intraperitoneally or by aerosol. *Purpose*. I intend to identify histopathological changes that occur after experimental infection and assess whether any of these models are good mimics of human clinical disease. *Experimental Design*. Experimental animals were infected with approximately 10 LD_{50} of *B. mallei* or *B. pseudomallei* and groups of animals were sampled daily. *Results.* Similarities between BALB/c mice and Syrian hamsters intraperitoneally infected with *B. mallei* included a pyogranulomatous inflammatory response in both species and a tropism for infection in reticuloendothelial (RE)-rich tissues (such as spleen, lymph nodes, bone marrow, and liver). In mice the infection was generally limited to RE tissues, whereas in hamsters, infection was widely disseminated. The characteristic histopathologic change in mice was pyogranulomatous inflammation, which generally lacked discrete organization. Also, necrosis was never extensive and hemorrhage was rarely present. In hamsters, however, discrete pyogranulomas with central necrosis and persistence of leukocytic karyorrhectic debris were typical, and the inflammation was frequently accompanied by hemorrhage. In hamsters, we frequently observed septic thrombi in many tissues. However, in mice, septic thrombi were never observed. In animals intraperitoneally infected

with *B. pseudomallei*, lesions were similar, but more severe.

In BALB/c mice given *B. mallei* by aerosol, prominent lesions in the nasal cavity included an acute inflammatory cell infiltrate, erosion, and ulceration of both respiratory and olfactory epithelium and a serocellular exudate. Involvement of the olfactory tract and olfactory lobe of the brain was present in all mice by day 4. When comparing aerosol infections caused by *B. mallei* to those caused by *B. pseudomallei*, *B. mallei* infection was more contained and seeded fewer tissues; the areas of inflammation contain a higher or equal percentage of macrophages than neutrophils, there was less hemorrhage and thrombosis, and microorganisms were not visualized in H&E stained tissues. *Conclusions.* Although hamsters are too susceptible to infection to be good models of human glanders and melioidosis, aspects of the infections in mice are similar to those found in humans.

NOTES