2015 FDA Science Forum

Emerging Technologies • May 27 – 28, 2015

U.S. Food and Drug Administration

Contents

A Message from FDA's Acting Commissioner

Stephen Ostroff, M.D.
Acting Commissioner, FDA

It is with great pleasure that we welcome you to FDA's 2015 Science Forum. As you know, science is all about change. And since our last public Science Forum in 2006, FDA has made extraordinary advances in responding to and leading the many changes being driven by new science and emerging technologies.

One obvious change since our last forum is that we've established our new headquarters at White Oak—and we're proud to be holding our public Science Forum here at our new research campus. This state-of-the-art facility brings together the laboratories of FDA's Centers for Biologics, Drugs, and Devices to promote the cross-cutting collaboration FDA scientists needed to assess and develop new technologies, many of which will become the regulated medical products of the future.

And FDA recently celebrated the completion of our White Oak Biodefense Laboratory, a high-tech lab complex housing over 500 researchers, and supporting the development of critical medical products, such as influenza vaccines, and treatments for global diseases like Ebola, HIV, tuberculosis, and malaria.

The campus also houses our Center for Tobacco Products (CTP), created in 2009 with the mission of reducing the public harm caused by tobacco products. Our CTP scientists reflect the range of scientific disciplines needed to review tobacco product applications, inform regulations, conduct tobacco regulatory research, and mount educational campaigns.

But strengthening our scientific infrastructure is just a part of the broader advances we're making to encourage and lead research into new medical therapies and new methods to ensure food safety. As the pace of scientific discovery has accelerated in recent years, major advances in basic science were not effectively translating into real-world products to benefit patients and consumers. To address the challenge, FDA launched successful new initiatives, like Critical Path, Sentinel, and Medical Countermeasures to identify ways to drive innovation and incorporate emerging technologies into all we do to protect and promote the public health.

And in 2011, FDA issued a Strategic Plan for Advancing Regulatory Science—the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of FDA-regulated products. The eight scientific priority areas outlined in that plan frame the sessions of this Science Forum. They are FDA's blueprint for speeding innovation, improving regulatory decision-making, and getting safe and effective products to people in need.

During the next two days, you'll hear FDA scientists and national scientific experts discuss regulatory science challenges and successes in more than 30 panel discussions and

presentations. Our poster sessions will showcase the range of cutting-edge research underway at FDA and show how we're positioning ourselves to evaluate emerging technologies. We look forward to your continuing contributions in collaboration with FDA to help us close the regulatory science gaps we've identified at this Forum.

Thank you for your participation and for helping drive innovation in this critical field of public health. I hope you have an enjoyable and productive Forum.

Bio of Stephen Ostroff, M.D.

Dr. Stephen Ostroff, M.D., is FDA's acting commissioner. As the top official of the Food and Drug Administration (FDA), Dr. Ostroff is committed to strengthening programs and policies that enable the agency to carry out its mission to protect and promote the public health.

"It's a singular honor to be given the opportunity to represent the people of FDA who every day dedicate their work to assure safe and effective medical products, foods, and cosmetics and to mitigate the health consequences of tobacco products," says Dr. Ostroff.

Before being named acting commissioner, Dr. Ostroff served as FDA's chief scientist. In this capacity, he was responsible for leading and coordinating FDA's cross-cutting scientific and public health efforts. The Office of the Chief Scientist works closely with FDA's product centers, providing strategic leadership and support for FDA's regulatory science and innovation initiatives.

Dr. Ostroff joined FDA in 2013 as chief medical officer in the Center for Food Safety and Applied Nutrition and senior public health advisor to FDA's Office of Foods and Veterinary Medicine.

Before that he served as deputy director of the National Center for Infectious Diseases at the Centers for Disease Control and Prevention (CDC), where he was also acting director of CDC's Select Agent Program. While at CDC he focused on emerging infectious diseases, food safety, and coordination of complex outbreak response. He retired from the Commissioned Corps of the U.S. Public Health Service at the rank of Rear Admiral (Assistant Surgeon General). Dr. Ostroff was also the director of the Bureau of Epidemiology and acting physician general for the Commonwealth of Pennsylvania and has consulted internationally on public health projects in South Asia and Latin America.

Dr. Ostroff graduated from the University of Pennsylvania School of Medicine in 1981 and completed residencies in internal medicine at the University of Colorado Health Sciences Center and preventive medicine at CDC.

A Message from FDA's Acting Chief Scientist

Luciana Borio, M.D.
Acting Chief Scientist, Office of the Chief Scientist, OC, FDA

We are delighted to welcome you to FDA's 2015 Science Forum. Over the course of the next two days, you'll be able to explore with us some of the exciting scientific research underway at FDA.

As you know, science is at the heart of everything we do – it underpins all of our regulatory decisions. And as scientific breakthroughs lead to the development of innovative, new medical therapies, FDA sometimes faces new challenges and questions about how to regulate them. This is where FDA research comes in.

Many of FDA's 10,000 scientists are at work in our research laboratories – here at White Oak and across the nation – evaluating how emerging technologies are affecting FDA-regulated products. FDA then integrates the results of this research into our regulatory review process, enabling our reviewers to make science-based decisions about a product's benefits and risks. One example you'll hear about at our Forum is the research FDA social and behavioral scientists are conducting to examine the relationship between e-cigarette use and conventional cigarette smoking among young adults in the U.S.

FDA research has also contributed to our understanding of why a drug sometimes produces negative side effects in some people, but not in others, once it goes on the market. Thanks to FDA scientists, we now know why some African Americans are significantly more likely to develop antibodies against a drug used to treat hemophilia A. The discovery of genetic variations that seem to cause this response in certain individuals is an important step toward using precision medicine to develop a treatment for this disease.

And you might be surprised to learn that FDA scientists also invent, contributing to the development of new discoveries and products to keep our food and drugs safe. One of these inventions, the CDx Device, will be presented at the Forum by the FDA inventor. This device is being used at our ports of entry to screen for counterfeit drugs and contaminated products. In an increasingly globalized economy, the CDx Device and other cutting-edge technologies are proving crucial to FDA's ability to ensure the safety and quality of food and drug imports.

But to be able to deliver on the promise of these emerging technologies, collaboration is essential. Nowhere was this more clearly demonstrated than in FDA's recent collaborative response to the Ebola epidemic in West Africa. It highlighted FDA's commitment to the principle of a science-based decision-making process and to close cooperation and coordination with all our partners in government, industry, clinicians, and our counterparts in West Africa in responding to public health emergencies.

We believe this year's Forum will give all of you

a closer look at the many projects underway at FDA and provide you with a deeper understanding of regulatory science research, its challenges, and its opportunities for collaboration. And we look forward to sharing with you some of the exciting scientific advances we're making with our partners in the scientific community.

Bio of Luciana Borio, M.D.

Dr. Luciana Borio is FDA's acting chief scientist. In this capacity, she is responsible for leading and coordinating FDA's cross-cutting scientific and public health efforts.

The Office of the Chief Scientist works closely with FDA's product centers, providing strategic leadership and support for FDA's regulatory science and innovation initiatives, including the Advancing Regulatory Science Initiative, the Critical Path Initiative, scientific professional development, scientific integrity, and the Medical Countermeasures Initiative (MCMi).

Since 2011, Dr. Borio has served as the assistant commissioner for counterterrorism policy and director of the Office of Counterterrorism and Emerging Threats (OCET) in the Office of the Chief Scientist at FDA. In this capacity, Dr. Borio provided leadership, coordination, and oversight for FDA's national and global health security, counterterrorism, and emerging threat portfolios and led the MCMi.

Dr. Borio has been instrumental in coordinating FDA's response to the 2009 H1N1 influenza pandemic and continues to oversee FDA's preparedness and response activities for emerging threats, such as the avian influenza A (H7N9) virus and the West Africa Ebola epidemic.

Before joining FDA as a medical reviewer in 2008, Dr. Borio served as a senior associate at the University of Pittsburgh Medical Center – Center for Biosecurity, assistant professor of medicine at the University of Pittsburgh, and advisor on biodefense programs for the U.S. Department of Health and Human Services. Dr. Borio received her M.D. from the George Washington University, and continues to practice medicine at The Johns Hopkins Hospital.

FDA Science Forum Agenda

May 27, 2015 – Day 1

8:30 – 8:40 AM Introduction
Remote Access: https://collaboration.fda.gov/fdascienceforum/

8:40 – 8:50 AM Welcome by FDA Acting Chief Scientist, Luciana Borio, M.D.

8:50 – 9:00 AM Remarks by FDA Acting Commissioner, Stephen M. Ostroff, M.D.

9:00 – 10:15 AM Poster Sessions 1 & 2 and Break

1. Ensure FDA Readiness to Evaluate Innovate Emerging Technologies
2. Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

10:15 – 12:00 PM Concurrent Sessions 1 & 2

Session 1: Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

Great Room Section B, *Remote Access:* https://collaboration.fda.gov/fdascienceforum/

Session Chair: **Steven K. Pollack, Ph.D.,**
Director, Office of Science and Engineering Laboratories, CDRH, FDA

10:15 – 10:45 AM Breakthroughs in Imprint Lithography and 3D Additive Fabrication

Joseph M. DeSimone, Ph.D.
Chancellor's Eminent Professor of Chemistry and Chemical Engineering, University of North Carolina at Chapel Hill and North Carolina State University; Co-Founder and CEO, Carbon 3D

10:45 – 11:06 AM Single Cell Methods in Cell Product Characterization

Malcolm Moos, M.D., Ph.D.
Medical Officer, CBER, FDA

11:06 – 11:28 AM Field Portable Devices – Taking the Laboratory to the Sample

Mark Witkowski, Ph.D.
Chemist, Trace Examination Section, Forensic Chemistry Center, ORA, FDA

11:28 – 11:50 AM The Intersection of Personalized Cardiac Therapies, Cell Based Diagnostics and Multi-Variate Physiological Monitoring

David G. Strauss, M.D., Ph.D.
Medical Officer, CDRH, FDA

11:50 – 12:00 PM Q&A Session

Session 2: Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

Great Room Section C, *Remote Access:* https://collaboration.fda.gov/fdascienceforum3/

Session Chair: **Lee Zwanziger, Ph.D.,**
Senior Science Policy Analyst, Risk Communication Staff, Office of Planning, Office of Policy, Planning, Legislation, and Analysis, Office of Policy, OC, FDA

10:15 – 10:45 AM Lessons Learned from Applying Epidemiology, Cultural Competency and Health Literacy Research to Address Health Disparities

Olivia Carter-Pokras, Ph.D.
Associate Professor, Epidemiology and Statistics, University of Maryland School of Public Health

10:45 – 11:01 AM E-Cigarette Use and Cigarette Smoking Behavior among U.S. Young Adults: A Mixed Methods Study

Blair N. Coleman, Ph.D., M.P.H.
Epidemiologist, CTP, FDA

11:01 – 11:17 AM Experimental Study of Patient Information Prototypes

LT. Oluwamurewa (Murewa) Oguntimein, M.H.S, C.H.E.S.
Senior Regulatory Management Officer, Social Scientist, Office of Research and Standards, Division of Therapeutic Performance, CDER, FDA

11:17 – 11:33 AM Incorporating Patient Preferences into Regulatory Decision Making

Telba Irony, Ph.D.
Chief, General and Surgical Devices Branch, Division of Biostatistics and Office of Device Evaluation, CDRH, FDA

11:33 – 11:50 AM Recent Findings from Nonhuman Primates on the Long-Term Adverse Behavioral Effects of General Anesthesia When Given During Early Brain Development

Merle G. Paule, Ph.D., A.T.S.
Director, Division of Neurotoxicology, NCTR, FDA

11:50 – 12:00 PM Q&A Session

12:00 – 1:00 PM Lunch

1:00 – 2:45 PM Concurrent Sessions 3 & 4

Session 3: Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security

Great Room Section B, *Remote Access:* https://collaboration.fda.gov/fdascienceforum/

Session Chair: **Rakesh Raghuwanshi, M.P.H.,**
Scientific Program Manager, Office of Counterterrorism and Emerging Threats, OC, FDA

1:00 – 1:30 PM Enhancing Preparedness Through Novel Partnerships for IT Innovation: USCIIT-PREP

J. Perren Cobb, M.D., F.C.C.M, F.A.C.S.
Medical Director, Surgical Intensive Care Unit, and Vice-chair, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston Massachusetts; Associate Professor of Anesthesiology and Associate Professor of Surgery, Harvard Medical School, Boston Massachusetts

1:30 – 1:51 PM Electrophysiological Biomarkers of Brain Injury

Cristin Welle, Ph.D.
Neurophysiologist, Office of Science and Engineering Labs, CDRH, FDA

1:51 – 2:13 PM Pandemic Influenza Preparedness: Development of Novel Technologies For In-Depth Evaluation Of Vaccine Efficacy And Long-Term Memory During H7 Clinical Trials

Surender Khurana, Ph.D.
Staff Scientist, Office of Vaccines Research and Review, CBER, FDA

2:13 – 2:35 PM Filovirus Detection and Threat Mitigation

Steven Wood, Ph.D.
Biologist, Office of Science and Engineering Laboratories, CDRH, FDA

2:35 – 2:45 PM Q&A Session

Session 4: Implement a New Prevention-Focused Food Safety System to Protect Public Health

Great Room Section C, *Remote Access:* https://collaboration.fda.gov/fdascienceforum3/

Session Co-Chairs: **Palmer A. Orlandi, Jr., Ph.D.**,
Acting Chief Science Officer and Research Director, OFVM, FDA, and David G. White, M.S., Ph.D., Acting Director of the Office of Resource Planning and Strategic Management, OFVM, FDA

1:00 – 1:30 PM Food Safety Systems in the Americas: A Perspective from the Pan-American Health Organization (PAHO)

Enrique Pérez-Gutiérrez, D.V.M., M.Sc., M.P.V.M., Ph.D.
Senior Advisor, Foodborne Diseases and Zoonosis, Pan-American Health Organization

1:30 – 1:46 PM GenomeTrakr: A Pathogen Databases to Build a Global Genomic Network for Pathogen Traceback and Outbreak Detection

Ruth E. Timme, Ph.D.
Research Microbiologist, Molecular Methods and Subtyping Branch, CFSAN, FDA

1:46 – 2:02 PM The Nexus of Food Safety, Animal Health and Antimicrobial Resistance

Patrick McDermott, M.S., Ph.D.
Director, National Antimicrobial Resistance Monitoring System (NARMS), CVM, FDA

2:02 – 2:18 PM The Use of DNA Barcoding to Prevent Species-Specific Foodborne Illness and Detect Seafood Fraud

Jonathan Deeds, Ph.D.
Research Biologist, Office of Regulatory Science, CFSAN, FDA

2:18 – 2:35 PM Non-Targeted Screening Methods for Identification of Chemical Hazards of Public Health Concern

Tim Croley, Ph.D.
Supervisory Chemist, CFSAN, FDA

2:35 – 2:45 PM Q&A Session

2:45 – 3:45 PM Poster Sessions 3 & 4 and Break

3. Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security
4. Implement a New Prevention-Focused Food Safety System to Protect Public Health

3:45 PM End of Day 1

May 28, 2015 – Day 2

8:30 – 8:35 AM Keynote Introduction: **Carol Linden, Ph.D.**,
Director, Office of Regulatory Science and Innovation

Remote Access: https://collaboration.fda.gov/fdascienceforum2/

8:35 – 9:15 AM Cancer Genomes and the Wars Against Cancer

Bert Vogelstein, M.D.
Director, Ludwig Center at Johns Hopkins University
Investigator, Howard Hughes Medical Institute

9:15 – 10:15 AM Poster Sessions 5 & 6 and Break

5. Support New Approaches to Improve Product Manufacturing and Quality
6. Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

10:15 – 12:00 PM **Concurrent Sessions 5 & 6**

Session 5: Support New Approaches to Improve Product Manufacturing and Quality

Great Room Section B, *Remote Access:* https://collaboration.fda.gov/fdascienceforum2/

Session Chair: **Sau (Larry) Lee, Ph.D.**
Acting Associate Director for Science, Team Leader for OPQ Botanical Review Team, Office of Pharmaceutical Quality, CDER, FDA, and
Carolyn Wilson, Ph.D., Associate Director for Research, CBER, FDA

10:15 – 10:45 AM Framework for Chemical Characterization

Ram Sasisekharan, Ph.D.
Alfred H. Caspary Professor of Biological Engineering and Health Sciences & Technology, Koch Institute, Department of Biological Engineering, Massachusetts Institute of Technology

10:45 – 11:01 AM Screening for Counterfeit Pharmaceutical Products Using the CDx Device in Ultraviolet, Visible, and Infrared Modes for Field and Laboratory Use

Nicola Ranieri
Research Biologist, Microscopy and Image Analysis,
Trace Examination Section, ORA, FDA

11:01 – 11:17 AM Methods for Detection of Allergens in Food and in the Processing Environment: Approaches and Challenges

Lauren Jackson, Ph.D.
Chief, Process Engineering Branch, Division of Processing Science & Technology, Office of Food Safety, CFSAN, FDA

11:17 – 11:33 AM Advanced Analytics and Data Integration for Biomolecule Characterization

Sau (Larry) Lee, Ph.D.
Acting Associate Director for Science, Team Leader for OPQ Botanical Review Team, Office of Pharmaceutical Quality, CDER, FDA

11:33 – 11:50 AM Using NMR to Assess Structure and Comparability

Daron Freedberg, Ph.D.
Senior Scientist, CBER, FDA

11:50 – 12:00 PM Q&A Session

Session 6: Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Great Room Section C, *Remote Access:* https://collaboration.fda.gov/fdascienceforum4/

Session Chair: **Lisa M. LaVange, Ph.D.**
Director, Office of Biostatistics, Office of Translational Sciences, CDER, FDA

10:15 – 10:45 AM Individualized Therapy as a Practical Aspect of Patient Care

Howard L. McLeod, Pharm.D.
Medical Director, DeBartolo Family Personalized Medicine Institute and Senior Member, Department of Cancer Epidemiology, Moffitt Cancer Center

10:45 – 11:06 AM Not in Our Stars but in Ourselves: The Pharmacogenetic Determinants of Immunogenicity of Therapeutic Proteins

Zuben E. Sauna, Ph.D.
Senior Staff Fellow, CBER, FDA

11:06 – 11:28 AM Pharmacogenomics and Biomarker-Based Drug Development

Michael Pacanowski, Pharm.D., M.P.H.
Associate Director for Genomics and Targeted Therapy, Office of Clinical Pharmacology, CDER, FDA

11:28 – 11:50 AM Statistical Evaluation of "Me-Too" Companion Diagnostic Tests for Selecting Therapies

Gene Pennello, Ph.D.
Team Leader and Mathematical Statistician (Biomedical), Division of Biostatistics, CDRH, FDA

11:50 – 12:00 PM Q&A Session

12:00 – 1:00 PM Lunch

1:00 – 2:00 PM Poster Sessions 7 & 8 and Break

7. Modernize Toxicology to Enhance Product Safety
8. Harness Diverse Data through Information Sciences to Improve Health Outcomes

2:00 – 3:45 PM Concurrent Sessions 7 & 8

Session 7: Modernize Toxicology to Enhance Product Safety

Great Room Section B, *Remote Access:* https://collaboration.fda.gov/fdascienceforum2/

Session Chairs: **Donna Mendrick, Ph.D.**
Associate Director for Regulatory Activities, NCTR, FDA,
and James Weaver, Ph.D., Research Pharmacologist,
Office of Applied Regulatory Science, CDER, FDA

2:00 – 2:30 PM Human Microlivers for Disease Modeling

Sangeeta Bhatia, M.D., Ph.D.
John J. and Dorothy Wilson Professor of Health Sciences
and Technology and Electrical Engineering and Computer
Science, Massachusetts Institute of Technology

2:30 – 2:46 PM Discovery and Analytical Validation of System Biology Translation Biomakers of Toxicity

Richard D. Beger, Ph.D.
Branch Chief, Biomarkers and Alternative Models Branch, NCTR, FDA

2:46 – 3:02 PM Nonclinical Development of Neurotoxicity Biomarkers Using In Vivo MRI

Serguei Liachenko, M.D., Ph.D.
Director of Bioimaging, NCTR, FDA

3:02 – 3:18 PM Replacing the Clinical Thorough QT Study with a Panel of In Vitro Assays and Computational Integration

Norman Stockbridge, M.D., Ph.D.
Division Director, Division of Cardiovascular and Renal
Products, Office of New Drugs, CDER, FDA

3:18 – 3:35 PM Humanized Hepatic Mice: In Vivo Model to Predict Human-Specific Immunotoxicity, Drug Metabolism and Hepatotoxicity

Kristina E. Howard, D.V.M., Ph.D.
Research Veterinary Medical Officer, Division of Applied Regulatory Science,
Office of Clinical Pharmacology, Office of Translational Sciences, CDER, FDA

3:35 – 3:45 PM Q&A Session

Session 8: Harness Diverse Data through Information Sciences to Improve Health Outcomes

Great Room Section C, *Remote Access:* https://collaboration.fda.gov/fdascienceforum4/

Session Chairs: **Eric Donaldson, Ph.D.**
Clinical Virology Reviewer, Division of Antiviral Products, CDER, FDA, and Roger G. Perkins, M.S., Senior Advisor, Division of Bioinformatics and Biostatistics, NCTR, FDA

2:00 – 2:30 PM Transforming Trillions of Points of Data into Diagnostics, Therapeutics, and New Insights into Disease

Atul Butte, M.D., Ph.D.
Director, Institute for Computational Health Sciences and Professor of Pediatrics, University of California, San Francisco

2:30 – 3:45 PM Panel Discussion: Next Generation Sequencing Technology at FDA

Carolyn Wilson, Ph.D.
Associate Director for Research, CBER, FDA

Hugh A. Rand, Ph.D.
Bioinformatics Team Lead, CFSAN, FDA

Heike Sichtig, Ph.D.
Principal Investigator/Regulatory Scientist, CDRH, FDA

Weida Tong, Ph.D.
Director, Division of Bioinformatics and Biostatistics, NCTR, FDA

3:45 – 4:00 PM Break

4:00 – 4:15 PM Closing Remarks and Adjourn

Carol Linden, Ph.D.
Director, Office of Regulatory Science and Innovation

https://collaboration.fda.gov/fdascienceforum2/

External Speaker Bios

Session 1:
Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

Joseph M. DeSimone, Ph.D.

Chancellor's Eminent Professor of Chemistry and Chemical Engineering, University of North Carolina at Chapel Hill and North Carolina State University; Co-Founder and CEO, Carbon 3D

Dr. Joseph M. DeSimone is a prolific inventor, serial entrepreneur and eminent scholar. DeSimone is the Chancellor's Eminent Professor of Chemistry at the University of North Carolina at Chapel Hill, and William R. Kenan, Jr. Distinguished Professor of Chemical Engineering at North Carolina State University and of Chemistry at UNC. DeSimone is also an adjunct member at Memorial Sloan-Kettering Cancer Center. Currently DeSimone is on leave from the university and has assumed the CEO role at Carbon3D in Silicon Valley. DeSimone has published over 300 scientific articles and has over 150 issued patents in his name with over 80 patents pending.

DeSimone is one of fewer than twenty individuals who have been elected to all three branches of the National Academies: Institute of Medicine (2014), National Academy of Sciences (2012) and the National Academy of Engineering (2005). He is also a member of the American Academy of Arts and Sciences (2005). DeSimone has received over 50 major awards and recognitions including the 2015 Dickson Prize from Carnegie Mellon University; 2014 Industrial Research Institute Medal; 2014 Kathryn C. Hach Award for Entrepreneurial Success from the ACS; 2013 Fellow of the National Academy of Inventors; 2012 Walston Chubb Award for Innovation by Sigma Xi; the 2010 AAAS Mentor Award in recognition of his efforts to advance diversity in the chemistry PhD workforce; the 2009 NIH Director's Pioneer Award; the 2009 North Carolina Award; the 2008 $500,000 Lemelson-MIT Prize for Invention and Innovation; the 2007 Collaboration Success Award from the Council for Chemical Research; the 2005 ACS Award for Creative Invention; the 2002 John Scott Award presented by the City Trusts, Philadelphia, given to "the most deserving" men and women whose inventions have contributed in some outstanding way to the "comfort, welfare and happiness" of mankind; the 2002 Engineering Excellence Award by DuPont; and the 2002 Wallace H. Carothers Award from the Delaware Section of the ACS.

DeSimone, an innovative polymer chemist, has made breakthrough contributions in fluoropolymer synthesis, colloid science, nano-biomaterials, green chemistry and most recently 3D printing. DeSimone is the co-founder of several companies including Miceli Technologies, Bioabsorbable Vascular Solutions, Liquidia Technologies and Carbon3D. DeSimone received his BS in Chemistry in 1986 from Ursinus College in Collegeville, PA and his Ph.D. in Chemistry in 1990 from Virginia Tech.

Session 2:
Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

Olivia Carter-Pokras, Ph.D.

Associate Professor, Department of Epidemiology and Statistics, University of Maryland School of Public Health

Dr. Olivia Carter-Pokras is an Associate Professor in Epidemiology at the University of Maryland School of Public Health. A health disparities researcher for 3 decades in the Federal government and academia, Dr. Carter-Pokras has been recognized by the Governor of Maryland, Surgeon General, Assistant Secretary for Health, and Latino Caucus of the American Public Health Association for her career achievements to improve health care quality for Latinos, improve racial and ethnic data, and develop health policy to address health disparities. While at the University of Maryland, she has focused her research, service and education efforts to support translation of epidemiologic research into policy and practice to improve Latino population health.

Dr. Carter-Pokras is an elected fellow of the American College of Epidemiology and a member of the American Public Health Associations Science Board. She currently chairs the American College of Epidemiology's Policy Committee, and has served on the Institute of Medicines Advancing Pain Research, Care, and Education Committee.

A long-time member of Montgomery County's Latino Health Steering Committee, Dr. Carter-Pokras conducts health assessments of Latinos in Baltimore and Montgomery County in close partnership with local government and community based organizations. She has led NIH funded research projects to develop cultural competency and health literacy curricula, and address oral health of Latino and Ethiopian children and their mothers. Dr. Carter-Pokras is a Co-Investigator for the University of Maryland's PATIENTS program to support patient-centered outcomes research. She has published 64 peer-reviewed publications which have been cited 3165 times, and her research has played a critical role in national recognition of health disparities experienced by Latinos. Dr. Carter-Pokras lectures on chronic disease epidemiology, epidemiologic methods, cultural competency and health disparities to public health students and health professionals.

Session 3: Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security

J. Perren Cobb, M.D., F.C.C.M., F.A.C.S.

Medical Director, Surgical Intensive Care Unit, and Vice-chair, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston Massachusetts

Associate Professor of Anesthesiology and Associate Professor of Surgery, Harvard Medical School, Boston Massachusetts

Dr. J. Perren Cobb is Medical Director of the Surgical ICU and Vice-Chair for Critical Care, Department of Anaesthesia, Critical Care, and Pain Medicine at Massachusetts General Hospital. At the Medical School he holds the rank of Associate Professor of Anaesthesia and of Surgery, and Affiliate Faculty at the Harvard Stem Cell Institute. Dr. Cobb is also founding Director of the U.S. Critical Illness and Injury Trials Group (USCIIT), which fosters investigator-initiated hypothesis testing and strategic planning at the national level for critical illness and injury research. His academic interest is systems approaches to quality improvement and emergency preparedness.

Dr. Cobb was an undergraduate at Vanderbilt University and received his medical degree from the University of Louisville School of Medicine. He trained in General Surgery at the University of California-San Francisco and completed fellowships in critical care at the NIH and the University of Pittsburgh. His early research focus was the treatment of sepsis; he worked nationally with trauma collaborators to develop a novel sepsis diagnostic, the riboleukogram, which uses contemporary genomics and microfluidics technology to track the host response to injury and infection. Dr. Cobb's work has been supported by the National Institutes of Health, the U.S. Office of the Assistant Secretary for Preparedness and Response, the American Association for the Surgery of Trauma, the Society of Critical Care Medicine, and the Barnes Jewish Hospital Foundation. His awards include the Research Scholarship Award of the American Association for the Surgery of Trauma, the Founders Grant for Critical Care Research of the Society of Critical Care Medicine, the George H. A. Clowes, Jr. Memorial Research Career Development Award of the American College of Surgeons, and the 2nd Annual Critical Care Medicine Distinguished Alumnus of the University of Pittsburgh. Dr. Cobb is a former President of the Association for Academic Surgery.

Session 4:
Implement a New Prevention-Focused Food Safety System to Protect Public Health

Enrique Pérez-Gutiérrez, D.V.M., M.Sc., M.P.V.M., Ph.D.

Senior Advisor, Foodborne Diseases and Zoonosis, Pan-American Health Organization

Dr. Enrique Perez received his D.V.M. from the National University of Costa Rica, a Master in Preventive Medicine from the Federal University of Minas Gerais of Brazil, a Master in Veterinary Preventive Medicine from the University of California in Davis and his PhD in Epidemiology from the University of Utrecht in the Netherlands. In 2001, Dr. Perez joined the Pan-American Health Organization (PAHO/WHO) responsible for providing technical cooperation in the development of risk-based, sustainable integrated food safety systems; promoting international coordination between health and agriculture sectors; and promoting and carrying out research in food safety and foodborne diseases. He is actively involved in WHO-GFN network and PulseNet Latin America and the Caribbean network. He is actively engaged in strengthening country capacity in surveillance of food borne diseases, burden of foodborne of diseases studies, risk assessment and antimicrobial resistance projects along the Americas.

Keynote Speaker

Bert Vogelstein, M.D.

Director, Ludwig Center at Johns Hopkins, Investigator, Howard Hughes Medical Institute, The Sidney Kimmel Cancer Center at Johns Hopkins University

Dr. Bert Vogelstein was the first scientist to determine the molecular basis of a common human cancer. He and his colleagues have demonstrated that colorectal tumors result from the gradual accumulation of genetic alterations in specific oncogenes and tumor suppressor genes. His group's discovery and analysis of these genes and their functions represent a landmark in the application of molecular biology to the study of human disease. His work on colorectal cancers forms the paradigm for much of modern cancer research, with profound implications for diagnostic and therapeutic strategies in the future.

Dr. Vogelstein and his colleagues were also the first to map cancer genomes and use genome-wide sequencing to identify the basis of a hereditary form of cancer. His team has determined the genetic landscapes of more than a dozen tumor types, and together with their earlier studies, this work has provided the conceptual basis for what is now called personalized or precision medicine.

Dr. Vogelstein has won numerous awards over the last several years for his pioneering studies on the pathogenesis of human cancer. These include the The Bristol Myers Squibb Award for Distinguished Achievement in Cancer Research, the American Cancer Society Medal of Honor, The Gairdner Foundation International Award in Science, The Dickson Prize from the University of Pittsburgh, the Passano Award, the William Allan Award from the American Society of Human Genetics, the Richard Lounsbery Award from the National Academy of Sciences, the Louisa Gross Horwitz Prize from Columbia University, the Harvey Prize in Human Health from the Technion, the Charles S. Mott Prize from the General Motors Cancer Research Foundation, and the Pasarow Award from the Robert J. and Claire Pasarow Foundation. Most recently, he was one of 11 recipients of the inaugural Breakthrough Prize in Life Sciences.

He was elected to the American Academy of Arts & Sciences as well as the National Academy of Sciences, USA in 1992, the American Philosophical Society in 1995 and became a fellow of the American Association for the Advancement of Science in 2006. His advisory roles have included Chairmanship of the National Research Council Committee on the Biological and Biomedical Applications of Stem Cell Research and the Board of Scientific Counselors of the National Human Genome Research Institute. He has also held editorial positions at Science, Molecular Cell, Cancer Cell and The New England Journal of Medicine.

Dr. Vogelstein's pioneering studies of the genetic causes of human cancer have placed him among the most influential biomedical in the world. According to the Institute for Scientific Information in Philadelphia, the papers he has published since he began his work in the mid- 1970's have been cited more often than those of any other scientist, in any discipline.

Session 5:
Support New Approaches to Improve Product Manufacturing and Quality

Ram Sasisekharan, Ph.D.

Alfred H. Caspary Professor of Biological Engineering, Department of Biological Engineering, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

Dr. Sasisekharan has been a professor of Biological Engineering and Health Sciences & Technology at MIT since 1996 and is Director of the Harvard-MIT Division of Health Sciences & Technology and Alfred H. Caspary Professor of Biological Engineering & Health Sciences and Technology. His research has led to over 175 publications and over 80 patents. He is a founder of Momenta Pharmaceuticals (NASDAQ: MNTA), Cerulean Pharma (NASDAQ: CERU), and Visterra Pharmaceuticals. He has won many national and international awards; among them are both the Burroughs Wellcome and Beckman Foundation Young Investigator Awards. In addition, he received the 1998, 1999, 2000, and 2001 CaPCure Awards from the CaPCure Foundation, was honored with the Princess Chulabhorn Gold Medal Award in 2007, and received a National Institutes of General Medical Sciences MERIT Award in 2010 and 2015. He was elected the fellow of US National Academy of Inventors (NAI) in 2015. He received his B.S. in Physical Sciences from Bangalore University, his M.S. in Biophysics from Harvard University, and his Ph.D. in Medical Sciences from Harvard Medical School.

Session 6:
Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Howard L. McLeod, Pharm.D.

Medical Director, DeBartolo Family Personalized Medicine Institute and Senior Member, Department of Cancer Epidemiology, Moffitt Cancer Center

Dr Howard McLeod is Medical Director of the DeBartolo Family Personalized Medicine Institute, moving to Moffitt Cancer Center in September, 2013. He is also a Senior Member of the Division of Population Sciences. Most recently he was the Fred Eshelman Distinguished Professor and Founding Director of the UNC Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill. Dr McLeod has a Bachelors Degree in Pharmacy from the University of Washington and a PharM.D. from the Philadelphia College of Pharmacy and Science. He was a Clinical Research Fellow at St Jude Children's Research Hospital and a postdoctoral fellow at the Beatson Institute/University of Glasgow. Dr McLeod is chair of the NHGRI eMERGE network external scientific panel and a former member of FDA committee on Clinical Pharmacology and the NIH NHGRI Advisory Council. Since 2002, Dr McLeod has been vice chair for Pharmacogenomics for the NCI clinical trials cooperative group CALGB/ALLIANCE, overseeing the largest oncology pharmacogenomics portfolio in the world. Dr McLeod is also a 1000 talent scholar of China and a Professor at Central South University in Changsha, China. He directs the Pharmacogenetics for Every Nation Initiative, which aims to help developing countries use genetic information to improve National Drug Formulary decisions. Howard has published over 475 peer reviewed papers on pharmacogenomics, applied therapeutics, or clinical pharmacology and continues to work to integrate genetics principles into clinical practice to advance individualized medicine.

Session 7:
Modernize Toxicology to Enhance Product Safety

Sangeeta Bhatia, M.D., Ph.D.

John J. and Dorothy Wilson Professor of Health Sciences and Technology & Electrical Engineering and Computer Science, Massachusetts Institute of Technology

Dr. Sangeeta Bhatia is the John J. and Dorothy Wilson Professor at MIT's Institute for Medical Engineering and Science and Koch Institute for Integrative Cancer Research, an HHMI Investigator, and a member of the National Academy of Engineering. Her lab focuses at the intersection of engineering, medicine, and biology to leverage miniaturization tools from the world of semiconductor manufacturing to impact human health with micro- and nanotechnology. Dr. Bhatia's findings have produced high-throughput-capable human microlivers which model human drug metabolism, liver disease, and interaction with pathogens, and are used to develop new drugs and detect potential toxicities. Her group also develops nanoparticles and nanoporous materials that can be designed to assemble and communicate to diagnose and treat a variety of diseases, including cancer. She has appointments at Brigham & Women's Hospital, Broad Institute, Harvard Stem Cell Institute, and MIT's Ludwig Center. Dr. Bhatia received her B.S. from Brown University, M.S. and Ph.D. from MIT, M.D. from Harvard and completed graduate and post-doctoral training at MGH. Before MIT, she was tenured faculty at UCSD, and worked in industry at Pfizer, and others.

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Session 8: Harness Diverse Data through Information Sciences to Improve Health Outcomes

Atul Butte, M.D., Ph.D.

Director, Institute for Computational Health Sciences and Professor of Pediatrics, University of California, San Francisco

Dr. Atul Butte is the new Director of the new Institute of Computational Health Sciences (ICHS) at the University of California, San Francisco, and a Professor of Pediatrics. Dr. Butte trained in Computer Science at Brown University, worked as a software engineer at Apple and Microsoft, received his M.D. at Brown University, trained in Pediatrics and Pediatric Endocrinology at Children's Hospital Boston, and received his PhD from Harvard Medical School and MIT. Dr. Butte has authored nearly 200 publications, with research repeatedly featured in Wired Magazine, the New York Times, and the Wall Street Journal. Dr. Butte is also the principal investigator of ImmPort, the archival and dissemination repository for clinical and molecular datasets funded by the National Institute of Allergy and Infectious Diseases. In 2013, Dr. Butte was recognized by the White House as an Open Science Champion of Change for promoting science through publicly available data. Other recent awards include the 2014 E. Mead Johnson Award for Research in Pediatrics, 2013 induction into the American Society for Clinical Investigation, the 2012 FierceBiotech IT "Top 10 Biotech Techies", and the 2011 National Human Genome Research Institute Genomic Advance of the Month. Dr. Butte is also a founder of three investor-backed data-driven companies: Personalis, providing clinical interpretation of whole genome sequences, Carmenta, discovering diagnostics for pregnancy complications, and NuMedii, finding new uses for drugs through open molecular data.

Session 1: Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

Steven K. Pollack, Ph.D., Session Chair

Director, Office of Science and Engineering Laboratories, CDRH, FDA

Dr. Steven Pollack is the Director of the Office of Science and Engineering Laboratories (OSEL), the applied research arm of FDA's Center for Devices and Radiological Health. Before this, he held the position of Director of the Division of Chemistry and Materials Science within OSEL. He obtained his Ph.D. in physical organic chemistry at the University of California, Irvine and his B.S. in chemistry from the State University of New York at Albany. He has held research and management positions at American Hospital Supply Corporation, Allergan Pharmaceuticals and Springborn Laboratories before returning to academia for a postdoctoral fellowship at the University of Massachusetts at Amherst. He has held faculty positions at the University of Cincinnati in Materials Science and Engineering and at Howard University, where he was Professor of Chemistry. His academic work included polymer physics, polymer synthesis, and molecular modeling. Immediately before coming to FDA, he was a research chemist and a Deputy Laboratory Head in the Center for Bio/Molecular Science and Engineering at the U.S. Naval Research Laboratory where he worked in the areas of molecular electronics, nanotechnology, and biothreat detection. He is author of more than 75 papers, 3 patents, and 2 book chapters in the areas of computational chemistry, polymer science, and nanotechnology.

Malcolm Moos, M.D., Ph.D.

Medical Officer, CBER, FDA

Dr. Malcolm Moos, Jr. is a Medical Officer in the Center for Biologics Evaluation and Research and supports regulatory decisions in the emerging area of cellular therapy. His current research interests are to define how evaluating the status of major cell signaling pathways (BMP, Wnt, etc.) can be used in conjunction with recent developments in systems biology to characterize cell-based products more accurately and to improve product design. His awards include the Harvey W. Wiley Medal, the Center Director's Targeted Research Award (twice), the Center Director's Distinguished Service Award, and thirty-one others. He received a B.S from Stanford University and M.D. and Ph.D. from University of Minnesota.

Mark Witkowski, Ph.D.

Chemist, Trace Examination Section, Forensic Chemistry Center, ORA, FDA

Dr. Mark Witkowski was a Postdoctoral Fellow at D.O.M. Associates International Inc. in Manhattan Kansas from 1992 to 1994. He primarily worked with Near Infrared (NIR) and FT-IR instrumentation to develop methods for medical and geological applications. In 1994, Dr. Witkowski joined Warner-Lambert (WL) Pharmaceutical Company where he developed process analytical methods (NIR, GC) applicable to WL pharmaceutical manufacturing processes. He worked with WL corporate security to develop and implement a forensic testing program for suspect counterfeit WL pharmaceutical products. Dr. Witkowski joined FDA's Forensic Chemistry Center in 2000 as a specialist in vibrational spectroscopy. Currently, he is a chemist in the Trace Examination Section primarily using FT-IR, Raman, optical microscopic and micro-analytical techniques to analyze forensic samples, such as counterfeit pharmaceuticals, tampered/adulterated food and pharmaceutical products, and a variety of other trace evidence. Dr. Witkowski has testified multiple times in state, federal, and international courts of law. He helped develop and implement field deployable methods using Raman, FT-IR and a novel instrument developed at the FCC called the CDX. Dr. Witkowski is a co-chairman of the Portable Devices Technical Advisory Group (TAG) for the analysis of food products.

David G. Strauss, M.D., Ph.D.

Medical Officer, CDRH, FDA

Dr. David Strauss is an accomplished physician-scientist with a proven track record of leadership in translational regulatory science, spanning a spectrum of molecules and ion channels, stem cell based assays, large animal experiments, simulations, clinical trials including pharmacokinetic-pharmacodynamic modelling,

clinical biomarkers, personalized medicine, meta-analyses of clinical trials, and comparative effectiveness and outcomes studies using national registries and Medicare data. He established and continues to lead a 12-person intramural Cardiovascular Translational Research Group of engineers, laboratory scientists, clinical investigators and epidemiologists. Dr. Strauss's research is focused on: 1) predicting which patients will benefit and which are more likely to be harmed from implantable defibrillators and pacemakers; 2) studying advanced physiological monitoring medical devices to reduce "alarm fatigue" and improve prediction of hemorrhagic and septic shock; and 3) improving the assessment of arrhythmias and personalized medicine. He has applied novel electrocardiographic device methods to 34 clinical drug trials submitted to FDA, and led and managed two prospective clinical trials to assess novel electrocardiographic methods. Dr. Strauss is leading a study of commercially available induced pluripotent stem cell-derived cardiomyocytes and leading personalized medicine investigations of the ability of common genetic variants and patient-specific stem cell-derived cardiomyocytes to predict personalized drug response and perform a "clinical trial in a dish." He has published 73 peer-reviewed journal articles and book chapters, and one book. His work has appeared in the *Journal of the American Medical Association, Journal of the American College of Cardiology*, and the *American Journal of Cardiology.*

Session 2: Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

Lee Zwanziger, Ph.D., Session Chair

Senior Science Policy Analyst, Risk Communication Staff, Office of Planning, Office of Policy, Planning, Legislation, and Analysis, Office of Policy, OC, FDA

Dr. Lee Zwanziger joined the Office of Planning in 2007 as the founding designated federal officer (executive secretary) for FDA's Risk Communication Advisory Committee, and now works to promote risk communication and social and behavioral sciences at FDA. She previously worked in FDA's Center for Drug Evaluation and Research in the Safety Policy and Communication Staff, the Executive Secretariat Team, and the Advisors and Consultants Staff. Her experience outside FDA includes service as an adjunct or visiting part-time professor of science and technology studies at Virginia Tech University, staff work for the President's Council of Bioethics, and directing studies at the National Academies' Institute of Medicine on projects ranging from institutional review boards and data privacy to the anthrax vaccine. She came to FDA from Peace Corps service, teaching biological and physical sciences and medical ethics in Malawi's national school of health sciences. She holds graduate degrees in History and Philosophy of Science (Ph.D., University of Pittsburgh) and Biological Sciences (M.S., University of Alabama in Huntsville).

Blair N. Coleman, Ph.D., M.P.H.

Epidemiologist, CTP, FDA

Dr. Blair N. Coleman is an Epidemiologist in the Office of Science/ Center for Tobacco Products (CTP). Dr. Coleman has a doctorate in Behavioral and Community Health from the School of Public Health at the University of Maryland-College Park; a master's of public health from the Department of Behavioral Science and Community Health at the University of Florida; and a B.A. in psychology from DePaul University. Before joining FDA in 2012, Dr. Coleman completed the Oak Ridge Institute for Science Education (ORISE) Fellowship Program at the Centers for Disease Control and Prevention in the Office on Smoking and Health. At CTP, Dr. Coleman is involved in several surveillance activities that examine patterns of tobacco use and assists with projects using qualitative and quantitative research methodologies to explore attitudes, beliefs, and norms surrounding tobacco use behaviors. Dr. Coleman is focusing on mixed methods approaches to examining e-cigarette use among young adults in the United States.

Oluwamurewa Oguntimein, M.H.S, C.H.E.S.

Senior Regulatory Management Officer, Social Scientist, Office of Research and Standards, Division of Therapeutic Performance, CDER, FDA

Lt. Murewa Oguntimein is a U.S. Public Health Service Officer who serves as a Senior Regulatory Manager and Social Scientist for the Office of Research and Standards, Division of Therapeutic Performance (DTP). She is responsible for developing human factor study guidances for generic drug-device combination products. In addition, she reviews and responds to controlled correspondences and pre-ANDA meeting requests for generic inhalation products and drug-device combination products. She also serves as a Project Officer for several GDUFA-sponsored grants and contracts. Before joining DTP, Ms. Oguntimein was a social science analyst in Office of Medical Policy (OMP). She provided expertise in health literacy, consumer study research, patient and health education, program implementation and evaluation. Before joining OMP, Ms. Oguntimein, worked as a social science analyst in the Division of Nonprescription Clinical Evaluation and as a program analyst in the Office of Women's Health at FDA. In addition, she worked as a research coordinator for four years at The Johns Hopkins Center for Restless Leg Syndrome. She received her bachelor's degree in Psychology with a concentration in Biopsychology from the University of Maryland Baltimore County (UMBC). She also received her Master's in Health Science from the Johns Hopkins Bloomberg School of Public Health (JHSPH). While at JHSPH she also received a certificate in Health Communication and Health Financial Management. She is currently in a Ph.D. Behavioral and Community Health program at the University of Maryland School of Public Health. She is also Certified Health Education Specialist.

Telba Irony, Ph.D.

Chief, General and Surgical Devices Branch, Division of Biostatistics and Office of Device Evaluation, CDRH, FDA

Dr. Telba Irony is the Chief of the General and Surgical Devices Branch in the Division of Biostatistics and is currently on detail at the Office of Device Evaluation at the Center for Devices and Radiological Health. She received her Ph.D. from the University of California at Berkeley and was on the faculty of the George Washington University. She joined the Center for Devices to help implement the use of Bayesian methods in Medical Device Clinical Trials. She worked on several National Science Foundation grants on Bayesian Statistics and produced more than 50 peer-reviewed articles on Bayesian methods. Telba is a fellow of the American Statistical Association and an elected member of the International Statistical Institute. She was instrumental in the preparation of the following FDA guidance documents: "The Use of Bayesian Statistics in Medical Device Clinical Trials," and "Factors to Consider When Making Benefit-Risk Determinations in Medical Device Premarket Approval and De Novo Classifications." Telba Irony is currently leading the Decision Analysis initiative at CDRH, which involves Benefit: Risk assessments, "Patient Preference Elicitation," and Decision Tools for CDRH's Innovation Pathway.

Merle Paule, Ph.D., A.T.S.

Director, Division of Neurotoxicology, NCTR, FDA

Dr. Merle Paule received his Bachelor of Science degree in Biochemistry and his Ph.D. in Pharmacology and Toxicology at the University of California at Davis after which he conducted post-doctoral studies in Behavioral Pharmacology and Toxicology at the University of Arkansas for Medical Sciences. In 1983 he began work at FDA's National Center for Toxicological Research in Jefferson, Arkansas, where he remains today. In 2000 Merle attained certification as one of FDA's Senior Biomedical Research Scientists and in 2005 became the Director of the Division of Neurotoxicology at NCTR. Dr. Paule has played a major role in developing an automated system for monitoring multiple complex brain functions in nonhuman primates, children, and rodents. These functions include learning, short-term memory, motivation, color and position discrimination and time perception and are used as measures for determining the effects of drug and other chemical exposures. Use of similar or identical behavioral tasks across species serves to facilitate the interspecies extrapolation of exposure data and thus, the risk assessment process. Merle has served as an elected officer or appointed committee member in several prestigious scientific societies, including Past President of the Behavioral Toxicology Society, the Neurobehavioral Teratology Society and the Neurotoxicology Specialty Section of the Society of Toxicology. Dr. Paule is a member of several other scientific societies, including the Society for Neuroscience, the Society of Toxicology and the American Society for Pharmacology and Experimental Therapeutics. He is a reviewer for several scientific journals and sits on the editorial boards of Neurotoxicology, Neurotoxicology and Teratology and the Journal of Toxicology and Environmental Health. Merle has published over 200 research articles and 30 book chapters and holds adjunct professorships at the University of Arkansas for Medical Sciences in the Departments of Pharmacology and Toxicology and in Pediatrics. Dr. Paule is a Fellow in the Academy of Toxicological Sciences and in the International Behavioral Neuroscience Society.

Session 3: Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security

Rakesh Raghuwanshi, M.P.H., Session Chair

Scientific Program Manager, Office of Counterterrorism and Emerging Threats, OC, FDA

Rakesh Raghuwanshi is the lead scientific program manager in the Office of Counterterrorism and Emerging Threats (OCET), U.S. Food and Drug Administration (FDA). In this capacity, he manages FDA's Medical Countermeasures Initiative (MCMi) intramural regulatory science portfolio, which helps support regulatory decision-making for medical countermeasures (MCMs). Since 2011, Mr. Raghuwanshi has led the review and evaluation of MCM-related regulatory science projects. With the assistance of a dynamic interagency steering committee, he ensures that the projects are scientifically feasible and aligned with the U.S. Department of Health and Human Services (HHS) Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) priorities. He also led the planning and execution of FDA's MCMi Regulatory Science Symposia from 2012 to 2014. Mr. Raghuwanshi serves as a voting member of FDA's Institutional Animal Care and Use Committee (IACUC). He earned a B.A. in Economics and Political Science from UCLA, an M.P.H. from the Johns Hopkins Bloomberg School of Public Health, and a Graduate Certificate in Biohazardous Threat Agents and Emerging Infectious Diseases from Georgetown University.

Cristin Welle, Ph.D.

Neurophysiologist, Division of Biomedical Physics, Office of Science and Engineering Labs, CDRH, FDA

As the principal investigator of FDA's Neural Implant Lab in the Division of Biomedical Physics in the Center for Devices and Radiological Health, Dr. Cristin Welle directs a team of scientists in the development of test platforms to evaluate the long-term safety and reliability of neural interface devices in small animal model systems. The lab investigates invasive neural recording electrodes used in neuroprosthetic systems and novel electrode technology for the detection of traumatic brain injury. The goal is to contribute to the scientific knowledge base required to speed innovative neural device development and regulatory review. The Neural Implant Lab receives funding from the DARPA RE-NET and HAPTIX Programs and FDA's Medical Countermeasure and Critical Path initiatives and collaborates with government, academic, and industry researchers. Dr. Welle received her Ph.D. in Neuroscience from the University of Pennsylvania in 2010. In addition to her current research efforts, she provides subject matter expert consulting reviews for neurological device submissions to the Office of Device Evaluation and participates in internal and external efforts to facilitate the regulatory process for neural technology, including working groups, conference planning committees, and peer-review panels for the VA, DARPA and FDA. She served as a project lead for FDA Public Workshop on Brain-Computer Interface (BCI) Devices for Patients with Paralysis and Amputation held in November 2014.

Surender Khurana, Ph.D.

Staff Scientist, Office of Vaccines Research and Review, CBER, FDA

Dr. Surender Khurana began his post-doctoral research in biochemistry studying the molecular mechanisms of antibody responses generated by HIV infection. In 2002, he came to FDA, joining the Laboratory of Retrovirus Research at CBER. There, he developed the first assay for differential diagnosis of true HIV infections in the face vaccine-generated antibodies. This assay (HIV-SELECTEST) addressed the problem of vaccine-induced sero-positivity during clinical trials.

Following an NIH Request for Proposal, the assay was further developed and validated, after which it was licensed by FDA and implemented in HIV vaccine trials.

Since 2007, Dr. Khurana has worked in the Division of Viral Products at FDA. In response to H5N1 and potential influenza pandemics, he worked to better understand how vaccine adjuvants and vaccine modalities improve immunity against influenza.

Dr. Khurana developed the novel scientific technologies of whole genome phage display libraries and the measurement of antibody affinity maturation in polyclonal human sera following

vaccination or infection, using surface plasmon resonance. Based on this work, he developed a novel serodiagnostic assay to detect the H5N1 influenza virus that could be used for surveillance.

Building on his experience during the 2009 H1N1 (swine flu) pandemic, he has been developing improved assays for measuring the potency of influenza vaccines. Recently, he has started a new research program on elucidating humoral immune responses and pathogenesis generated following respiratory syncytia virus (RSV) and ebola infection that can help to develop RSV and Ebola vaccines. His extensive research on HIV and influenza has won him several awards, honors, and recognition worldwide.

Steven Wood, Ph.D.

Biologist, Office of Science and Engineering Laboratories, CDRH, FDA

Dr. Steven Wood has established a biodefense research program that incorporates the broader scope of FDA's public health mission and bioterrorism mandates. Specifically, he is interested in the rapid detection of FV and threat mitigation. He has a Ph.D. from the University of Connecticut and received post-doctoral training at the Medical College of Virginia and New York University. He joined CBER and then moved to CDRH. In parallel, Dr. Wood developed a research program that is focused on the interplay of immunology/vascular biology and medical devices. He is also an inter-agency oncology task force mentor. Dr. Wood also provides biocompatibility consults for cardiovascular and coagulation devices.

Session 4: Implement a New Prevention-Focused Food Safety System to Protect Public Health

Palmer Orlandi Jr., Session Co-Chair

Acting Chief Science Officer and Research Director, OFVM, FDA

Dr. Palmer Orlandi received an A.B. in chemistry from Lafayette College and a Ph.D. in biochemistry from the University of Kentucky. He joined FDA in 1997 after his postdoctoral research training at the Walter Reed Army Institute of Research and the National Institutes of Health. After 11 years as a principal investigator at FDA's Center for Food Safety and Applied Nutrition he joined the Division of Field Science in the Office of Regulatory Affairs as a science coordinator where he developed collaborative analytical methods programs for FDA field labs and the Food Emergency Response Network (FERN). From 2012 to 2015, he was the senior science advisor to the chief science officer in FDA's Office of Food and Veterinary Medicine. In this capacity, Dr. Orlandi served to integrate science and research efforts across all elements of FDA's foods program. He also ensured that research and laboratory programs were aligned with the needs of regulatory field labs as they supporeted the agency's evolving food safety mission. He is currently the acting Chief Science Officer and Research Director in the Office of Foods and Veterinary Medicine. He received a commission as an officer in the US Army in 1981 and since 1991 has been an officer in the Commission Corps of the Public Health Service.

David G. White, M.S., Ph.D., Session Co-Chair

Acting Director of the Office of Resource Planning and Strategic Management, OFVM, FDA

Dr. David White is the Director of the Office of Resource Planning and Strategic Management in FDA Office of Foods and Veterinary Medicine. His previous positions at FDA include acting Chief Science Officer / Research Director in FDA Office of Foods and Veterinary Medicine, Director of the Office of Research at FDA's Center for Veterinary Medicine, Director of the Division of Animal and Food Microbiology and Program Director of FDA National Antimicrobial Resistance Monitoring System headquartered in Laurel, MD. Before his position at FDA, Dr. White was an assistant professor in the Department of Veterinary and Microbiological Sciences at North Dakota State University. During that time he was also section chief of Diagnostic Microbiology at the North Dakota Veterinary Diagnostic Laboratory.

Dr. White is a past member of the sub-committee of Veterinary Antimicrobial Susceptibility Testing, CLSI and the Ad hoc group on Antimicrobial Resistance, Office International des Epizooties, Paris, France and a founding member of the Reservoirs of Antibiotic Resistance (ROAR). He also served on several USDA extramural and intramural research panels, including the USDA office of science quality review panel, National Program 108 Action Plan on Food Safety as well as on review panels for other agencies, including the National Institutes of Health, Centers for Disease Control and Prevention, USDA National Research Initiative Competitive Grants Program, and Department of Defense. Dr. White is an editor of the 2005 book *Frontiers in Antibiotic Resistance*, ASM Press, Washington, D.C. and is on the editorial board of Foodborne Pathogens and Disease and Antimicrobial Agents and Chemotherapy. He previously served as co-chair of both FDA Antimicrobial Resistance Steering Committee, U.S. Interagency Task Force on Antimicrobial Resistance and the re-established OIE Ad Hoc Group on Antimicrobial Resistance. Most recently he served as the U.S Delegate to the Codex Ad Hoc Intergovernmental Task Force on Antimicrobial Resistance.

Dr. White received his bachelor's degree from the University of Vermont, his Master of Science from the University of Kentucky, and his Ph.D. from the Pennsylvania State University. He also was a post-doctoral fellow in the Center for Adaptation Genetics and Drug Resistance at Tufts University School of Medicine.

Ruth E. Timme, Ph.D.

Research Microbiologist, Molecular Methods and Subtyping Branch, CFSAN, FDA

Dr. Ruth Timme is a Research Microbiologist at FDA's Office of Regulatory Science. She received her Ph.D. in 2006 in plant biology at The University

of Texas at Austin. Her research background focuses mainly on utilizing comparative genomics and phylogenetics methods to answer evolutionary questions. Although her training is in botany, her published research spans a diversity of organisms, including sunflowers (Helianthus), Dinoflagellates, Charophyte green algae, and Salmonella. At FDA she coordinates the dataflow for GenomeTrakr and implements phylogenomic methods for tracking foodborne pathogens through the U.S. food supply.

Patrick McDermott, M.S., Ph.D.

Director, National Antimicrobial Resistance Monitoring System (NARMS), CVM, FDA

Dr. Patrick McDermott is Director of the U.S National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria at FDA. He is a microbiologist by training, who has conducted research on antibiotic resistance for over 20 years. He represents FDA on the U.S. government's Interagency Task Force on Antimicrobial Resistance and on the Transatlantic Task Force on Antimicrobial Resistance. He is a member of the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) and the WHO Global Foodborne Infections Network (GFN).

Jonathan Deeds, Ph.D.

Research Biologist, Office of Regulatory Science, CFSAN, FDA

Dr. Jonathan Deeds is a research biologist in FDA's Center for Food Safety and Applied Nutrition (CFSAN) Office of Regulatory Science, where he is a research coordinator and subject matter expert in the areas of seafood safety and labeling. Dr. Deeds holds degrees in Biology from the University of Dayton (B.S., 1995), in Environmental Toxicology from the University of Louisiana at Lafayette (M.Sc., 1997) and in Marine Estuarine and Environmental Science from the University of Maryland (Ph.D., 2003). He began his FDA career in 2003 in the old Office of Seafood researching the sources and fate of natural toxins that accumulate in commercial seafood to develop better management controls. He currently leads or participates in several research projects for the development of methods for the detection of marine biotoxins in various fish and shellfish products to prevent consumer illness and for the implementation of updated methods for the species identification of FDA regulated seafood products. For this educational activity, he will be discussing the development and use of state-of-the-art DNA sequencing methodology now being used by the Agency to identify seafood products involved in outbreaks of illness, to develop and refine species-specific hazards controls, and to confirm product labeling to detect seafood substitution and fraud.

Timothy Croley, Ph.D.

Supervisory Chemist, CFSAN, FDA

Dr. Tim Croley is the Chief of the Spectroscopy and Mass Spectrometry Branch at FDA's Center for Food Safety and Applied Nutrition. Research in the branch is focused on new technologies and their application to food safety and food defense. Some specific applications include allergens, gluten, economically motivated adulteration, bacterial identification and software solutions for high resolution mass spectrometric screening. Tim's research interests are in the application of mass spectrometry to non-targeted screening, intact protein mass spectrometry to characterize bacteria, protein toxins, and development of novel extraction methods in complex matrices. Tim received his bachelor's degree in Chemistry with a minor in Biology from the University of Kentucky, a Ph.D. in Analytical Chemistry from Mississippi State University, and was a Post-doctoral Fellow at Trent University in Peterborough, Ontario.

Keynote Introduction

Carol D. Linden, Ph.D.

Director, Office of Regulatory Science and Innovation, OC, FDA

Dr. Carol Linden is the Director of the Office of Regulatory Science and Innovation in the Office of the Chief Scientist, Office of the Commissioner, FDA. She oversees a broad array of both intramural and extramural programs focused on bringing understanding of the latest in scientific and technological advances to the process of regulating products that support the health of the American public. Before assuming this position, Dr. Linden was the Principal Deputy Director of the Office of the Biomedical Advanced Research and Development Authority (BARDA) in the Office

of the Assistant Secretary for Preparedness and Response, Department of Health and Human Services. Her duties included oversight of advanced development and acquisition programs for Project BioShield medical countermeasures for CBRN threats as well as pandemic influenza vaccines, drugs, diagnostics and infrastructure. From October 2006 through April 2008 she also served as the Acting Director of BARDA, responsible for a doubling in the size of the office and implementation of the legislation that established the office.

In 2009, Dr. Linden co-chaired with the Department of Defense the Working Group on Strengthening the Biosecurity of the United States, which was mandated by an Executive Order, and produced a report with recommendations submitted to the White House. She also played an active role in the Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight, which made recommendations to support improvements in eight areas. More recently, she provided expertise to the Federal Experts Security Advisory Panel under the Executive Order "Optimizing the Security of Biological Select Agents and Toxins in the United States, and co-chaired a 2014 working group under the FESAP to review opportunities for improvements to the Select Agent Program.

Dr. Linden previously served as the Senior Scientist for the Office of Research and Development in the Science and Technology Directorate of the Department of Homeland Security, overseeing treaty and regulatory compliance as well as international collaborations. Immediately before this position, she served as Deputy Director of the Office of Research Programs. Before joining the Department of Homeland Security, Dr. Linden was the Scientific Director for the Defense Threat Reduction Agency (DTRA) Chemical and Biological Defense Directorate from mid-2003 until spring of 2004. Before her detail to DTRA, she served as the Director for the Department of Defense Medical Chemical and Biological Defense Research Programs for over 3 years, managing all aspects of the joint services medical Chemical and Biological Defense Program. Dr. Linden served a critical function in coordinating the working relationship between the technology base and advanced development, facilitating the transition of candidate vaccines, diagnostic technologies and therapies to the developer.

Dr. Linden obtained her bachelor's degree in biology from Bryn Mawr College, and a Ph.D. from the University of California Los Angeles in molecular biology. She conducted postdoctoral research at the California Institute of Technology and University of Maryland before joining the research staff at the U.S. Army Medical Research Institute of Infectious Diseases, where she subsequently served as the Chief, Research Plans and Programs.

Session 5: Support New Approaches to Improve Product Manufacturing and Quality

Sau (Larry) Lee, Ph.D., Session Co-Chair

Acting Associate Director for Science, Team Leader for OPQ Botanical Review Team, Office of Pharmaceutical Quality, CDER, FDA

Dr. Sau (Larry) Lee is the acting Associate Director for Science within the Office of Pharmaceutical Quality (OPQ) and the acting Team Leader of the OPQ Botanical Review Team. Dr. Lee and the OPQ Scientific and Research Staff are leading the effort in advancing OPQ research in manufacturing science, complex drug substances and products containing nanomaterials; as well as developing the regulatory policy and scientific standards that include computational and modeling tools that support quality review and inspection in OPQ. Dr. Lee joined the Office of Generic Drugs (OGD) in 2005 as a chemical engineer. In 2012, he was promoted to team lead of the peptide review group. This group specialized in Chemistry, Manufacturing, and Controls (CMC) reviews of Abbreviated New Drug Applications (ANDAs) for complex drug substances and products. During this time, he also led a team to evaluate OPQ's vision for a team-based product and process/ facility quality assessment approach for the Office of Pharmaceutical Quality (OPQ) TAG Integrated Team-based Review Pilot. He also co-led the Risk Based Review Pilot, which aimed to increase the review quality and efficiency of injectable products. In early 2013, he was promoted to Expert Regulatory Scientist in recognition of his expertise in evaluation of complex drug substances and products. Dr. Lee received a B.S. degree in Chemical Engineering from the University of Virginia with a minor in Materials Science and a Ph.D. in Chemical Engineering from Princeton University.

Carolyn Wilson, Ph.D., Session Co-Chair

Associate Director for Research, CBER, FDA

Dr. Carolyn Wilson is the Associate Director for Research at CBER, FDA. As ADR, Dr. Wilson ensures that CBER's research is relevant, high quality and provides CBER with the appropriate scientific expertise, tools, and data to support regulatory decision-making and policy development. Dr. Wilson's responsibilities include leading FDA's Genomics Working Group and CBER's Medical Counter-Measure Regulatory Science Initiative. Dr. Wilson maintains her laboratory program studying retroviruses that are either used as vectors for gene therapy clinical trials or are of concern in the xenotransplantation setting. Dr. Wilson joined the Division of Cellular and Gene Therapies at the Center for Biologics Evaluation and Research of FDA in 1993. As a researcher-reviewer in DCGT, she reviewed INDs and developed policy and guidance documents in two novel product areas: gene therapy and xenotransplantation. Dr. Wilson holds a Ph.D. in Genetics from The George Washington University.

Nicola Ranieri

Research Biologist, Microscopy and Image Analysis, Trace Examination Section, ORA, FDA

In 1989, Mr. Nicola Ranieri joined FDA's Forensic Chemistry Center (FCC). With over 25 years in the agency, he has made many technical contributions to the FCC laboratory and FDA. He began his career working with computers in developing and implementing conversions of conventional to digital imaging capabilities for the FCC Laboratory. He developed, designed, and implemented computerized 2D image analysis (IA) methods for particle sizing, counting with statistical results, and surface profilimetry instrumentation for the detection of counterfeit product packaging, pharmaceutical tablet analysis, and in for classification of puncture holes in plastic bottles and on "rubber stoppers" used to assist in numerous felony product tampering cases. He is an expert in toolmark analysis of characterization of pharmaceutical tablets, and punctures in plastic and rubber pharmaceutical stoppers and in food/beverage products. He introduced print identification technology to the FCC laboratory, FDA CSO's and OCI Special Agents, and to multiple international forensic FDA partners. Mr. Ranieri established, implemented and still maintains today "imaging records" of Video-Time- Lapse time studies of various products which are still used today for consumer complaint cases. During

this time he also designed and is the principal key person in the development and implementation of a photo documentation image library system the Photo Documentation System (PDS).

The PDS is used at FDA's International Mail Facilities (IMF) and OCI Headquarter. In the last decade, he has been the principal FCC and FDA researcher and analyst in the detection of counterfeit pharmaceuticals using 2D and 3D IA, print process identification, and CDx technology. His creativity, imagination, and inventive ability have been crucial in the development and widespread implementation of the CDx technology. The hand-held device was identified and ranked as the Secretary of Health and Human Services's (HHS) top pick for scientific achievement in the 2013 HHS Innovates Award. He has patent submissions and is the lead inventor for five versions of CDx technology (CD1, CD2, CD3, CD3+, and CD4). As a DHRD Cadre member he is the leading contributor in FDA's course IM214 for the use of the CDx. Mr. Ranieri has been an integral part of FDA's hand-held technology transfer between FDA and Corning Inc. His contributions have had a profound impact to the the FCC laboratory, FDA, and within the last two years have reached the World Health Organization (WHO), a global level with a profound impact on identification of counterfeit anti-malarial drugs. He is the FCC Trace Examination Section (TES) imaging authority. His work has been on forensic samples related to product tampering, counterfeiting, diversion, fraud and counter-terrorism. He is one of FCC experts for forensic analysis by electron microscopy and is FCC's only Italian translator (fluent in Italian).

Lauren Jackson, Ph.D.

Chief, Process Engineering Branch, Division of Processing Science & Technology, Office of Food Safety, CFSAN, FDA

Dr. Lauren Jackson is Chief of the Process Engineering Branch at the Food and Drug Administration (FDA)/Division of Food Processing Science and Technology (DFPST), located in Bedford Park, IL. This division of FDA/CFSAN is part of the research consortium and FDA Center of Excellence, the National Center for Food Safety and Technology (NCFST). Dr. Jackson received her B.S. in Food Science from Cornell University and her M.S. and Ph.D., both in Food Science, from the University of Wisconsin-Madison. Her expertise is: the effects of processing on food constituents and contaminants, food allergen control, the stability of biothreat agents, and the analysis and detection of chemical contaminants and constituents in food. Her main focus has been on understanding the effects of processing on the formation and destruction of natural toxins in food. She also is one of FDA's subject matter experts on cleaning and other measures for controlling allergens in food manufacturing facilities. Dr. Jackson is actively involved in several committees for the Institute of Food Technologists (IFT) and the American Chemical Society (ACS), and is a member of both societies as well as International Association for Food Protection (IAFP). She is a Scientific Editor for the Journal of Food Science and the Journal of Food Protection.

Darón Freedberg, Ph.D.

Senior Scientist, CBER, FDA

Dr. Darón Freedberg received his Bachelor's degree in Chemistry from the University of California, San Diego in 1990. He earned his Ph.D. under the mentorship of Professors Frank A.L. Anet and Craig A. Merlic in Organic Chemistry at the University of California, Los Angeles in 1994, where he used NMR spectroscopy to study deuterium isotope effects in conformational analysis and also on the NMR detection of helium-3 in buckeyballs. He was then a post-doctoral fellow with Dr. Dennis A. Torchia at the NIH working on NMR spectroscopy of HIV protease-inhibitor complexes. Throughout his career, Dr. Freedberg always had an interest in structures of glycans. This was a natural fit for FDA, where he now works on using NMR to better understand glycan structure-function relationships..

Session 6: Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Lisa M. LaVange, Ph.D., Session Chair

Director, Office of Biostatistics, Office of Translational Sciences, CDER, FDA

Dr. Lisa LaVange is Director of the Office of Biostatistics in the Office of Translational Sciences, Center for Drug Evaluation and Research (CDER), US Food and Drug Administration (FDA). As Director, she oversees approximately 180 statistical reviewers and staff members involved in the development and application of statistical methodology for drug regulation. She is a member of the PDUFA V steering committee and serves on the CDER Antibacterial Drug Development Task Force. Before joining FDA, Dr. LaVange was Professor and Director of the Collaborative Studies Coordinating Center (CSCC) in the Department of Biostatistics, Gillings School of Global Public Health at the University of North Carolina at Chapel Hill (UNC), where she served as Principal Investigator (PI) of the coordinating centers for several large-scale multi-center clinical trials, epidemiology studies, and patient registries. Before joining academia, Dr. LaVange spent 10 years in the pharmaceutical industry and 16 years in non-profit research. She is a Fellow of the American Statistical Association, served as President of the Eastern North American Region of the International Biometric Society (IBS; 2007), and served on the IBS Executive Board (2013-2014). She was formerly co-editor of the Journal of Pharmaceutical Statistics and editor-in-chief of the ASA-SIAM book series.

Zuben E. Sauna, Ph.D.

Senior Staff Fellow, CBER, FDA

Dr. Zuben Sauna has been a research reviewer with the US Food and Drug Administration since 2009. He is associated with the Department of Hematology where he is involved with the review of plasma-derived and recombinant coagulation proteins that are used as therapeutics. Dr. Sauna also leads a research laboratory that focuses on the next generation of therapeutic proteins and in pharmacogenomics. A key focus of his research activities has been to understand the pharmacogenetic basis of the immune response to protein therapeutics, which can significantly affect the efficacy and safety of these drugs. His laboratory uses the coagulation Factor VIII as a model and exploits a combination of computational, in vitro and ex vivo approaches to understand why some individuals and/or sub-populations develop inhibitory antibodies while others do not. Dr. Sauna has over 50 research papers in high-impact journals such as Nature Biotechnology, Nature Medicine and Nature Reviews Genetics to his credit. He received his Ph.D. from Poona University, India, with subsequent training at the National Cancer Institute, Bethesda, MD.

Michael Pacanowski, Pharm.D., M.P.H.

Associate Director for Genomics and Targeted Therapy, Office of Clinical Pharmacology, CDER, FDA

Dr. Michael Pacanowski is the Associate Director for Genomics and Targeted Therapy in the Office of Clinical Pharmacology at FDA. His team of translational scientists is charged with advancing the use of genomic and other biomarker innovations to maximize individualization in drug development. To that end, Dr. Pacanowski oversees a program focused on reviewing investigational new drugs, developing policies and processes, engaging stakeholders, and conducting regulatory science research. Dr. Pacanowski received his Pharm.D. from the Philadelphia College of Pharmacy and his M.P.H. from the University of Florida. He completed a residency in clinical pharmacology at Bassett Healthcare in Cooperstown, NY, and a clinical research fellowship in cardiovascular pharmacogenomics at the University of Florida.

Gene Pennello, Ph.D.

Team Leader and Mathematical Statistician (Biomedical), Division of Biostatistics, CDRH, FDA

Dr. Gene Pennello is a Team Leader and Expert Mathematical Statistician in Diagnostics Statistics Branch II of the Division of Biostatistics, Center for Devices and Radiological Health, FDA. He has been with the Agency since 1998. Before joining FDA, Dr. Pennello was a postdoctoral training fellow at the National Cancer Institute, Division of Cancer Epidemiology and Genetics. He has a Ph.D. in Statistics from Oregon State University (1993), a Master's degree in Statistics from the University of California at Davis, and two Bachelor of Science degrees also from UC Davis, in Statistics and in Computer Science and Mathematics. Dr. Pennello has experience in FDA review of in vitro diagnostic tests, including those that measure biomarkers for prognostic or predictive claims and has published several papers on biomarker evaluation. Some of his statistical interests include Bayesian analysis, multiple comparisons, and missing data

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Session 7: Modernize Toxicology to Enhance Product Safety

Donna Mendrick, Ph.D., Session Co-Chair

Associate Director for Regulatory Activities, NCTR, FDA

Dr. Donna Mendrick is the Associate Director of Regulatory Activities at the National Center for Toxicological Research (NCTR). Before this she was the Director of the Division of Systems Biology at NCTR. Dr. Mendrick's FDA committee assignments include the Senior Science Council, Tox21, and ICCVAM. She is FDA's ad hoc member of the Advisory Committee to the National Institutes of Environmental Sciences. Dr. Mendrick was an Assistant Professor of Pathology at Harvard Medical School and Brigham and Women's Hospital. She then joined Human Genome Sciences and led multiple project teams, toxicity studies, pharmacology studies, etc. Just before joining FDA, she was a Scientific Fellow and Vice President of Pharmacogenomics at Gene Logic where she oversaw pharmacogenomics and spearheaded its toxicogenomics effort. Dr. Mendrick has many years of experience in the fields (in alphabetical order) of histology, immunology, pathology, pharmacogenomics, pharmacology, toxicology and toxicogenomics employing small molecule drugs, recombinant therapeutic proteins and monoclonal antibodies. She is past President of the National Capital Area Chapter of the Society of Toxicology and currently is the Chair of the Contemporary Concepts in Toxicology (CCT) committee, a member of the Scientific Liaison's Governance Committee and a member of the planning committee for the Future Tox III CCT meeting.

James Weaver, Ph.D., Session Co-Chair

Research Pharmacologist, CDER, FDA

Dr. James Weaver obtained B.A. and M.S. degrees from the University of Vermont and a Ph.D. from the Department of Microbiology & Immunology, University of Louisville in 1987. After a postdoctoral position in the Department of Biochemistry at Uniformed Services University of the Health Sciences, he joined the Center for Drug Evaluation & Research, FDA as a staff fellow in 1989 and became a Research Pharmacologist in 1994. He is currently doing biomarker and immunotoxicology research in the Division of Applied Regulatory Science. Publication areas include: the role of complement in streptococcal arthritis, Raman spectroscopy of protein secondary structure, surface acting anti-HIV agents, biology of multidrug resistance, evaluation of transgenic mouse models for carcinogenicity testing, vascular injury by phosphodiesterase inhibitors, flow cytometry methods, and in vivo and in silico immunotoxicology.

Richard D. Beger, Ph.D.

Branch Chief, Biomarkers and Alternative Models Branch, NCTR, FDA

Dr. Richard Beger is the Branch Chief of Biomarkers and Alternative Models Branch at NCTR. This Branch consists of metabolomics, proteomics and alternative models research teams that focus on discovering and evaluating biomarkers of disease and injury. He received his Ph.D. in theoretical biophysics from Purdue University in 1991 and joined the National Center for Toxicological Research (NCTR), US FDA, in Jefferson, AR in 1998. He has been a member of Society of Toxicology and Metabolomics Society since 2004 and is an author of over 115 publications and 7 book chapters. After arriving at NCTR, he initiated research activities using NMR-based and MS-based metabolomics methods to identify non-invasive and tissue-based metabolic biomarkers of drug toxicity, drug efficacy, disease status, and precision medicine.

Serguei Liachenko, M.D., Ph.D.

Director of Bioimaging, NCTR, FDA

Dr. Serguei Liachenko is Director of Bioimaging in the Division of Neurotoxicology, Office of Research, National Center for Toxicological Research. His current research involves nonclinical bio-imaging in pharmaceutical industry and government settings focusing on magnetic resonance to investigate the efficacy and toxicity imaging biomarkers in the areas of neuroscience, inflammation and cardiovascular medicine to support drug discovery and development. He studied biochemistry at the Russian State Medical University and pharmacology at the National Center of Biologically Active Compound Safety in Russia.

Norman Stockbridge, M.D., Ph.D.

Division Director, Division of Cardiovascular and Renal Products, Office of New Drugs, CDER, FDA

Dr. Norman Stockbridge received his M.D. and PhD (physiology) from Duke University. He did basic science research before joining the Division of Cardiovascular and Renal Products in 1991. He has served as the Division Director since 2004.

Kristina E. Howard, D.V.M., Ph.D.

Research Veterinary Medical Officer, CDER, FDA

Dr. Kristina Howard received her veterinary degree from the Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA. She completed her doctoral training in Immunology at North Carolina State University, Raleigh, NC, where she was appointed to faculty in 2004. Dr. Howard has worked with a wide variety of animal models in research focused on immunotoxicity, viral pathogenesis and vaccine development. Since joining FDA in 2010, she has used immune humanized mice to assess the safety of biological drug products. Her research is focused on evaluating the ability of humanized mouse models to better predict the safety of small and large molecule drug products in humans

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Session 8: Harness Diverse Data through Information Sciences to Improve Health Outcomes

Eric Donaldson, Ph.D., Session Co-Chair

Clinical Virology Reviewer, Division of Antiviral Products, CDER, FDA

Dr. Eric Donaldson received his B.S. degree in Microbiology and Immunology from Montana State University where he used computational biology to identify and characterize endogenous retroviruses in the human genome. He received his Ph.D. in Microbiology and Immunology from University of North Carolina where he used computational biology and structural bioinformatics to model and predict coronavirus and norovirus evolution, and to inform empirical testing in the lab. Dr. Donaldson gained expertise in Next Generation Sequencing (NGS) as a member of the faculty in the Department of Epidemiology at the University of North Carolina where he used this technology to identify novel coronaviruses in bats, to study the evolution of norovirus infection in immunocompromised patients, to sequence a simian hemorrhagic fever virus genome, to identify novel hemorrhagic viruses in baboons, and to identify pathogens present in fatal human respiratory infections of unknown etiology. This work allowed Dr. Donaldson to gain experience with the entire process of obtaining sequence samples, preparing them for sequencing using multiple next generation sequencing platforms, and analyzing the complex data sets generated from this emerging technology. Dr. Donaldson has led the effort in developing a NGS analysis pipeline and review procedures for analyzing antiviral drug resistance data for the Division of Antiviral Products. He currently serves as a CDER representative on FDA Genomics Working Group and is chairing a subcommittee that is developing a Best Practices document that will provide information on the best practices for submitting NGS data to the Agency. Dr. Donaldson has over 50 publications in peer-reviewed journals.

Roger Perkins, M.S., Session Co-Chair

Senior Advisor, Division of Bioinformatics and Biostatistics, NCTR, FDA

Roger Perkins is the Senior Advisor in the Division of Bioinformatics and Biostatistics at the U.S. Food and Drug Administration's (FDA) National Center for Toxicological Research (NCTR). During his 20+ years at NCTR, he has co-authored some 90 manuscripts involving computational science and predictive modeling for toxicological endpoints and biomarker discovery. Past research areas include development of chemometric and QSAR predictive models for endpoints associated with endocrine activity, development of phenotype-anchored knowledge bases, development of integrative databases used for high throughput data interpretation, and determination of best practices for predictive model development from microarray and next generation sequencing data. During the last two years, he has been involved in a number of research projects employing Bayesian latent variable methods such as topic modeling for data modeling and information retrieval, including the development of software for use of the methods in a regulatory environment. He also serves on several FDA governance boards and committees involving IT, scientific computing and high performance computing. He is currently a board member of the Midsouth Computational Biology and Bioinformatics Society and a member of the Arkansas Bioinformatics Consortium's Steering Committee. Before joining FDA, he was Director of the U.S. Navy's Advanced Scientific and Engineering Supercomputer Center, and before that a program manager and nuclear codes specialist at the National Science Foundation's San Diego Supercomputer Center. He was trained as a nuclear engineer at the University of Florida and spent more than a decade as a reactor physicist in fast breeder reactor and fusion reactor research and development.

Carolyn Wilson, Ph.D., Panelist

Associate Director for Research, CBER, FDA

Dr. Carolyn Wilson is the Associate Director for Research at CBER, FDA. As ADR, Dr. Wilson ensures that CBER's research is relevant, high quality and provides CBER with the appropriate scientific expertise, tools, and data to support regulatory decision-making and policy development. Dr. Wilson's responsibilities include leading FDA's Genomics Working Group and CBER's Medical Counter-Measure Regulatory Science Initiative. Dr. Wilson maintains her laboratory program studying retroviruses that are either used as vectors for gene therapy clinical trials or are of concern in the xenotransplantation setting. Dr. Wilson joined the Division of Cellular and Gene Therapies at the Center for Biologics Evaluation and Research of FDA in 1993. As a researcher-reviewer in DCGT, she reviewed INDs and developed policy and guidance documents in two novel product areas: gene therapy and xenotransplantation. Dr. Wilson holds a Ph.D. in genetics from The George Washington University.

Hugh A. Rand, Ph.D., Panelist

Bioinformatics Team Lead, CFSAN, FDA

Dr. Hugh Rand has a B.S. in chemistry and a Ph.D. in applied mathematics. After completing his formal education, Dr. Rand spent 15 years in the biotech industry working for Immunex and Amgen. He worked on a wide variety of applied bioinformatics problems in the area of immunology and oncology, with problems ranging from searching for immunomodulatory proteins in viral genomes to assessing the likelihood of detecting patients with depleted T-cell subsets in phase II clinical trials. In 2013 he joined FDA-CFSAN as the team leader for the bioinformatics group charged with supporting Next Generation Sequencing (NGS) for FDA-CFSAN. This team provides NGS analysis of the food-borne pathogen samples that FDA receives, and ensures that the NGS data is submitted to the NCBI. In concert with the Division of Microbiology at FDA-CFSAN, this team has been a key driver and support group for the Genome Trakr project – which uses WGS of cultured pathogens as part of real-time surveillance for the rapid detection and analysis of outbreaks of food-borne illnesses.

Heike Sichtig, Ph.D., Panelist

Principal Investigator/Regulatory Scientist, CDRH, FDA

Dr. Heike Sichtig is a principal investigator and lead technical regulatory scientist in FDA's Office of In Vitro Diagnostics and Radiological Health in the Division of Microbiology Devices. She is leading the highly collaborative effort on developing FDA-ARGOS (formerly MicroDB): FDA dAtabase for Regulatory Grade micrObial Sequences. She joined the Division of Microbiology Devices in 2012 and is primarily focusing on enabling next generation sequencing (NGS) based technologies for clinical diagnostics. She is part of a multidisciplinary team that is developing and implementing the concepts for validation and evaluation of NGS-based microbiological diagnostic devices. Dr. Sichtig obtained a B.S. / M.S. in somputer science/statistics from Kean University in 2002 and 2003, and a Ph.D. in biomedical engineering from Binghamton University in 2009. She did her postdoctoral training at the University of Florida/Genetics Institute in Gainesville FL in pathogen signatures, transcriptional regulation and epigenetics. Dr. Sichtig received the Commissioner's Special Citation award in 2014 and several others related to FDA review work.

Weida Tong, Ph.D., Panelist

Director, Division of Bioinformatics and Biostatistics, NCTR, FDA

Dr. Weida Tong is Director of Division of Bioinformatics and Biostatistics at FDA's National Center for Toxicological Research (USFDA-NCTR). He has served a science advisory board member for several large projects involving multiple institutes in Europe and USA. He also holds several adjunct positions at universities, including Associate Professor at UMDNJ/Rutgers University and UAMS, full professor at UALR and, more recently, guest professor at Zhejiang University. His division at FDA is to develop bioinformatic methodologies and standards to support FDA research and regulation and to advance regulatory science and personalized medicine. The most visible projects from his group are (1) development of FDA bioinformatics system ArrayTrackTM suite, to support FDA review and research on pharmacogenomics; (2) leading the effort on the Microarray Quality Control (MAQC) consortium to develop standards for translational science

and personalized medicine; and (3) development of liver toxicity knowledge base (LTKB) for drug safety; and (4) in silico drug repositioning. In addition, his group also specializes in molecular modeling and QSARs with specific interest in estrogen, androgen, and endocrine disruptor. Dr. Tong has published more than 200 papers and book chapters.

Concurrent Session 1: Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

Joseph M. DeSimone, Ph.D.

Chancellor's Eminent Professor of Chemistry and Chemical Engineering, University of North Carolina at Chapel Hill and North Carolina State University; Co-Founder and CEO, Carbon 3D

Breakthroughs in Imprint Lithography and 3D Additive Fabrication

There is a renaissance underway today in research that is being fueled by the DIY (do-it-yourself) culture that is generally referred to as the "Makers Movement". The maker culture exploits new tools for fabrication and encourages invention and rapid prototyping. Such tools in combination with an innovative mindset will make major impacts in many fields, including in tissue engineering. This lecture will describe breakthroughs in the Makers Movement-including an off-shoot of imprint lithography used to mold individual particles, and a pioneering advance in 3D additive manufacturing that is rapid, continuous and no longer layer-by-layer.

Malcolm Moos, M.D., Ph.D.

Medical Officer, CBER, FDA

Single Cell Methods in Cell Product Characterization

Development of cell-based therapies has been hindered by the difficulty of identifying cellular characteristics that predict in vivo performance reliably, an essential step in developing tests for use in control of the manufacturing process, for release of finished products for clinical use, and meeting other regulatory requirements. Cells are complex analytically, and many products being evaluated for therapeutic potential contain more than one cell type. This difficulty is compounded by the fact that many analytical methods are performed on material from a sample of many cells—so-called "population-average methods"—rather than on single cells. These methods are likely to obscure differences between similar cell types and miss the presence of rare cell types altogether. Since comparatively minor fractions of a heterogeneous cell population may be responsible for significant biological actions, including both therapeutic and deleterious effects, population-average methods may have limited utility in evaluating cell preparations for safety and potential therapeutic effectiveness. This study proposes that concepts from developmental, cell, and systems biology, and control theory, in combination with emerging analytical tools, may offer an approach to analysis of cell populations with discriminating power sufficient to help overcome these obstacles that will be of use not only in developing improved testing strategies, but in design of improved products and manufacturing processes.

Mark Witkowski, Ph.D.

Chemist, Trace Examination Section, Forensic Chemistry Center, ORA, FDA

Field Portable Devices – Taking the Laboratory to the Sample

In the last 10 years, traditional laboratory instrumentation has been reduced in size for the purpose of analyzing samples outside the laboratory. Conversely emerging novel technologies have been developed during this same period with the sole purpose of analyzing samples only in the field. The combining of miniaturized traditional laboratory instrumentation with novel technologies has taken sample field analysis to a new level of sophistication and data quality. This presentation will give an over view of field portable instrumentation, discuss strategies for analyzing samples in the field and present case studies of sample field analysis.

David G. Strauss, M.D., Ph.D.
Medical Officer, CDRH, FDA

The Intersection of Personalized Cardiac Therapies, Cell Based Diagnostics and Multi-Variate Physiological Monitoring

Individual patients respond differently to medical therapies. Personalized medicine uses a diagnostic test to predict which patients will benefit and which are likely to be harmed by therapies. This research is studying personalized medicine for heart devices and drugs to ensure FDA's readiness for multiple innovative, emerging technologies. This talk will cover 3 key areas of FDA research.

1) Predicting which patients will benefit and which are more likely to be harmed from implantable defibrillators and pacemakers. Cardiac resynchronization therapy is a special pacemaker for heart failure patients that reduces mortality, however not all patients benefit and significant risks exists. Through a combination of simulation/modeling, patient-cohort studies, individual-patient meta-analyses of clinical trials and postmarket studies with Medicare and national medical device registry data, the study developed and tested improved diagnostic criteria to predict individual patient benefit and understand sex differences.

2) Studying clinical biomarkers and patient-specific induced pluripotent stem cells for predicting individualized risk of drug-induced arrhythmias. Drug-induced arrhythmias have led to multiple drug withdrawals from the market and risk-stratification for individual patients remains a challenge. Through simulations/modeling, combined analysis of preclinical and clinical trial data submitted to FDA and prospective FDA-sponsored clinical trials, the research is studying novel electrocardiographic biomarkers to better predict risk. In addition, the research is studying the ability of patient-specific induced pluripotent stem cell derived cardiomyocyte diagnostic assays to predict clinical trial results by developing patient-specific cells from 20 subjects in FDA-sponsored clinical trial. The clinical trial will then be reperformed in the laboratory to determine if iPS cells can predict personalized response.

3) Multi-variate physiological monitoring to predict critical clinical events such as hemorrhagic and septic shock, while reducing alarm fatigue. Traditional vital signs and physiological monitors are poor predictors of clinical events and result in a plethora of false alarms, resulting in alarm fatigue, which has been identified as the top medical device hazard. Through a combination of clinical and large animal studies, the research is studying and developing regulatory guidance for next generation multi-variate physiological monitoring devices.

This intramural FDA regulatory science research is pushing the envelope of science and positioning FDA to evaluate emerging technologies.

Concurrent Session 2: Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

Olivia Carter-Pokras, Ph.D.

Associate Professor, Department of Epidemiology and Statistics, University of Maryland School of Public Health

Lessons Learned from Applying Epidemiology, Cultural Competency and Health Literacy Research to Address Health Disparities

There is increasing recognition of the need to pay greater attention to social determinants of health, such as education, health literacy and immigration status to achieve the World Health Organization's definition of health: "complete physical, mental and social well-being." Our nation's health goals (Healthy People 2020) now include the creation of social and physical environments that promote good health for all, as well as the achievement of health equity, elimination of disparities and improvement of health of all groups. Public health and other behavioral and social sciences research are critical to achieving these goals since they help improve our understanding of psychosocial, behavioral, community and societal influences on health in the general population; help predict illness; and help people change behaviors and understand treatments, and manage illness. This presentation will draw upon lessons learned by public health researchers engaged in epidemiologic, cultural competency and health literacy research to address health disparities and related social determinants of health. Cross-cutting lessons and successful elements for synthesizing epidemiologic and other evidence into a rational basis for policy and practice will be discussed. There remains a need for researchers to improve measures, ask policy-relevant questions, and facilitate the translation and dissemination of their research.

Blair Coleman, Ph.D., M.P.H.

Epidemiologist, CTP, FDA

E-Cigarette Use and Cigarette Smoking Behavior among U.S. Young Adults: A Mixed Methods Study

The prevalence of electronic cigarette (e-cigarette) use is rapidly increasing in adults and youth; however, little is known about the public health impact of their use. A debate over e-cigarettes has emerged in the public health literature; one side recognizes the potential benefit of these products as a harm reduction tool, while others argue e-cigarette use may delay or deter smoking cessation due to dual use of these products or increase the risk of initiation of conventional cigarettes or other tobacco products among previous nonsmokers (Lee et al., 2013; Pepper and Brewer, 2013). Drawing on the Theory of Planned Behavior, this research focused on attitudes, beliefs, and perceived social norms of e-cigarettes, as well as openness to conventional cigarette smoking among young adult users of the product. Using a mixed methods approach, a secondary data analysis was conducted using the 2012-2013 National Adult Tobacco Survey (NATS) as well as focus group data collected in five cities across the U.S. To better understand the relationship between e-cigarette use and cigarette smoking among young adults aged 18-29. Quantitative analyses of NATS found non-cigarette smoking young adults who have tried e-cigarettes were more likely to report openness to cigarette smoking in the future compared to those who have not tried e-cigarettes (AOR= 2.4; 95% CI= 1.7-3.3). However, qualitative findings suggest that young adult exclusive e-cigarette users were less interested in conventional cigarette smoking, and overwhelmingly described negative aspects to cigarette smoking that appeared to become more salient as a result of their e-cigarette use. Findings from this research provide additional insight on this complex relationship, as well as attitudes, beliefs, and perceived social norms surrounding e-cigarette use. These findings provide a basis for further exploration of the association between e-cigarette use and conventional cigarette smoking.

Oluwamurewa Oguntimein, M.H.S, C.H.E.S.

Senior Regulatory Management Officer, Social Scientist, Office of Research and Standards, Division of Therapeutic Performance, CDER, FDA

Experimental Study of Patient Information Prototypes

Patients need easy access to up-to-date and accurate information about their medicines to use them safely and effectively. Patients are currently receiving multiple pieces of paper with their prescription drugs from the pharmacy containing information that is developed and distributed through various sources. Written prescription drug information is provided through a voluntary effort, Consumer Medication Information, as well as through FDA mandated use of Medication Guides and Patient Package Inserts. These written documents can be difficult to read and understand, duplicative and overlapping, incomplete or contradictory. Medication Guides reportedly are not being distributed and Consumer Medication Information is not meeting patient needs as determined by the distribution and quality goals of Public Law 104-80; that 75% of patients must receive useful written information about their medication by year 2000 and 95% by year 2006. FDA is considering a new regulation to require all prescription products to have Patient Medication Information, a single document standardized in content and format. The source of information in Patient Medication Information would be FDA approved labeling. To help inform the regulations, FDA created Patient Medication Information prototypes and contracted with RTI International in late 2010 to conduct an experimental study to assess the content and format of these prototypes. This presentation will briefly discuss the experimental study of patient information prototypes including the purpose of the study, description of the prototypes, the study phases, and the results.

Telba Irony, Ph.D.

Chief, General and Surgical Devices Branch, Division of Biostatistics and Office of Device Evaluation, CDRH, FDA

Incorporating Patient Preferences into Regulatory Decision Making

On March 2012, the Center for Devices and Radiological Health at FDA issued a guidance document listing the factors considered when the Center makes Benefit – Risk determinations for approval of medical devices. A ground-breaking factor described in the guidance is “Patient Tolerance for Risk and Perspective on Benefit”. The Center recognizes that considering patient preferences is essential because only patients live with their medical conditions and consequences of the choices they make for their own care. Until now, this factor has not been formally considered in the regulatory setting. Through an example, this research will demonstrate how to obtain quantitative evidence on how patients weigh benefits against risks and how this evidence could be used in regulatory decision making.

Merle Paule, Ph.D., A.T.S.

Director, Division of Neurotoxicology, NCTR, FDA

Recent Findings from Nonhuman Primates on the Long-Term Adverse Behavioral Effects of General Anesthesia When Given During Early Brain Development

Our increasing ability to keep premature infants and compromised neonates alive is resulting in an ever-increasing population in our nation’s neonatal intensive care units. Part of this success lies in the increased number of complicated surgical and other interventions that are brought to bear in this already-at-risk population, many of which are carried out under various forms of anesthesia and/or sedation, often in combination with other therapeutics and often for protracted periods. Concerns over the potential adverse effects of these kinds of exposures prompted the conduct of studies addressing this issue. Initial rodent and other animal data clearly showed that general anesthesia produces neurotoxicity in developing animals in the form of increased neural cell apoptosis (programmed cell death). These findings were replicated in nonhuman primates prompting the need to know if such effects also resulted in altered brain function like that observed in rodent

models. The NCTR Operant Test Battery, a tool consisting of several tasks or games designed to engage specific brain functions including visual discrimination, motivation, learning and short-term memory, was used to assess the effects of early exposure to general anesthetics in rhesus monkeys. The study has now shown that a single 24 hour episode of ketamine-induced or a single 8 hour episode of isoflurane/nitrous oxide-induced general anesthesia leads to deficits in several brain functions including learning, motivation and, in the case of ketamine, concept formation. These data are troubling since performance of these same OTB tasks by children is correlated with IQ, is often indistinguishable from that of monkeys, and is sensitive to clinical entities such as ADHD, depression and anxiety. In addition, the monkey model has proven to be highly predictive of drug effects in humans. The long-term cognitive impairments noted here provide additional evidence that a single episode of general anesthesia occurring during a sensitive period of brain development can result in seemingly permanent deficits in brain function in primates. Supported by CDER/FDA and NCTR/FDA.

Concurrent Session 3: Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security

J. Perren Cobb, M.D., F.C.C.M., F.A.C.S.

Medical Director, Surgical Intensive Care Unit, and Vice-chair, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston Massachusetts

Associate Professor of Anesthesiology and Associate Professor of Surgery, Harvard Medical School, Boston Massachusetts

Enhancing Preparedness Through Novel Partnerships for IT Innovation: USCIIT-PREP

Optimal outcomes in response to public health emergencies require rapid feedback on how well medical countermeasures (MCMs) work to protect and/or treat affected individuals and their families. This information is used by clinicians in the field to guide therapy and by public health agencies responsible for mobilizing the necessary resources at both the regional and national levels. The overarching goal of USCIIT-PREP is to facilitate development of MCM's to protect against threats, specifically, select public health emergencies. The overarching goal of this FDA Program is to enhance preparedness by developing and implementing strategies to assess, evaluate, and monitor medical countermeasure safety, performance, and patient compliance in response to a public health emergency. Influenza was chosen as the prototypic test case as it is one of the most predictable and serious public health threats. Moreover, the communication systems, infrastructure, data analysis and reporting algorithms, and sample collection and processing protocols that USCIIT-PREP develops for seasonal influenza could be applied directly to protect against other threat agents, including pandemic influenza (such as 2009 pH1N1), emerging respiratory viruses (such as H7N9 or MERS-CoV), and other biothreats agents such as inhalational anthrax. This work is also important because USCIIT-PREP uniquely catalyzes communication and builds infrastructure across the care continuum (pre-hospital to rehab, adult and pediatric), linking HHS agencies, academic medical centers, community medical centers, critical illness and injury professional organizations, and industry. Working with FDA, ASPR/BARDA, CDC, and NIH, USCIIT-PREP has developed four specific focus areas to foster innovation and catalyze change:

- Rapid communication during an event that bridges expertise across federal agencies, academic medical centers, community hospitals, public health centers, professional health organizations, and industry
- Standardization of clinical data architecture, vocabularies, and electronic case report forms (eCRF's) consistent with federal regulatory standards
- Prepositioning of consensus clinical protocols for research and human subjects review approval before an event
- Creating North American and international research collaboratives to promote clinical best practice, rigorous research review, and data sharing during emergencies.

In conjunction with HHS partners, USCIIT-PREP will hold short-period incidence studies and national exercises annually to test the feasibility of the eCRF, data gathering and analytical capabilities, and the communication and reporting infrastructure. This is essential to maintain a "warm base" of 20 or more clinical trial sites with pre-positioned protocols that may be nationally exercised to maintain a steady state of preparedness, or be used during an actual public health emergency.

Cristin Welle, Ph.D.

Neurophysiologist, Division of Biomedical Physics, Office of Science and Engineering Labs, CDRH, FDA

Electrophysiological Biomarkers of Brain Injury

Traumatic brain injury (TBI) presents a significant challenge to civilian and military medicine. In the U.S., each year there are over 1.5 million TBIs, resulting in 50,000 deaths. It is estimated that approximately 80% of these injuries are classified as mild TBI (mTBI). In the military, TBI is one of the major causes of mortality and morbidity, with up to 20% of injured Service Members diagnosed with TBI. Despite the prevalence of TBI, and the public health consequences, there are currently

no FDA-approved diagnostics for TBI. This is due to a lack of understanding regarding appropriate biochemical or physiological biomarkers of brain injury. However, recently literature suggests that changes in brain waves, or electroencephalogram (EEG), may indicate TBI. The study has measured changes to brain signals in an animal model of traumatic brain injury. Through the use of a non-invasive, spatially localized brain injury model, quantitative changes in EEG were evaluated that occur at acute and chronic timepoints following the application of injury. This work has identified multiple alterations of EEG characteristics that are associated with injury. The use of EEG as a diagnostic modality opens up new possibilities for the development of novel medical devices. EEG headsets, such as those currently used in commercial gaming systems, could be used as portable, field deployable TBI diagnostic devices. This could allow injured civilians or Service Members to be quickly and accurately screened for a brain injury following exposure to a blast or projectile head wound, greatly advancing the state-of-the-art diagnosis and care of TBI.

Surender Khurana, Ph.D.

Staff Scientist, Office of Vaccines Research and Review, CBER, FDA

Pandemic Influenza preparedness: Development of novel technologies for in-depth evaluation of vaccine efficacy and long-term memory during H7 clinical trials

Rapid response against influenza viruses with pandemic potential requires development of effective vaccines. In the case of H5N1 and H7N9 immune responses to the inactivated vaccines (MIV) were very poor, due to lack of pre-existing immunity. Novel adjuvants including Oil-in-water and cage-like particles were evaluated in human trials and found to significantly increase virus neutralizing titers, heterosubtypic immunity, and afforded dose sparing. Several prime-boost approaches including priming with DNA, LAIV, or replicating Ad4 also resulted in desired seroconversion rates after the MIV boost. New molecular tools have been developed to better understand the humoral responses to influenza vaccines. Using Whole Genome Fragment Phage Display Libraries (GFPDL) and Surface Plasmon Resonance (SPR) technologies it was observed that oil-in-water adjuvants (MF59, AS03) induced epitope spreading from HA2 to HA1 in the hemagglutinin (HA), when compared with unadjuvanted or aluminum-adjuvanted inactivated H5N1 vaccines. Furthermore, a significant increase in the binding avidity of antibodies to properly folded HA1 was measured in SPR, which correlated with broadening of cross clade neutralization. Similarly, increased binding avidity was observed in sera from clinical trial of oral Ad4HAVtn followed by H5N1 MIV boost. In the case of H7N9, virus like particle (VLP) vaccine combined with ISCOMATRIX, the unadjuvanted vaccine elicited low antibody binding to the native HA1 that was focused on the C-terminus, while the ISCO-adjuvanted vaccine induced high binding titers to the HA1 receptor binding domain and significant affinity maturation that correlated with neutralization titers. For prime boost approach in the H7N7 vaccine trials, H7N7 LAIV-primed subjects, but not control subjects, generated strong hemagglutination-inhibiting and neutralizing antibody responses to the H7N7 MIV. The study found that the quantity, epitope diversity, and affinity of H7 head-specific antibodies increased rapidly in only H7N7 LAIV-primed subjects after the MIV. However, all cohorts generated a vigorous, high affinity HA2 stalk-specific antibody response. Consistent increases in circulating memory B cell frequencies after the MIV reflected the specificity of high affinity antibody production. These technologies can be adapted to many other vaccines. They will improve our understanding of the mechanisms of adjuvant activity, and help to select optimal vaccine modality, adjuvant, and vaccination protocols against emerging diseases.

Steven Wood, Ph.D.

Biologist, Office of Science and Engineering Laboratories, CDRH, FDA

Filovirus Detection and Threat Mitigation

Filovirus (FV) has two members that have been identified: Marburgvirus and Ebolavirus and both are lethal. The Ebola outbreak in Africa in 2014 also infected patients and primates in the U.S. Species of Ebola include Zaire, Sudan, Bundabyo, Tai and Reston. FV infections induce a dramatic viremia. FV targets the immune system and survival hinges on outwitting the virus. A concerted defense is mediated by a combination of antibodies, hunger-

killer T cells and immune regulators (cytokines) and all are needed to survive FV infection. Rapid identification of FV is crucial for patient treatment, survival and epidemic management. Next generation, chip based, sequencing (NGS) was applied for identification of FV. Importantly, NGS can quickly identify new viral species as well as antigenic drift of the viruses. For threat mitigation, a model FV vaccine is being used to follow the generation of hunter-killer T-cells, which are crucial to surviving infection. Rapid means of identifying FV and the use of this technology have been developed to unravel the cellular and molecular responses that are altered during the infection. This insight is critical for regulating and evaluating new technologies for FV detection and treatment and can be applied to other viral agents such as coronavirus that causes Middle Eastern Severe Acute Respiratory Syndrome (MERS) that is on the biothreat event horizon. Research is supported by Defense Advanced Research Products Agency (DARPA), Biodefense and Research Development Agency (BARDA), National Institute of Allergy and Infectious Diseases (NIAID), Medical Counter Measures Initiative (MCMI), Office of Women's Health (OWH), CDER, and CDRH. Biodefense and Emerging Infections Reagent Repository of the American Type Culture Collections, G. Kaplan, K Konduru and D. Diamond have supplied reagents.

Concurrent Session 4: Implement a New Prevention-Focused Food Safety System to Protect Public Health

Enrique Pérez-Gutiérrez, D.V.M., M.Sc., M.P.V.M., Ph.D.

Senior Advisor, Foodborne Diseases and Zoonosis, Pan-American Health Organization

Food Safety Systems in the Americas: A perspective from the Pan-American Health Organization (PAHO)

This presentation is based on the institutional experiences for ensuring food safety in the Americas. It will address the situation of the food control systems in Latin America and the Caribbean, using as framework, the international viewpoint developed by FAO/WHO, in particular focusing in public health aspects of the food safety system. The presentation will cover how the countries respond to the challenges in food safety that are tied to the evolution and transformation of food security, agricultural and food industry, international commerce and the demands of consumers, all which necessitate greater efforts to consolidate public policies and institutions that are trustworthy and credible in terms of risk prevention. Countries need to confront the challenge of food safety as a public good based on State decisions and, as such, doing investments on prevention and organizational strengthening of food control systems. It is clear that the level of investments in the food safety system in each country has aspects particular to that country, varying on the types of laws and regulations to the way that the public health, veterinary services and plant health services are organized. It is believed that all countries in the region have developed some minimum core capacities to protect public health by reducing the risk of food borne illness. Also, those capacities are contributing to economic development by maintaining consumer confidence in the food system and for domestic and international trade as well as tourism. Nevertheless, given that the development of the food control systems in the region emerged in response to international commerce or tourism, also a fragmented institutionalization exits, creating double standards (one food safety system for exports and one for the domestic market), which are neither effective nor efficient in solving public health challenges. PAHO is working with Member Countries in developing food safety systems strategically oriented and structured around national food control programs that manag·e food safety without interfering with the development of the agricultural, food industry and commerce. This means, no matter what organizational structure model is used, food control systems must be supported by institutions that can overcome conflicts caused by competing responsibilities, fragmentation of surveillance and lack of coordination; as well capable of filling the gaps pertaining to specialized personnel, availability of resource, technological modernization, and responsibilities in public health protection.

Ruth E. Timme, Ph.D.

Research Microbiologist, Molecular Methods and Subtyping Branch, CFSAN, FDA

GenomeTrakr: A Pathogen Databases to Build a Global Genomic Network for Pathogen Traceback and Outbreak Detection

This study demonstrates how with the selection of proper data quality and use of data filtration techniques one can use desktop NGS sequencer data into a combined analysis. Multiple data analysis pipelines are tested to document the ability of some pipelines to combine draft genomes of bacterial data for phylogenetic clustering to provide leads in outbreak investigations of foodborne pathogens. This study outlines how these tools will be implemented to create a pathogen detection network where state and federal public health agencies can share data to build a publicly available and transparent reference data-base with data deposited into a public genomic database (NCBI). Herein are described the components of the NGS pathogen network that includes studies and current integration among a pilot consisting of state public health laboratories (AK, AZ, FL, HI, MD, MI, MN, NM, NY, VA, SD, WI and WA) as well as federal laboratories. Details of the successes and failures will be provided concerning communication, coordination, data acquisition, assembly, storage, and analysis.

Several recent case studies will be reported on this initial pilot study. The hardware and software implemented allows us to compare and cluster complete genomes of thousands of taxa at a time, and the software outputs daily phylogenetic trees for source tracking of food and environmental isolates. Herein are reported enhanced molecular epidemiological insights gained by comparative analysis of Salmonella, E. coli, and Listeria genomes previously deemed indistinguishable by conventional subtyping methodologies. These results demonstrate an important investigative role for WGS tools within a regulatory environment while highlighting the novel additional insights provided to epidemiological investigations through comparison to a reference database. To read more about FDA's Salmonella, E. coli, and Listeria genomics efforts see: GenomeTrakr network where >10,000 unpublished draft genomes for food safety into the SRA database have been released.

Patrick McDermott, M.S., Ph.D.

Director, The National Antimicrobial Resistance Monitoring System (NARMS), CVM, FDA

The Nexus of Food Safety, Animal Health and Antimicrobial Resistance

The use of antimicrobial agents in food animals can select for resistant bacterial pathogens that may be transmitted to humans via the commercial meat supply. To track this resistance, FDA coordinates the National Antimicrobial Resistance Monitoring System (NARMS). NARMS is a partnership between FDA, the Centers for Disease Control and Prevention, and the USDA, which collects information on antibiotic resistance in food borne bacteria from retail meats, human clinical cases and food producing animals, respectively. Isolates from each source are characterized and compared to understand and describe the movement of resistant bacteria and their genes from the production environments where antibiotics are commonly used, through the food supply to human infections. NARMS has evolved and expanded over the years to incorporate new sampling designs and analytical methods. The advent of affordable whole genome sequencing technologies will provide unprecedented details on the constellation of resistance genes in food borne organisms, and the strain types harboring them. This will provide more definitive information about emerging bacterial resistances, and the impact of interventions designed to limit or prevent resistance spread. NARMS data also are used to evaluate the risks associated with new animal antibiotics to help ensure that antimicrobials remain effective for protecting human and animal health. In this way, resistance surveillance at FDA is a key component of prevention-based food safety policies for protecting public health.

Jonathan Deeds, Ph.D.

Research Biologist, Office of Regulatory Science, CFSAN, FDA

The Use of DNA Barcoding to Prevent Species-Specific Foodborne Illness and Detect Seafood Fraud

In 1914 the agency that would later become FDA received an inquiry if dyed whitefish eggs could be labeled as caviar, leading to one of the Agency's earliest policies on seafood labeling. FDA has worked since its inception to provide consistent and scientifically sound recommendations about acceptable market names for seafood. Seafood labeling is required to be truthful and not misleading. Truthful labeling requires identifying seafood species using an acceptable market name. FDA provides guidance to industry on the development and use of acceptable market names for seafood sold in interstate commerce. Incorrect use of an established acceptable market name that results in the labeling being false and/or misleading can result in the product being misbranded. In addition, due to its incredible diversity, the control strategies for hazards associated with various seafood products are species-specific. Correct labeling for species is essential to the proper implementation of FDA's Hazard Analysis Critical Control Point (HACCP) regulation. In recent years there have been numerous reports of seafood in the U.S. being labeled with an incorrect market name which has had negative impacts both on the seafood industry and on consumer confidence in seafood. In response to this issue, CFSAN initiated Project Fish SCALE (Seafood Compliance and Labeling Enforcement) which is a multi-faceted approach to address FDA issues with seafood labeling and species identification. At the heart of this project is the updating of FDA's species identification capabilities to state-of-the-art forensic techniques using DNA sequencing.

Protocols, reference standards, and other training materials generated through this project are now being used in FDA Office of Regulatory Affairs Regional Field Laboratories across the country whenever the identity of a seafood product needs to be determined. In addition, these materials are being used by other domestic and international agencies as well as by private laboratories that directly service the seafood industry. The data generated has allowed FDA to respond to claims of mislabeling and fraud, take regulatory action against non-complaint seafood producers and distributors, and has enhanced our ability to rapidly respond to several illness outbreaks involving seafood.

Timothy Croley, Ph.D.

Supervisory Chemist, CFSAN, FDA

Non-targeted Screening Methods for Identification of Chemical Hazards of Public Health Concern

To manage the globalization of the food supply, methods need to be developed for monitoring potential contaminants. One promising strategy has been to explore chemical screening methods centered on high resolution mass spectrometry and data processing algorithms that allow for simultaneous targeted and non-targeted analyses. The complexities and diversity of food matrices augment the difficulty in developing and implementing this approach, especially in a high-throughput manner. This presentation focuses on the analytical considerations needed for performing this type of screening. In particular, the impact of chromatography and mass spectrometry conditions are examined. In addition, the discussion will include data quality issues that could impact successful identification of an unknown chemical. Finally, the presentation will include the application of novel data processing algorithms to these data, and a discussion of the process challenges to minimize errors in the detection, chemical formula generation, and potential identification of chemical species.

Keynote Speaker

Bert Vogelstein, M.D.

Director, Ludwig Center at Johns Hopkins University, Investigator, Howard Hughes Medical Institute, The Sidney Kimmel Cancer Center at Johns Hopkins University

Cancer Genomes and the Wars Against Cancer

Genome-wide sequencing efforts have revealed the genomic landscapes of the vast majority of human cancer types. For most cancers, this landscape consists of a small number of mountains (genes altered in a high fraction of tumors) and a much larger number of hills (genes altered infrequently). Only ~1% of human genes, when altered by mutation, appear to be able to promote or "drive" tumorigenesis. It is now known that a typical tumor contains ~three driver gene mutations plus 30 to 100 non-synonymous mutations in other genes, called passengers, that confer no selective growth advantage. The driver genes all seem to function through a small number of universal pathways that determine cell fate, cell survival, or genome maintenance, and more detailed knowledge of these pathways is the key to enhanced understanding of cancer. But even now, the new, comprehensive knowledge of the genetic basis of human tumors provides guidelines for battling the disease on many fronts. The opportunities for improved therapies with conventional and non-conventional agents, as well as the prospects for improved primary and secondary cancer prevention, will be discussed – along with the obstacles that must be overcome to achieve success.

Concurrent Session 5: Support New Approaches to Improve Product Manufacturing and Quality

Ram Sasisekharan, Ph.D.

Alfred H. Caspary Professor of Biological Engineering, Department of Biological Engineering, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

Framework for Chemical Characterization

The Sasisekharan Laboratory at MIT has extensive experience characterizing complex biopharmaceutical mixtures in terms of their structural attributes and how these attributes impinge on their function. A central component of the approach is the ability to employ a 'systems' framework that has been developed over the past decade or so in integrating diverse datasets pertaining to complex biopharmaceutical mixtures. This framework has permitted us to incorporate orthogonal datasets including analytical and functional data to get to the bottom of structure-function relationships of such complex mixtures. In his presentation, Dr. Sasisekharan will outline the overall concepts related to this systems approach as well as use specific examples to illustrate the key elements of this approach and the extensive experience to build a robust set of integrated tools and algorithms to determine the extent of characterization required for establishing equivalence for therapeutics that are used in the clinic.

Nicola Ranieri

Research Biologist, Microscopy and Image Analysis, Trace Examination Section, ORA, FDA

Screening for Counterfeit Pharmaceutical Products using the CDx Device in Ultraviolet, Visible, and Infrared Modes for Field and Laboratory Use

FDA's Office of Regulatory Affairs, Forensic Chemistry Center developed the Counterfeit Detection Device (CDx) and methodology to rapidly screen for counterfeit pharmaceutical dosage forms and packaging in a non-destructive manner. Although traditional methods of detecting counterfeit products are effective, they require expensive, sophisticated, and frequently bulky instrumentation, with testing performed in a laboratory by highly trained operators. The CDx is an inexpensive, rugged, portable, hand-held, electronic device allowing 'real-time' rapid screening results in the field and laboratory. It is simple to use and does not require special technical training. The battery-operated device uses LEDs to emit different wavelengths of light in the spectrum from ultraviolet to infrared. The visual response of the product to the wavelengths is captured using either still photos or video acquisition. The user can then use stored images of authentic products or packaging for comparison to the suspect product/packaging. The CDx is used to examine both the finished dosage form as well as the packaging (tablets, capsules, powders, and packaging on inks, papers, and covert markings). The device has also demonstrated utility in aiding in the examination of products that have been tampered, diverted, re-labeled, or re-glued. The CDx has been used to analyze numerous counterfeit products including commonly consumed pharmaceuticals. The CDx is being used nationally at a number of ports of entry, where inspectors screen drugs, associated packaging, and cosmetics to identify counterfeit, falsified, and unapproved products. The device is also being used internationally, including in the United Kingdom, Canada, Laos, and Ghana. The advantages of the device are being applied on a global scale to help improve the quality of medicines in areas with the greatest counterfeit drug problems, where bad or counterfeit products have been directly linked to adverse health consequences.

Lauren Jackson, Ph.D.

Chief, Process Engineering Branch, Division of Processing Science & Technology, Office of Food Safety, CFSAN, FDA

Methods for Detection of Allergens in Food and in the Processing Environment: Approaches and Challenges

Celiac disease is non-IgE-mediated hypersensitivity to gluten, a class of water insoluble proteins found in wheat, barley, rye, and possibly oats, while food allergies are an IgE-mediated response to certain

food proteins. Current estimates are that 1% of the US population suffers from celiac disease, while 4% is afflicted with food allergies. As therapeutic approaches are not currently available, adhering to a strict gluten-free/allergen-free diet is the only treatment for individuals with celiac disease or those with food allergies. Consumers with celiac disease or food allergies rely on food labels to accurately disclose the presence of allergenic ingredients and those containing gluten. However, undeclared gluten/allergens can inadvertently appear in foods through incorrect labelling, improper handling of rework, cross-contact during manufacture and insufficient equipment cleaning procedures. Sensitive and accurate analytical methods are needed to detect and quantify the presence of gluten and allergens in finished foods, beverages, ingredients and in the food processing environment. However, allergen and gluten detection and quantitation depend greatly on the type and nature of the allergen, the food matrix and the whether and how the food has been processed. Analytical tools available to detect allergenic proteins or foods include immunochemical approaches, DNA detection methods, and LC-MS/MS techniques. The choice of method used depends on the purpose of the test, the food matrix, the extent and manner in which the food is processed, the turn-around time, portability and cost. All methods must be properly validated to ensure confidence in the results. New approaches are currently under development to improve the reliability of analytical results, and for simultaneous detection of multiple allergens. This presentation will cover the approaches and challenges associated with allergen and gluten detection in food and in the food processing environment. In addition, collaborative projects between FDA, the food industry and academia aimed at improving allergen and gluten detection will be discussed.

Sau (Larry) Lee, Ph.D.

Acting Associate Director for Science, Team Leader for OPQ Botanical Review Team, Office of Pharmaceutical Quality, CDER, FDA

Advanced analytics and data integration for comparative biomolecule characterization: A case study of generic enoxaparin

To develop follow-on (or generic) versions of drug products containing a heterogeneous mixture of molecules or macromolecules (e.g., proteins), the manufacturer will need to demonstrate similarity (or sameness) of these molecules to those present in the reference products. With advances in analytics, characterization of these complex molecules at the molecular level becomes possible. To reply on the in vitro approaches to demonstrate similarity (or sameness) for complex molecules and minimize the need for in vivo studies, it will be critical to determine and understand the extent to which in vitro chemical and/or biological characterization assays employed can characterize the sameness (or similarity) between the reference and follow-on (or generic) drug products for the complex molecules. Such an in vitro-oriented approach is best illustrated through FDA's experience with generic enoxaparin. In the review of enoxaparin under the Abbreviated New Drug Application (ANDA) pathway, FDA developed a scientifically rigorous approach based on five criteria: first, equivalence of physicochemical properties; second, equivalence of heparin source material and mode of depolymerization; third, equivalence in disaccharide building blocks, fragment mapping and sequence of oligosaccharide species; fourth, equivalence in biological and biochemical assays; and finally, equivalence of in vivo pharmacodynamic profile. In addition to fulfillment of these criteria, FDA also used in vitro, ex vivo and model animal data to ensure there was no increased immunogenicity risk of the generic enoxaparin product relative to the brand name product. The approval of the highly complex enoxaparin product using this framework under the ANDA pathway represents a major development. It indicates that analytical and scientific advancements may in certain cases allow the elimination of unnecessary in vivo testing in animals and humans.

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Daron Freedberg, Ph.D.

Senior Scientist, CBER, FDA

Using NMR to Assess Structure and Comparability in Vaccines and Therapeutics

NMR spectroscopy is a powerful analytical that can be used to obtain atomic-level details on biomolecules, including vaccines and protein therapeutics. While many glycan functions are known, the structural causes for their function remain undiscovered. Therefore, there has been an effort to establish glycan structure-function relationships using NMR spectroscopy. We have uncovered helices in bacterial polysaccharides and changes in structures upon calcium binding. We have also detected hydrogen bonds, which help hold these glycans together and give them their shapes. Finally, I will describe some of the methods we have used to characterize protein therapeutics under final container conditions and without resorting to isotopic labels.

Concurrent Session 6: Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Howard L. McLeod, Pharm.D.

Medical Director, DeBartolo Family Personalized Medicine Institute and Senior Member, Department of Cancer Epidemiology, Moffitt Cancer Center

Individualized Therapy as a Practical Aspect of Patient Care

Personalized genomic medicine is finally becoming a real part of oncology, infectious disease, and mental health, with implications for therapy selection, treatment avoidance, dosing, and risk prediction. However, there are many treatments for which there are few tools for individualized selection based on patient-specific attributes. Discovery strategies are needed to more thoroughly understand the pharmacological basis for drug toxicity, efficacy, and resistance, especially in patients with cancer. The presence of clinically predictive germline variants has also opened the hope that objective predictors of patient toxicity will be in the future. This will allow for the development of robust risk/benefit models, whereby decisions between apparently equal treatment options can be made for an individual patient. These probabilistic strategies are important ways to make pharmacogenomic findings of relevance to modern cancer care. There is a need for personalized medicine approaches to also go beyond DNA, to include biomarkers that reflect the patients current situation. While this can include proteomic or metabolomics strategies, blood level guided therapy remains an underexplored clinical tool. It is also clear that there are many barriers to clinical application. These include expanding the science to understanding the pathways of genes that regulate a drug's activity. There are also critical non-science issues, such as integration of new tests into health systems, changing old habits to allow application of new data, and the reality that the cost of both testing and the therapeutic options are a key driver in health care. As the scientific evidence matures, we must think beyond our favorite aspect of translational science if we are to overcome the many obstacles to delivering more careful selection of cancer therapy.

Zuben E. Sauna, Ph.D.

Senior Staff Fellow, CBER, FDA

Not in Our Stars but in Ourselves: The Pharmacogenetic Determinants of Immunogenicity of Therapeutic Proteins

Recent estimates put the value of prescription drugs that are either ineffective or unsafe in individual patients at upwards of $100 billion per year. The implications of identifying at risk patients and drug development based on the genetic characteristics of individual patients are thus immense. Understanding the genetic basis of drug responses (pharmacogenetics) thus has the potential to revolutionize the practice of medicine. Here, we apply pharmacogenetic principles to immunogenicity which is the development of anti-drug antibodies to therapeutic proteins. Immunogenicity is a significant impediment to development and licensure of any therapeutic protein and limits the clinical utility of many approved products. There is increasing evidence that patient related genetic factors can play an important role in why some individuals develop inhibitory anti-drug antibodies while others do not. Moreover, as most native proteins do not make for good drugs a new generation of therapeutic-proteins, which have been engineered to improve product attributes or to enhance process characteristics, are rapidly entering the drug development pipeline. Engineered proteins inevitably require the generation of so-called neo-epitopes which do not exist naturally and are thus potentially immunogenic. Here we demonstrate how emerging computational and experimental techniques can potentially be used to assess the immunogenicity risk posed by neo-epitopes to the patient population as well as to specific patients and ethnic groups. We also provide examples of replacements therapeutic proteins that present different risks to patients based on their genotype. Finally we discuss patient specific genetic risk factors for immunogenicity in the context of a recent controversy with respect to a marketed engineered Factor VIII drug product and the discontinuation of the development of a Factor VIIa analog in Phase III clinical trials.

Michael Pacanowski, PharM.D., M.P.H.

Associate Director for Genomics and Targeted Therapy, Office of Clinical Pharmacology, CDER, FDA

Pharmacogenomics and Biomarker-Based Drug Development

This presentation will provide an overview of precision drug development, highlighting recent approvals, FDA initiatives, and the evolving regulatory framework.

Gene Pennello, Ph.D.

Team Leader and Mathematical Statistician (Biomedical), Division of Biostatistics, CDRH, FDA

Statistical Evaluation of "Me-Too" Companion Diagnostic Tests for Selecting Therapies

A companion diagnostic test is one that is essential for the safe and effective use of a therapeutic product. A common indication for a companion diagnostic is to select patients for a particular therapy. For example, KRAS PCR test kits are being used to select colorectal cancer (CRC) patients for cetuximab if the test does not detect KRAS mutations in a CRC tissue sample. Typically, FDA approval of a therapy together with its companion diagnostic test (CD) is supported by randomized clinical trials (RCTs) of the therapy. To evaluate the clinical utility of the CD, therapeutic efficacy is evaluated in patient subgroups with test negative and test positive results, the putative predictors of response or non-response to therapy. After approval of the first CD, a "me-too" or follow-on CD (FCD) may be developed for the same indication. However, to support the FCD, another RCT for the therapy may not be feasible given the RCTs already conducted for it. Further, the sponsor of the FCD may not have access to samples archived from the existing RCTs and thus would not be able to test them with the FCD. Instead, an FCD is often supported by a study of agreement of FCD and CD test results on samples external to the existing trials. However, the level of agreement deemed necessary for approval of the FCD is difficult to determine without consideration of its clinical implications. In this conceptual talk, I'll discuss a statistical model for indirectly estimating the clinical utility of the FCD by combining FCD-CD agreement data with trial summary data on the clinical utility of the CD. Model assumptions can ensure that estimates of utility (e.g., difference in hazard ratio between test result subgroups) cannot be better for the FCD than the CD and deteriorates as FCD disagreement with CD increases. Even if this indirect approach indicates that the FCD is approvable, a post-approval clinical study may be warranted to obtain direct evidence of clinical utility of the FCD. I'll explore some feasible post-market study designs.

Concurrent Session 7: Modernize Toxicology to Enhance Product Safety

Sangeeta Bhatia, M.D., Ph.D.

John J. and Dorothy Wilson Professor of Health Sciences and Technology & Electrical Engineering and Computer Science, Massachusetts Institute of Technology

Human Microlivers for Disease Modeling

Human tissues are composed of collectives of cells that cooperate in a healthy microenvironment to exhibit physiological functions. Conversely, dysregulated microenvironments are a hallmark of disease. Our laboratory focus is on interactions between cells and their microenvironment that occur on the length scales of receptor interactions (10 nm) to multicellular interactions (100 µm). We leverage engineering tools that have been created by the semiconductor community to speed rates of computation through miniaturized manufacturing capabilities. These micro- and nanotechnology tools, by virtue of their spatial resolution, enable the precise synthesis, interrogation, and perturbation of tissue microenvironments. Thus, my laboratory aims to dissect the role of the tissue microenvironment in both health and disease using engineering tools. Specifically, we focus on tissue microenvironments of clinical importance in hepatology and oncology, and we seek to translate our findings into new therapies for patients. The hepatocyte, the parenchymal cell of the liver, is responsible for most of the vital functions of this organ; however, the hepatocyte phenotype is exquisitely sensitive to isolation from microenvironmental cues in vivo. My research program uses microtechnology tools and biomaterials to synthesize 2D and 3D hepatic microenvironments to study determinants of cell fate and function and then perturb and interrogate these to model human disease. These efforts have produced high-throughput-capable human microlivers which model human drug metabolism, liver disease, and interactions with pathogens, and are used to develop new drugs and detect potential toxicities. Using these platforms, some of our recent achievements have been the discovery of small molecules that drive proliferation of adult hepatocytes and maturation of stem-cell derived progeny to enable sourcing of human hepatocytes, and the development of the first high-throughput model systems to study hepatotropic pathogens (e.g. liver stage malaria and hepatitis viral infections).

Richard D. Beger, Ph.D.

Branch Chief, Biomarkers and Alternative Models Branch, NCTR, FDA

Discovery and Analytical Validation of System Biology Translation Biomakers of Toxicity

Systems biology technologies which include transcriptomics, proteomics and metabolomics are being used to discover biomarkers and aid our understanding of health-to-disease status. These technologies can be used to evaluate biofluids obtained non-invasively from non-clinical studies of drug toxicity; the potential biomarkers thus identified can be subsequently evaluated in patients that have displayed drug toxicities to determine whether they are translatable injury biomarkers. These translatable biomarkers need to be analytically validated in both the non-clinical and clinical studies. Examples of translatable biomarkers of acetaminophen induced liver injury will be shown. It is important to compare these new translatable biomarkers to an established translational biomarker (ALT) to understand their clinical value. Evaluating these biomarkers with many timepoints in both non-clinical and clinical samples can aid our understanding of the kinetics of the biomarkers compared to ALT. Ex vivo imaging of tissues can be used to discover location of drugs, drug metabolites and location of metabolomic or proteomic biomarkers related to disease or toxicity. This imaging information can be used to enhance the sensitivity from the other imaging techniques (i.e., MRI/MRS) and be combined with the current non-clinical gold standard – histopathology.

Serguei Liachenko, M.D., Ph.D.

Director of Bioimaging, NCTR, FDA

Nonclinical development of neurotoxicity biomarkers using in vivo ***MRI***

Modern in vivo imaging technologies like magnetic resonance imaging (MRI) have attained an important role in medical research due to low invasiveness and ability to provide functional information about biological systems. Such information could be obtained from the same subject repeatedly and with the least possible interference, which makes in vivo imaging a unique and indispensable tool to support drug safety evaluation and other toxicological research. MRI was used in non-clinical settings to probe the changes in living rat brain following exposure to one of ten classical neurotoxicants. The specific response (change in T2 relaxation) was identified and the investigation of its specificity and sensitivity against current gold standard (histopathology) is initiated. This approach may lead to the development of the sensitive non-invasive early biomarker of neurotoxicity, which can significantly improve the quality of the drug safety research.

Norman Stockbridge, M.D., Ph.D.

Division Director, Division of Cardiovascular and Renal Products, Office of New Drugs, CDER, FDA

Replacing the Clinical Thorough QT Study with a Panel of In Vitro Assays and Computational Integration

A large community effort is underway to remedy the high false-positive rate of proarrhythmia assessment that is based today largely upon the hERG assay and the "Thorough QT study", described in ICH S7B and E14, respectively. The replacement leverages a high degree of understanding of the fundamental basis of proarrhythmia initiation and the ability to explore in vitro the effects of drugs on various types of human ventricular ion channels. When drugs affect more than one ion channel type, it may be difficult to predict by direct inspection of these data what the net effect would be in the heart. However, these data can be integrated to produce the net effect on the cardiac action potential. This model can then be interrogated to determine how proarrhythmic a drug is. The overall effort will be described, with particular eM.P.H.asis on aspects of the development effort that are being undertaken in laboratories of CDRH and CDER.

Kristina E. Howard, D.V.M., Ph.D.

Research Veterinary Medical Officer, CDER, FDA

Humanized Hepatic Mice: In Vivo Model to Predict Human-Specific Immunotoxicity, Drug Metabolism and Hepatotoxicity

At present, significant gaps exist in our ability to evaluate and accurately predict human drug metabolism and drug-induced toxicities in animal models. For small molecule therapeutics, preclinical testing is confounded by significant differences in hepatic metabolism between animals and humans. As a result, the metabolite profiles derived from animal studies may be inconsistent with human responses, and potential toxicities associated with human-specific metabolites can be missed. In addition, the ability to test biologics for safety and efficacy in animal models is limited by differences in biological receptors between species. The potential contribution of human-specific immune responses to the development of immunotoxicity and tissue injury hampers the assessment of product quality. It is now possible to create mice with a highly humanized liver and/or an engrafted human immune system. Such models are now being evaluated for their ability to better predict clinical outcomes. Published studies have demonstrated the ability of humanized hepatic mice to detect liver injury induced by fialuridine, which was responsible for unpredicted severe liver injury during clinical trials. This presentation will provide background on these innovative chimeric animal models and their potential applicability in assessing pre-clinical hepatoxicity.

Concurrent Session 8: Harness Diverse Data through Information Sciences to Improve Health Outcomes

Atul Butte, M.D., Ph.D.

Director, Institute for Computational Health Sciences and Professor of Pediatrics, University of California, San Francisco

Transforming Trillions of Points of Data into Diagnostics, Therapeutics, and New Insights into Disease

With the end of the United States NIH budget doubling and completion of the Human Genome Project, there is a need to translate genome-era discoveries into clinical utility. The difficulties in making bench-to-bedside translations have been well described. The nascent field of translational bioinformatics may help. Dr. Butte's lab at Stanford builds and applies tools that convert trillions of points of molecular, clinical, and epidemiological data -- measured and often released to the public by researchers and clinicians over the past decade and now colloquially termed "big data" -- into diagnostics, therapeutics, and new insights into disease. Several of these efforts have been spun out into biotech companies. Dr. Butte, a bioinformatician and pediatric endocrinologist, will highlight his lab's work on using publicly-available molecular measurements to find new uses for drugs and evaluating patients presenting with whole genomes sequenced.

Session 1: Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

(Posters 1-66 are located in Section A)

1. Overview of Regulatory Review of Proton Therapy Devices at FDA

Abbineni, Gopal, FDA/CDRH/NMRT; Bhullar, Amarjeet, FDA/CDRH/NMRT; Jung, William, FDA/CDRH/NMRT; O'Hara, Michael, FDA/CDRH/NMRT

Plain Language Synopsis: Proton therapy is an advanced radiation treatment machine which uses proton beam to destroy cancer cells while sparing surrounding healthy tissues. Sponsors typically choose traditional 510 (k) submission pathway to bring the technology to market. Owing to the bulkiness of the device; its high energy radiations; and its dependence on software, FDA pays close attention to Radiation, Electrical, and Mechanical safety and software issues to fulfill FDA mission and improve public health and safety.

Abstract: Proton therapy is an advanced radiation treatment technique which uses proton beam to destroy tumor cells while minimizing radiation exposure to surrounding healthy tissues. A proton therapy device consists of a power supply system, an accelerator (e.g. synchrotron or cyclotron) to accelerate the proton beam, a beam transport system to extract, bend and focus the proton beam, a beam delivery system which directs the proton beam to the patient using gantry, and treatment nozzle, and other necessary control and treatment planning systems. Proton therapy is best suited to treat tumors at critical sites (base of skull; spine tumors; prostate; melanoma of eye, brain tumors, and pediatric tumors, etc). Currently there are 14 proton therapy centers in operation in the United States and at least 10 are under construction. FDA is particularly interested in this technology because 1) The technology allows physicians to control where the proton releases bulk of its cancer killing energy as a result can effectively treat cancer patients; 2) The patient is exposed to little or no radiation dose beyond the tumor site (no exit dose); and 3) The technology significantly reduces the amount of radiation to healthy tissues resulting in less adverse long term radiation effects. Because of its potential benefit in treating cancer patients, FDA played important role in reviewing this technology. Since 1987 FDA has reviewed 24 proton therapy submissions from various sponsors. Sponsors typically choose the traditional 510 (k) submission pathway to bring the technology to market. Due to the bulkiness and complexity of the device; its high energy radiations; and its dependence on software, FDA's primary concern of safety areas are Radiation, Electrical, and Mechanical safety and software issues. In conclusion, FDA efforts in bringing this technology to market is it would increase the number of cancer patients who may be able to benefit from the proton therapy.

2. Comprehensive Translational Assessment of Human Induced Pluripotent Stem Cell Derived Cardiomyocytes Using High-Throughput Optical Imaging Assays

Blinova, Ksenia, FDA/CDRH/OSEL; Stohlman, Jayna, FDA/CDRH/OSEL; Chan, Dulciana, FDA/CDRH/OSEL; Smith, Godfrey, University of Glasgow; Hortigon, Maria, Clyde Biosciences; Rodriquez, Victor Zamora, Clyde Biosciences; Vicente, Jose, FDA/CDRH/OSEL; Johannesen, Lars, FDA/CDRH /OSEL; Galeotti, Loriano, FDA/CDRH/OSEL; Stockbridge, Norman, FDA/CDER/OND; Strauss, David, FDA/CDRH/OSEL

Plain Language Synopsis: New biotechnology enables human stem cells to be generated from patient skin or blood cells. These cells can then be turned into any type of cells, including heart cells. We are studying if these cells can predict the patient response to drugs using optical imaging laboratory tests.

Abstract: Background: The ability to generate human induced pluripotent stem cells (iPS) and differentiate these cells into patient-specific iPS-cardiomyocytes promises a new paradigm in drug development, toxicity screening and personalized medicine. In line with FDA's strategic plan for regulatory science, it is critical for FDA to ensure readiness to evaluate this innovative emerging technology. The goal of this study was to determine if iPS-cardiomyocyte laboratory assays can predict the effect of therapies in humans. Methods: Two commercially available human iPS-cardiomyocyte lines were studied (Cellular Dynamics and Axiogenesis). iPS cardiomyocytes

were plated on 96-well plates for optical measurements using voltage-sensitive dyes on CellOPTIQ platform (Clyde Biosciences). All recordings were done in triplicates with negative, vehicle and positive controls on each plate. Twenty-five drugs and three drug combinations were studied for acute effects (20min -5h) and six drugs for chronic (>24h) effects. Drug-induced iPS-cardiomyocyte action potential duration (APD) prolongation and arrhythmia development were compared to clinical results from two FDA-sponsored clinical trials (8 drugs) and from FDA label information on QT prolongation and risk of the arrhythmia torsade de pointes [TdP]. Results: Quantitative concentration-dependent analysis comparing iPS-cardiomyocytes to clinical trial results demonstrated good correlation between drug-induced APD prolongation and clinical QT prolongation. iPS-cardiomyocytes had greater APD prolongation than clinical QT prolongation with some drugs (dofetilide, quinidine). Of nineteen drugs with an FDA label of QT prolongation, fifteen exhibited APD prolongation after acute drug exposure in iPS-cardiomyocytes. One of the non-concordant drugs was assessed in chronic experiments (amiodarone) and caused APD prolongation at 24h. None of the 6 drugs without QT prolongation caused APD prolongation. Of fourteen drugs with TdP risk on FDA label, nine caused arrhythmias in iPS-cardiomyocytes. Discussion/Future Work: Optical imaging of iPS-cardiomyocytes with voltage sensitive dyes is an emerging technology for high-throughput toxicity assessment for drug-induced arrhythmias. There is good concordance between iPS-cardiomyocyte and clinical studies, with potential to improve the assessment of arrhythmias in the assay. In the following year we will investigate further the effect of drugs on patient-specific iPS-cardiomyocytes generated from subjects in FDA-sponsored clinical study to determine if stem cells can predict personalized drug response.

3. Femtosecond Laser Device Safety in Ophthalmic Surgical Procedures: Evaluating the Effect of Critical Laser Parameters on Laser-induced Visible and Ultraviolet Nonlinear Harmonic Generation in Corneal Tissue

Calhoun, William, FDA/CDRH/OSEL; Weiblinger, Richard, FDA/CDRH/OSEL; Beylin, Alexander, FDA/CDRH/OSEL; Ilev, Ilko, FDA/CDRH/OSEL

Plain Language Synopsis: We address concerns regarding harmonic generation, a possibly phototoxic side effect, induced in corneal tissue by femtosecond lasers during common ocular surgical procedures. We also compare conventional FSLs with the emerging class of FSLs with respect to harmonic generation.

Abstract: A specific goal of FDA/CDRH Optical Therapeutics and Medical Nanophotonics Laboratory (OTMN Lab) is to develop a firm understanding of emerging optical therapeutic devices, focusing on associated safety and efficacy concerns. This is accomplished through quantitative in-vitro and ex-vivo experimental studies that investigate the fundamental laser-tissue interactions generated by such devices. Femtosecond laser (FSL) devices are rapidly emerging as unmatched tools for clinical therapies due to their precision and reduced energy characteristics. The large impact that this advanced technology is having on the medical field is demonstrated by its use in several of the most commonly performed surgical procedures in the world, specifically, LASIK, cataract surgery and corneal transplantation to achieve photodisruption, the predominant tissue cutting mechanism, FSLs must generate very high optical intensities, which also generate nonlinear optical effects (NOEs) such as second and third harmonic generation (SHG and THG, respectively). NOEs cause a modification of the optical properties of a susceptible material due to an intense optical field, often generated by ultra-short pulsed lasers. Harmonic generation (HG) is one example of a NOE that could impact therapeutic efficacy and patient safety by changing the laser parameters responsible for the therapeutic effect and by generating phototoxic UV light (THG) and intense visible light (SHG). Coincidentally, corneal tissue, which has seen the largest application of FSL based therapies, strongly generates NOEs due to its highly organized and oriented, dense collagen structure. We have investigated how critical FSL parameters such as pulse energy, pulse repetition rate, and numerical aperture influence HG duration, intensity and efficiency in corneal tissue. Experimental results demonstrated corresponding increases in HG intensity with increasing repetition rate and numerical aperture. HG duration

decreased with increasing repetition rate and pulse energy. The data also demonstrated a significant difference in HG between FSL parameters closely representing the two most common classes of FSL therapeutic devices. These results, as well as those from ongoing studies, will determine if FSL stimulated HG poses a safety concern in commonly performed FSL based ophthalmic surgeries, as well as provide a guide for the safe development and use of future devices.

4. Direct Determination of Glyphosate, Glufosinate, and AMPA in Soybean and Corn by Liquid Chromatography/Tandem Mass Spectrometry

Chamkasem, Narong, FDA/ORA/SRL; Morris, Cynthia, FDA/ORA/SRL; Harmon, Tiffany, FDA/ORA/SRL

Plain Language Synopsis: Glyphosate and glufosinate are non-selective herbicides used for the control of a broad spectrum of weeds. Glyphosate is the most applied pesticide in the world. FDA is not currently monitoring this analyte due to the lack of proper method. GAO audit has reported this deficiency of FDA pesticide residue program. The proposed method will enhance FDA lab capacity and quickly response to the GAO's findings.

Abstract: A simple high-throughput liquid chromatography/tandem mass spectrometry (LC/MS) method was developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in soybean and corn using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. Two grams of sample was shaken with ten milliliters of water containing ethylenediaminetetraacetic acid disodium salt (Na2EDTA) and acetic acid for 10 min to precipitate protein. After centrifugation, the supernatant was passed thru an Oasis HLB SPE to retain suspended particulates and non-polar interferences. The sample was directly injected and analyzed in 6 min by LC/MS with no sample concentration or derivatization steps. Two multiple reaction monitoring (MRM) channels were monitored in the method for each target compound to achieve true positive identification. Three internal standards corresponding to each analyte were used to counter matrix suppression effect. Linearity of the detector response with a minimum coefficient of determination (R2) of more than 0.995 was demonstrated in the range of 10 to 1000 ng/mL for each analyte.

5. Polony Detection and Genotyping of Hepatitis A Virus from Frozen Raspberries Possibly Implicated in an Acute Case of Hepatitis A

Chen, Haifeng, FDA/CFSAN; Hu, Yuan, FDA/ORA; Kulka, Michael, FDA/CFSAN; Elkins, Chris, FDA/CFSAN

Plain Language Synopsis: The polony method was designed to be an effective tool with the ability to simultaneously detect and genotype HAV embedded in the food matrix in a single assay.

Abstract: Hepatitis A virus (HAV) is one of the leading causes of foodborne infections. HAV can be transmitted by ingestion of contaminated food or water as well as direct contact with HAV-infected individuals through the fecal-oral route. It is highly critical to develop rapid and sensitive detection methods for HAV in food samples to prevent and control HAV-related foodborne diseases. In this study, we described a polymerase colony (polony) method for simultaneous detection and genotyping of HAV from frozen raspberries that possibly implicated in an acute case of hepatitis A.RNA extraction was performed on frozen raspberries (collected in New Hampshire outbreak, July 2013) by ultracentrifugation concentration and QiaAMP viral RNA mini kit. Polony assay was used to detect and genotype the virus. Additional analysis using nested reverse transcription PCR (RT-PCR) was completed to confirm the presence of HAV, and obtain the amplicon sequence information for phylogenetic analysis and confirmation of virus genotype. HAV was detected and identified as sub-genotype IB in the frozen raspberries using polony approach. This approach amplifies multiple individual cDNA molecules reverse transcribed from viral RNA to produce immobilized colonies within a thin acrylamide gel on a microscope slide. The resulting polonies were then genotyped by single base extensions with fluorescent dye-labeled nucleotides. This sample was also tested positive for HAV by nested RT-PCR. Phylogenetic sequence analysis of the nested RT-PCR amplicon from the sub-genomic region of VP1/P2A confirmed that the virus belonged to sub-genotype IB.

6. Developing Chemometric Methods in Comparing 2D NMR Spectra of Similar Drug Products

Chen Kang, FDA/CDER/OPQ/OTR/DPA; Li Feng, University of Maryland Baltimore County, Department of Mathematics and Statistics; Ghasriani Houman, FDA/CDER/OPQ/OTR/DPA; Brinson Robert G., NIST/IBBR; Adams Kristie M., USP; Aubin Yves, Health Canada; Freedberg Darón I., FDA/CBER/OVRR; Marino John P., NIST/IBBR; Keire David A., FDA/CDER/OPQ/OTR/DPA

Plain Language Synopsis: Protein higher order structure (HOS) is a CQA for biosimilar approvals. Solution state 2D NMR spectrum is the only high-resolution biophysical method in comparing HOS. Chemometric methods are developed to quantify the difference between collected NMR spectra of protein therapeutics, allowing comparisons on inter-lot and originator-follow on products.

Abstract: The higher order structure (HOS) of protein therapeutics is a critical quality attribute of protein therapeutics. In developing biosimilar products, it is crucial to compare HOS between the protein API within the originator and the biosimilar drugs. Solution state 2D NMR spectroscopy is probably the only high-resolution analytical tool for HOS comparison. However, the measured 2D spectra could be highly similar to each other among "biosimilar" products. Chemometric methods have to be developed to differentiate such highly similar NMR spectra quantitatively. Using filgrastim as an example, a 175-residue protein drug version of the native human protein granulocyte colony-stimulating factor (GCSF),1,2 we showed the 2D 1H-15N HSQC NMR spectra with reasonable single to noise ratio (S/N) can be collected using a standard room temperature probe equipped 600 MHz NMR spectrometer. The measured filgrastim products are from the originator Amgen and the three Indian-sourced follow-on manufactures (i.e., Biocon, Intas and Dr. Reddy's) as well as an in-house prepared GCSF. We then examined two chemometric comparison methods, the Euclidean distance based on spectra-grid and the sequential nearest neighbor (SNN) graph-invariant. Both chemometric methods established the similarity in the decreasing order of Dr. Reddy's > Intas > Biocon > the in-house prepared G-CSF. In addition, both Intas and Dr. Reddy's sample are near or within the Amgen inter-lot difference. Further numeric tests demonstrated the results of the spectra-grid comparison is very sensitive to the spectra reference or alignment, a subtle variation of 0.005 ppm is dramatic enough to change the order of similarity. On the other hand, the SNN graph-invariant method is independent of reference or alignment changes. Therefore we demonstrated the general applicability of solution NMR and the robustness of the graph-invariant method. The results provide an alternative or orthogonal avenue for comparing HOS for biosimilar drug products or drug products after manufacture changes. Therefore, SNN graph method is a facile tool for protein higher order structure comparisons via 2D NMR spectra.

7. Harmonization of Outputs of Diagnostic Devices to a Risk of Disease Scale: Methods and Evaluation

Chen, Weijie, FDA/CDRH/OSEL/DIDSR; Sahiner, Berkman, FDA/CDRH/OSEL/DIDSR; Samuelson, Frank, FDA/CDRH/OSEL/DIDSR; Pezeshk, Aria, FDA/CDRH/OSEL/DIDSR; Petrick, Nicholas, FDA/CDRH/OSEL/DIDSR

Plain Language Synopsis: Many diagnostics (biomarkers, algorithms) intend to predict risk of disease using a classifier score. We investigate methods for transforming classifier scores that are often on an arbitrary scale onto the risk/probability of disease scale and investigate assessment methods to evaluate the calibration of estimated risks to true risks.

Abstract: Many medical diagnosis devices use a biomarker or a model combining multiple biomarkers to diagnose a condition or predict risk of disease (e.g., computer-aided diagnosis systems in imaging, genetics/genomic based risk assessors, etc.). The outputs of many such devices are numerical scores on some arbitrary scale; the diagnostic meaning of such scores can be unclear. This may render the interpretation of a diagnostic or comparison among different diagnostics difficult, thus leading to suboptimal patient treatment. There are emerging devices that provide an estimate of risk of disease, but the evaluation criteria are not standardized. In this project, we investigated a semiparametric and a nonparametric method for transforming diagnostic scores to a clinically meaningful risk of

disease (or probability) scale with an appropriate confidence interval estimate. A second focus of this study is development of methods and tools for assessing the calibration of risk, i.e., measuring how well estimated risk/probabilities agree with actual observed risk. We developed simulation models to validate and compare our scale transformation methods. We also used simulations to investigate the statistical properties (e.g., sample size requirement) of assessment criteria (mean square error, Brier score) for the evaluation of scale transformation methods. Our simulation studies show that scale harmonization and calibration evaluation can be achieved with a reasonable patient sample size. The assessment methods and software tools developed in this project prepare CDRH for evaluating innovative biomarker/algorithm-based diagnostic devices for diagnosis, prognosis prediction, risk assessment, and personalized therapy selection.

8. Detection and Quantification of Gluten during the Fermentation of Beer using Lateral Flow Devices

Cheng, Raymond, FDA/CFSAN/ORS; Panda, Rakhi, FDA/CFSAN/ORS; Cho, Chung Y., FDA/CFSAN/ORS; Garber, Eric A.E., FDA/CFSAN/ORS

Plain Language Synopsis: This project focuses on the development and evaluation of rapid, field deployable analytical methods for the detection of intact and fermented / hydrolyzed gluten to complement the laboratory-based methods currently used to support the Food Allergen Labeling and Consumer Protection Act of 2004 and the Gluten-Free Regulation of 2013.

Abstract: The accurate declaration of gluten on food labels helps prevent people with Celiac Disease (CD) from unnecessary adverse reactions. To assure such accuracy, FDA relies on analytical methods. Though many methods exist for the accurate detection of intact gluten in food, reliable assays for the detection of gluten in foods that have undergone fermentation or processes that might induce hydrolysis are lacking. Antibody-based assays, such as ELISAs and lateral flow devices, constitute one class of analytical methods commonly employed for the detection of food allergens and gluten. Though not as sensitive as ELISAs, lateral flow devices (LFDs), are field deployable, rapid, and inexpensive. LFDs employing a sandwich format rely on the ability of two antibodies to bind to the target gluten protein. However, peptides generated from the hydrolysis of gluten proteins may not be long enough to accommodate the binding of two antibodies, while still being long enough to contain the immunopathogenic sequence that causes the celiac response. An alternative to the sandwich format is a competitive configuration, in which the presence of a single epitope on a peptide is sufficient to generate the necessary competition, with immobilized gluten, for gold labeled antibodies. As the concentration of gluten in the sample increases, the intensity of the band observed in the LFD decreases. Five sandwich and one competitive LFD were used to follow the presence of gluten in sorghum-based beers made containing 0, 20, and 200 µg/mL gluten. Also included in the brewing process was a proline endopeptidase, Brewers Clarex®, designed to cleave immunopathogenic sequences. The amount of intact and hydrolyzed gluten present in the sorghum beer made with 20 µg/mL gluten could not be accurately quantified. The sorghum beer samples prepared with 200 µg/mL gluten analyzed using the sandwich LFDs displayed a decrease in detectable gluten during fermentation with the addition of Clarex® enhancing the loss. In contrast, the competitive LFDs displayed no change in detectable gluten unless Clarex® was included in the brewing process. It is evident from the LFDs that the competitive format showed potential for detecting both intact and hydrolyzed gluten.

9. Towards the Complete Apicoplast Genome of the Foodborne Parasite Cyclospora Cayetanensis

Cinar, Hediye Nese, FDA/CFSAN/OARSA; Gopinath, Gopal, FDA/CFSAN/OARSA; Choi, Dajung, FDA/CFSAN/OARSA; Lee, Seulgi, FDA/CFSAN/OARSA; Murphy, Helen, FDA/CFSAN/OARSA; DaSilva, Alexandre, FDA/CFSAN/OARSA

Plain Language Synopsis: Cyclospora cayetanensis is a parasite responsible for large food related outbreaks around the world, including the USA. Genomic sequence data are required to develop molecular methods for detection and differentiation of outbreak strains contaminating the food supply. Very little sequence information is available for this organism due to limitation in sample resources, and technical difficulties. We

will sequence the Cyclospora cayetanensis genome using advanced technologies.

Abstract: Cyclospora cayetanensis is a coccidian parasite responsible for food and water-related outbreaks around the world, including the most recent ones involving over 900 persons in 2013 and 2014 outbreaks in the USA. Detection of low level contaminations in foods, and traceback investigation during outbreaks require the use of sensitive molecular methods. Despite advances in genome technologies, little sequence information is available for C. cayetanensis. The absence of animal models or in vitro infection models makes genome sequencing of this parasite very challenging. Multicopy organellar DNA such as mitochondrion and apicoplast genomes have been particularly informative for detection and genetic traceback analysis in other parasites. Apicoplast is a non-photosynthetic plastid, with its own genome, found in most Apicomplexa, including C. cayetanensis. We sequenced the C. cayetanensis genomic DNA obtained from stool samples from patients infected with Cyclospora in Nepal using a metagenomics approach on Illumina platforms. By bioinformatically filtering out the metagenomic reads of non-coccidian origin sequences and concentrating the reads by targeted alignment, we were able to obtain contigs containing Eimeria-like mitochondrial, apicoplastic and some chromosomal genomic fragments. A mitochondrial genome sequence was assembled and submitted to Genbank by our group recently. Currently, we have assembled and mapped ~60 % of the estimated 35 kb apicoplast genome sequence including an inverted repeat region which contains duplicated SSU and LSU rRNA genes as well as duplicated tRNA genes. Overall, the mapping pattern of C. cayetanensis is evolutionarily conserved, with high similarity to the apicoplast genome structure of Eimeria spp. The remaining gaps are being sequenced from PCR amplicons, amplified with primers designed on the basis of our draft assembly sequence. An annotated draft sequence of the C. cayetanensis apicoplast genome will be presented, and the phylogenetic relationship with other apicomplexans will be discussed.

10. Development of Non-Clinical Test Methods for Evaluating Durability and Degradation in Fully Absorbable Cardiovascular Stents

Dreher, Maureen, CDRH/OSEL/DAM; Nagaraja, Srinidhi, CDRH/OSEL/DAM; Lu, Qijin, CDRH/OSEL/DAM; Malinauskas, Richard, CDRH/OSEL/DAM; Batchelor, Benjamin, University of Texas at Dallas

Plain Language Synopsis: Fully absorbable cardiovascular stents are an emerging technology designed to address adverse late stent thrombosis. Significant methodological challenges exist for evaluating their durability and degradation reliably, and standards are not available to assess performance. This project develops improved methods to study durability and degradation in fully absorbable cardiovascular stents.

Abstract: To reduce the adverse event of late stent thrombosis following implantation of drug eluting cardiovascular stents, medical device developers have begun to investigate absorbable materials for the stent platform. The absorbable stent platform is intended to degrade over time but must also provide radial support during healing to maintain vessel patency and withstand cyclic loading. The stent's ability to perform these functions has traditionally been evaluated through a combination of computational modeling and in vitro fatigue testing for metal, permanent devices. However, absorbable stents pose unique challenges for conducting these evaluations due to their sensitivity to environmental conditions (e.g., temperature) and their intended degradation. Standard testing strategies for addressing these challenges do not exist and standards that are available for the evaluation of metallic stents have limited applicability to the absorbable counterpart. These challenges result in difficulties obtaining reliable safety and performance information pre-clinically. Therefore, the overall objective of this project was to develop improved testing methods for evaluating the durability of fully absorbable cardiovascular stents while simultaneously simulating degradation of the device. In this project, we developed multiple test setups that allow for durability assessment of absorbable stents and from their implementation, propose tangible strategies for conducting these long term tests so that they can provide reliable safety and performance information. We

studied two test models, tubular stent mimics and generic diamond-shaped stent sub-units, to identify important interactions between mechanical loading and degradation that affect performance. Specifically, when exposed to pulsating flow consistent with in vivo conditions during degradation, tubular absorbable stent mimics degraded at significantly slower rates than that measured for control devices under static conditions. In addition, diamond stent sub-units loaded under tensile fatigue conditions during degradation exhibited different changes in mechanical properties as compared to samples degraded under control conditions. This project directly addressed significant limitations of standard test methods for cardiovascular stents when applied to the emerging absorbable technology. The methods and results generated from this project have enabled CDRH to review incoming data effectively and provide sponsors with meaningful guidance on how to gather relevant non-clinical safety information.

11. Improved Methodologies for Testing and Evaluating Visual Contrast Sensitivity in Clinical Trials for Ophthalmic Drugs and Devices

Drum, Bruce, FDA/CDRH/ODE/DOED; Hilmantel, Gene, FDA/CDRH/ODE/DOED

Plain Language Synopsis: Commercially available tests of visual contrast sensitivity are known for poor reliability and biased results due to subjects' inability to complete all measurements. We describe methods to improve the precision and accuracy of test results and avoid the biases resulting from missing measurements.

Abstract: Visual contrast sensitivity assessment is important for the clinical evaluation of many ophthalmic devices and drugs. It provides visual function information that is not captured by other visual function tests. Unfortunately, commercially available contrast sensitivity tests are known for poor accuracy and reliability. Contrast sensitivity is assessed by finding the lowest contrast needed to detect alternating light and dark bar patterns (sine-wave gratings) of varying bar widths (spatial frequencies). The just-detectable contrast (contrast threshold) is found by choosing the target grating from two or three alternatives. If the grating is undetectable patients still have a 50% or 33% chance of guessing correctly and biasing the result. Also, user instructions allow patients to say no grating is visible, making the threshold dependent on their subjective criteria. Statistical analyses of results are subject to bias and increased variability when some patients fail to see the highest available contrast. Manufacturers recommend imputing arbitrary values for unmeasurable sensitivity values, but this distorts means and variability estimates, rendering them uninterpretable. We propose the following changes: Test Procedures: The patient must complete the test twice, using different versions to prevent response memorization; The patient must choose a stimulus even if the response is a guess; For each spatial frequency, the lowest contrast with both responses correct is defined as the threshold. These changes reduce variability by limiting the probability of a correct guess to 25% for a two-alternative test and 11% for a three-alternative test. Statistical Analysis: Unmeasurable thresholds are designated < the lowest measurable sensitivity; Means that include unmeasurable values are designated < the calculated value, and corresponding variability estimates are designated > the calculated value; Reports of statistical analyses must include the number and % of unmeasurable values; Biased means may be replaced with medians, which are unbiased as long as fewer than half of the thresholds are unmeasurable. These changes increase the reliability of contrast sensitivity test results and minimize the impact of biases arising from unmeasurable thresholds.

12. The Presence of Undeclared Food Allergens in Cumin

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Plain Language Synopsis: In late 2014, millions of dollars of food were recalled due to the presence of undeclared food allergens in cumin. A combination of analytical methods, including mass spectrometry, PCR, species-specific sequencing, Next-Generation DNA Sequencing using universal plant primers, and antibody-based technologies

(i.e., ELISA, Western blot analysis, and a novel xMAP® multiplex assay) were employed to study the presence of food allergens in cumin.

Abstract: Beginning in the autumn of 2014, millions of dollars of food were recalled in the United States for undeclared peanut, attributed to cumin used in the manufacture of the products. These voluntary recalls resulted from contract laboratory analyses performed as a result of a recall requested by the Canadian Food Inspection Agency (CFIA) of a product that tested positive in a survey for undeclared peanut and almond. The contractual laboratory confirmed the ELISA data generated by CFIA and identified cumin as the source of the peanut and almond. In response to the possible health risk to consumers, CFSAN decided to explore in detail the questions raised regarding the presence of undeclared peanut and almond in cumin. A combination of analytical methods, including mass spectrometry, PCR, species-specific sequencing, Next-Generation DNA Sequencing using universal plant primers, and antibody-based technologies (i.e., ELISA, Western blot analysis, and a novel xMAP® multiplex assay) were employed. The antibody-based technologies confirmed the results observed by CFIA and the contractual lab but also indicated the presence of other tree nuts. The DNA-based technologies detected the presence of multiple peanut-derived genes (e.g., Ara h1, Ara h2, Ara h3, and mRNA agglutinin) in addition to other genetic material indicative of possible cross contamination. Global proteomic screening by mass spectrometry identified the presence of peanut-specific peptides from the Ara h 1, Ara h 2, and Ara h 3 proteins. Though all of the analytical methods were unequivocal about the presence of peanut, the differences between the antibody-based methods and the DNA- and mass spectrometric-based methods raised questions regarding secondary sources of inadvertent cross-contamination and the likelihood of homologous, cross-reactive proteins. With the incidence of people with multiple food allergies at approximately 30 % and rising, the ability to ascertain the presence of multiple food allergens and reliably distinguish between homologous, cross-reactive proteins is increasingly important. The use of mass spectrometry, DNA-, and antibody-based technologies provides a multi-faceted approach for addressing this complex problem.

13. Optimization of Label-free Chemiresistive Biosensor for Low Level Detection of E. coli

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Plain Language Synopsis: Optimization of the design and fabrication of flexible, label free biosensor based on chemiresistive technique and the integration to a printed circuit board for a chip level packaging, to be employed in a real-time monitoring system.

Abstract: The Food Safety Modernization Act specifically addresses incorporating technological advances in carrying out the US Food and Drug Administration's (FDA) mission to protect the public. However, even with overall improvements in safety and regulation of food, FDA still conducts considerable numbers of recalls and safety alerts due to foodborne pathogens. Hence, a rapid, cost-effective, and early detection biosensor would be a valuable tool not only for the food industry but also for regulatory bodies. We present new developments in fabrication of a flexible, label free biosensor based on chemiresistive technique for the detection of Escherichia coli (e.g., E. coli O157), responsible for numerous food-borne and water-borne infections worldwide. The biosensor is constructed on a fiber like matrix of melt spun polypropylene (membrane) due to its strength and its high surface area character. It is fabricated by synthesizing chemically sensitive conductive, functionalizable polymeric thin film via oxidative chemical vapor deposition (oCVD) technique to immobilize the analyte detecting molecules. The degree of functionalization versus the bacterial attachment is investigated as well as integration of a wireless monitoring system. To achieve this the membrane was employed to a printed circuit board (PCB) for a chip level packaging. The degree of functionality was carried out by co-polymerizing 3, 4-ethylenedioxythiphene (EDOT) and 3-thiopheneethanol (3-TE) under various monomer ratio (EDOT:3-TE), where the EDOT provides high conductivity and the 3-TE provides the -OH functional groups to be cross-linked with p-maleimidophenylisocyanate for the antibody attachment. Two-point probe measurement

was performed to quantify the sensitivity of the resistive response upon exposure to analyte at systematically varied bacterial concentration. The verification of the functional co-polymer thin films were carried out by Fourier transform infrared (FTIR), UV-visible, X-ray photoelectron spectroscopies (XPS), and by scanning electron microscopy (SEM). And, a higher bonding of E. coli with higher functional groups on the sensor was revealed from Green-light, and SEM. Real time detection and monitoring of E. coli with a lower than 10 cfu/mL level has been measured by Two-point probe in this biosensor configuration.

14. Development and Evaluation of Microarray Assay Suitable for Molecular Epidemiological Surveillance of West Nile Virus

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Plain Language Synopsis: We have developed new tools which are suitable for large-scale analysis of genetic variation of WNV circulating in the U.S. and for monitoring of their detectability by screening assays used to guarantee the safety of blood supplies. The developed methods can be applied for monitoring of other emerging and re-emerging pathogens.

Abstract: Since its first recognition in the U.S. in 1999, West Nile virus (WNV) has become endemic in the Western Hemisphere. WNV is primarily transmitted by mosquito bites, but can also be transmitted by blood transfusion. WNV is estimated to have infected >4 million humans in the US causing >41,000 cases of severe disease and >1,700 deaths reported to CDC. Concerns with the dramatic spread of WNV prompted a detailed investigation of the genetic evolution of WNV in search of causes for its rapid adaptability. Additional concerns were raised by the potential for mutations in the WNV genome to affect the performance of diagnostic and screening assays, viral pathogenesis and therapeutic approaches. Thus, the investigation, identification and detection of variants that may appear in the course of WNV outbreaks are extremely important and can only be achieved by the genomic characterization of new WNV isolates in a timely fashion. To address this need, we have developed a next generation microarray-based assay capable of direct analysis of genetic variation in field specimens. The assay detects mutations by hybridizing biotinylated samples to oligoprobes, covalently immobilized on slides, covering the target region of the WNV genome with subsequent silver staining. Assay evaluation was performed using WNV isolates from the US epidemic seasons, 1999-2013. The new assay detected unambiguously all mutations previously identified by traditional sequencing analysis. In addition the new assay eliminated the need for viral isolation by tissue culture, because viral RNA can be isolated directly from plasma using magnetic beads loaded with specific oligoprobes. The identified mutants can be further sequenced using classical Sanger or next generation sequencing methods to determine position and character of mutations. The use of the described microarray for an initial screening of WNV isolates can notably reduce the time required for analyses of circulating genetic variants as well as sequencing related expenses. We expect that this new assay will be a valuable tool for rapid epidemiological surveillance of WNV during annual outbreaks.

15. A New Approach for the Detection of Nanoparticle-Induced Cytotoxicity In Vitro

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Plain Language Synopsis: Available evidence suggests that nanoparticles (NPs) can interfere with many well-established cytotoxicity assays, resulting in false positive and false negative outcomes. We demonstrate here an alternative approach for assessing NP-induced cytotoxicity in vitro. The method has several advantages and has the potential to be used in high-throughput toxicity screening.

Abstract: Emerging materials utilizing nanoparticles (NPs) are being increasingly introduced into consumer products. Conventional in vitro cytotoxicity assays are often used as initial screens to determine safety for many NP-based materials. Since evidence is available showing that NPs can interfere with conventional in vitro assay mechanisms, there is a need to develop

alternative approaches for the safety evaluation of NPs. We report here a new approach for the in vitro assessment of nanoparticle (NP)-induced cytotoxicity using Fluoro-Jade C (FJ-C), which has been extensively used in in vivo neurotoxicity studies to label dead and dying neurons. Different types of cell cultures including rat brain micro-vessel endothelial cells (rBMVECs), bovine BMVECs, mouse neural stem cells (NSCs), and human neuroblastoma SH-SY5Y cells were examined, and potential cytotoxic effects of silver (Ag)- and gold (Au)-nanoparticles were assessed. NP-induced cytotoxicity was determined by a significant increase in the number of FJ-C labeled cells. In addition to various doses of Ag- and Au-NPs, two well-known toxicants, cadmium and thimerosal, were employed in vitro to induce cell death and served as positive controls. Two common in vitro cytotoxicity assays, lactate dehydrogenase (LDH) and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assays, were also conducted in parallel to the FJ-C labeling using the same batch of cell cultures and the results were compared. Our results suggest that the FJ-C-based approach can provide an accurate assessment of NP-exposure induced cytotoxicity. To further use this new approach, we addressed an important question as to whether Ag ions released from the AgNPs were contributing to their observed cytotoxic effects. We examined using rBMVECs the effects of cell culture media in which AgNPs had been maintained for 24 hrs at 37oC and then removed by ultra-centrifugation. The results of all three assays (FJ-C, LDH and XTT) demonstrated that once AgNPs were removed from the culture media and despite the presence of Ag ions there was no observed cytotoxicity, suggesting that the observed adverse effects were attributed to AgNPs rather than Ag ions. Thus, the new FJ-C labeling approach has the potential to augment if not replace currently used in vitro cytotoxicity assays following NP-exposure.
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16. Comparative Proteomic Analysis of Secreted Excreted Antigens of Three Strains of Trypanosoma Cruzi

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Plain Language Synopsis: We analyzed proteins released by three different strains of the blood parasite *Trypanosoma cruzi*, causing Chagas disease. We identified proteins unique to each strain, which could be associated with disease severity and proteins secreted by all strains, which could be used to develop new diagnostics and/or vaccines for this disease.

Abstract: Trypanosoma cruzi, the blood borne protozoan parasite causing Chagas disease, infects an estimated 10 million people mostly in Latin America and poses a threat to blood supplies in endemic and non-endemic countries such as the U.S. Different strains of parasite are reported to display differences in growth rates, infectivity, tissue tropism, antigenic composition, virulence and morbidity in animal models and susceptibility to chemotherapeutic drugs. Due to the diversity in the clinical expression of infection there have been studies to find biological, biochemical and genetic differences among strains. The identification of proteins secreted by the infective stage of parasite in blood may contribute to a better understanding of the infection process and lead to the development of new diagnostics, drugs and/or vaccines. To that end, we analyzed the secretome of three strains of *T. cruzi*: Columbiana (resistant to benznidazole and of genotype I); 0704 (isolated by hemoculture from a US blood donor and of genotype I); and Tulahuen (genotype VI). In our animal model, C57BL/6 mice showed high susceptibility to the Columbiana strain, with blood parasitemia reaching 1.2x10e6 parasites/ml. The 0704 isolate is less virulent, with maximum parasitemia of 2.8x10e5 parasites/ml and the reference strain Tulahuen results in no detectable parasites in blood by microscopy. Culture supernatants of *T. cruzi* infected NIH-3T3 cells, containing trypomastigote excreted/secreted antigens were collected, concentrated and trypsinized. The resulting peptides were analyzed by quantitative 2D nano-LC MS/MS. Results of proteomic analysis identified 1,438 proteins from Tulahuen; 1,157 from 0704, and 973 from Columbiana. The percentage of proteins having signal peptide and transmembrane motifs were 57.4%, 62.3% and 65%, respectively. Further, the

secretomes of Columbiana and 0704 strains were the most similar (same genotype). This secretome analysis also revealed unique proteins which could represent virulence factors and conserved proteins which could be exploited for diagnostic and vaccine purposes.

17.Development of a New Flow Cytometry Assay for Dengue Serology based on a Protein-Bead Array

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Plain Language Synopsis: This project aims to develop a flow cytometry-based assay that would have high sensitivity and specificity in the detection of anti-DENV antibodies, with the goal of reducing the time elapsed from appropriate diagnosis to implementation of early clinical interventions that minimize the risk of developing severe dengue.

Abstract: Dengue is the most important re-emerging mosquito-borne viral disease worldwide. This disease is caused by infection with any of the four serotypes of dengue virus (DENV) and human exposure to any of them can cause a clinical spectrum that range from a mild, flu-like illness known as dengue fever (DF), to a life-threatening form called severe dengue (SD). SD is potentially fatal, if early supportive therapy is not available, it is observed in endemic areas, and usually follows infection with a different serotype (heterotypic infection). The development of SD has been linked to the presence of pre-existing, non-neutralizing anti-dengue IgG antibodies produced in the first infection. There is no specific treatment for DENV, however, early diagnosis helps with timely initiation of supportive therapy to manage the cardinal symptoms of SD. Serologic tests are needed to confirm cases of dengue but these often take several days to be completed, thus jeopardizing proper patient care. The main goal of this study is the development of a rapid highly sensitive and specific test for dengue infection capable of supporting early diagnosis and able to be more efficacious to expeditiously detect all DENV serotypes. This test is based on Antigen-coupled Beads for the identification of antibodies anti-DENV in plasma and serum samples from infected individuals. For this, we covalently coupled DENV-1 and -4 envelope proteins to polystyrene beads. We standardized the bead-based array using equal volumes of coated-beads mixed with diluted positive specimens in 96-well plates, which after incubation are then washed and secondary antibodies are added. The samples are acquired in FacsCanto instrument and data are analyzed using FlowJo software. At this stage, we are testing the assay performance with confirmed DENV-positive plasma and serum samples from blood donors infected with DENV and the results are promising. The next step is to bind DENV-2 and -3 envelope proteins to polystyrene beads aiming simultaneous detection of anti-DENV IgG antibodies directed against all serotypes in a single clinical sample. Our approach is a protein-bead array for DENV (termed by us DenguePlex) based on flow cytometry that would be a cost effective, comprehensive, single platform for both diagnosis and patient monitoring.

18. Evaluating the Credibility of Computational Fluid Dynamics (CFD)-based-hemolysis Prediction Models using Benchmark Medical Device Geometries

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Plain Language Synopsis: Computational Fluid Dynamics modeling has shown promise in evaluating the risk of hemolysis (red blood cell damage) in blood contacting medical devices. This study evaluates the credibility of different CFD models in predicting hemolysis by comparing simulation results to experimental velocity fields and hemolysis data obtained using benchmark flow geometries.

Abstract: Damage to red blood cells (hemolysis) due to high fluid shear stresses is a safety concern with blood contacting medical devices such as heart valves and blood pumps. To investigate this issue, device sponsors typically perform in vitro hemolysis experiments by recirculating animal blood through their devices in flow loops. In recent years, computational fluid dynamics (CFD) modeling has been used to design and to predict hemolysis potential in medical devices. A variety of blood damage models (power-law, strain-based, and energy dissipation) have been

used by modelers to predict hemolysis. However, no studies have been performed that compare these different types of hemolysis models to one another and to bench experiments. The objective of this study is to use inter-laboratory velocity and hemolysis experimental data obtained in benchmark flow geometries to evaluate the accuracy of CFD predictions. Fluid flow through a benchmark flow nozzle model was simulated using commercial CFD software (Ansys Inc.). Two different hemolysis prediction methods, namely power-law and strain-based models, were evaluated in this study. Blood was assumed to be Newtonian with a viscosity of 3.5 cP and a density of 1056 kg/m3. CFD simulations and bench experiments were performed for Reynolds numbers ranging between 3500 and 8000 in the throat of the nozzle. The SST k-omega computational model (a form of the Reynolds-Averaged-Navier-Stokes [RANS] model) was used to simulate turbulent flow. The model parameters in the RANS equations were adjusted so that the velocity data from the CFD simulations matched that obtained experimentally using quantitative flow measurements After the computational implementation of the hemolysis equations was verified using standard analytical benchmarks such as pipe and Couette flow problems, hemolysis predictions from the CFD simulations were compared to inter-laboratory experimental blood damage results using animal blood flowing through the nozzle. Our preliminary results indicate that both the power-law and the strain-based models failed to accurately predict the experimental hemolysis data for most of the flow conditions. This suggests that for CFD to be successfully used as a regulatory tool, more realistic and accurate hemolysis models need to be developed and validated using in vitro blood damage experiments.

19. Quality Control Metrics Improve Repeatability and Reproducibility of Single-nucleotide Variants Derived from Whole Genome Sequencing

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Plain Language Synopsis: We developed five quality control metrics which were demonstrated to improve both repeatability and reproducibility of genetic variants detected from whole genome sequencing and whole exome sequencing data and can be easily adopted in down-stream association analysis.

Abstract: Although many quality control (QC) methods have been developed to improve quality of SNVs in SNV-calling, QC methods for use subsequent to SNP-calling have not been reported. We developed five QC metrics to improve quality of SNVs using the whole genome sequencing data of a monozygotic twin pair from Korean Personal Genome Project. The QC metrics improved both repeatability between the monozygotic twin pair and reproducibility between SNV-calling pipelines. We demonstrated the QC metrics improve reproducibility of SNVs derived from whole genome sequencing and whole exome sequencing data. The QC metrics are calculated based on the reference genome used in the alignment without accessing the raw and intermediate data or knowing the SNV-calling details. Therefore, the QC metrics can be easily adopted in down-stream association analysis.

20. Oximetry System Performance Assessment with Acetal/POM Phantoms Incorporating Hemoglobin Calibration Standards and Customized Saturation Levels

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Plain Language Synopsis: We have developed an approach for evaluation of near-infrared spectroscopy (NIRS) systems with solid polyoxymethylene (POM) phantoms enclosing hemoglobin solutions of commercially available co-oximetry controls or a cuvette of gradually desaturating hemoglobin solution. Our findings indicate that these approaches may be suitable for basic accuracy testing in NIRS-based devices.

Abstract: Near-infrared spectroscopy (NIRS) has been widely used as an effective non-invasive technology for cerebral oximetry and hemodynamic evaluations. NIR light can penetrate as deeply as a few centimeters, and is altered by

the spectral absorption signatures of absorbing molecules. Analysis of externally detected NIR diffuse reflectance spectra enables quantitative evaluation of endogenous or exogenous chromophores. Clinical acceptance of NIRS devices is enhanced by the fact that devices can be compact, portable, and relatively inexpensive. However, NIRS devices incorporate different light sources, detectors, illumination/collection geometries, and processing algorithms, so performance can vary. Therefore, the development of standardized phantom-based test methods will greatly improve the ability to evaluate device performance in an objective, quantitative manner. International consensus standards for NIRS devices are in the early stages of development. Such standards have the potential to greatly facilitate device development, quality control, clinical quality assurance, as well as regulatory evaluation. Evaluation of devices based on NIRS for detection of hemoglobin (Hb) content and oxygenation have previously involved tissue-simulating phantoms incorporating artificial dyes or flow systems. The purpose of the current project is to develop simple, effective techniques for NIRS performance evaluation with potential to become standard test methods. We have developed and evaluated two techniques based on cuvette inserts in solid turbid phantoms for measuring commercially-available Hb oximetry reference standards and custom-formulated oxy/deoxy-Hb solutions. Both approaches incorporate solid acetal, or polyoxymethylene (POM), a tissue-simulating matrix material proposed in a recent draft standard. As a first step, POM optical properties and their stability over time were characterized using an inverse-adding-doubling approach, and measurements with a spectrophotometer and integrating sphere. Two fiberoptic-probe-based NIRS systems were then used to measure relative differences in oxy- and deoxy-Hb concentration between Hb reference solutions as well as gradual variations in Hb solution saturation after the addition of yeast. Our preliminary results indicated that simple phantom-based approaches based on commercially available polymers and inclusions containing Hb standards, or controlled oxygenation levels, may be useful for benchtop assessment of essential performance in NIRS systems as well as a variety of other biophotonic devices.

21. Development and Characterization of Optically and Acoustically Tunable Tissue-simulating Phantoms for Evaluating Photoacoustic Imaging Systems for Breast Cancer Detection

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Plain Language Synopsis: This project funded by FDA Office of Women's Health involves developing optically and acoustically tunable tissue-simulating phantoms, for quantitative performance assessment of photoacoustic tomography (PAT) systems designed for breast cancer detection. The novel polymer material developed allows more biologically realistic assessment of system performance than prior PAT phantom materials.

Abstract: This project, funded by FDA Office of Women's Health, aims to develop phantom-based method to evaluate an emerging breast cancer imaging technology, photoacoustic tomography (PAT). PA delineates blood vessels based on infrared light absorption by hemoglobin in organs such as the breast. By detecting thermal expansion induced acoustic waves caused by the deposited optical energy, PAT images several centimeters depth with high spatial resolution. PAT has great potential for early breast cancer diagnosis by imaging blood vessels surrounding a tumor and by measuring the increased oxygen extraction from blood in those vessels. However, the lack of a well characterized breast mimicking phantom with realistic acoustic and optical properties has hindered PAT system development and testing. The project goal is to develop breast tissue-simulating phantom material that allows quantitative assessment and comparison of PAT systems. We have developed a new phantom material consisting of polyvinyl chloride plastisol (PVCP) gel and two plasticizers, dipropylene glycol dibenzoate and diethylene glycol dibenzoate. The addition of a carbon-based plastic colorant and titanium dioxide yields optical absorption and scattering similar to human tissue. The optical properties were characterized using spectrophotometry. The acoustic properties were measured using transmission method with two broadband single element transducers with

a center frequency of 7.5MHz. The results show that the newly developed phantom material has tissue relevant acoustic attenuation and a sound speed of about 1540 m/s, overcoming the low sound speed problems (about 1400 m/s) and attenuation reported commercial PVCP reported in PAT literature. Phantoms incorporating blood-filled channels (0.5 mm diameter) covering a range of depths were used to assess differences in system penetration depth for the two types of phantoms. A clinical linear array with a center frequency of 7.5MHz suitable for breast imaging was used as acoustic detector. The penetration depth is 21.6mm in our phantom and 26.2 mm in the commercial PVCP phantom. The extra 20% penetration depth in the latter phantom is due to its lower acoustic attenuation. We conclude that our novel PAT phantoms with breast tissue relevant optical and acoustic properties may enable more accurate assessment of PAT system performance.

22. Size-Dependent Tuning of Horseradish Peroxidase Bioreactivity by Gold Nanoparticles

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Plain Language Synopsis: The overall goal of this research is to develop tools to evaluate the chemical behavior of nanomaterials in vitro. We are evaluating the chemical reactivity of nanomaterials, as a function of size and composition, in model systems that will ultimately help us assess the safety and efficacy of nanomaterials in foods and cosmetics.

Abstract: Molecules with diverse biological functions, such as heme peroxidases, can be useful tools for identifying potential biological effects of gold nanoparticles (AuNPs) at the molecular level. Here, using UV-Vis, circular dichroism, dynamic light scattering, and electron spin resonance spectroscopy, we report tuning of horseradish peroxidase (HRP) bioactivity by reactant-free AuNPs with diameters of 5, 10, 15, 30 and 60 nm (Au-5nm, Au-10nm, Au-15nm, Au-30nm and Au-60nm). HRP conjugation to AuNPs was observed with only Au-5nm and Au-10nm prominently increasing the α-helicity of the enzyme to extents inversely related to their size. Au-5nm inhibited both HRP peroxidase activity toward 3,3',5,5'-tetramethylbenzidine and HRP Compound I/II reactivity toward 5,5-dimethyl-1-pyrroline N-oxide. Au-5nm enhanced HRP peroxidase activity toward ascorbic acid and HRP Compound I/II reactivity toward redox-active residues in the HRP protein moiety. Further, Au-5nm also decreased the catalase- and oxidase-like activities of HRP. Au-10nm showed similar, but weaker, effects, while Au-15nm, Au-30nm and Au-60nm had no effect. Results suggest that AuNPs can size-dependently enhance or inhibit HRP bioreactivity toward substrates with different redox potentials via a mechanism involving extension of the HRP substrate access channel and decline in the redox potentials of HRP catalytic intermediates.

23. Profiling Immunogenicity and Safety of Adjuvanted Pandemic Influenza Vaccine Formulated at Different Dosages in Mice

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Plain Language Synopsis: We use systems biology approach to investigate the immunogenicity and safety profile of adjuvanted H5N1 vaccine formulated in different dosages in a mouse model. We have found that an improper ratio of antigen to adjuvant in a formulated vaccine could not only affect vaccine efficiency but also enhance vaccine reactogenicity.

Abstract: Avian influenza continues to possess imminent threat to the public health. The timely availability of effective vaccines is now an urgent mission for pandemic preparedness. Adjuvants are a group of biological or chemical substances that act to accelerate, prolong, or enhance antigen specific immune responses in animals or humans when used in combination with an antigen. The antigen sparing effect of adjuvants is especially attractive since this property could potently make more vaccines available in a short time. However, an improper ratio of antigen to adjuvant may adversely affect vaccine efficiency as well as increase vaccine reactogenicity. Systems biology is a new approach to systematically monitor host immune responses and vaccine

reactogenicity. The current study was designed to investigate the immunogenicity and safety profile of an MF59-like adjuvanted H5N1 vaccine in a mouse model using systems biology approach. Mice were intramuscularly administered with H5N1 monovalent vaccine formulated with an MF59-like adjuvant at various dosages. Antibody development, cytokine levels and T/B cell profiling were monitored at different time points. Administration of H5N1 vaccine formulated at different dosages of adjuvant resulted in no difference in antigen specific antibody titers. However, mice administered with H5N1 vaccine formulated in a higher dosage of adjuvant generally showed enhanced levels of inflammatory cytokines than those immunized with the vaccine formulated in a lower dosage of adjuvant. Immunization of H5N1 vaccine formulated in a higher dosage of adjuvant promoted the migration of CD4+ T cells in the blood circulation while had no impact on CD8+ T cells. It appeared that administration of H5N1 vaccine formulated in a higher dosage of adjuvant led to reduced circulation of B cells. Overall the migration of both CD4+ and CD8+ T cells into the lung was reduced in mice immunized with H5N1 vaccine formulated in a higher dosage of adjuvant. Our data suggest the ratio of antigen to adjuvant affects lyM.P.H.ocyte trafficking. Optimizing the ratio of antigen to adjuvant in a formulated vaccine is critical for mounting robust immune responses against pandemic influenza.

24. Development of a Reference Virus Database (rVDB) to Enhance Next-generation Sequencing Data Analysis for Evaluating Safety of Biologicals

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Plain Language Synopsis: Adventitious viruses are the most important safety concern in biologicals. The use of novel cell substrates, such as tumorigenic and tumor-derived cell lines, has introduced a greater challenge for detecting unknown and unexpected viruses. Advanced virus detection technologies, such as next-generation sequencing, are a promising tool for broad virus detection.

Abstract: Background: Next-generation sequencing technologies are a promising tool to detect known and novel viruses for cell substrates and product safety. However, the bioinformatics analysis is a challenge since currently available databases are redundant, not properly annotated, or not inclusive of all virus and virus-like sequences. This gap was recognized as a priority unmet need by the Advanced Virus Detection Technologies Interest Group (AVDTIG), which is comprised of scientists from industry, government agencies including regulatory, academia, and technology service providers. Therefore, our laboratory, in discussions with the AVDTIG members, undertook to create a new viral sequences reference database that would be less redundant and include all virus and virus-like elements (e.g. retrotransposons). Methods: Initially, a list of keywords was generated to search GenBank for "positive selection" of all virus-related and virus-like sequences. The selected sequences were then tagged based upon "negative selection" words to identify cellular and non-viral elements for further manual examination. The chosen viral-related entries were clustered based on percent identity to reduce redundancy. Results: Our database includes virus, virus-related, and virus-like sequences, representing complete virus genome and partial sequences, including endogenous retroviruses. The database is still being tested for further refinement and development. The current version is being tested for accuracy and robustness. We found that it captures sequences that would have been lost since they were misannotated with host taxonomy. It also runs significantly faster than interrogating the entirety of GenBank sequences due to absence of host cell sequences. We have found the most efficient format to present the sequence data and the ideal clustering method and percent identity cut-off to reduce redundancy without loss of unique sequences. Conclusion: We have designed a maintainable reference viral sequence database (rVDB), which after initial testing, will be made public through NCBI to allow scientists to perform more rapid NGS data analysis. The database is meant to be updated monthly to include new viral sequences added to GenBank. The availability of the database will facilitate NGS investigations of biologicals for "unwanted" viruses with increased efficiency. Further accurate annotation will produce more confident and relevant results of NGS data analysis.

25. Oxygen Levels Regulate ADAMTS13 Expression and Function in vitro and in Children with Congenital Heart Disease

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Plain Language Synopsis: Children with cyanotic heart disease, while long recognized for their predisposition toward of clot formation and thrombotic complications, have few targeted prophylactic options and there remains an incomplete mechanistic understanding for their thrombophilia. Here, we investigated whether low oxygen tension-- a clinical feature of cyanotic heart disease--might directly regulate the cellular secretion of ADAMTS13, an emerging type of anticoagulant recently shown to be deficient in this population. We found ADAMTS13 to be directly modulated in a dose and time dependent fashion by environmental hypoxia.

Abstract: Background: Patients with cyanotic heart disease are recognized for their remarkable predisposition for stroke and thrombosis, but there currently is a lack of mechanistic insight and targeted prophylactic intervention. It was recently suggested that the Von Willebrand Factor cleaving protease ADAMTS13, an emerging biopharmaceutic anticoagulant, may be deficient in this population. Seeing that hypoxia is a prominent clinical feature of patients with cyanotic heart disease, we investigated the impact of environmental hypoxia on the secretion and proteolytic activity of ADAMTS13 both in vitro and in vivo. Methods and Results: In a cohort of patients (n=24) with congenital heart disease and varying levels of cyanosis, a positive correlation (p=0.023) between plasmatic ADAMTS13 activity and blood oxygen saturation within the central venous system was identified. Based on this observation, we exposed human cell lines expressing ADAMTS13 to normobaric hypoxia and carried out expression and activity measurements. We found the extracellular expression and activity of ADAMTS13 to be significantly reduced at 4, 8 and 24 hours in an oxygen dependent manner. Hypoxia-induced modification to ADAMTS13 secretion was fully reversible upon re-oxygenation. Rats maintained under hypoxic conditions similarly showed a decrease in circulating VWF-cleaving activity (99.4% vs 70.0%, p=0.009). Additionally, the ADAMTS13 molecule synthesized under hypoxia shows decreased thermostability, suggesting that in addition to impaired cellular secretion, the protein may be physically altered. Interestingly, in vitro experiments with hypoxia mimetics demonstrated that changes in secretion during hypoxia are independent of the transcriptional effect of HIF-1α. Conclusions: These results provide evidence for the modulation of ADAMTS13 by hypoxia. This finding has potential clinical implications for patients with low hemoglobin oxygen saturation who may have deficient plasmatic ADAMTS13 function.

26. Quantification of Steady-State Temperature Rise within the Eye due to Ultrasound Insonation

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Plain Language Synopsis: The Soft-tissue Thermal Index (TIS) is related to the potential for temperature rise in soft tissues in the body due to ultrasound exposure, but the TIS is inaccurate for ophthalmic exposure. To address this safety concern an experimental study of temperature rise within the lens due ultrasound exposure was performed.

Abstract: The CDRH-recognized Output Display Standard (ODS) defines acoustic output indices that are available for display so that the operator can monitor and adjust the ultrasound exposure level. One of these indices, called the Soft-tissue Thermal Index, or TIS, is related to the potential for temperature rise in most soft tissues in the body. However, because of the unique properties of the eye such as high absorption of ultrasound in the lens and orbital fat, and no cooling blood supply to the lens, vitreous or aqueous humor, the TIS is inaccurate for ophthalmic exposure. To address this potential safety issue, FDA has placed

restrictions in its regulatory ultrasound guidance on the available output levels for ophthalmic exams. Based on a theoretical study, the global maximum dreated acoustic intensity (ISPTA.3) is < 50 mW/cm2. Although the model and calculations are more exact than those used to derive the TIS, the results still are based on a simplified model. An experimental assessment of this limit needs to be undertaken to ensure safe use while not restricting clinical utility. To this end, an ex vivo experimental study of temperature rise within the porcine lens due ultrasound exposure was performed. Using the interpolated value at this intensity and measured under steady state conditions, the average temperature rise and the maximum temperature rise over twenty samples were 0.09°C and 0.23°C respectively. A 1°C temperature rise was not obtained until an ISPTA.3 of approximately 320 mW/cm2 was used. This current data, alongside in situ intensity values may be used to support the current FDA ultrasound guidance.

27. Longitudinal Stability and Reliability of Optogenetic Neuromodulation In Vivo

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Plain Language Synopsis: Optogenetics has the potential to drive the development of new classes of therapeutics by combining genetics and optics to control well-defined events within specific cells. We are studying the reliability and stability of these neuromodulation methods. This understanding is critical to translational application of this technology.

Abstract: The term “optogenetics” refers to a rapidly expanding toolkit, comprising both device and biologic components, for using light to manipulate cellular function at the molecular level. Optogenetics is revolutionizing the study of brain circuits, and is driving the development of new classes of therapeutics. We are employing optogenetics to study the health of neurons near implanted devices as well as the general reliability and stability of these neuromodulation methods. Such topics are under-represented in the scientific literature, but are critical to translational application of this biotechnology. Here we report on an in vivo optogenetic assay to detect changes in neuronal function near implanted microelectrode arrays. Single-shank optrodes (NeuroNexus) were implanted into the motor cortex of adult male B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J mice. Following one week recovery, weekly recordings were performed from awake mobile mice. Each recording consisted of 10 minutes of spontaneous activity, followed by optical stimulation (473 nm, 10 mW power) with 5 second trains (5, 10, 20, 50, and 100 ms pulses at 1, 5, 10 and 40 Hz). Recordings were made for 7-20 months while spontaneous and optically-evoked activities were quantified to assay for the presence of viable neurons. Enhanced multiunit activity was detected in response to chronic optical stimulation. At early time points, optically driven spiking persisted for 5-10 ms after stimulus onset; longer pulses did not drive activity for the duration of the pulse. In contrast, after many weeks, neurons were able to sustain firing up to 100 ms. This effect was frequency-dependent and not observed for all stimulation paradigms. These data suggest that neuronal circuitry is altered proximal to chronically implanted optogenetics systems. Published reports differ on the histologic effects on neurons near implants, and very little data exists which can distinguish between potential cellular toxicological effects and circuit remodeling effects. Work is ongoing to dissociate the contributions of multiple potential causes of these changes. Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

28. Benchmarking Assessment of Open Source and Newly Released Salmonella Mutagenicity (Q) SAR Models for Potential Use Under ICH M7

Stavitskaya, Lidiya, FDA/CDER/OTS/OCP; Minnier, Barbara, FDA/CDER/OTS/OCP; Kruhlak, Naomi, FDA/CDER/OTS/OCP

Plain Language Synopsis: Under the ICH M7 regulatory guideline, pharmaceutical sponsors may submit (Q)SAR model predictions for bacterial mutagenicity to FDA/CDER to qualify

drug impurities as non-mutagenic. The guideline, however, does not specify the use of any particular model and requires that FDA/CDER pass judgment as to whether a given model is fit-for-purpose. This poster describes the evaluation of five open source and commercially-available (Q)SAR models with which FDA/CDER had no prior experience to assess the quality and reliability of data generated by them.

Abstract: The current draft of the International Conference on Harmonisation (ICH) M7 guideline describes the use of (quantitative) structure-activity relationship ((Q)SAR) models during drug safety evaluation. The guideline specifies that an expert rule-based methodology and a statistical-based methodology should be applied and the results should be reviewed with expert knowledge to provide additional supportive evidence on the relevance of the prediction. The guideline, however, does not specify the use of any particular model, but instead recommends that the models meet the general definition of statistical or rule-based methodologies, and allow the identification of structural alerts. In our previous studies, we reported the construction of QSAR models using commercial software with prediction accuracy ranging from 82% to 84% in cross-validation and 73% to 76% in external validation. More importantly, when the models were applied in combination, sensitivity of 91% and negative predictivity of 79% was achieved, which are key parameters in the use of these models under ICH M7. In this study, we evaluated the performance of two freely-available, open source (Q)SAR programs, Toxtree and OECD Toolbox and three newly-released, commercial (Q)SAR programs, Leadscope Expert Alert System, Sarah Nexus, and Symmetry as potential candidates for qualifying pharmaceutical impurities. To effectively assess their performance, an in-house Salmonella mutagenicity database of 3979 compounds and a highly-curated version of the Hansen dataset of 3700 compounds were used for benchmarking. These sets are comprised of drug-like and industrial chemical examples containing a variety of functional groups as well as less-common atoms. These assessments will improve and facilitate the ability of FDA/CDER to interpret the quality and reliability of in silico data submitted under ICH M7 for the qualification of pharmaceutical.

29. Importance of Comprehensive Reporting of (Q)SAR Assessments Under ICH M7

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Plain Language Synopsis: Under the ICH M7 regulatory guideline, pharmaceutical sponsors may submit (Q)SAR model predictions for bacterial mutagenicity to FDA/CDER to qualify drug impurities as non-mutagenic. This poster describes the general processes, expert analysis steps, and reporting formats that could be used by sponsors for documenting comprehensive (Q)SAR assessments performed in accordance with ICH M7.

Abstract: Pharmaceutical synthesis involves the use of reactive chemicals, such as reagents and intermediates, which often continue to be present at low levels in a finished drug substance and have the potential to present a mutagenic risk to patients. Regulatory agencies recommend that these impurities be controlled to an acceptable level (e.g., Threshold of Toxicological Concern) or shown to lack mutagenic potential. Mutagenicity assessment may be performed through an empirical Ames test or, under the newly finalized International Conference on Harmonisation (ICH) M7 guideline, through the use of two complementary (Q)SAR methodologies. Under ICH M7, pharmaceutical sponsors are submitting (Q)SAR model predictions for bacterial mutagenicity to regulators to qualify impurities as non-mutagenic. Historically, the content of these submissions has varied from a simple, single sentence conclusion to an in-depth discussion of the predictions with supporting raw model data. Although the content of a (Q)SAR report may differ depending on the complexity of the prediction, all reports should ideally include key information such as the version of software and models used, raw model predictions, and an explanation of any conclusions that are based on expert interpretation of the (Q)SAR data, particularly if those conclusions differ from the raw model output. Expert interpretation, or the application of expert knowledge, can enhance the overall accuracy of predictions. Specific examples of expert knowledge include verifying that the test

chemical is within a model's applicability domain, confirming that there is an adequate number of relevant training set examples for a structural alert, and examining structurally analogous chemicals with known empirical data from external sources. This poster will describe the general processes, expert analysis steps, and reporting formats that could be used for regulatory (Q) SAR assessments under ICH M7 through the presentation of non-proprietary examples.

30. Examining the Effect of Pulse-Oximeter Alarm Settings on Alarm Fatigue in an Intensive Care Unit Patient Population

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Plain Language Synopsis: The pulse oximeter is one of the primary sources of alarms contributing to hospital alarm fatigue, a top medical device hazard. We analyzed the effect of varying pulse oximeter alarm settings on alarm rates in an ICU patient population to understand the need for personalized settings and 'smart' alarm algorithms.

Abstract: Background: In acute hospital settings there can be up to 350 alarms per patient per day, which leads to caregiver desensitization to alarms and alarm fatigue. Alarm fatigue has been listed as a National Patient Safety Goal from the Joint Commission and the top medical device hazard by ECRI Institute. The most frequent alarms are for low oxygen saturation (SpO2). A 90% oxygen saturation threshold with a 10 second delay (i.e., saturation needs to be below 90% for at least 10 consecutive seconds) is generally used as a default setting for alarming a pulse oximeter. There is limited evidence to support these settings as optimal. We retrospectively examined pulse oximeter alarm setting effects on alarm rates in an intensive care unit (ICU). Methods: We conducted an analysis of 962 previously collected ICU patient records containing SpO2 data sampled at 1 Hz for at least 1 hour (44,900 hours of SpO2 recordings) obtained from the Multiparameter Intelligent Monitoring in Intensive Care II Database collected from Beth Israel Deaconess Medical Center, Boston, MA. We simulated SpO2 alarm rates by applying different oxygen saturation thresholds (84%, 86%, 88% and 90%) and time delays (10-50 seconds) as alarm triggers. We analyzed variability of alarm frequency over an entire ICU stay for records with a minimum of 12 hours of SpO2 data. Results: By modifying the alarm settings from the 90% SpO2 threshold and 15 second delay to 88% SpO2 threshold and 25 second delay, the overall simulated alarm rate decreased 62% (from 0.93 alarms/hour to 0.35 alarms/hour). A limited number of patient records accounted for most alarms; specifically, 10% of patient records were responsible for 57% of alarms at the 90%-15 second delay and 67% of alarms at the 88%-25 second delay. The percentage of patient records with alarm rates greater than 1 alarm/hour for at least 50% of their ICU stay dropped from 17% (90%-15 second delay) to 4.6% (88%-25 second delay). Conclusion: These findings support that personalized alarm settings are needed for pulse oximeters used in ICU populations. However, by lowering the alarm sensitivity, there is greater risk to responding timely to true alarms.

31. Whole Genome Sequences of Epstein-Barr Viruses Associated with Burkitt LyM.P.H.oma Tissues

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Plain Language Synopsis: We reported nine newly sequenced EBV genomes from primary South America and West Africa Burkitt LyM.P.H.oma (BL) biopsy tissues and analyzed the sequences diversity on a whole-genome level among these EBVs. BL-specific variations pattern in LMP-1 were identified in EBVs of both West Africa and South America BL tumors.

Abstract: Epstein-Barr virus (EBV), first discovered from Burkitt's lyM.P.H.oma (BL), is a class 1 carcinogen that is now associated with a wide range of hematologic and epithelial cancers, including lyM.P.H.oma nasopharyngeal carcinoma (NPC), gastric cancer, Hodgkin lyM.P.H.oma and some AIDS-associated B cell lyM.P.H.oma. Although almost all BL cases from Africa and NPC in China are EBV-positive, consistent with a direct role of EBV in tumor causation, the precise nature of the mechanisms of causation remains to be

elucidated. The goal of this study is to obtain a comprehensive assessment of EBV genomes in BL tissues, and to determine how these sequences differ from EBV genomes in NPC tumors, matched non-tumor reservoir of same individuals and from EBV genomes in healthy individuals from same regions. We conducted non-biased, high-throughput genomic sequencing of four South America BL samples and five West Africa BL samples. EBV genomes in the BL biopsies were assembled and analyzed using the WT-EBV (type 1) as a reference. Average EBV genome copies ranging from 27.8 to 95.4 per cell were found in the examined BL tissues. All of the 9 BL-EBV genomes belong to Type I EBV and showed little evidence of strain heterogeneity within each tumor in consistent of monoclonal nature of BL tumor. Phylogenetic analysis with all published EBV genomes revealed EBVs from 8 out of 9 BL tumors, except VGO, were closely related and clustered together as a group utterly separated away from those of NPC-associated EBVs. There were considerable variations in genomes of BL-EBV ranging from 517-1160 variations when compared with the genome sequence of WT-EBV. Most variations found were single nucleotide variations (SNVs) with as much as one third of the variations resulted in amino acid changes. Non-synonymous variations highlighted 2 hyper-variable coding regions in EBNA-1 and LMP-1 genes. There was a unique pattern with consistent 9 combined point mutations/variations observed in LMP-1 N-terminus of 8 BL-EBVs (except VGO) irrespective of geography. Our genomic sequencing study of BL tumor biopsies could facilitate the discovery of gene variations with pathogenicity significance in BL-associated EBVs.

32. A Study of the Immune Response to Treatment with Fc-fusion Drug Using a Mouse Model of Hemophilia B and Recombinant Fc-fusion FIX

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Plain Language Synopsis: We compared the immune responses of rFIX or rFIX-mFc fusion-protein in a Hemophilia B mouse model. The rFIX induced a higher IgE response than rFIX-mFc. High IgE levels can lead to anaphylaxis, known to be a rare but severe adverse event associated with the treatment of Hemophilia B patients.

Abstract: Bioengineered proteins are rapidly becoming the norm in drug development. The fusion of a therapeutic protein with the Fc-region of an antibody is a platform technology that is used to increase the circulating half-life of protein and peptide therapeutics. Two such recombinant fusion proteins, coagulation factor VIII Fc (rFVIIIFc) and factor IX Fc (rFIXFc) were recently approved by FDA. The primary purpose of developing the Fc fusion proteins was to extend the circulating half-life resulting in less treatment burden to patients, who would benefit from fewer infusions. However, bioengineered proteins could have consequences with respect to immunogenicity. Using a mouse hemophilia B (HB) model we compared the immune responses to infusions of the recombinant human FIX (rFIX) and a recombinant chimeric human FIX - mouse Fc (hFIX-mFc) fusion protein. HB mice received weekly IV injection of ~3.6 nmole/kg of rFIX or hFIX-mFc for 5 weeks. Anti-FIX IgG and inhibitory Ab were measured for all treated and untreated mice. In addition, levels of IgE and cytokines were also tested at the end of the treatment. Splenic cells were used to perform ex-vivo studies to determine FIX-specific T and B cell functional and memory responses. Our results showed that, following IV treatment with rFIX or hFIX-mFc, there are no statistically significant differences in the titers of anti-FIX IgG Ab. However, a significantly larger proportion of mice treated with rFIX developed elevated titers of total IgE which correlated with a Th2 bias following rFIX. In contrast, hFIX-mFc induced a Th1-like response and no increase in IgE levels. Our study suggests that in mice hFIX-mFc fusion protein promotes a Th1-like response and is less likely than rFIX to induce an IgE response, which may lead to anaphylaxis, a rare but severe adverse event associated with the treatment of HB patients.

33. Single Lab Validation of 13-plex Luminex Suspension Array to identify 11 Clinically Relevant STEC O serogroups, eae and aggR Virulence Factors

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Plain Language Synopsis: Here we present data to support adoption of a method modification to the 11-plex Luminex Suspension Array described in BAM chapter 4a. The addition of 2 important virulence factor targets will improve FDA's ability to distinguish between pathogenic STEC and STEC that are not likely to cause disease.

Abstract: BAM chapter 4a section R describes a Luminex array to identify the 11 most clinically relevant STEC O serogroups (O26, O45, O91, O103, O104, O111, O113, O121, O128, O145, and O157). While O serogroup identification is important because of the high correlation of some serogroups with illness, serogroup information alone may not be sufficient to make regulatory decisions. STECs require adherence factors to attach to host cells to cause severe illness, and therefore, presence of other virulence factors are also needed to better indicate pathogenic potential. The Luminex suspension array was therefore modified to include two additional virulence factor targets (eae and aggR) to comprise a 13-plex assay. The new 13-plex Luminex suspension array will improve differentiation of pathogenic STEC from non-pathogenic STECs without adversely affecting O serogroup identification nor requiring any additional time, effort, or training. Preliminary data from two laboratories (SFL and CFSAN) have shown accurate O serogroup identification and eae/aggR presence in 12 ATCC reference strains representing the 11 STEC O serogroups, E. coli strains representative of Enteroaggregative E. coli (5 strains), and 15 eae allelic variants (18 strains). This method modification involves minimal change to the original validated method, only the amount of primer mix used in the PCR reaction, settings on the Bio-plex instrument, and preparation of primer mix and 100x bead pool (most likely prepared in a commercial kit). Here we present data from a single lab validation study in one additional ORA laboratory (PRL-NW). 22 blind pure cultures (11 STEC O serogroups, 4 eae positive strains, 4 aggR positive strains, and 3 non-toxigenic E. coli) were analyzed.

34. Global Crisis Response at the Forensic Chemistry Center: Strategies, Challenges, and Innovations When Outbreaks Occur Half a World Away

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Plain Language Synopsis: FDA's Forensic Chemistry Center has provided key analytical findings during many outbreaks around the world involving illness, injury, and death related to foods and drugs. Some case studies will be used to highlight the importance of various aspects of effective crisis response from the analytical laboratory perspective.

Abstract: Over the last two decades, the Forensic Chemistry Center (FCC) has been asked to provide laboratory support to the investigation of multiple outbreaks across the globe involving illness, injury, and death related to foods and drugs. Partnering with the Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and other U.S. and international agencies, FCC has played a critical role in these investigations. The analytical challenges presented by each crisis are as diverse as their geographic origin. From diethylene glycol-contaminated cough syrup in Panama to melamine-tainted pet food raw materials from China, effective response requires a broad array of analytical tools and strategies. Various targeted and non-targeted screening methods have been developed at FCC and used extensively for these types of forensic examinations. A recent incident involved the death of 73 people and hospitalization of dozens more in the village of Chitima, Mozambique. The victims had consumed varying amounts of pombe, a traditional homemade beer made by repeated boiling/reducing of a corn, water, and flour mixture with sugar or yeast to speed fermentation. Symptoms included vertigo, asthenia, dyspnea, vomiting, and diarrhea but were not suggestive of a particular etiology. The challenges involved with this crisis will be used to highlight the importance of various aspects of response including sample provenance, availability of controls, selection of methods, and background investigation.

35. Top-Down Mass Spectrometry for the Identification of Serovar Specific Salmonella Protein Biomarkers

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Plain Language Synopsis: Bacterial detection methods based on instrumental analysis of chemical signatures (mass of biomarkers) from bacteria (proteins, DNA, lipids, and metabolites) by mass spectrometry are sensitive, specific, and flexible; a single instrument can be used for many targets at one time and new bacterial targets can be easily accommodated.

Abstract: to facilitate typing, epidemiology, and trace-back of Salmonella contamination in the food supply, a minimum of serovar level differentiation is required. Bacterial detection methods that require selection of probe based assays are limited by probe selection. The probe can only detect what it was designed to target. Intact protein mass spectrometry (MS) of bacterial protein lysates offers an inherently multiplexed measure of the mass of soluble proteins in their intact state. This non-targeted MS based method provides a high-throughput and relatively unbiased snapshot of the expressed proteins in a wide range of bacterial samples and is amenable to both screening and targeted analysis. Because a broad range of protein masses are measured in a single experiment, this technique facilitates the differentiation of closely related bacteria as well as the detection of unsequenced nonsynonymous single-nucleotide polymorphisms (SNPs) and plasmid proteins that may be specific to a given strain, with no need for prior knowledge of the bacteria. The masses that are determined to be different between strains are subsequently identified by top-down mass spectrometry. Because the mass of the intact proteins are measured, a sequenced genome is not required for identification specific biomarker proteins. The strength of our novel top-down MS platform is that it can rapidly identify novel combinations of strain specific protein biomarkers for bacterial identification without the need for prior genome sequencing or probe selection. Identification and validation of biomarkers at an expressed protein level is integral to rapid screening development and to determining which proteins are subject to environmental pressure. An additional advantage of a top-down mass spectrometry based platform for analysis of bacterial proteins is that we identify protein post-translational modifications that may be relevant to functional changes that occur to proteins present in different environments. A variation of this experiment based on MALDI-MS has recently received FDA approval as a clinical diagnostic device. However, that method is not able to achieve identification of bacteria beyond the sub-species level. The presented top-down mass spectrometry platform represents a significant step forward in specificity for differentiation of closely related bacterial serovars by MS.

36. Rapid Identification and Quantification of an Adulterant Oil in Extra Virgin Olive Oil

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Plain Language Synopsis: A difficult issue facing FDA and other regulatory agencies has been the relative ease to adulterate extra virgin olive oil (EVOO) with lower-grade olive oils or seed or nut oils while meeting the physical and chemical property limits of various established standards. To date, there is no screening method that can rapidly authenticate EVOO, identify the nature and quantify the concentration of an adulterant in commercial EVOO products. The proposed rapid (< 5 min) near infrared and chemometrics methodology has successfully been used to identify and quantify an adulterant in commercial EVOO products.

Abstract: While FDA has jurisdiction over deceptive label declarations often found with adulterated extra virgin olive oils (EVOO), the Agency is also mandated with the protection of the US public against the intentional adulteration of foods. A new rapid (<5 min) and novel Fourier transform near infrared (FT-NIR) spectroscopic procedure and chemometric analysis are described to screen

for the authenticity of EVOO and to determine the nature and amount of an adulterant oil (e.g., soybean oil, hazelnut oil, palm olein, or refined olive oil) in EVOO. To identify the type and determine the quantity of an adulterant, gravimetric mixtures were prepared by spiking an EVOO with different concentrations of each adulterant. Based on FT-NIR spectra, four partial least squares (PLS) calibration models were developed for four specific groups of adulterants each with a characteristic FA composition, namely high-linoleic acid (LA) oils, high-oleic acid (OA) oils, palm olein (PO), or refined olive (RO) oils. Using these different PLS calibration models for prediction, plots of predicted vs. gravimetric concentrations of an adulterant in EVOO yielded linear regression functions with four unique sets of slopes, one for each group of adulterants. Four corresponding slope rules were defined that allowed for the determination of the nature and concentration of an adulterant in EVOO products by applying these four calibration models. The standard addition technique was used for confirmation.

37. Simple Functionalization Strategies for Enhancing Nanoparticle Separation and Recovery with Asymmetric Flow Field Flow Fractionation

Mudalige, Thilak, FDA/ORA; Qu, Haiou, FDA/ORA; Sánchez-Pomales, Germarie, FDA/CDRH; Sisco, Patrick, FDA/ORA; Linder, Sean, FDA/ORA

Plain Language Synopsis: Engineered nanoparticles are used in a large number of consumer products. To evaluate the quality of these products, methods for the characterization of nanoparticle size and size distribution are essential. Here we developed a method to improve resolution and recovery of a size based separation technique.

Abstract: Due to the increasing use of engineered nanomaterials in consumer products, the US Food and Drug Administration has determined that the development of robust, reliable, and accurate methodologies to characterize nanoparticles in consumer products is needed to ensure that the agency is ready to regulate these products. It is well documented that adsorption, biodistribution, metabolism, excretion, and toxicity of nanoparticles are dependent on their size, thus the determination of size and size distribution are essential for complete characterization. Furthermore, size-based separation techniques are preferred for those samples consisting of nanoparticles with a wide size distribution or multimodal populations. Methods that can separate and determine the size of nanomaterials in samples that contain polydispersed and/or multimodal nanoparticle populations are of particular interest. Asymmetric-flow field flow fractionation (AF4) has shown promise for the separation of nanoparticles with wide size range distributions; however, low analyte recoveries and decreased membrane lifetimes, due to membrane fouling, have limited its application. Herein, we report a straightforward strategy to minimize membrane fouling and improve nanoparticle recovery by functionalizing the nanoparticle surface, as well as that of the AF4 membrane. Gold nanoparticles (AuNP) were stabilized through functionalization with a phosphine molecule, whereas the surface of the membrane was coated with a negatively charged polystyrenesulfonate polymer. Improved nanoparticle separation was demonstrated by analyzing AuNP reference materials of different sizes (e.g., 10, 30, and 60 nm), obtained from the National Institute of Standards and Technology (NIST). We were able to obtain recoveries of 99.1 (±0.5) %, and detection limits as low as 6 µg/kg using this method. Furthermore, the stability of the polymer coating and its specificity toward minimizing membrane fouling were demonstrated. The method reported in this study is a robust size based separation technique with excellent recovery and resolution (Anal. Chem., 2015, 87 (3), pp 1764–1772).

38. Correlation Between In-vitro and In-vivo Corrosion in Cardiovascular Stents

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Plain Language Synopsis: The objective of this study is to correlate bench corrosion resistance of a medical device with in vivo corrosion. By studying devices manufactured with different

processes, the results of this study provides enhanced understanding of manufacturing effects on performance and will improve the acceptance criteria for corrosion resistance measured non-clinically.

Abstract: There is a public health need to understand the effects of corrosion on nickel containing medical devices due to the potential loss of mechanical integrity and nickel toxicity/sensitization. This is particularly important for Nitinol devices where nickel is approximately half of the elemental composition and its corrosion resistance is highly dependent on manufacturing. A recent FDA public workshop identified the lack of data correlating proposed acceptance criteria for a device's corrosion resistance from in vitro tests to evidence of corrosion in vivo as a barrier to timely review and approval of devices. This study directly addresses this knowledge gap by studying corrosion resistance, both in vitro and in vivo, of Nitinol cardiovascular stents manufactured with different processes. The overall goal of this project is to assess whether current non-clinical corrosion testing correlates to observed in vivo corrosion. In this study, four groups of Nitinol stents of a generic design were manufactured by one of four prescribed processes, each resulting in a different surface type. Each group has distinct corrosion resistance ranging from low (not acceptable) to excellent (acceptable) resistance. Stents from each group were evaluated for their corrosion resistance and tendency to leach nickel in vitro. In vitro results confirm that the different manufacturing processes produce stents with different corrosion resistances ranging from low to excellent and demonstrate that the degree of nickel leaching out of the device is dependent on manufacturing process. Stents with the lowest corrosion resistance yielded nickel leach values that were moderately high as compared to all other groups, while stents with 2 different surface types and excellent corrosion resistance were found to yield both low and high amounts of nickel leaching. Additional stents from each of the four groups were implanted into the iliac artery of minipigs for 6 months and then explanted. Interim Scanning Electron Microscopy image analysis of explanted stents suggests evidence of in vivo pitting corrosion that was dependent on the surface type. Combined, these data provide enhanced understanding of the effect of manufacturing on corrosion properties and generate data that will improve the acceptance criteria for corrosion resistance.

39. Effects of a Proline Endopeptidase on the Detection and Quantification of Gluten during the Fermentation of Beer

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Plain Language Synopsis: Antibody- and mass spectrometric-based methods were used to detect and quantify hydrolyzed gluten during beer fermentation. Reduction in gluten concentrations was observed as fermentation proceeded with additional losses generated by adding proline endopeptidase, an enzyme marketed as a method to degrade gluten.

Abstract: Celiac disease is an immune-mediated enteropathy caused by the ingestion of gluten proteins. Currently, the only effective treatment for celiac disease is to follow a strict gluten-free diet. In 2013, FDA required food bearing the labeling claim "gluten-free" to contain less than 20 ppm gluten. It was also recognized that fermented and hydrolyzed foods lack a scientifically valid method to comply with this regulation. In this study, the reliability of antibody-based methods to detect and quantify gluten during the fermentation process associated with beer production was evaluated. Further, we evaluated the effect of proline endopeptidase (PEP), an enzyme marketed to degrade immunopathogenic sequences suspected of causing celiac disease, on the detection and quantification of gluten. Sorghum beer containing 0, 20, and 200 µg/mL (ppm) wheat gluten was brewed, with or without the addition of PEP at the start of fermentation. Samples collected throughout the brewing process were analyzed by five ELISAs and western blot. The final product of the 20 and 200 ppm beer samples displayed a reduction in the detectable gluten concentration by 80% and 66%, respectively. PEP treatment increased the loss of gluten to 99% and 98%, respectively. Western blot analysis confirmed the results of the ELISA. Mass spectrometry was used to investigate the profile of hydrolyzed gluten peptides in the beer samples. Using a global

proteomics approach, numerous wheat gluten peptides were detected in the final product of the 20 and 200 ppm beer. Research is underway to detect and characterize hydrolyzed gluten peptides throughout the fermentation process, determine whether immunopathogenic epitopes are present, develop a method to assess the extent of protein hydrolysis, and quantify gluten subjected to fermentation and hydrolysis.

40. Establishing Optimal Characterization Methods for Nanosized Generic and Brand Drug Comparison

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Plain Language Synopsis: Techniques for measuring nanomaterials have vague limitations on which types and sizes of materials they can measure. Our study evaluated a number of complex drug formulations with several methods and outlined their practical limitations and pitfalls, making the results especially useful for comparing generic and brand drug formulations containing nanomaterials.

Abstract: More than twenty New Drug Application (NDA) drug products using various nanotechnology platforms including liposomes, micelles, nanocrystals, and nanoparticles have been approved by FDA. There have also been generic versions approved for some of these drugs (e.g., Doxorubicin HCl liposomal injection; Doxil). The Office of Generic Drugs (OGD) has received numerous inquiries on guidance related to demonstrating bioequivalence (BE) specifically for nano-sized drug products. To determine proper recommendations, laboratory investigations into ideal characterization methods for nanomaterials are necessary. Physicochemical properties of nano drug products, such as size, pH, and surface charge have a drastic effect on drug safety and efficacy and its behavior in the appropriate biological context. In our study, a number of techniques, such as Laser Diffraction (LD) and Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM) were evaluated for their capability to measure nanoparticle size distributions. Tests were performed with drug formulations such as liposomes, nanoemulsions, and coated metallic nanoparticles. The applicability of characterization techniques was strongly dependent on material type, size, and size distribution. Certain dispersion media were incompatible, e.g. a 10% serum protein solution skewed results with an IV metallic nanoparticle, while simulated tear fluid (STF) with albumin had a small increase in apparent size of an ophthalmic emulsion. Zeta potential measurement with DLS changed the stability and pH of sensitive materials, such as an oil nanoemulsion. Viscosity was an important parameter used in DLS measurements and varied when an ophthalmic emulsion was diluted with water or measured at body (37°C) or room temperature (25°C). User specified calculation models on the Laser Diffraction instrument were shown to introduce significant variability around 100 nm or less. TEM was the only technique that could distinguish both the size and shape of nanoparticles, but required extensive sample preparation. The advantages and limitations of each technique were carefully examined and DLS was determined to be a relatively quick technique for many material types within the nanoscale size range, but showed difficulty when measuring polydispersed samples. The study additionally served as a benchmark for regulatory approaches, data reporting, and characterization requirements in generic drug applications.

41. Evaluation of the Performance of a Handheld NIR Device for the Rapid Screening of Chemical Adulterants

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Plain Language Synopsis: Cases of fraud of extra virgin olive oils (EVOO) have long been reported by the media and in the scientific literature. To date, there is no screening method that can rapidly authenticate commercial EVOO products. The proposed rapid (< 5 min) near infrared and chemometrics methodology has successfully been used to screen commercial EVOO products for authenticity using a handheld near infrared device.

Abstract: While FDA has jurisdiction over deceptive label declarations often found with adulterated extra virgin olive oil (EVOO), the Agency is also mandated with the protection of the US public against the intentional adulteration of foods.

A priority for FDA has been the development of novel, rapid, non-targeted screening tools for the detection of economically motivated adulteration (EMA) of food products such as EVOO based on rapid, high-throughput methods and instrumentation including portable or hand-held devices for field use. The performance of a handheld NIR device was evaluated and compared to that of a benchtop FT-NIR spectrometer. Univariate and multivariate statistical analyses of observed NIR spectra were used to screen commercial products that were labeled extra virgin olive oil (EVOO), and provided a rapid and presumptive indication of authenticity. Olive oil spectra were rapidly measured in the transmission mode using disposable glass tubes without any sample preparation. Discriminant power plots and interclass distances were used to find the optimal spectral range for chemometric analysis. Analysis of spectral data was carried out by using univariate analysis (conformity index) and, for classification, multivariate analysis which included principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA). These screening tools allowed the discrimination between authentic EVOO and blends spiked with adulterants. Using Q residual vs. Hottelling's T2 plots it was possible to classify commercial EVOO products. These results indicate that the handheld NIR device is suitable for the routine screening of EVOO for the rapid discrimination between products presumed to be authentic and those that would require further determination of quality and purity.

42. Comparing Diagnostic Image Reading Performance in Laboratory and Clinical Studies

Samuelson, Frank, FDA/CDRH/OSEL; Abbey, Craig, UC Santa Barbara; He, Xin, FDA/CDRH/OSEL

Plain Language Synopsis: Ratios or percent changes are good ways to compare diagnostic statistics among pre-clinical laboratory imaging studies and subsequent clinical observation studies. Some performance metrics, such as expected utility and AUC, are consistent across both laboratory and clinical studies.

Abstract: Before using a new diagnostic imaging device in a clinical study on a large number of patients, it is common to perform controlled laboratory studies with a limited number of readers and cases comparing the new device to the standard of care. These studies measure the performance of a set of readers using the devices to diagnose a set of patients enriched with a high prevalence of diseased cases. After the acceptance of a new technology into widespread clinical use, observational studies of performance in the population are conducted, which measure the actual clinical performance. Ideally the pre-clinical laboratory study will accurately predict the results of the observational study. We want to know which measures of reader performance in such a pre-clinical study are predictive of future observational clinical studies. In this presentation we compare measures of reader performance from several pre-clinical laboratory studies and large clinical studies in screening mammography. Comparing performance measures in controlled laboratory studies and observational clinical studies can be difficult because these studies have very different disease prevalences, and in general the prevalence in the clinical studies is not known. To avoid this problem we use ratios of performance statistics between modalities within any one study, similar to the method of Baker and Pinsky (2001). We examine ratios or percent changes in false positive rates and true positive rates. Using simple assumptions and models, we also examine changes in expected utility and area under the ROC curve (AUC). We find that utilizing ratios is a very useful way to compare performance measures from multiple studies. We find that the percent changes in some performance metrics, such as expected utility and AUC are consistent across both laboratory and clinical studies, even across studies with greatly different prevalence. Changes in other metrics, such as the false positive rate, true positive rate, and recall rate can differ greatly between laboratory and clinical studies.

43. Development of RNA Aptamers that Bind Specifically to *Trypanosoma cruzi* Infected Cells

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Plain Language Synopsis: Chagas disease, caused by the parasite *Trypanosoma cruzi*, can be transmitted by blood transfusion. Prescribed drugs

have side effects and might not reach therapeutic levels in tissues. We developed small RNA molecules, called aptamers, binding specifically to the surface of infected cells to be used for targeted drug delivery.

Abstract: Chagas disease, caused by *Trypanosoma cruzi*, can be transmitted by blood transfusion and is a major concern for blood safety in non-endemic countries. Two forms of the parasite are found in the mammalian host, the extracellular trypomastigotes and intracellular amastigotes. Benznidazole and Nifurtimox, the two prescribed drugs, have significant side effects and may not reach therapeutic levels in deep tissues where surviving intracellular amastigotes can cause persistent infection. One approach to increase therapeutic effect of the drugs is to perform targeted drug delivery to tissues harboring amastigotes. Intracellular parasites are known to express antigens on plasma membrane of infected host cells. To identify ligands that recognize these membrane antigens, we used the Systematic Evolution of Ligands by Exponential enrichment method to develop aptamers against *T. cruzi* infected 3T3 cells. The specificy of these RNA aptamers arise from sequence dependent folding and generation of stable three dimensional structures that can bind to targets of interest. A random library containing 1015 unique sequences, at a concentration of 100 nM, was allowed to interact with non-infected 3T3 cells. The supernatant was recovered and allowed to interact with infected 3T3 cells. After washing the bound RNA molecules were recovered and amplified by RT-PCR. This process was repeated iteratively for 15 rounds with increasing stringency. The library recovered at round 15 was cloned and sequenced. Phylogenetic analysis indicated 7 major sequence families had been enriched. The major families represented 47.2% of the total clones sequenced with aptamers ranging from 169-180 bases in length. These aptamers were shown to bind with high specificity to a parasite cell extract in Enzyme Linked Aptamer assays. Additional studies are being performed to determine the specificity and binding characteristics of these 7 aptamers to *T. cruzi* infected 3T3 cells. We are developing aptamer ligands that can bind specifically to *T. cruzi* infected cells. These aptamers can be used for targeted drug delivery to infected host tissues therefore maximizing the therapeutic benefit and minimizing the toxic side-effects of current anti-*T. cruzi* drugs. These aptamers specific for infected cells can also be used for in-vivo imaging and may be of diagnostic value.

44. Effects of 25 FDA Approved Tyrosine Kinase Inhibitors on Rat Liver Mitochondria

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Plain Language Synopsis: Tyrosine kinase inhibitors (TKIs) are a group of new anti-cancer drugs. This study tested all the 25 FDA-approved TKIs and found that some TKIs can damage the functions of mitochondria, the powerhouse of the cell. This helps explain why some TIKs cause serious harm to the liver.

Abstract: FDA has approved 25 tyrosine kinase inhibitors (TKIs), with six of them having black box warnings for hepatotoxicity (BBW-H) in the product labeling. The mechanisms and risk factors for TKIs-induced hepatotoxicity are unknown. Here the 25 TKIs were tested in isolated rat liver mitochondria. The oxygen consumption rate, inner membrane potential, inner membrane permeability transition, outer membrane integrity, and reactive oxygen species were measured, as these endpoints are emerging as novel in vitro assays to predict a drug's potential to induce clinical hepatotoxicity and have been adopted by pharmaceutical industries to screen drug candidates to avoid hepatotoxicity at the early development stage. At concentrations equal to 100-fold peak blood concentrations (Cmax), 14 TKIs were found to induce at least one type of mitochondrial damage, with only one drug, ceritinib, affecting outer membrane integrity. Among the six TKIs with BBW-H, mitochondrial damage was induced by regorafenib, lapatinib, pazopanib and sunitinib, but not ponatinib or idelalisib. Statistical analysis showed that mitochondrial liability at 100-fold Cmax did not correlated well with these TKIs' hepatotoxicity potential observed in clinical trials. However, at clinically relevant concentrations (equal to Cmax), three TKIs including regoragenib, sorafenib and pazopanib were found to induce mitochondrial damage, indicating that mitochondrial dysfunction likely contributes to the hepatotoxicity of some TKIs. Preliminary

data with primary rat hepatocytes showed that ceritinib induced cytochrome c release in isolated mitochondria at concentrations >20-fold Cmax and in hepatocytes at about 2-fold Cmax, indicating that the biotransformation process may play a role in ceritinib hepatotoxicity. Investigations are under way to examine the effects of these TKIs on hepatocytes from multiple species. These data provide novel mechanistic insights into TKIs induced hepatotoxicity but cast some doubts on the generalized application of using mitochondrial liability to predict drug hepatotoxicity in humans.

45. Development of Bioluminescent *Trypanosoma cruzi* Parasites Expressing NanoLuc Luciferase to Study Chagas Disease Pathogenesis

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Plain Language Synopsis: The mechanisms that determine *T. cruzi* distribution and pathological outcomes of Chagas Disease (CD) are poorly understood. To better study acute and chronic CD progression in infected animals without killing them, we generated transgenic parasites expressing the NanoLuc™ luciferase reporter that will be used in real time imaging studies.

Abstract: Trypanosoma cruzi is the etiological agent of Chagas disease (CD), which affects 10 million people in Central and South America. CD acute phase occurs shortly after an initial infection, and is followed by a chronic stage which causes cardiac and/or gastrointestinal disorders. There is no effective treatment or vaccine to control the disease. During the chronic phase, the level of parasitemia in blood is very low and parasites are thought to remain in different tissues. The mechanisms that determine parasite tropism and pathological outcomes of CD are poorly understood. Real-time bioluminescence imaging constitutes a non-invasive powerful tool to monitor pathogen dissemination and tissue distribution within infected animals. To better study chronic CD progression and host-parasite interactions in vivo, we developed transgenic parasites expressing the NanoLuc™ (NLuc) luciferase reporter. This novel small, ATP independent luciferase variant is a highly stable 19-kDa protein that possesses ~150-fold-greater specific activity than both Renilla and firefly luciferases. Transgenic Colombiana strain parasites were generated after transfection of early log-phase wild-type epimastigotes with a plasmid containing the NLuc gene reporter (pBEX-Nluc). NLuc expressing epimastigotes were obtained by selection in media with G418. Infection of murine 3T3 cells with stationary phase epimastigotes resulted in production of luminescent intracellular amastigotes and release to the culture supernatant of trypomastigotes also expressing Nluc activity, indicating that modified parasites could complete a full life cycle in vitro as the parental wild-type strain. Swiss Webster mice were infected with 5,000 tissue culture-derived trypomastigotes. *In vivo* imaging studies will be performed to define the parameters of the assay and determine the suitability of the NLuc expressing parasites to monitor parasite burden and distribution during the chronic stage of infection. We are currently in the process of generating bioluminescent parasites from a *Trypanosoma cruzi* strain isolated from a US blood donor (0704). We plan to compare the tissue distribution of the Colombiana and 0704 strains, as well as to study regression of the infection during treatment with the anti-parasite drug Benznidazole.

46. Assessing the Biological Impact of Degradants Evolving from Biodegradable Polymeric Medical Devices

Skoog, Shelby, FDA/CDRH/OSEL/DBCMS; Guo, Ji, FDA/CDRH/OSEL/DBCMS; Casey, Brendan, FDA/CDRH/OSEL/DBCMS

Plain Language Synopsis: Novel medical uses of biodegradable polymers have raised new questions of their potential hazard to local tissue environments. The goal of this study is to develop a better understanding of how these materials affect the body through assessment of polymer degradation behavior and evaluation of polymer degradant biocompatibility.

Abstract: Biodegradable polymers are widely used in a variety of medical devices. Novel use of these materials in medical devices, including Class III cardiovascular devices, has resulted in new risks as these biodegradable polymers break down in the human body. Due to these new risks, it is critical that FDA develop an enhanced,

quantitative understanding of how these materials affect local tissue responses to improve the regulatory processing. Unfortunately, assessing biocompatibility of these materials and the risks they pose to patients is quite complex since these materials are constantly evolving in the body. Furthermore, evaluating biodegradation of these materials in a physiologically relevant procedure in the laboratory is challenging. The main focus of this research is to assess the biocompatibility of degradants (fragments of polymers that have degraded form the parent polymer material) evolving from biodegradable polymers widely used in medical products. Model biodegradable polymeric materials (PLGA, PLA, & PCL) were degraded in vitro in a physiologically relevant process. Throughout degradation of the materials, we systematically characterized the polymer degradation behavior and assessed the in vitro biocompatibility of degradants evolving from the biodegradable polymers. Degradant species were identified and quantified using liquid chromatography - mass spectrometry and gel permeation chromatography. The influence of the degradants on cytotoxicity was assessed using a variety of assays evaluating cell viability, inflammation, oxidative stress, and cell morphology. The effect of the degradants on blood compatibility was investigated using endpoints such as hemolysis, coagulation cascade activation, and platelet activation/aggregation. The intent of this research is to provide a better understanding of the biocompatibility of degradants which arise from biodegradable polymers, specifically if they pose any previously undetected risks. This information will hopefully: 1) enable FDA to better assess the safety of medical products which contain biodegradable materials, and 2) result in the development of testing procedures which can be used by industry to accurately assess the safety of their products. This work is intended to clarify and expedite the regulation pathway for products incorporating biodegradable materials, which in turn will bring safe and effective products to the market faster.

47. Validation of Functional MRI and Quantitative EEG as Clinical Biomarkers

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Plain Language Synopsis: The emerging technologies of functional magnetic resonance imaging (fMRI) and quantitative electroencephalography (qEEG) can lead to new clinical biomarkers to diagnose neurological disorders and guide treatment. Current FDA research is examining ways to validate fMRI and QEEG and minimize sources of variance.

Abstract: CDRH continually faces new kinds of diagnostic technologies and needs to evaluate scientific evidence in support of the claims that sponsors would like to make. Two such technologies under study in OSEL/DBP are functional magnetic resonance imaging (fMRI) and quantitative electroencephalography (qEEG). Clinical biomarkers based on these emerging technologies have the potential to diagnose neurological disorders and guide and evaluate treatment, including non-device (i.e., pharmaceutical) treatments. Currently, fMRI is used is to assist in presurgical planning for tumor resection or epilepsy surgery. Recently, FDA cleared two qEEG devices - one to assist with attention deficit hyperactive disorder (ADHD) diagnosis, the other to assist with traumatic brain injury diagnosis. Ways to validate fMRI and qEEG include developing quality assurance (QA) metrics, assessing bias and precision across analysis platforms, and evaluating techniques to improve reproducibility. The development of a QA metric is needed to validate the use of fMRI on a personalized, individual patient basis. We are investigating the use of a breath-hold or resting state run to calculate a QA metric that predicts fMRI contrast-to-noise ratio better than temporal signal-to-noise ratio, the traditional metric. This new fMRI assessment tool could help validate the use of fMRI as a biomarker in clinical applications. The development of simulated fMRI data can be used to validate fMRI analysis techniques across clinical MRI centers and assess issues of bias and precision between diagnostic software packages. We are currently collaborating with the Radiological Society of North America (RSNA) Quantitative Imaging in Biomarkers Alliance (QIBA) to develop simulated fMRI data sets. This work can be used to describe the abilities and limitations of fMRI for presurgical planning. A recent medical device submission incorporating

qEEG has been cleared for use as confirmatory support when assessing children believed to have ADHD. However, FDA has not cleared a qEEG biomarker to stand on its own as a diagnostic tool. We are investigating the effect of factors such as subject movement, data acquisition techniques, and data processing algorithms on reproducibility of qEEG metrics. This research has the potential to stimulate development of qEEG biomarkers and ensure effective medical innovation.

48. Computational Modeling of Intravascular High-Intensity Therapeutic Ultrasound (HITU) Fields and Bioeffects

Soneson, Joshua, FDA/CDRH/OSEL

Plain Language Synopsis: Emerging applications of high-intensity therapeutic ultrasound (HITU), such as intravascular HITU for treating thrombolysis and hypertension, require careful review to ensure safety and efficacy. Computational models can provide insight and alert reviewers to pitfalls in the development and review processes. Here, a computational model specific to intravascular HITU is discussed and results are presented.

Abstract: While ultrasound has been commonplace in diagnostic imaging applications for decades, its therapeutic use is also starting to gain traction. At higher output levels, ultrasound can be used to disrupt or destroy tissue using mechanical, thermal, or both of these effects simultaneously. One main advantage of HITU is that it can be focused from a source outside the body, and inasmuch can be a totally noninvasive treatment modality. FDA has cleared or approved devices for the noninvasive ablation of uterine fibroids, cosmetic and ophthalmic applications, and the minimally-invasive ablation of cardiac tissue. Recently, catheter-based intravascular delivery of therapeutic ultrasound has been proposed to treat thrombolysis in acute myocardial infarctions as well as drug-resistant hypertension via renal denervation. In these applications the ultrasound field propagates radially from an axisymmetric source, as opposed to the axially-directed focused ultrasound “beams” used in previous applications. In anticipation of device submissions, a computational model capable of predicting the acoustic field, cavitation potential, temperature elevation, and thermal damage in tissue has been developed. Relevant to this geometry, the wide-angle parabolic wave and bio-heat transfer equations are discretized using finite differences and implemented in Matlab for rapid computation and convenient graphical output. The code accounts for multiple medium layers, so fluid cooling of the catheter and different tissue layers are accurately modeled. Developing software such as this allows FDA reviewers to become familiar with new applications early in the development process.

49. The Introduction of Pathogen Reduction Technology in the U.S.

Storch, Emily, FDA/CBER/OBRR/DHCR/CRB; Jain, Nisha, FDA/CBER/OBRR/DHCR/CRB

Plain Language Synopsis: Pathogen reduction is a novel technology for the reduction of bacterial, viral, and parasitic contamination of blood components. The INTERCEPT Blood System from Cerus is the first pathogen reduction system approved to treat plasma and single donor apheresis platelets to reduce the risk of transfusion-transmitted infections.

Abstract: Pathogen reduction is a novel technology for the reduction of bacterial, viral, and parasitic contamination of blood components. Various products have been in development for several decades. Mechanisms under continuing development include riboflavin and ultraviolet light, methylene blue, and amotosalen and ultraviolet light. The Cerus INTERCEPT System for the Photochemical Inactivation of viruses, bacteria, parasites and leukocytes, was recently approved by the Office of Blood Research and Review for both plasma and apheresis platelets. INTERCEPT is the first pathogen reduction system approved to treat plasma and single donor apheresis platelets to reduce the risk of transfusion-transmitted infections. Premarket approval was granted for INTERCEPT plasma Dec 16, 2014 and for INTERCEPT platelets Dec 18, 2014. INTERCEPT uses the synthetic psoralen derivative amotosalen HCL (S-59) a photoactive compound, and long-wavelength ultraviolet (UVA) radiation, at 320 - 400 nm. Blood components are treated with amotosalen which intercalates into helical regions of DNA or RNA, then subsequent application of UV illumination causes covalent cross-linking of amotosalen-bound nucleic acids. These interactions prevent further replication. Unreacted amotosalen and

residual photo-reactive byproducts are then removed when blood components are passed through a compound adsorption device. Plasma treated by INTERCEPT averages 1 amotosalen adduct every 89 base pairs in leukocytes, and for platelets, averages 1 amotosalen adduct for every 83 base pairs. INTERCEPT products are contraindicated for patients with a history of hypersensitivity reactions to amotosalen or other psoralens. A Phase III trial, the SPRINT study evaluating INTERCEPT platelets was conducted in 645 subjects in the US. The primary endpoint was incidence of Grade II bleeding, which was 58.5% for INTERCEPT platelets and 57.5% in control, p = .001, therefore meeting noninferiority. In the trial platelets treated with INTERCEPT demonstrated lower Corrected Count Intervals (CCIs) however, CCIs met clinically effective levels (11.1 x 103 with INTERCEPT platelets vs. 16.0 x 103 for control). Patients in the INTERCEPT group received more platelet transfusions (8.4 INTERCEPT vs 6.2 control, p < .001). There was a statistically significant increased rate of respiratory reactions in the INTERCEPT cohort, but there was no difference in the proportion of patients who experienced transfusion reactions.

50. Identification of Human-pathogenic Molds isolated from Environmental Surveillance Samples Collected from Four Compounding Company Settings by DNA Sequencing of Internal Transcribed Spacer Region

Sulaiman, Irshad, FDA/ORA/SRL; Jacobs, Emily, FDA/ORA/SRL; Simpson, Steven, FDA/ORA/SRL; Kerdahi, Khalil, FDA/ORA/SRL

Plain Language Synopsis: We recently developed and validated an internal transcribed spacer (ITS) region based sequencing protocol for rapid species identification of molds. This newly developed method can be used in FDA's environmental monitoring program in assessing good manufacturing practices of public health importance.

Abstract: We recently developed and validated a DNA sequencing protocol for species identification of human-pathogenic molds targeting internal transcribed spacer (ITS) region. We successfully used this method in analyzing environmental swabs collected from a Compounding Center known to cause 2012 multistate fungal meningitis outbreak in the 20 states of the United States. The fungal meningitis was a result of contaminated preservative-free MPA steroid injections. We characterized 26 environmental swabs collected from various locations of the manufacturing premises to determine a possible cause of the outbreak, and identified 10 distinct fungal species. In this follow-up environmental surveillance study, we have used this method and characterized a total of 198 environmental swabs for the detection of pathogenic indicator mold species from four Compounding Company premises located in the Southeast, Southwest and Western region of the United States. These samples were initially analyzed by conventional microbiologic protocols for the fungal isolation. The ITS regions of isolated fungal isolates were sequenced using ABI 3500 XL Genetic Analyzer, and data was analyzed using BioEdit and Geneious programs. Of the swabs examined, only 25 swab samples were found positive for fungi. Data analysis on the generated ITS sequences confirmed the presence of following fungal species in the environmental swabs examined: (i) Pestalotiopsis cocculi from the location SER1; (ii) Epicoccum sp. and Trichaptum biforme from the location SER2; (iii) Nigrospora sphaerica and Fusarium sp. from the location SWR1; and (iv) Curvularia hawaiiensis, Fusarium chlamydosporum, Penicillium chrysogenum, Penicillium commune, and Preussia sp. from the location WR1. All of the generated ITS fungal sequences matched 100% with the published sequences available in GenBank, except that the sequence of Curvularia hawaiiensis revealed a 2-point-mutation and matched 99% with published ITS sequence (GenBank Accession No. KF897857). Furthermore, we have also used this novel method in examining unopened containers of Greek yogurt from the recalled batch to determine mold contamination in the product. Thus, the ITS regions is a good genetic marker. It can be used in the environmental monitoring program for the presence of indicator fungal species and assessing good manufacturing practices of the compounding area of public health importance.

51. Global Toxicological Evaluation of Human Cellular Response to Silver Nanoparticles using Quantitative Mass Spectrometry

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Plain Language Synopsis: There is a need to understand the potential health impact of novel chemistries and materials used in medical products beyond the current basic toxicology assays. In this study, we use discovery proteomics to provide such global information about the potential impact of silver nanoparticles on human cells.

Abstract: Introduction: Silver (Ag) in both ionic and nanomaterial form is known for its antimicrobial properties and has been widely used in various food and medical applications. Silver nanoparticles (AgNPs) have emerged as an important class of nanomaterials for a wide range of medical devices, but there has been concern over possible Ag-induced toxicity. Various studies demonstrate that common mechanisms of Ag-induced toxicity include oxidative stress, DNA damage and apoptosis. Individual toxicity assays are specific and robust but they lack in providing global proteome information. Here we aim to quantitate changes in human cells upon treating with AgNPs by using SILAC based metabolic labeling and discovery proteomics, to identify differentially regulated pathways and to develop enhanced methodologies for toxicological screening of emerging materials in medicine. Methods: Human monocyte cells (Thp-1) were chosen based on relevance as a human model of response to a biomaterial implants and were cultured in SILAC RPMI 1640 media for labeling. Both light and heavy isotope labeled cells were activated to macrophage-like cells using phorbol 12-myristate 13-acetate (PMA) and treated with varying dosages of 10 nm AgNPs (test article), AgNO3 (silver ion control), and 10 nm gold nanoparticles [AuNPs (nanoparticle control)]. After exposure to the various agents, peptide samples were prepared from cell lysates. Samples were subjected to LC-MS/MS analysis on an Agilent 6540 QTOF LC/MS system with a nanospray interface. The MS/MS data were searched against the Swissprot protein database using Agilent Spectrum Mill search engine for protein identification and differential expression quantitation. Results: In this preliminary study, we were able to identify and quantify proteomic changes due to AgNP treatments for over 100 proteins simultaneously. Our initial results indicated varying effects of ionic and nano silver treatment on protein expression levels, with treatments generally causing expression level changes between 0.1 and 3 fold, suggesting unique effects of nanoparticulate silver compared to ionic silver at similar doses. These studies are contributing to optimization of dosing, sample processing, LC/MS parameters, and data analysis protocols that will permit identification and confirmation of unique protein markers in cells indicative of changes in cell function and behavior.

52. Detection of Salmonella from Cloves by Modified-Culture-Method and three PCR Methods

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Plain Language Synopsis: Spices are ready-to-eat foods that pose considerable risk to the public safety and have been implicated in Salmonella outbreaks in the recent years. This study attempts to develop an efficient culture detection method and select an effective PCR protocol for Salmonella in cloves.

Abstract: Introduction: Spices are typically added to ready-to-eat foods and present a unique challenge to food safety. Many spices, including cloves, have antimicrobial properties and current methods for detection of Salmonella from these food ingredients are not as sensitive as they are for foods that do not contain inhibitors. Purpose: to improve the Bacteriological Analytical Manual (BAM) for detection of Salmonella from cloves by modifying the culture assay and integrating it with real-time PCR platforms. Methods: A modified culture method was compared with the reference BAM method. For the modified-method, 25g of cloves were rinsed in 225ml of tryptic-soy-broth for 60s and the rinsate was pre-enriched. The BAM was followed for the remainder of the assay. Six trials with two clove cultivars and three

Salmonella serotypes were conducted. Each trial was comprised of 40 dry-inoculated samples (10-810 CFU/g); 20 samples were processed by BAM-method and 20 by the modified-method. Pre-enrichment cultures from the modified-method were used for comparison of the effectiveness of FDA-PCR, ABI-MicroSEQ® and GeneDisc® for the detection of Salmonella, with three DNA different extraction methods. Results: With the modified-method, for the six trials 100%, 30%, 100%, 90%, 95% and 80% of the 20 samples were positive for Salmonella. With the BAM-method in the same trials, 75%, 0% 30%, 10%, 15%, and 25% of the samples were positive; respectively. There was 100% correlation among all the DNA extraction methods for the various PCR platforms examined. The molecular data matched the cultural data. In conclusion, the modified culture method was more sensitive than the BAM method for the detection of Salmonella in cloves. All three PCR methods were equally effective for detection of Salmonella from cloves. Significance: This modified culture method will substantially improve the detection of Salmonella from cloves and can potentially be applied to other spices in the future.

53. Proposed Designs for Controls for microRNA Measurement Technologies

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Plain Language Synopsis: MicroRNA is a class of small noncoding RNA with potential as sensitive biomarkers of drug-induced adverse events. Controls are needed because poor agreement can be seen between microRNA measurement technologies. Controls made from 32 microRNAs and designed to mimic a standard curve successfully reproduced known differences in performance among technologies.

Abstract: MicroRNA, a class of small noncoding RNA that regulate gene expression at the translational level, have shown potential as sensitive biomarkers of drug-induced toxicity and disease. microRNA is profiled in biofluids and tissue using fixed-content (pre-plated quantitative polymerase chain reaction assays and microRNA microarrays) or open (next generation RNA sequencing) platforms. Because there can be poor correlation between different microRNA measurement technologies, there is a need to develop universal standards, calibrators, and/or reference materials. Spike-in controls (e.g., the External RNA Control Consortium (ERCC) controls) have demonstrated utility for benchmarking the performance (sensitivity, specificity, dynamic range, limit of detection, and accuracy) of mRNA profiling technologies, especially next generation RNA sequencing. We prototyped spike-in control designs for microRNA profiling technologies that produce heterologous standard curves and span the dynamic range of platforms, similar in design to the ERCC controls. The controls are comprised of 32 microRNAs selected for identical sequence between human, rat, and mouse, and presence on common fixed-content profiling platforms. 32 microRNAs were commercially synthesized, distributed into 8 equimolar pools, and mixed together in a modified Latin square design that spanned 4 log units at 5-fold increments. The spike-in controls were assayed on four types of microRNA profiling platforms and produced metrics that reflected known differences in performance among platforms like the undercounting of homopolymer-containing microRNA due to sequencing errors. The results from these pilot tests provide the basis for further development of microRNA profiling controls that can be used across a range of measurement technologies and will help inform the development of community-wide microRNA controls.

54. Investigation of Quartz Crystal Microbalance (QCM) for Determination of Viability of Hepatitis A Assay Eluate

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Plain Language Synopsis: Currently FDA viral assay(s) have tedious or non-existent means of verification of the viability of recovered virus. Employing Quartz Crystal Microbalance (QCM) techniques to measure changes in a virus negative control compared to a virus positive sample will provide viability status of the sample in minutes.

Abstract: The critical measure of biological regulatory assays is that they are capable of being used to protect the public from exposure to pathogens. For bacterial pathogens an increase in cell number is generally accepted to infer

viability, thus the ability to cause disease. For viral pathogens a measurable increase in particle number gives incomplete information about the ability to confer disease to a susceptible individual. The proportion of viral replicates incapable of producing disease varies depending on the virus. Researchers have reported that a large proportion of viral replicates may be incapable of producing infectivity in a susceptible host. While PCR and RT-PCR can be used to show an increase in amount of viral nucleic acid and infer viral genome number, it provides no information about infectivity. To date, the only way to assess that ability in vitro is to perform a plaque assay which typically requires days to visualize/assess. For some viruses such as Hepatitis A Virus (HAV) strain HM175 plaque assay may be extensively prolonged or not even possible. We are investigating the ability of Quartz Crystal Microbalance (QCM) techniques to rapidly assess the viability of the non-pathogenic HAV virusHM175 from eluate(s) of the BAM HAV method. The quartz crystals employed in QCM have a piezoelectric character which allows measurement of nano changes in mass which occur on the surface of the crystals. Researchers have used QCM to specifically trap viruses but QCM has not been used to verify the growth of viral numbers within a live cell or cell population. We are employing QCM to measure frequency changes in minutes when a cell line, Fetal Rhesus Kidney (frhk) cells are inoculated with viral assay extract spiked with HAV. Frequency measurement changes of a spiked sample can be compared to frequency measurements from frhk cells which have not been spiked with HAV. When mass changes of the spiked sample versus the unspiked sample occur, viral growth (viability) is occurring. This tool can be used to quickly verify the potential infectivity of a regulatory viral assay isolate. Ongoing efforts are exploring additional species of viral viability targets.

55. GDUFA API (Mixture) and Amorphous Solid Dispersion (ASD)

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Plain Language Synopsis: Amorphous solid dispersion is used as an example to illustrate GDUFA API and FDF definitions and CMC scientific review considerations.

Abstract: Amorphous solid dispersion (ASD) is one of the innovative technologies to enhance solubility, dissolution, and oral bioavailability of poorly water soluble Active Pharmaceutical Ingredient (API). ASD is usually prepared by combining a water-soluble polymer with drug substance to form a single-phase amorphous mixture that stabilized the amorphous drug substance. Spray-drying or melt extrusion process is generally used for large scale ASD production. For purposes of self-identification and payment of fees, Generic Drug User Fee Amendments of 2012 (GDUFA) defines API and Finishes Dosage Form (FDF) differently from the way they have been defined traditionally. For example, a mixture of a drug substance with excipient(s) when the drug substance is unstable or cannot be transported on its own may be filed as an API under GDUFA , while in all other cases, combination of a drug substance with another component of a drug product would be considered as an FDF. GDUFA API and GDUFA FDF incur different filing fees and annual facility fees. Therefore, it is important to distinguish GDUFA API vs. GDUFA FDF. A few examples of API/excipient mixtures are presented to clarify the difference of these definitions. ASD may be filed as GDUFA API (mixture). As presented, CMC scientific review considerations for ASD GDUFA API are similar to those of general API, but also pose some unique challenges due the nature of being a mixture.

56. An Update of Botanical Drug Development in the United States: Status of Applications

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Plain Language Synopsis: Unique features of botanical drugs present significant challenges to both sponsors and CDER reviewers. We analyzed internal IND records on botanicals and profiled trends of submission over the last 15 years. The data provide a vision for the Agency on how well we're dealing with this special category of products that are ranked high in public attentions.

Abstract: We conducted a survey of botanical drug applications across the therapeutic divisions within CDER, FDA since 1999. The objective is to evaluate the status and trend of botanical IND submissions under the current U.S. regulatory environment. The overall number of botanical submissions are as follows: 1999: 21(including Pre-IND: 0), 2000: 20(4), 2001: 24(3), 2002: 21(5), 2003: 40(9), 2004: 33(11), 2005: 44(6), 2006: 33(11), 2007: 46(7), 2008: 41(14), 2009: 37(10), 2010: 37(10), 2011: 41(8), 2012: 38(5), 2013: 59(8), 2014: 46(11), 2015: 8 (3). The trend shows a surge in number of submissions after the publication of Agency's Botanical Guidance in 2001, followed by steady submissions, ranging between 40-50 annually (excepting 2013 that peaked at 59). Among the 602 submissions totaled so far, 79% (476) are submitted in original IND and the rest 21% (126) in pre-IND format initially. From the therapeutic perspectives, more than 26% of the INDs have been focused on the unmet cancer-related therapy areas (161), followed by dermatology (71), GI (50), antiviral (43), endocrine/metabolism (37), reproductive & urology (36), neuropharmacology (36), pulmonary/allergy/rheumatology (30), cardiovascular (28), anti-infective (21) and ophthalmology (10), etc. In the last 5 years (2009-2014), 20 out of 217 INDs were placed on clinical holds, indicating ≥90% of the original INDs were evaluated as safe to proceed for the initial human trials. This 10% rate of clinical holds is significantly less than those in the pre-botanical guidance era, during which 34% were placed on clinical hold, suggesting that the regulatory environment for botanical drug development has been becoming more transparent, consistent and harmonious. In summary, based on our analysis of the 602 INDs submitted, the Agency has undertaken a proactive role in this unique drug category in effectively coaching sponsors for botanical drug development, and as a result, two products, namely kunecatechins (Veregen) and crofelemer (Fulyzaq), each achieved and met the human efficacy/safety standards for a new drug, and were approved for market uses in the United States.

57. Quantitative Scoring System on Severity of Toxicity and Time-Dependent Emergence of Target Organs of Toxicity: An Alternative Approach to Non-Clinical Data Risk Analysis of IND and NDA

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Plain Language Synopsis: The often voluminous & complicated toxicology data contained in INDs and NDAs present significant challenges to reviewers. We proposed a novel approach to regulatory science review processes, in providing an efficient means for in-depth analysis & investigation of sponsor's toxicology data. It is in line with Agency's mission in playing a proactive role in expedited new drug review. It is also within the domain of current FDA Science Forum's theme on "Ensure FDA Readiness to Evaluate Innovative Emerging Technologies" and fits for key FDA Regulatory Science Priority Area.

Abstract: Analysis of non-clinical toxicology data submitted to CDER, FDA during new drug development life-cycle presents significant challenges to the primary reviewers, due to amount and complexities of data contained in the applications. We developed a scoring system to provide a more objective means to rank severity of histopathology findings and a spreadsheet method to analyze time-dependent emergence of target organs of toxicity. The scoring system incorporates incidence rate and severity of a particular toxicity from affected animals among treatment groups and converts the values into a weighted score, by using the following formula: Sum of severity scores (1=minimal, 2=slight, 3=moderate, 4=marked and 5=severe) from all affected animals/Total number of affected animals. Because the scoring system employees raw data from individual animals instead of relying on sponsor's summary table, it provides more objective and integrative view of a specific toxicity. In addition, it allows quantitative analysis for possible trend or dose-response relationship of a specific finding. The treatment duration-dependent target organ analysis involves a spreadsheet system with specific toxicity targets listed under the row category, while the treatment duration (time) is proportionally represented and arranged by successive columns on the horizontal coordinate. A toxicity target emerged in one study

but not in other shorter study durations can be highlighted by a milestone sign at that time point and supplemented with auxiliary information such as Threshold Dose/NOAEL/NOAEL's AUC (TNA), number or % of subjects affected, to facilitate an overview. A separate spreadsheet summarizes all studies with column headings: Target, Key Findings of Target, Species, Duration of Study, Doses, TNA, whereas the rows list line-items of targets. We believe that in lieu of these alternative means of analysis and presentation of toxicity data, the methods not only significantly facilitate secondary and tertiary review processes during IND development but also provide a new dimension in toxicity management that may shed insights into the understanding of the toxicity mechanism of new drug products.

58. Sequencing Quality Control (SEQC) - An FDA-led Consortium Effort for Assessing RNA-Seq

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Plain Language Synopsis: Emerging methodologies such as next-generation sequencing (NGS) contribute to our understanding of disease and health. The FDA-led community-wide Sequencing Quality Control (SEQC) project aimed to address the technical performance issues for NGS. The poster provides an overview and main conclusions of the SEQC project.

Abstract: Emerging methodologies such as next-generation sequencing contribute to our understanding of disease and health. Rapid progress over the last few years have moved these technologies from an exploratory to an applied stage, and an increasing amount of data derived from such approaches is received by regulatory agencies supporting the evidence for the safety and efficacy of new medical products. The realization has spawned a number of FDA efforts to use these technologies through integrated bioinformatics within inter-center and cross-community collaborations. This poster is to discuss how the FDA-led community-wide MicroArray Quality Control (MAQC) makes an attempt to address the technical performance issues for these emerging biomarker technologies. Specifically, the third phase of MAQC, also known as the SEquencing Quality Control (SEQC) project, developed a comprehensive plan to assess the power and limitations of NGS with a substantial effort to compare RNA-Seq with microarrays (a mature transcriptomic technology). The project involved >180 participants from ~80 organizations. Importantly, the project generated large RNA-Seq data sets covering a broad range of biological samples (clinical, non-clinical and reference samples) with which many critical issues of applying RNA-Seq in clinical and safety evaluation were evaluated and discussed. The poster provides an overview and main conclusions of the SEQC project.

59. Optimization of Next Generation Sequencing using the Illumina MiSeq for Detection of Human Norovirus from Stool Samples

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Plain Language Synopsis: The purpose of this study was to optimize the sequencing protocol for NoV detection on the Illumina MiSeq platform, and the results show the importance of sample preparation optimization, RNA isolation and library generation for NGS detection of norovirus from stool samples.

Abstract: Introduction: Next generation sequencing (NGS) is a promising approach for detection and identification of Norovirus (NoV), the leading cause of gastroenteritis in the U.S. This has been challenging by the relative low or gapped target mapping due to nucleic acid contamination, effect of sample preparation and biased viral genomic coverage. Purpose: The purpose of this study was to optimize the sequencing protocol for NoV detection on the Illumina MiSeq platform. Method: Norovirus-containing stool samples were suspended in 10% phosphate-buffered saline (suspension), centrifuged at 9,000 x g for 3 min (supernatant), and the supernatant was filtered through a 0.22 µM membrane filter (filtrate). Viral RNA was isolated from each of these three different preparations (suspension, supernatant and filtrate) with three different kits available in our lab: QIAamp viral RNA kit, RNAqueous kit and RNeasy mini kit. NoV RNA was quantified with real time RT-PCR and libraries were generated with same amount of RNA for each sample with and without oligo(dT) selection. Sequencing was performed on MiSeq and Genomic Workbench

was employed for the data analysis. Results: (1) The viral RNA yield with the RNAqueous kit was significantly ($p<0.05$, $n=3$) lower than from the other two kits; the highest viral RNA yield was obtained with the QIAamp viral RNA kit. (2) The filtrate, supernatant and suspension of the same stool sample showed similar sequencing outcomes, according to the total reads number, viral reads number, and percentage of viral specific reads. (3) The same mapping pattern - with more reads mapping towards the 3'-end, was shown for all oligo(dT) selected samples, but not for non-oligo(dT) selected samples. Significance: These results show the importance of sample preparation optimization, RNA isolation and library generation for NGS using Illumina MiSeq for detection of Norovirus from stool samples.

60. Evaluation of Electrophysiological Biomarkers for the Diagnosis of Mild Brain Injury in a Novel Mouse Model

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Plain Language Synopsis: This abstract reports two major findings in an MCMi supported project. First, we successfully produced mild brain injury in mice by using high intensity focused ultrasound. Second, we identified signature changes of brain waves after brain injury. These changes can potentially serve as diagnostic biomarkers for mild brain injury.

Abstract: Currently there are no widely-used diagnostic or therapeutic medical products for mild brain injury, partly due to the lack of objective biomarkers for measuring outcomes. Electrophysiological indices, such as quantitative EEG (qEEG), have the potential to serve as biomarkers for neural damage. Recent advances in EEG recording technology may soon allow for rapid, non-invasive signal detection on inexpensive and portable platforms. However, the signature changes in neural activity that accompany mild brain injury need to be established. In our study, we identify electrophysiological signatures of mild brain injury in a novel mouse model. Controllable high-intensity focused ultrasound (HIFU) pulses that mimic blast overpressure waves were used to produce brain injury in mice. HIFU-induced brain injury was evaluated by immunohistochemical markers for microglial (Iba-1) and astrocyte activation (GFAP), as well as by locomotor behavioral testing with a rotarod. Elevated expression of Iba-1 and GFAP was observed in the cortex, corpus callosum and hippocampus after HIFU exposure, suggesting a diffuse neuroinflammatory response. In addition, animals subjected to HIFU exposure ($n=8$) exhibited significantly poorer performance in the rotarod behavioral tests than those with a sham exposure ($n=8$) up to 1month post-injury, indicating a chronic functional impairment in locomotor ability. Brain activities were recorded with a 16-channel epidural micro-electrocorticography (μECoG, Neuronexus) array implanted on the primary motor cortex. Periodic recordings (twice per week) from freely moving animals were performed for 4 consecutive weeks before and after HIFU or sham exposure. The frequency content of all electrophysiological signals was quantified by multitaper fast fourier transform (FFT) following artifact rejection (Chronux toolkit). Our preliminary μECoG signals showed a long-term reduction in power indelta (1-4 Hz) band; and a trend of reduction in signal coherence between electrodes after brain injury ($n=6$ for HIFU and 4 for sham group). Our preliminary data suggest HIFU effectively produced brain injury quantifiable by changes in EEG spectral content. EEG has the potential to serve as a brain injury biomarker for use in rapid brain injury diagnosis with portable technologies.

61. Performance Characteristics of the AmpliSeq Cancer Hotspot Panel v2 in Combination with the ION Torrent Next Generation Sequencing Personal Genome Machine

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Plain Language Synopsis: Next generation sequencing is a relatively new technology that

is becoming increasingly widespread. Only one system/reagent kit has come through CDRH for approval, but more are expected. We wanted hands-on experience with the product, the output, data generation and interpretation, and to see how well the system (PGM/cancer panel) performs.

Abstract: Next-Generation Sequencing is a rapidly advancing technology that has both research and clinical applications. For many cancers, it is important to know the precise mutation(s) present, as specific mutations could indicate or contra-indicate certain treatments as well as be indicative of prognosis. Using the Ion Torrent Personal Genome Machine and the AmpliSeq Cancer Hotspot panel v2, we sequenced two pancreatic cancer cell lines, BxPC-3 and HPAF-II, alone or in mixtures, to determine the error rate, sensitivity, and reproducibility of this system. The system resulted in coverage averaging 2000x across the various amplicons and was able to reliably and reproducibly identify mutations present at a rate of 5%. Identification of mutations present at a lower rate was possible by altering the parameters by which calls were made, but at the expense of an increase in erroneous, low-level calls. The panel was able to identify known mutations in these cell lines that are present in the COSMIC database. In addition, other, novel mutations were also identified that, in a clinical setting, may prove useful. The system was assessed for systematic errors such as homopolymer effects, end of amplicon effects and patterns in NO CALL sequence. Homopolymer or positional effects in mutation identification were not found. However, the NO CALL frequency varied by mutation type, with deletion mutations having the highest rate of NO CALLs (6.91%). Lower rates of NO CALLs were found in multi-nucleotide polymorphisms (2.42%), single nucleotide polymorphisms (1.08%) and insertions (0.53%). Overall, the system is adequate at identifying the known, targeted mutations in the panel at a rate of 5%, as well as having the ability to identify possibly useful novel mutations. However, the rate of NO CALLs, particularly in deletion mutations, could lead to missed mutations and must be specifically monitored.

62. A Custom Tiling Microarray for Rapid Detection and Genotyping of Norovirus

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Plain Language Synopsis: Microarray holds the potential of simultaneously detecting and characterizing multiple foodborne viruses without sequence information. We evaluated the sensitivity, specificity, and accuracy of a custom tiling microarray for detection and genotyping of norovirus. Microarray, coupled with NextGen sequencing, is an effective method for rapid detection and discovery of foodborne viruses.

Abstract: Norovirus (NoV) is the most common pathogen causing foodborne outbreaks in the US. The detection and identification of virus contaminants are essential for investigation and prevention of foodborne illnesses. In the absence of a suitable cell culture system, molecular methods, such as microarray, have been applied to detect and genotype causative viral agents. The purpose of this study was to evaluate the sensitivity, specificity, and accuracy of a custom tiling microarray for detection and genotyping of norovirus. The tiling microarray, which contains overlapping 25-mer oligonucleotide probes covering partial genomes of common foodborne viruses, was custom designed and manufactured by Affymetrix. Viral RNA was prepared from clinical samples or in vitro transcription. To assess the sensitivity, we commercially synthesized a NoV target representing exact match for a selected long probeset on the array. As expected, the array correctly identified the corresponding GII.4 strain as top hit. The current detection limit for a perfect-match unamplified norovirus target is 1.6E+03 genome copies. The specificity of the array was demonstrated by simultaneously detecting all viruses from an RNA mixture of hepatitis A virus, coxsackievirus, and norovirus. Microarray could clearly discriminate each virus with no cross reactions. An accuracy test was performed with three clinical samples from patients with acute gastroenteritis. One stool sample, containing GII.4 2006_Minerva, was detected as a GII.4 strain. For stool sample 2014_SW-S3, the numbers of positive individual probes specific to NoV and GII.4 were slightly higher than those of other species and

genotypes, a weak indication of a GII.4 strain. For vomit sample 2014_SW-V4, no positive signals showed up in the long probe set analysis; yet short probe set analysis suggested a GII.8 genotype. The microarray findings were further validated by NextGen Sequencing (NGS). As expected, NGS matched both the 2006_Minerva and 2014_SW-S3 samples to GII.4. Nevertheless, the 2014_SW-V4 sample was mapped to GII.7 genogroup, which is not represented on the array. In summary, we evaluated the sensitivity of Affymetrix Tiling Microarray with minimum detectable concentrations for pure unamplified viral RNA transcripts. The array was capable of detecting and genotyping norovirus from multi-virus and clinical samples with reasonable specificity and accuracy.

63. "One-Test-Fits-All" Approach for Detection and Identification of Influenza Virus Infections Using Nanomicroarray and NGS Assays

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Plain Language Synopsis: A novel method can ultrasensitively detect and simultaneously differentiate any influenza viruses from a single sample in a single test.

Abstract: Conventional methods for detection and discrimination of influenza viruses are time consuming and labor intensive. It is critical to develop accurate methods for their rapid characterization, prevention and treatment. We report a "one-test-fits-all" approach using nanomicroarray for screening, and next-generation sequencing (NGS) assays for extensive identification of influenza virus infection or co-infections. The nanomicroarray was developed to target hemagglutinin, neuraminidase, and matrix genes to identify influenza A and B viruses. PCR mega-amplicons synthesized by using a paired set of degenerate universal primers for whole-genome of influenza viruses were detected and confirmed using NGS. Influenza infections including A(H3N2), A(pdH1N1), influenza B virus, and A(H3N2 and pdH1N1) virus co-infections were identified in nasopharyngeal swab specimens in a single test run. Bioinformatics studies reveal their comprehensive genetic composition and provide matrix reporting information for diagnosis and characterization of novel virulence and drug resistance markers in these specimens. Furthermore, analytical sensitivity studies demonstrated that the NGS sequencing-based diagnostics can achieve ultrasensitive detection of influenza genomes. The current diagnostic platform allows for extensively identifying any unknown influenza viruses in a single test, simultaneously detecting and discriminating multiple influenza virus infections in a single specimen, and effectively monitoring changes in circulating influenza viruses that may have pandemic potential, thus facilitating diagnostics and antiviral treatment in the clinical setting and protection of the public health. (Disclaimer: This article/speech reflects the views of the author and should not be construed to represent FDA's views or policies)

64. Surveillance Using Whole Genome Sequencing Effectively Predicts Antimicrobial Resistance Phenotypes in Campylobacter

Zhao, Shaohua, FDA/CVM/OR/DAFM; Chen, Yuansha, FDA/CVM/OR/DAFM; Mukherjee, Sampa, FDA/CVM/OR/DAFM; Li, Cong, FDA/CVM/OR/DAFM; Young, Shenia, FDA/CVM/OR/DAFM; Lam, Claudia, FDA/CVM/OR/DAFM; Tyson, Gregory, FDA/CVM/OR/DAFM; Folster, Jason, CDC/Division of Foodborne, Waterborne, and Environmental Diseases; Whichard, Jean, CDC/Division of Foodborne, Waterborne, and Environmental Diseases; McDermott, Patrick, FDA/CVM/OR/DAFM

Plain Language Synopsis: Whole-genome sequencing (WGS) is being explored as a method that may provide a single, comprehensive, and cost-effective approach to define the resistance mechanisms and predict antimicrobial resistance phenotypes. The objective of this study was to define antimicrobial resistance genotypes and to evaluate the correlation between resistance phenotype and genotype using WGS. Our study showed that WGS has great sensitivity and specificity to predicted resistance phenotype and could be used as a routine method for antimicrobial resistance surveillance program.

Abstract: Introduction: Antimicrobial resistance in Campylobacter spp. from the food supply is a global public health concern. Whole-genome sequencing (WGS) is being explored as a method that may provide a single, comprehensive, and cost-effective approach to define the resistance mechanisms and predict antimicrobial resistance phenotypes. Purpose: The objective of this study was to define antimicrobial resistance genotypes and to evaluate the correlation between resistance phenotype and genotype using WGS. Methods: One hundred-fourteen Campylobacter isolates (n=82 C. coli and n=32 C. jejuni) recovered by the National Antimicrobial Resistance Monitoring System (NARMS) were selected in this study. Resistance phenotypes were determined by broth microdilution with nine antimicrobials. Epidemiological cut-off values (ECOFF) were used to interpret the antimicrobial susceptibility data. Genomic DNA was sequenced as paired-end reads using Illumina MiSeq. Previously reported antibiotic resistance genes were downloaded from GenBank to a database designed and housed at FDA. Resistance genotypes were determined using assembled WGS sequences through BLAST analysis with the cutoff set at 50% sequence length and 85% aa identity to known resistance proteins. Results: A comprehensive resistance genotype was identified for each of 114 isolates. Eighteen resistance genes, including tetO, blaOXA-61, catA, lnuC, aph(2'')-Ib, aph(2'')-Ic, aph(2')-If, aph(2'')-Ig, aph(2'')-Ih, aac(6')-Ie/aph(2'')-Ia, aac(6')-Ie/aph(2'')-If, aac(6')-Im, aadE, sat4, ant(6'), aad9, aph(3')-Ic, aph(3')-IIIa plus mutations in three house-keeping genes (gyrA at position 86, 23S rRNA at position 2074 and 2075) were identified by WGS. Overall, there was a high correlation between phenotypic resistance to a given drug and presence of one or more genes expected to confer resistance to that drug. Correlation was 100% for tetracycline, ciprofloxacin/nalidixic acid, and erythromycin. A few discrepancies were observed for gentamicin, azithromycin, clindamycin, and telithromycin; the correlation between phenotype and genotype for these drugs ranged from 95.3% to 98.7%. All isolates were susceptible to florfenicol, and no genes associated with florfenicol resistance were detected. Significance: The resistance mechanism for each of nine antimicrobials tested was defined. Overall, there was a high correlation (>95%) between antimicrobial susceptibility phenotype and genotype. WGS shows promise as a useful tool for antimicrobial resistance surveillance programs.

65. Formulation and Performance of Danazol Nano-crystalline Suspensions and Spray Dried Powders

Zolnik, Banu S., FDA/CDER; Kumar, Sumit, University of Connecticut; Jog, Rajan, University of Connecticut; Shen, Jie, University of Connecticut; Burgess, Diane J., University of Connecticut; Nakissa, Sadrieh FDA/CDER – currently affiliated with FDA/CFSAN

Plain Language Synopsis: The objective of this study was to understand and optimize selection of dosage forms for nanotechnology enabled drug candidates based on their in vivo and in vitro performance.

Abstract: Purpose: The aim of this study was to formulate and evaluate in vitro and in vivo performance of differently sized liquid and spray-dried powders of danazol nanocrystalline suspensions. Methods: A DoE approach was used to optimize stabilizer concentration and formulate danazol (BCS class II) nano-crystalline suspensions and dry powders via wet milling followed by spray drying. Solubility studies were performed to select the best stabilizer combinations. Dowfax 2A1 with either PVP40 or HPMC E3 were the best stabilizer combinations. Particle size, PXRD, contact angle measurement and in vitro dissolution were used in characterization of the liquid and powder formulations. Results: The liquid nano-crystalline suspensions followed particle size-dependent dissolution rates i.e. faster dissolution for smaller crystals. The spray-dried nano-crystal powders did not show fast dissolution profiles compared to the liquid nano-crystalline suspension. The poor dissolution of the spray-dried powder correlated to its high LogP value (LogP 4.53) and poor wetting (determined via contact angle measurement). In vivo bioavailability studies showed superior performance of the liquid nano-crystalline suspensions compared to other milled and un-milled formulations. This study indicates that drug candidates with high LogP values and contact angles may not be suitable for formulation as dry nano-crystals. Conclusions: This study shows the importance of the molecular properties of

poorly soluble drug candidates (BCS class II/IV) on appropriate selection of drug candidates for nano-crystalline formulation. Drug candidates with poor wetting properties (due to high LogP and high contact angle) formulated as nano-crystalline suspensions may not be appropriate for drying processing.

66. Nanotechnology Enabled Formulations: In Vitro and In Vivo Performance of Itraconazole Nanocrystals

Zolnik, Banu S., FDA/CDER; Kumar, Sumit, University of Connecticut; Jog, Rajan, University of Connecticut; Shen, Jie, University of Connecticut; Burgess, Diane J., University of Connecticut; Nakissa, Sadrieh FDA/CDER – currently affiliated with FDA/CFSAN

Plain Language Synopsis: The objective of this study is to understand how nanotechnology enables low solubility drugs to become more bioavailable. Systematic approach was used to make different formulations with different particle size ranges. Each formulation was tested in vivo and in vitro. This study provided insight for improving the absorption of low soluble drugs.

Abstract: Purpose: The objectives of the present study were to formulate and optimize different sized liquid and solid nano-crystalline formulations and evaluate their in vitro and in vivo performance to determine the effect of particle size on the oral bioavailability of solid nano-crystalline formulations. Methods: Itraconazole, categorized as BCS class II, was selected for this purpose. Itraconazole has a solubility of 4-5 µg/ml at pH 1.2 with good membrane permeability. A Netzsch media mill was used to mill the macro-suspension. Wet milling followed by spray drying was used to reduce the particle size and achieve non-aggregating crystalline powder formulations. A DoE approach was used to optimize the milling formulation critical parameters i.e. stabilizer concentrations. Multiple linear regression analysis and ANOVA were employed during DoE. After formulation optimization, different sizes of crystalline formulations were evaluated for in vitro and in vivo performance. The in vitro dissolution experiments were performed utilizing USP apparatus II and the oral bioavailability of the formulations was investigated using a male Sprague -Dawley rat model. Results: Based on drug solubility studies, PVP40 and SLS were selected for wet milling processing. Mannitol was chosen as the auxiliary excipient for spray drying processing. In vitro dissolution utilizing a USP apparatus II, showed superior release profiles for both liquid and nano-crystalline powder formulations compared to coarse-sized and un-milled formulations. Significantly, the oral bioavailability of nano-crystalline formulations with particle size of 280 nm was more than 20 times that of the un-milled formulation. Whereas, the nano-crystalline formulation with particle size of 750 nm showed only a 2.8 times increase in bioavailability compared to the un-milled formulation. Conclusions: This study provides insight for formulation of spray dried nanocrystalline formulation of poorly soluble drugs. In addition, it is shown that within the nano-size range very significant differences in oral bioavailability occur.

Session 2: Strengthen Social and Behavioral Science to Help Consumers and Professional Make Informed Decisions about Regulated Products

(Posters 67-73 are located in Room 1406 and Posters 74-77 are located in Room 1408)

67. Correction of Overstatement and Omission in Direct-to-Consumer Prescription Drug Advertising

Aikin, Kathryn J., FDA/CDER/OMP/OPDP; Betts, Kevin R., FDA/CDER/OMP/OPDP; O'Donoghue, Amie C., FDA/CDER/OMP/OPDP; Rupert, Douglas J., RTI International; Lee, Philip K., RTI International; Amoozegar, Jacqueline B., RTI International; Southwell, Brian G., RTI International

Plain Language Synopsis: We examined the effect of corrective direct-to-consumer advertising on perceptions of drug risks, benefits, product attitudes, and future behavioral intentions. The corrective ad was able to correct a claim overstating drug benefit, but was less effective at correcting omitted risk information. The corrective ad also affected product perceptions and intentions.

Abstract: Purpose: Little experimental evidence exists regarding corrective television advertising as a remedy for misleading direct-to-consumer prescription drug ads. We examined how exposure to a simulated violative ad for a fictitious prescription drug and a corresponding corrective ad shaped viewer perceptions, understanding, and intended behavior. Method: We recruited 1,057 adults who self-identified as being diagnosed with asthma from a nationally representative online panel. Participants were randomly assigned to one of four conditions: violative ad only, corrective ad only, violative then corrective ad, or control (a reminder ad for the product). After viewing the ad(s), participants answered questions assessing their perceptions of the ad claims, perceptions of product risks and benefits, and attitudes and intentions toward the product. Results: Participants who saw the violative ad alone were more likely to rate the overstatement of efficacy claim as accurate compared to those who saw the violative and corrective ads or only the corrective ad, but they did not differ in accuracy ratings of the omission of risk claim from those who saw the corrective or both the violative and corrective ads. Participants who saw the violative ad perceived the ad to be more truthful than those who saw both the violative and corrective ads. Participants who viewed the violative ad recalled more benefits and fewer risks than those who saw only the corrective ad or both the violative and corrective ads. Participants who saw the violative ad perceived the drug as more likely to be effective, more likely to produce strong effects, less risky, and that the risks would be less serious than those who saw the corrective or both the violative and corrective ads. Participants who viewed the violative ad perceived the drug as more effective and less risky compared to other asthma drugs, had more positive future intentions about the product, and had a more positive attitude toward the product than did those who saw the corrective ad or both the violative and corrective ads. Conclusion: The results suggest corrective advertising may be useful for correcting misleading drug impressions, but may be less effective at addressing risk omission.

68. The Long and Short of it: Literacy, Language and Label Comprehension of OTC Drug Facts

Baro, Elande, FDA/CDER/OMPT/CDER/OTS/OB/DBIV; Cohen, Barbara, FDA/OC/OPP/OPL/RCS; Higgins, Karen, FDA/CDER/OMPT/CDER/OTS/OB/DBIV; Komo, Scott, FDA/CDER/OMPT/CDER/OTS/OB/DBIV

Plain Language Synopsis: People of all literacy levels must understand OTC Drug Facts Labels. We take an in-depth look at data from two label comprehension studies. We explain what factors drive the differences in comprehension among literacy groups. We conclude with some advice.

Abstract: FDA regulations require that the labeling of Nonprescription Drug Products be understood by the ordinary individual as well individuals of low comprehension. As a result, it is imperative to evaluate whether subjects of different literacy backgrounds are able to understand key sections of the Drug Facts Label (DFL) including indication, directions for use, warnings and adverse reactions. We look at the Label Comprehension Studies for two Non-Prescription Drugs that have been approved

in the last several years. These studies test the "potential consumer's ability to understand the label messages and to apply the information on the label to hypothetical individuals with varying demographic and medical characteristics". After a brief assessment of the predictors of literacy, we identify the main factors that are associated with differences in comprehension of the DFL among literacy groups. Long sentences and numbers in the label are often associated with a decrease in comprehension rate at any level of literacy. Although females have an overall better comprehension of the DFL, the low literate females perform worse than the low literate males in the dosing instructions. In addition to evaluating comprehension of the label, we assess whether some aspects of the questionnaire itself, such as direct or scenario questions, question length and numbers in the question may differentially affect the performance across literacy groups. An important finding is that low literates may be able to pinpoint the right answer, without necessarily understanding the message on the label, when the same number appears both in the label and in the questionnaire. Moreover, for any literacy group, longer questions and direct questions are associated with an increase in the probability of an incorrect answer. Since the studies attempt to assess whether consumers can correctly apply their comprehension of the label to a hypothetical real life scenario, careful consideration to question wording needs to be considered. We conclude with some recommendations for the design of future label comprehension studies.

69. Harnessing Patient Preference from Social Media

Ho, Martin, FDA/CDRH/OSB; Irony, Telba, FDA/CDRH/OSB; Nguyen, Mimi, FDA/CDRH/OCD; O'Callaghan, Kathryn, FDA/CDRH/OCD; Saha, Anindita, FDA/CDRH/OCD

Plain Language Synopsis: We conducted a sentiment analysis of social media posts about treatment for obesity and epilepsy to obtain potential patient preferences data from the Internet. The study has informed CDRH of patient sentiments in social media. It also allowed CDRH to compare the results with those of a traditional survey.

Abstract: Social media has become a popular medium for individuals to express their opinions. After conducting a patient preferences survey on weight loss devices, CDRH explored using sentiment analysis to harness patient preferences from unstructured posts of social media for comparison with the survey results. Sentiment analysis is an evolving technology that applies text analytic to analyze a document and infer the author's sentiment about a topic of interest, such as a medical treatment. This is a collaboration between CDRH and SAS to capture web-based patient sentiments on the benefits, risks, and other attributes of medical treatment to treat obesity and epilepsy. The study consists of the following steps: (1) Searched for self-reported symptom-treatment-outcome content from a number of sites based on defined terms and concepts, which used to identify posts made by individuals on websites where communities are discussing issues related to the treatment of obesity or epilepsy. These terms/concepts were then determined whether a specific comment would be collected and made part of the corpus of comments used in analysis. (2) Performed signal-to-noise processing of the document corpus (veracity scoring) by applying a set of lexicons and linguistic tools to pre-process the highly unstructured documents to eliminate those deemed as not useful or relevant to the objective and saved the resulting set of comments into a single table used in the analysis to evaluate patient preference and risk tolerance of medical procedures/devices for the treatment and management of patients. (3) Identified and categorized the posts from the processed corpus of data to specific therapies/devices using data. Identified the common and reoccurring topics related to obesity/epilepsy and its treatment including comments related to risk/benefit issues associated with specific therapies. (4) Applied text analytic methods to determine patient sentiment and polarity as a function of the content and context of collected posts. It would allow the system to analyze the timing and frequency of posts related to specific "attributes" related to the state of patients' conditions, the application of medical and surgical therapies and an analysis of comments related to risk and benefit. (5) Built a visual dash board that captures patient sentiments for subsequent incremental data.

70. Comparing Food Label Use and Understanding among English-dominant Hispanics, Spanish-dominant Hispanics, and Other Consumers

Liu, Sherry, FDA/CFSAN/OAO; Lin, Chung-Tung Jordan, FDA/CFSAN/OAO

Plain Language Synopsis: Spanish-dominant Hispanics were less likely to read the food label when buying a food product for the first time, use serving size information, use the label to compare different food items, and use the label to see how high or low the food is in certain nutrients than English-speaking non-Hispanics.

Abstract: While some Hispanics living in the United States use the English language exclusively or more often than Spanish (English-dominant Hispanics), other Hispanics in the United States predominantly use the Spanish language in their daily lives (Spanish-dominant Hispanics). This study examined food label use and understanding of label information among Spanish-dominant Hispanic, English-dominant Hispanic, and English-speaking non-Hispanic groups. The analytic sample (n=2,804) consisted of adults aged 18 years or older in the United States from probability-based online consumer panels KnowledgePanel® and KnowledgePanel LatinoSM. Sampled panel members were invited by email to complete a survey online from October 2 thru October 25, 2012. Participants were categorized into Spanish-dominant Hispanic, English-dominant Hispanic, and English-speaking non-Hispanic population groups and asked how often they read the food label and use the label information in different ways. Participants were also asked to perform calculations (e.g., calories, percent daily value) and to select the correct meaning of the terms "serving size" and "percent daily value." Logistic regression was used to examine the relationships between population group and each of the study outcomes. Adjusting for covariates, Spanish-dominant Hispanics were less likely to read the food label and use serving size information than English-speaking non-Hispanic and English-dominant Hispanic population groups. Spanish-dominant Hispanics were also less likely to use the label to compare different food items with each other and to see how high or low the food is in things like calories, salt, vitamins, or fat than English-speaking non-Hispanics. Similar food label use behaviors were reported by English-dominant Hispanics and English-speaking non-Hispanics. Spanish-dominant and English-dominant Hispanic groups were less likely to correctly calculate caloric intake than English-speaking non-Hispanics. English-dominant Hispanics were also less likely to understand the meaning of the term "percent daily value" than English-speaking non-Hispanics. Different perceptions, attitudes, and behaviors can impact individuals' health outcomes and public health information and education may be more effective if targeted to different subgroups' needs. These findings suggest that additional strategies for educating Hispanics, particularly Spanish-dominant Hispanics, on how to use nutrition information on the food label to help make informed dietary choices are needed.

71. Healthcare Provider Perceptions of Direct-to-Consumer Advertising: A National Survey of Primary Care Physicians, Specialists, Physician Assistants, and Nurse Practitioners

O'Donoghue, Amie, FDA/CDER/OMP/OPDP/IO; Kelly, Bridget, RTI International; Geisen, Emily, RTI International; Aikin, Kathryn, FDA/CDER/OMP/OPDP/IO; Betts, Kevin, FDA/CDER/OMP/OPDP/IO; Southwell, Brian, RTI International; West, Suzanne, RTI International; Chowdhury, Dhuly, RTI International

Plain Language Synopsis: We examined how primary care physicians, specialists, nurse practitioners, and physician assistants felt about direct-to-consumer (DTC) advertising of prescription drugs. Findings suggest some important differences between provider types in terms of attitudes toward DTC advertising and the effects of DTC advertising on patients and patient-provider communication.

Abstract: Direct-to-consumer (DTC) advertising of prescription drugs has increased substantially in the last 15 years. At the same time, the role of physician extenders (nurse practitioners and physician assistants) has become increasingly important. We conducted a nationally representative online survey of 2,008 providers, including primary care physicians, specialists, nurse practitioners, and physician assistants. Respondents answered questions regarding their attitudes toward DTC advertising and their beliefs

about its effects on patients and patient-provider communication. Results showed that overall, provider attitudes toward DTC advertising were mixed, although providers felt that DTC advertising was more beneficial (45%) than harmful (29%) to patients. A substantial portion of providers (42%) indicated that patients have refused prescriptions because of the side effects described in DTC ads. Physician extenders had more positive views of DTC advertising than primary care physicians. Primary care physicians reported more pressure to prescribe advertised medications than specialists or nurse practitioners. These findings suggest some important differences between provider types in terms of attitudes toward DTC advertising and the effects of DTC advertising on patients and patient-provider communication.

72. The Interagency *Listeria monocytogenes* in Retail Risk Assessment: A Mathematical Tool to Identify Efficient Risk Mitigations In Retail Delicatessens

Pouillot, Régis, FDA/CFSAN/OAO; Gallagher, Daniel, Virginia Polytechnic Institute and State University; Dennis, Sherri, FDA/CFSAN/OAO; Kause, Janell, USDA/FSIS

Plain Language Synopsis: Practices in retail delicatessen may lead to the contamination of food with *Listeria monocytogenes*, a deadly bacteria. We developed a computer model that mimics a deli to inform risk managers about the most effective strategies to reduce the risk of illness in this setting.

Abstract: Listeria monocytogenes (Lm) is a foodborne pathogen, leading to a rare but frequently fatal disease. Scientific literature suggests that ready-to-eat foods (RTE) sliced or prepared in the delicatessen (deli) of retail food stores may contribute to a significant number of listeriosis cases. Nevertheless, little was known about how Lm contamination of RTE foods occurs in retail delis. In this context, risk assessment provides a useful framework to integrate scientific research and data and to evaluate the public health implications of changes in food safety practices and policies. A mathematical model that mimics an operating deli of a retail food store tracks the occurrence and number of *Listeria monocytogenes* (Lm) that may potentially be present in the environment and on ready-to-eat (RTE) foods and estimates the risk of listeriosis from consumption of the RTE foods commonly prepared and sold in those delis. The model is used for an in-depth evaluation of the factors that contribute to an increased risk of listeriosis, and to evaluate the public health impact of various interventions under six different baseline conditions that may characterize a retail deli and the RTE products it serves. The results of the study suggests reducing the risk of listeriosis associated with RTE foods prepared and served in retail deli operations is dependent upon i) the control of the bacterial growth through the use of growth inhibitors or through better temperature control in retail delis, ii) the control of cross contamination during the routine operation of the retail deli, iii) the control of contamination at its source, notably the control of the environmental contamination and niches, and the control of contamination on incoming products, whether or not those products support Lm growth, and iv) continuous sanitation. The results also confirm a deli slicer can be a primary site for Lm cross contamination. This quantitative risk assessment serves as a "virtual laboratory" to evaluate the public health impact of changing retail deli practices and interventions. The predictions generated by this model improves our understanding of the complex factors that impact the risk of listeriosis from consumption of RTE products prepared in the retail deli, informs risk managers about the relative impact of potential mitigation strategies, and encourage improvements to retail food safety practices to further control Lm in RTE foods.

74. Developing Audience Personas for Communicating Drug Safety Messages and Information

Rausch, Paula, FDA/CDER/OC/DHC; McCormack, Lauren, RTI International, Center for Communication Science; Taylor, Olivia, RTI International, Survey, Statistical and Computing Science; Lefebvre, Craig, RTI International, Center for Communication Science; Bann, Carla, RTI International, Center for Communication Science

Plain Language Synopsis: We conducted a large survey to better understand the kinds of information patients and HCPs need and desire related to safety concerns that arise with drugs after they're approved. We used that data to

develop four audience profiles that will help us better communicate drug safety information to the public.

Abstract: This study's purpose was to develop a set of research-based audience profiles that go beyond traditional demographic characteristics to include factors that affect information processing and decision making, and examine how these factors and other consumer characteristics can impact the effectiveness of communications about emerging post-market drug safety issues. To do this, we conducted a national cross-sectional internet panel survey of 1,244 consumers to define a set of drug safety information audience personas. We included a variety of measures drawn from key behavioral and communication theories, including health literacy; perceptions of relative risk, seeking control and subjective norms; self-appraisal of skill; information competency; self-efficacy; and medication use. To further understand audience behaviors and characteristics, we also examined comprehension, behavioral intentions, and other measures of effectiveness for two different randomly assigned versions of a drug safety message. Using Chi-squared Automatic Interaction Detector (CHAID) and latent class analysis, we identified four clusters of respondents that provided the foundation for the audience personas based on several key distinguishing characteristics, including age, race, education, health literacy, perceived seeking control, self-appraisal of skill, information insufficiency, self-efficacy, and medication use. Based on a linear regression model, the four personas differed on their level of comprehension, intentions to report symptoms to a health care professional, and intentions to look for more information about the medicine regardless of the DSC they reviewed. These statistically derived groups were transformed into the following personas: "Not Engaged" (12%), "Low Involvement Users" (29%), "Careful Users" (50%) and "Social Information Seekers" (9%). Significant differences on socio-demographic, risk information-seeking variables, and health literacy levels, were found among the four Personas. Significant differences in their responses to FDA drugs safety messages were also found, including how well they understood the content, if they would report experiencing the highlighted symptoms to their provider or FDA, and the likelihood of searching for more information. This study shows that relatively inexpensive web-based surveys, grounded in relevant theoretical constructs, can be used to develop clear and distinct "pictures" that can be used to visualize priority audience segments, and guide the development of drug safety messages, products and services.

75. Quantitative Information on Oncology Prescription Drug Websites: A Content Analysis

Sullivan, Helen, FDA/CDER/OMP/OPDP; Aikin, Kathryn, FDA/CDER/OMP/OPDP; Squiers, Linda, RTI International

Plain Language Synopsis: We examined 65 cancer-related prescription drug websites to see how drug benefits and risks are communicated to consumers and health care professionals. Most websites used numeric information. This was more likely for health care professional-directed webpages. Websites were more likely to present numeric information for all benefits than for all risks.

Abstract: Purpose: Prescription drug advertising, which is prevalent in oncology, can impact patient-physician interactions. Research has shown that including quantitative information about drug benefits and risks into direct-to-consumer (DTC) ads can increase consumer understanding, although some numeric and graphic formats are more useful than others. Our objective was to determine whether and how quantitative information about drug benefits and risks is presented to consumers and healthcare professionals on cancer-related prescription drug websites. Methods: We conducted a content analysis, reviewing 65 active cancer-related prescription drug websites in 2014. We assessed the inclusion and presentation of quantitative information for two audiences (consumers and healthcare professionals) and two types of information (drug benefit and risk). Results: All but one website (98.5%) presented some quantitative information. This information was more likely to appear on webpages directed at healthcare professionals (98.5%) compared to webpages directed at consumers (72.3%), $p < 0.001$. Overall, websites were equally likely to present quantitative information for benefits (96.9%) and risks (95.4%). However, the amount of the information differed significantly; both consumer-

directed and healthcare professional-directed webpages were more likely to have quantitative information for all benefits (consumer: 38.5%; healthcare professional: 86.1%) compared to all risks (consumer: 3.1%; healthcare professional: 6.2%), $p < 0.001$. The numeric and graphic presentation of the quantitative information also differed by audience and information type. Conclusion: Consumers have access to quantitative information about oncology drugs, and in particular about the benefits of these drugs. This has implications for patient-physician interactions.

76. Relationships between Food Label Use Behaviors and Adult Weight Outcomes

Zhang, Yuanting, FDA/CFSAN/OAO/DPHIA; Liu, Sherry T., FDA/CFSAN/OAO/DPHIA; Chen, John Tuhao, Department of Mathematics and Statistics, Bowling Green State University

Plain Language Synopsis: We examined the relationships between food label use behaviors and body mass index (BMI) using data from the 2008 Health and Diet Survey (HDS) and the 2007-2008 National Health and Nutrition Examination Survey (NHANES). We found that overweight and obese adults may use food labels differently than normal weight adults.

Abstract: The relationship between food label use and obesity is complex and not well understood. In this paper, we further examined the relationships between food label use and body mass index (BMI) using data from the 2008 Health and Diet Survey (HDS) and the 2007-2008 National Health and Nutrition Examination Survey (NHANES). Specifically, this study use propensity score techniques to validate previous studies on whether there is a causal relationship between food label use and weight status. Data from the 2008 Health and Diet Survey (HDS) and 2007-2008 National Health and Nutrition Examination Survey (NHANES) were used. Descriptive statistics, Chi-square tests and adjusted logistic regression models were used to examine the association between food label use behaviors and obesity. Odds ratios of food label use behaviors among overweight and obese individuals compared to normal weight individuals were estimated. Propensity score matching, which allows causal inferences with the observational data in modeling the effect of an intervention, was used to analyze HDS data. Finding from the unweighted HDS data showed that overweight ($p<.001$) and obese adults ($p<.05$) were more likely to use serving size and calorie information ($p<.001$) compared to normal weight adults. Similarly, unweighted data from the NHANES revealed that overweight adults were more likely to use food labels ($p<.10$) and serving size information ($p<.05$) than normal weight adults; obese adults were also more likely to use serving size information ($p<.10$). However, these differences in food label use disappeared in weighted models, which may suggest that these results are not generalizable to the overall U.S. population. In HDS analyses, obese adults were also less likely to pay attention to the food brands than normal weight adults ($p<.01$). Overall, overweight and obese adults may use food labels and label information differently than normal weight adults. According to our study, there may be some weak associations between the food label use behaviors and weight status. Overweight and obese consumers were more likely to read the food label information on calorie and serving sizes, but might not understand the meanings of the exact information.

77. Model Based Design and Safety Analysis of Medical Device User Interfaces

Zhang, Yi, FDA/CDRH/OSEL; Jones, Paul, FDA/CDRH/OSEL; Masci, Paolo, Queen Marry University of London

Plain Language Synopsis: This research applies model based engineering techniques to develop novel verification and hazard analysis methods, which help manufacturers establish the quality and safety in medical device user interface designs. Artifacts produced by these methods provide evidence for regulators to quickly and objectively assess the safety of devices' interaction with users.

Abstract: Designs of medical device Human Computer Interfaces (HCI) need to be robust and appropriately reactive to user actions. There is evidence that the HCI design of some devices on the market can cause use errors and erroneously process user input, which may subsequently lead to serious patient harm. Model based engineering (MBE) technology can be used to model HCI design decisions with mathematical precision. This technology can facilitate the development of HCI models that clearly define the device's interaction behavior with users; offering a

formal (mathematical) basis to reason about and verify the safety of the design. Automatic tool support is available to facilitate such reasoning and verification activities. Tool artifacts provide manufacturers and regulators an objective and scientific basis for assessing the safety of medical device user interfaces. The authors have successfully applied MBE techniques to the analysis of medical device user interfaces in two studies. In the first study, automatic model extraction was applied to the user interface software of a marketed infusion pump to produce a model that resembles the pump's use interaction behavior. Automated formal proving on the model uncovered several design flaws in the pump's user interface that could lead to severe consequences including the pump ignoring key presses that might cause patient overdose. In the second study, the authors captured the user interface software design common in medical devices with a generic user interface model. Based on this generic model, a hazard analysis technique was proposed that integrates human cognition process models and general interaction design principles to guide more comprehensive and systematic identification of design flaws in user interfaces. Preliminary experiments showed that this hazard analysis technique can identify 3 times more software-related hazards in user interface designs, compared to standard hazard analysis techniques.

Session 3: Facilitate Development of MCM to Protect Against Threats to US and Global Health and Safety

(Posters 1-35 are located in Section A)

1. Norovirus Infection Enhances in vitro Growth of *Salmonella enterica* while Co-infection Dampens Virus Survival

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Plain Language Synopsis: We determined that co-infection of cells with the foodborne pathogens Norovirus and the *Salmonella enterica* increases bacterial growth while reducing virus replication. Interestingly, prior viral infection also increases bacterial growth. Efforts in identifying the mechanistic events occurring during co-infection will improve diagnosis and treatment of disease caused by these pathogens.

Abstract: Co-infection of bacterial and viral pathogens often culminates in exacerbation of host response and enhances disease severity. Noroviruses are the major cause of non-bacterial gastroenteritis in humans contributing to greater than 50% of foodborne infections in the U.S.A. Noroviruses infect over 267 million people annually, and cause about 200,000 deaths in infants and the elderly, thereby representing a major public health threat. Similarly, *Salmonella enterica* is a human pathogen found in poultry food and infects 1.4 million people annually, resulting over 17,000 hospitalizations and 600 deaths. Due to increased percapita consumption of poultry and seafood, there is an augmented risk of co-infection of these pathogens, which may result in severe gastrointestinal disease in humans. Molecular mechanisms that govern the co-infection of these pathogens are yet to be explored. Since there is no established in vitro system to study human norovirus, we used murine norovirus as a surrogate since it causes robust infection and cytopathicity of RAW 264.7 macrophage cells. This cell line also supports robust growth of S. enterica inducing cytopathic effect. RAW 264.7 cells were infected at an MOI of 5 of murine NoV and S. enterica, and at 24 hours post infection, bacterial counts and virus titers were assessed. Co-infection resulted in statistically significant increase in bacterial growth, compared to the bacterial control. Interestingly, co-infection resulted in an approximately a two-log reduction of norovirus when compared to virus control as measured by the plaque assay. This observation was supported by the decrease in viral RNA levels measured by real-time RT-PCR. More importantly, prior exposure of macrophages to norovirus enhanced their susceptibility to S. enterica infection as demonstrated by a statistically significant increase in bacterial growth when compared to the bacterial control. Up regulation of macrophage extracellular adhesion molecules and proinflammatory cytokines that enhance bacterial infection may support these observations, and studies are underway to define the molecular basis of increased susceptibility to bacterial pathogenesis upon infection with norovirus. Identification of molecular markers will support the regulatory decisions of FDA in approving diagnostic tests and provide treatment guidelines in cases of co-infection of these pathogens in humans.

2. Collaborative Study for the Characterization of a Chikungunya Virus RNA Reference Reagent for Use in Nucleic Acid Testing

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Plain Language Synopsis: We developed and characterized in a collaborative study a CHIKV RNA Reference reagent to assist in the development and evaluation of CHIKV NAT assays for use in blood screening.

Abstract: Infections with the mosquito-borne chikungunya virus (CHIKV) can cause febrile illness or be asymptomatic. Laboratory diagnosis of CHIKV is often made with laboratory-developed nucleic acid tests (NAT) since there are no FDA-approved diagnostic or blood screening assays. A CHIKV RNA reference reagent (CHIKV-RR) consisting of cell culture-grown, heat-inactivated CHIKV diluted in human plasma was assessed by 8 laboratories in a collaborative study. The participants were asked

to test the CHIKV-RR using their NAT assay(s) by qualitative testing (determination of RNA end-point by testing log and half-log dilutions followed by calculation of estimated NAT-detectable units/mL, after adjustment for the volume of reagent used for testing), and by quantitative testing, when available. Results from the testing showed that the CHIKV-RR had an estimated overall mean of 7.56 log10 detectable units/mL, ranging from 6.2 log10 to 8.6 log10. The CBER/FDA CHIKV RNA Reference reagent for NAT has been characterized through a collaborative study and determined to have a concentration of 7.56 log10 detectable units/mL. This material is now available to assist assay development and public health laboratories worldwide upon request, and for use to formulate a lot release panel upon assay licensure.

3. Genetically Modified Live Attenuated L. donovani Parasites Induce Innate Immunity through Classical Activation of Macrophages that Directs Th1 Response in BALB/C Mice

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Plain Language Synopsis: We demonstrated the biomarkers of innate response by live attenuated parasites in macrophages from BALB/c mice. These biomarkers will be helpful in the evaluation of Leishmania vaccine efficacy.

Abstract: Visceral Leishmaniasis (VL) causes significant mortality and there is no effective vaccine. Previously, we have shown that, genetically modified L. donovani parasites (Ldp27-/-and LdCen-/-), hereafter described as live attenuated parasites, induced host protective adaptive immune response in various animal models (mice, hamster, dog). However the immunological basis of protection in terms of host innate response induced by these live attenuated parasites and the interaction of such response with the protective adaptive immune response has not been yet demonstrated. In this study, we demonstrate innate immune response upon infection with live attenuated parasites in macrophages from BALB/c mice both in vitro and in vivo. In vitro infection of macrophages with live attenuated parasites induced significantly higher production of pro-inflammatory cytokines (TNF-a, IL-12, IFN-g,IL-6), chemokines (MCP-1/CCL-2, MIP-1a/CCL-3,IP-10), ROS and nitric oxide concomitantly reducing anti-inflammatory cytokine (IL-10) and arginase-1 activity compared to infection with LdWT parasites suggesting dominant classically activated/ M1 macrophage response which in turn helps in presenting antigen to T cell as observed by robust CD4+T cell activation in vitro compared to LdWT infection. Similarly, in in vivo studies, parasitized splenic macrophages isolated from live attenuated parasite infected mice also demonstrated induction of M1 macrophage phenotype as indicated by up regulation of IL-1b, TNF-a, IL-12, iNOS2 and MIP-1a , and down regulation of genes associated with the M2 phenotype i.e. IL-10, YM1, Arg-1 and MRC-1 compared to LdWT infected mice. Furthermore, ex-vivo antigen presentation assay showed macrophages from live attenuated parasite infected mice induced more IFN-γ and IL-2 but significantly less IL-10 production by OVA specific CD4+T cells resulting in proliferation of host protective Th1 cells. These data suggest that live attenuated parasite infection promotes a state of classical activation (M1 dominant) in macrophages, leading to the generation of Th1 response that could lead to protective immunity in mice.

4. Ricin Detection: Tracking Active Toxin in Environmental Samples

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Plain Language Synopsis: This project aims at developing a sensitive and robust assay for the detection of active ricin toxin in contaminated environmental samples (e.g. facility surfaces and food matrices).

Abstract: Ricin is a naturally occurring protein toxin that has been linked to numerous antigovernment and terrorist reports. Because of its acute toxicity, ricin has been categorized as a category B biothreat agent by the US Center for Disease Control and Prevention (CDC). Acting as a ribosome inhibitor, ricin is composed of an A- and B-polypeptide chain. The B-chain of ricin is a carbohydrate binding protein which is required for cellular entry, while the A-chain is

an N-glycosidase responsible for inhibiting the ribosome by selectively removing an adenine base. Importantly, both peptide chains are necessary for toxicity. Our lab carries out a MCM funded project aimed at developing sensitive and robust methods for detecting biologically active ricin. In our current design, ricin will be captured from contaminated environmental samples using either DNA aptamers or semi-synthetic carbohydrate ligands both of which have been engineered to specifically bind to the B-chain of ricin with high affinity. Biologically active A-chain will subsequently be detected using either cell-based cytotoxicity assays or cell free enzymatic assays that can identify RNA/ribosome deadenylation. The anticipated outcomes of this project will advance the science and technology behind the nation's ability to recover from potential adverse impacts caused by the intentional release of ricin.

5. The Role of T-regulatory Cells to Control the Tacaribe infection in Mice

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Plain Language Synopsis: Hemorrhagic viruses that infect the brain can cause paralysis, convulsions, and death. Here we use a mouse model to show for the first time that increasing the frequency of suppressor regulatory T cells can improve the clinical outcome in neonatal mice with arena virus Tacaribe encephalitis.

Abstract: Viral hemorrhagic fevers such as Ebola, Lassa, Junin and Machupo are neurotropic viruses that cause a multi-system syndrome that includes vascular damage and encephalitis often resulting in death or long-lasting neurological sequelae. Neonates are at increased risk of viral encephalitis. Using Tacaribe arenavirus as a model of viral hemorrhagic fever in neonatal mice we studied the role of T cells in the pathogenesis of the disease. We show that the encephalitis in neonatal mice is caused not by the virus, but by infiltrating effector CD4+ and CD8+ T cells into the brain parenchyma. Interestingly, during infection regulatory T cells (Tregs) do not infiltrate the CNS. Since the absence of Tregs can lead to increased inflammation and tissue damage we explored the role of Tregs in TCRV induced encepahlytis in neonatal mice. We find that increasing the frequency of Tregs by transferring Tregs of naïve mice is sufficient to improve survival. Despite increased numbers the Tregs do not migrate into the CNS but are retained in the cervical lyM.P.H. nodes. We hypothesize that Tregs reduce the proliferation of effector CD8+T cells as well as the migration of CD8+T cells into the brain as we have detected overall fewer CD8+T cells in brains of infected mice that have increased numbers of Treg. Supporting ex-vivo studies show that the proliferation of virus specific memory CD8+T cells purified from mice primed and boosted with TCRV is suppressed by the addition of Tregs in the culture system. Thus, these results suggest that modulation of the Treg population can reduce the proliferation and migration of CD8+ T cells into the CNS during a TCRV infection reducing tissue damage and improving the survival.

6. Non-coding RNAs and Heme Oxygenase-1 in Vaccinia Virus Infection

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Plain Language Synopsis: We have identified several small unique ncRNAs in the total RNA isolated from vaccinia-infected primary human cells. This new class of ncRNAs is likely to provide a new mechanistic pathway of virus-host interaction, thus expanding our knowledge about the regulatory potential of ncRNAs in disease pathogenesis and in developing strategies for therapeutic interventions against poxvirus infection.

Abstract: Small nuclear RNAs (snRNAs) are <200 nucleotide non-coding uridylate-rich RNAs. Although the functions of many snRNAs remain undetermined, a population of snRNAs is produced during the early phase of infection of cells by vaccinia virus. In the present study, we demonstrate a direct correlation between expression of the cytoprotective enzyme heme oxygenase-1 (HO-1), suppression of selective snRNA expression, and inhibition of vaccinia virus infection of macrophages. Hemin induced HO-1 expression, completely reversed virus-induced host snRNA expression, and suppressed vaccinia virus infection. This involvement of specific virus-induced snRNAs and associated gene clusters

suggests a novel HO-1-dependent host-defense pathway in poxvirus infection.

7. Interferon-lambda is Produced by and Induces Autocrine Expression of Interferon-Stimulated Genes in Human Bronchial Epithelial Cells

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Plain Language Synopsis: This project examines the roles of two types of interferon proteins, produced by human respiratory epithelial cells in response to infection by a respiratory virus, to coordinate host cell antiviral gene expression. These data define a feedback mechanism involved in the well-known host antiviral response induced upon viral infection.

Abstract: Interferons play a key role in the innate immune response to viral infection. In this study, we determined which IFN genes are expressed by primary human bronchial epithelial (HBE) cells following infection with respiratory syncytial virus (RSV) strain A2. Infection of HBE cells with RSV induced co-expression of the IFN-lambda (Type III interferon) genes (IFNL1, IFNL2 and IFNL3) in a dose- and time-dependent manner. It also induced expression of the IFNB1 gene and low levels of the IFNA1 gene, but not other type-I IFN genes. Induction of IFNB1 and IFNL gene expression correlated with marked increases in the levels of IFN-beta and IFN-lambda protein in the culture supernatants. The newly expressed IFN-beta and IFN-lambda proteins in turn induced activation of STAT1 and STAT2 and subsequent expression of mulitple IFN-stimulated genes (ISGs), including MX1, OAS1, IFIT1, IFIT3, IFI44 and IRF7. Using neutralizing antibodies that selectively inhibit the activity of type-I versus type-III IFNs, we determined that ISG expression induced by RSV infection in HBE cells can be inhibited partially by either anti-type I or anti-type III IFN antibodies and almost completely by co-treatment with both types of antibodies together. Recombinant human IFN-lambda1 induced activation of STAT1 and STAT2 in naïve HBE cells, and induced expression of the same ISGs that were induced by viral infection. Furthermore, pre-treatment with recombinant human IFN-lambda1 decreased the ability of RSV to infect naïve HBE cells indicating that IFN-lambda1 may be a useful prophylactic agent to protect against infection by respiratory viruses that preferentially infect airway epithelial cells. These findings demonstrate that bronchial epithelial cells express significant levels of both type I and type III IFN following viral infection. Furthermore, the newly expressed IFN-beta and IFN-lambda proteins combine to mobilize a potent antiviral response in HBE cells by inducing autocrine expression of multiple ISGs.

8. Assembling a Field-Based DNA Diagnostic System Independent of Lab Instruments, Electricity, and Refrigeration

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Plain Language Synopsis: Numerous challenges exist for identifying harmful bacteria or viruses in locations lacking standard laboratory facilities ("the field"). The polymerase chain reaction (PCR) is a technology capable of detecting these agents. We have investigated whether instruments, reagents, and common resources could be adapted for detection of bacteria in "the field."

Abstract: Numerous challenges exist for utilizing DNA diagnostic technologies in locations lacking standard laboratory facilities. The polymerase chain reaction (PCR) is a technology ideally suited for the detection of targeted genomic fragments from bacteria, viruses, or higher-level species. The challenges in utilizing this technology in the field encompass sample preparation (obtaining target DNA in sufficient quality and quantity), amplification of the defined genetic target to detectable levels, and detection/quantification/identification of the target genome, ideally in the absence of a requirement for standard lab instruments, refrigeration, and electricity. Much of the published work on a "point of care" electricity-free PCR diagnostic system has focused on specially designed alternative amplification instruments powered by the sun or by a chemical reaction, often using an isothermal amplification technology (LAMP). These instruments are not generally available currently, are of questionable cost and utility, and have required preparation kits

and reagents not likely to be practical in a field environment. We have accepted this challenge and sought a simpler path: we asked whether available instruments, reagents, and common resources could be assembled into a viable pathway for detection of a common pathogen, *Salmonella enterica* Typhimurium, as a model. We have experimental data on: 1) simple methods for isolation of suitable quality DNA from the bacteria; 2) determination of the utility of a small, commercially available battery-operated PCR instrument, the Palm PCR, comparing its function with standard lab instruments for amplification reactions and limits of detection of target DNA; 3) stability of different Taq DNA polymerases at ambient temperature; 4) detection of amplified DNA using a common dye and a cell phone (still under active investigation). The challenges and solutions at each step will be outlined. The issues addressed may apply to the regulatory review of some devices designed for point of care diagnostics. This work demonstrates the potential for the use of existing, available reagents and instruments to create a viable DNA-based diagnostic method for use in the field. The methods developed could be adapted to test for the presence of various bio-threat agents, pathogens, or animal species using basic PCR technologies.

9. An Optimized Inter-Regional Model to Assess the Impact of Pandemic Influenza on the US Blood Supply

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Plain Language Synopsis: Severe disruptions in blood collections could prevent patients from receiving important medical care. Our inter-regional model simulates how a Pandemic Influenza may disrupt the US blood supply system. Our model helps decision makers better understand the dynamics of such complex events and reach well-informed decisions.

Abstract: Background: Severe disruptions in blood collections could prevent patients from receiving important medical care. Pandemic Influenza is an important example of an unpredictable event that poses a threat to the US blood supply and public health. Pandemic Influenza may have a major impact on the public because people have little or no immunity to the new virus. During Pandemic Influenza, blood collection may decrease significantly because many regular donors and blood collection personnel may be ill or caring for someone who is ill. This would reduce the US blood supply levels and possibly cause a shortage of blood and blood products. We assess the potential impact of Pandemic Influenza on US Blood Supply levels by modeling inter-regional variation of Pandemic Influenza trends in the US. Methods: We refined a previously developed Pandemic Influenza scenario (Simonetti et al. ref) by employing an Inter-regional Blood System (IRBS) in which we optimized the transfer of blood among the US regions by using a Neural Network Heuristic (NNH). We analyzed spatial and temporal variations of Pandemic Influenza by dividing the US into four regions to reflect the blood collection regional subdivision. Results: The NNH enhanced the overall efficiency of the IRBS, which consequently improved the response of the total national blood supply. We present a comparison between the results on the effect of Pandemic Influenza from a national versus an inter-regional model. Conclusions: Understanding how the complex dynamics of a Pandemic Influenza in its spatial and temporal variations could potentially impact the US blood supply levels may assist decision-makers and stakeholders to make informed decisions.

10. The Role of Female Hormones in Enhanced Susceptibility to Avian Influenza Infections in a Mouse Model

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Plain Language Synopsis: Statistics show that women are more susceptible to severe influenza illness than are men. Here, using a mouse model, we show that female mice were more susceptible than male to severe disease associated with a highly pathogenic influenza virus. This susceptibility positively correlated with levels of progesterone, a female hormone.

Abstract: Epidemiological data suggests that

women, particularly pregnant women, are more susceptible than men to influenza infections including highly pathogenic avian influenza (HPAI) H5N1 viruses. Vaccination is the most effective approach to prevent influenza-associated illness; however, because of misconceptions about severe complications of influenza infections and the safety of vaccines, many pregnant women have been reluctant to receive influenza vaccines. Physiologic and immunological changes associated with female hormone shifts during pregnancy or menstrual cycle can predispose women and pregnant women to infections such as HIV. Thus, understanding the role of female hormones in enhanced susceptibility of women and pregnant women to avian influenza viruses is crucial for developing effective intervention strategies against avian influenza. Addressing the specific needs of vulnerable populations including pregnant women in the public health response is the key in pandemic preparedness. Due to the ethical considerations about the safety of fetus, few clinical studies are designed to specifically address medical concerns and safety issues in pregnant women. Thus, we developed a mouse model to better understand the impact of avian influenza infections on gender and pregnancy and to try to identify the potential risk factors that account for the observed susceptibility. Our model studies have confirmed that female mice especially pregnant mice are more susceptible than male mice to avian influenza as indicated by more severe lung inflammation. Enhanced susceptibility of female and pregnant mice to avian influenza appeared to be positively correlated with serum progesterone levels while negatively correlated with serum estradiol levels. Mice implanted with estradiol pellets showed little weight loss while progesterone- and placebo-implanted mice showed similarly significant morbidity following a sublethal infection of an H5N1 vaccine candidate without polybasic HA cleavage site. Despite all hormone-implanted mice having similar pulmonary viral titers, progesterone- and placebo-implanted groups exhibited a stronger pro-inflammatory response, while pro-inflammatory cytokine secretion was significantly reduced in the lungs of mice implanted with estradiol pellets. These findings indicate that female hormones differentially regulate host immunity during avian influenza infection; thus developing safe and efficacious H5N1 pandemic vaccines should be specifically tailored to the needs of high-risk subjects such as pregnant women.

11. Non-Glycosylated G Protein Vaccine Protects against Homologous and Heterologous RSV Challenge while Glycosylated G Enhances RSV Lung Pathology and Cytokine Levels

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Plain Language Synopsis: The G attachment protein represents an important candidate for inclusion in an effective RSV vaccine. We evaluated the safety and protective efficacy of non-glycosylated vs. glycosylated RSV G protein vaccination in mice. The glycosylated G protein provided poor protection and enhanced lung pathology after RSV challenge, while non-glycosylated G protected mice suggesting that it could be developed as a protective vaccine against RSV disease.

Abstract: New efforts are under way to develop a vaccine against RSV that will provide protective immunity without the potential for vaccine-associated disease enhancement as was observed in infants following vaccination with formalin-inactivated RSV vaccine. In addition to the F fusion protein, the G attachment surface protein represents a target of neutralizing antibodies and thus represents an important vaccine candidate. However, glycosylated G protein expressed in mammalian cells was shown to induce pulmonary eosinophilia upon RSV infection in mouse model. In the current study, we evaluated in parallel the safety and protective efficacy of RSV A2 recombinant non-glycosylated G protein ectodomain (amino acids 67-298) expressed in E. coli (REG) vs. glycosylated G produced in mammalian cells (RMG) in a mouse RSV challenge model. Vaccination with REG generated neutralizing antibodies against RSV A2 in 7/11 BALB/c mice, while RMG did not elicit neutralizing antibodies. Total serum binding antibodies against the recombinant proteins (both REG and RMG) were measured by SPR and found to be >10-fold higher for REG compared with RMG-vaccinated animals. Complete reduction of lung viral loads after homologous (RSV-A2) and heterologous (RSV-B1) viral challenge was observed in 7/8

animals vaccinated with REG but not in RMG vaccinated animals. Furthermore, enhanced lung pathology and elevated Th2 cytokines/chemokines were observed exclusively in animals vaccinated with RMG (but not with REG or PBS) after homologous or heterologous RSV challenge. This study suggests that bacterially produced non-glycosylated G protein could be developed alone or as component of a protective vaccine against RSV disease.

12. Developing Peptide Mimotopes of Capsular Polysaccharides and Lipopolysaccharides Protective Antigens of Pathogenic Burkholderia Bacteria

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Plain Language Synopsis: This study is to address the specific science gap for medical countermeasures (MCMs) against biological threats of pathogenic B. mallei and B. pseudomallei by developing peptide-based mimotopes as vaccine candidates. The study offers MCM development against the potential devastating biothreats to U.S. and Global Health and Security

Abstract: Background: Burkholderia pseudomallei (BP) and Burkholderia mallei (BM) are the causative agents of melioidosis and glanders, respectively. Effective therapeutics and safe vaccines against these bacterial pathogens are urgently needed. Our lab was previously supported by DTRA/DoD to develop BP and BM-specific monoclonal antibodies (MAbs) and to evaluate their anti-BP and BM effects. After studying more than 100 MAbs developed, we identified 4 MAbs recognizing specifically capsular polysaccharides (PS) or lipopolysaccharides (LPS) of BP and BM possessed the high protective activities against lethal doses of intranasal BP and BM infections in mice. Bacterial PS and LPS protective antigens that usually induce only short-term immune response and possess toxicity may not be optimal vaccine candidates in humans. Methods: Using phage display panning against three different phage peptide libraries, we selected phage clones specifically recognized by each of the 4 protective MAbs. Candidate phage clones selected after at least 3 rounds of biopanning to ensure the validity of specific binding by each protective MAbs were sequenced to identify their insert peptide sequences. Results/Discussion: After examining by sequencing of a total of 240 candidate phage clones, we eventually identified 6 specific clones and their peptide inserts. Chemically synthesized peptides corresponding to those displayed by the 6 phage clones were conjugated to keyhole limpet hemocyanin (KLH) carrier protein and tested for their binding specificity of the respective protective MAbs. The study revealed that 4 of the 6 peptides functioned well as "mimotopes" of Burkholderia PS and LPS as demonstrated a high degree of specific competition against the binding of 3 protective MAbs to BP and BM. Our results suggest that the 4 selected peptide mimics corresponding to PS/LPS protective antigens of BP and BM could potentially be developed into peptide vaccines, when the public is facing un-expected, devastating biothreats of the pathogenic Burkholderia bacteria.

13. Respiratory Syncytial Virus-Induced Host IFN Signaling Differs between A549 and BEAS-2B Epithelial Cell Lines

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Plain Language Synopsis: A549 cells are highly infected by respiratory syncytial virus (RSV) while BEAS-2B cells limit RSV infection to just a few cells. Using these cell types we showed that early expression of certain signaling molecules including STAT2 may be critical for preventing viral spread.

Abstract: Intact host innate immunity, including interferons (IFN) and interferon stimulated genes (ISG), is critical for local control of respiratory syncytial virus (RSV). We compared the innate response to RSV by two respiratory epithelial cell lines, BEAS-2B and A549, to define critical innate components that control local epithelial spread of infection in vitro. BEAS-2B and A549 respiratory epithelial cell lines were infected with RSV expressing GFP (rgRSV) at low MOIs to observe the spread of infection and innate responses over time. Expression of IFNs and ISGs were measured using qRT-PCR and ELISA, and STAT1/2

phosphorylation was determined by western blotting. RSV infection and cellular localization of IRF9, STAT1 and STAT2 were observed by confocal microscopy. rgRSV spread throughout A549 cells, but BEAS-2B cells contained the RSV in foci of 10-15 cells. Both cell lines highly expressed IFN-β, IFN-λ1 and -λ2, but the A549 cells expressed more of these IFN and had higher levels of pSTAT1/STAT2. Paradoxically, the A549 cells expressed lower levels of classic antiviral ISG (e.g. ISG15, MX1), but higher levels of NF-kB associated genes (e.g. CCL2, CCL5). Finally, STAT2 was only detected within uninfected cells, and in BEAS-2B cells, more STAT2 localized to the nucleus early than in A549 cells. A balance between expression of NF-kB genes and ISG may determine local control of RSV infection by respiratory epithelial cells, which may be critically mediated by the kinetics of pSTAT2 nuclear localization.

14. Immunotherapy with a Monoclonal Antibody Inhibits Enteric Invasion and Lethal Sepsis in a Murine Model of Gastrointestinal Anthrax Infection

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Plain Language Synopsis: We herein demonstrate that a monoclonal antibody (mAb) targeting anthrax toxin provides protection in a murine model of gastrointestinal anthrax infection. The survival advantage imparted by this mAb is associated with a reduction of bacterial invasion into the intestinal mucosa and prevention of subsequent bacterial dissemination.

Abstract: Deliberate infection with weaponized *Bacillus anthracis* is a contemporary bioterrorism threat, highlighted by the postal-borne inhalational attacks of 2001, which resulted in several lethal pulmonary infections. However, it is generally regarded that the principal portal of access for *B. anthracis* in nature is by enteric entry. Moreover, it has been proposed that anthrax could be deliberately introduced as a contaminant to the food supply of humans or agricultural animals as an act of terror, one that would be potentially difficult to detect or guard against. Understanding disease pathogenesis and treatment strategies in the setting of gastrointestinal anthrax is therefore a worthwhile objective. A novel animal model of GI anthrax was recently developed by our group, in which vegetative *B. anthracis* (Sterne strain) bacteria are administered to A/J mice by oral gavage for the evaluation of experimental therapeutics. One such agent is a humanized recombinant IgG1-lambda monoclonal antibody (mAb)* targeting Domain IV of the protective antigen (PA) component of *B. anthracis* lethal toxin (LT) and edema toxin (ET). Anti-anthrax PA mAb therapy has been shown effective against inhalational anthrax in animals, however the potency of this therapeutic agent in the context of GI infection has yet to be ascertained. We hereby demonstrate the efficacy of passive immunotherapy with an anti-anthrax PA mAb in the setting of post-exposure prophylaxis of anthrax using our murine GI infection model. We show that mice orally gavaged with *B. anthracis* are protected from lethal sepsis by delivery of a single intravenous dose of PA mAb, when administered up to 48 hours following ingestion.

*Expired Raxibacumab which was cycled off the Strategic National Stockpile; biological activity was confirmed by in vitro assays

15. A Feasibility Study: Flexible Epidermal Electrodes for Brain Injury Detection in an Animal Model

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Plain Language Synopsis: We exposed the mouse brain to ultrasound that mimicked blasts, to produce controlled brain injury. We then showed that a novel flexible temporary-tattoo-like electrode technology is capable of detecting the brain injury. Such electrode technology enables the possibility of rapid and early brain injury detection at mass casualty events.

Abstract: Background: Following traumatic brain injury (TBI), there is a limited window of time during which early medical intervention can have a major positive impact on outcome.

However, clinical imaging to diagnose TBI is usually performed hours if not days after the injury. Such a delay in early TBI diagnosis and treatment may be shortened if there is an available biomarker that can be rapidly detected with portable diagnostic devices. Following severe TBI, abnormalities in somatosensory evoked potentials (SSEPs) correlate with a poor prognosis for survival (Anderson et al., 1984, Gaetz 2004), and these EEG-based SSEP signals present an attractive biomarker for portable detection of brain injury. While conventional EEG systems are bulky, cumbersome and susceptible to interference resulting from the slippage of electrodes on the scalp, recent innovations in flexible electronics have resulted in small, conformable, disposable epidermal electrodes that may act as sensors as part of a portable TBI diagnostic device. We aim to evaluate the feasibility of using novel flexible epidermal electrodes to detect TBI-induced changes in SSEPs. Methods: In anesthetized mice (N = 8), we exposed the brain to high-intensity focused-ultrasound pulses that mimicked blast overpressure, to produce controlled brain injury. Immediately before ultrasound-induced injury and up to one hour after, we electrically stimulated the median nerve and recorded SSEPs from the somatosensory cortex. Invasive epidural electrodes, with a high signal-to-noise ratio, were used in one group of animals as a comparison; non-invasive flexible epidermal electrodes were tested in another group of animals. Results: In the cohort implanted with standard epidural electrodes, the SSEP peak amplitudes were reduced immediately after ultrasound exposure. In the cohort recorded with epidermal electrodes, we obtained comparable SSEP dynamics, although with reduced SSEP peak amplitudes. Epidermal electrode recordings were sensitive to different levels of severity of brain injury produced by graded ultrasound exposure. Conclusions: Preliminary results indicate that novel flexible epidermal electrodes are capable of recording the dynamics of SSEPs resulting from brain injury. This new flexible electrode technology enables the possibility of rapid and early brain injury detection using portable, field-deployable devices.

16. A Quantitative Study on the Hemagglutination Inhibition (HI) Antibody Titer and the Protection against Influenza Infection

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Plain Language Synopsis: This work aims to use the antibody level (HI titer) as a means to predict influenza vaccine efficacy. Furthermore, the resulting model can estimate the efficacy based on not only the antibody level but also the number of flu viruses a person might be exposed to.

Abstract: Background: The objective of this research is to evaluate the relationship between HI titer in the host and the protection against influenza using modeling approaches. We expect that the results of this research will provide us an insight on whether HI titer is a good predictor of protection against influenza; and if it is, what the level of HI titer needed for a sufficient protection is. Methods: We first searched available data from human challenge studies that reported post-vaccination HI titer, challenge dose, and post-challenge influenza infection. We grouped the volunteers based on their HI titer levels. We assumed the relationship between challenge dose and infection rate (response) could be described by a beta-Poisson dose-response models that has been used for influenza virus. We estimated the model parameters for each HI titer group, and examined the dependency between host susceptibility represented by model parameters and HI titer. The dose-response model was further modified by incorporating such dependency and fit to the data set. Results: An exponential dependency between the model parameters and HI titer was identified. We incorporated this into the beta-Poisson dose-response model and fit it with all the available data sets. The parameters were estimated based on each data set and a range of the possible values were obtained. Our model estimates that, during a normal outbreak, the infection rate in individuals with HI titer of 40 or higher will be at least 50% less than that in people with HI titer of 5 or less, although it may not be true for exposure to extremely high doses. Conclusions: The modified models can be potentially used to identify the critical level of post-vaccination HI titer required for sufficient protection against influenza; and therefore,

enhance our ability to evaluate the efficacy and protection offered by future candidate influenza vaccines.

17.In vitro Antiviral Activity of Interferon (IFN)-Lambda Combined with IFN-Beta or Neuraminidase Inhibitor against Influenza A Viruses

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Plain Language Synopsis: Influenza viruses pose a continuous threat to humans. The currently available influenza antiviral drugs have a number of limitations; consequently there is a great need to identify additional antiviral strategies. Our studies provide support for the combined use of interferon plus Tamiflu (oseltamivir) as a potential means to block influenza virus replication in vivo.

Abstract: Influenza viruses pose a continuous threat to humans because of their ability to cross species barriers and adapt to new hosts. The 2009 influenza pandemic highlighted the rapidity with which an influenza virus can spread worldwide. The currently available influenza antivirals have a number of limitations; therefore, there is a need to identify other antiviral strategies, including novel drugs and drug combinations. Here, we examined the effects of IFN-beta (a type I interferon), IFN-lambda (a type III interferon), oseltamivir carboxylate (a neuraminidase (NA) inhibitor), and combinations of these agents against two seasonal H1N1 and H3N2 influenza A viruses in vitro. We observed that A/California/04/09 (H1N1) and A/Panama/2007/99 (H3N2) isolates were almost equally sensitive to the antiviral activity of IFN-beta and oseltamivir carboxylate in A549 and Calu-3 cells. In contrast, IFN-lambda exhibited substantially lower protective potential against the H1N1 strain (64-1030 fold, $P < 0.05$), and was ineffective against H3N2 virus in both cell lines. Three dimensional analysis of the drug-drug interactions revealed that IFN-lambda combined with either IFN-beta or NA inhibitor interacted in an additive or synergistic manner, respectively, to inhibit cell-associated yield of influenza A viruses in human epithelial cell lines. Overall, the present study demonstrated that anti-influenza agents with different mechanisms of action (i.e., a NA inhibitor combined with IFN-lambda) exerted a significantly higher ($P < 0.05$) synergistic effect compared to co-treatment with drugs that target similar signaling pathway (i.e., type I IFN combined with type III IFN) in vitro. Our findings provide support for the combined use of interferon plus oseltamivir as a potential means to block influenza virus replication in vivo.

18. T Cell Response Induces Inflammation and Neuronal Pathology Following Neonatal Arenavirus Infection

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Plain Language Synopsis: The development of BSL-2 systems to model encephalitis caused by ClassA pathogens allows for improved understanding of the pathogenesis of the disease as well as the evaluation of potential therapeutic targets. These studies use mice infected with Tacaribe virus to show that T cells play a key role in the disease and are a likely target for therapeutics approaches.

Abstract: Arenavirus such as Junin and Machupo are causative agents of viral hemorrhagic fevers and are designated as class A pathogens by the CDC. These neurotropic viruses cause a multi-system syndrome that includes vascular damage and encephalitis often resulting in death or long-lasting neurological sequelae. In these studies we use New World arenavirus, Tacaribe (TCRV), a BSL-2 agent, to model and study the encephalitis. Infection of neonatal C57BL/6 (B6) mice with neurotropic arenavirus Tacaribe (TCRV) results in seizures, hind limb paralysis and death within 15 days of challenge. Characterization of the neural response to the virus shows meningioencephalitis, with increased expression of CXCL10, IL-1, granzyme, IFNg and TNFa as well as T cell infiltration that esults in neuronal damage and disruption in the cerebellum by 10 days post-infection. Despite having similar viral loads in the brain, mice lacking T cells (CD3e-/-) show decreased inflammatory responses compared to WT mice and survive TCRV challenge. Moreover, transfer of CD4+ or CD8+ T cells from WT mice into CD3e -/- mice re-establishes encephalitis and death. TCRV-induced neuropathogenesis is T cell mediated and is dependent on T cells expressing MyD88, as MyD88-/- mice survived infection and infected CD3e-/- mice transferred with T cells from

MyD88-/- also survived. Unlike WT mice, brains of MyD88-/- mice displayed reduced inflammation and reduced T cell infiltration, despite comparable viral loads. This resulted in limited neural disruption in the cerebellum. Collectively, these findings show that T cells play a key role in the CNS pathology induced by arenavirus Tacaribe and that MyD88-mediated signalling is critical for this T cell response. Understanding the mechanisms underlying viral encephalitis helps the Agency identify correlates of protection and assess and manage the relative risk of new and existing therapies for acute hemorrhagic fevers, particularly since they may need to be licensed or stockpiled based on the animal rule mechanism.

19. Characterization of Donor Plasma Used for Intravenous Influenza Immune Globulin Production

Khalenkov, Alexey, FDA/CBER/OBRR; Sultana, Ishrat, FDA/CBER/OBRR; Scott, Dorothy, FDA/CBER/OBRR

Plain Language Synopsis: During the 2009 H1N1 pandemic (pdm09H1N1), Baxter Biolife initiated a unique program to collect plasma from donors who self-identified as having had influenza or having received the pdm09H1N1 vaccine. Plasma collected from self-identified donors was manufactured into immune globulin (FLUIGIV) product. We report preliminary epidemiological findings of this product related to donor age, sex, location, and type of influenza exposure (convalescent vs. vaccinated).

Abstract: Safe, effective, and timely therapeutics during an influenza pandemic are challenging due to time constraints for large-scale vaccine manufacturing, limited supplies of vaccines and drugs, and the potential for drug resistance. Immune globulins from convalescent or vaccinated donors are used to prevent or treat many viral diseases such as Hepatitis A, Hepatitis B, CMV, rabies, measles, vaccinia, varicella and others. In small or retrospective studies, influenza immune plasma from convalescent donors may have had benefit. During the 2009 H1N1 pandemic (pdm09H1N1), Baxter Biolife initiated a unique program to collect plasma from donors who self-identified as having had influenza or having received the pdm09H1N1 vaccine. More than 15,000 plasma units were collected from self-identified donors over 7 weeks, which was manufactured into immune globulin (FLUIGIV) using a licensed process. This FLUIGIV preparation protected SCID mice against lethal pdm09H1N1 infection. We obtained more than 250 plasma samples collected from these self-identified donors and contemporaneous unselected donors, to evaluate influenza antibody responses by hemagglutination inhibition (HAI). We report preliminary epidemiological findings related to donor age, sex, location, and type of influenza exposure (convalescent vs. vaccinated). Self-identified vaccinated and convalescent donor groups both had higher geometric mean titers against A/California/07/09 (H1N1) virus compared to the unselected plasma donor group. Antibody mean titers for both self-identified groups were also greater than 1:40, which in general is thought to be a protective level. We also found that approximately 20% of donors who did not self-report as being vaccinated or having history of flu infection had titers comparable to those in two other groups. Overall, collecting plasma from donors who self-identify themselves as vaccinated or being recovered from pandemic influenza could be a rapid and feasible approach for production of large quantities of FLUIGIV. Additional high titer donations could probably be selected if an inexpensive, rapid, virus-free test that measures neutralizing activity in plasma became available.

20. Exploring Antibody Repertoires and Affinity Maturation Against Pandemic Influenza Vaccines: Impact of Adjuvants and Prime-Boost Approaches in Humans

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Plain Language Synopsis: During the 2009 H1N1 pandemic (pdm09H1N1), Baxter Biolife initiated a unique program to collect plasma from donors who self-identified as having had influenza or having received the pdm09H1N1 vaccine. Plasma collected from self-identified donors was manufactured into immune globulin (FLUIGIV) product. We report preliminary epidemiological findings of this product related to donor age, sex, location, and type of influenza exposure (convalescent vs. vaccinated).

Abstract: Rapid response against influenza viruses with pandemic potential requires development of effective vaccines. In the case of H5N1 and H7N9 immune responses to the inactivated vaccines (IIV) were very poor, due to lack of pre-existing immunity. Novel adjuvants including Oil-in-water and cage-like particles were evaluated in human trials and found to significantly increase virus neutralizing titers, heterosubtypic immunity, and afforded dose sparing. Several prime-boost approaches including priming with DNA, LAIV, or replicating Ad4HA also resulted in desired seroconversion rates after the IIV boost. We have developed new molecular tools to better understand the humoral responses to influenza vaccines. Using Whole Genome Fragment Phage Display Libraries (GFPDL) and Surface Plasmon Resonance (SPR) technologies we observed that oil-in-water adjuvants (MF59, AS03) induced epitope spreading from HA2 to HA1 in the hemagglutinin (HA), when compared with unadjuvanted or aluminum-adjuvanted inactivated H5N1 vaccines in humans. Furthermore, a significant increase in the antibody affinity maturation to properly folded HA1 was measured in SPR, which correlated with broadening of H5N1 cross clade neutralization. Similarly, increased antibody binding affinity was observed in sera from clinical trial of oral Ad4HAVtn followed by H5N1 IIV boost. In the case of H7N9, the unadjuvanted virus like particle (VLP) vaccine elicited low Ab binding to the native HA1 that was focused on the C-terminus, while the ISCOMATRIX-adjuvanted VLP vaccine induced high binding titers to the HA1 RBD and significant affinity maturation that correlated with neutralization titers. These technologies can be adapted to many other vaccines. They will improve our understanding of the mechanisms of adjuvant activity, and help to select optimal vaccine modality, adjuvant, and vaccination protocols against emerging infectious diseases.

21. Ebola virus (EBOV) High Throughput BSL-2 Neutralization Assay Based on Replication-Competent Recombinant VSV Expressing the EBOV GP and the Green Fluorescent Protein (GFP)

Konduru, Krishnamurthy, FDA/CBER/DETTD/LEP; Kaplan, Gerardo, FDA/CBER/DETTD/LEP

Plain Language Synopsis: The BSL-2 FRNT is a simple method that only requires 1-day to determine EBOV GP neutralizing antibodies. Anti-GP neutralizing antibody titers assessed by the BSL-2 FRNT and EBOV BSL-4 PRNT are comparable. rVSV-EBOVgp-GFP could also be used to screen antibodies or inhibitors targeting EBOV cell entry.

Abstract: Background: Current epidemic in West Africa underscores the urgent need to develop Ebola virus (EBOV) vaccines, therapeutics, and diagnostics. The EBOV glycoprotein (GP) mediates viral cell entry and elicits protective cellular and humoral immune responses. Because passive immunization with EBOV neutralizing antibodies protected nonhuman primates (NHPs) against a lethal challenge and candidate vaccines based in GP elicit anti-EBOV neutralizing antibodies, EBOV neutralizing antibodies may provide a correlate of protection. Therefore, there is a need to analyze neutralizing anti-EBOV antibodies in preclinical studies and clinical trials to assess the efficacy of passive immunotherapies and vaccines. EBOV studies require BSL-4 containment presenting a logistical challenge for the evaluation of neutralizing antibodies. We hypothesized that replication-competent VSV-G-deleted recombinant VSV containing the EBOV GP and GFP (rVSV-EBOVgp-GFP) could be used to develop a rapid and simple biosafety level (BSL)-2 neutralization test. Methods: We constructed an rVSV-EBOVgp-GFP containing the Mayinga strain Zaire EBOV glycoprotein and GFP. rVSV-EBOVgp-GFP was neutralized with sera/plasma of guinea pigs or NHPs vaccinated with an EBOV candidate vaccine consisting of the EBOV glycoprotein fused to the Fc fragment of human IgG1 (EBOVgp-Fc). Using rVSV-EBOVgp-GFP, we developed a Fluorescence Reduction Neutralization test (FRNT) and compared it to the EBOV BSL-4 Plaque Reduction Neutralization Test (PRNT). Results: The FRNT is a simple assay that can be performed in 1-day using a flow cytometer, fluorescence plate reader, or fluorescence microscopy. We showed that sera/plasma from guinea pigs or NHPs vaccinated with EBOVgp-Fc but not control Fc neutralized rVSV-EBOVgp-GFP. A high degree of correlation was observed between the BSL-2 FRNT and EBOV BSL-4 PRNT neutralization assays. Conclusions: The BSL-2 FRNT neutralization assay could be used as a simple, rapid, and high-throughput test to analyze anti-EBOV neutralizing antibodies in clinical trials.

Additionally, our data suggest that rVSV-EBOVgp-GFP could also be suitable for screening antibodies and inhibitors targeting the EBOV cell-entry process.

22. Novel Approaches for 1st Response Treatment of Viral Encephalitis by Emerging Pathogens: Lessons from the Sindbis Model

Manangeeswaran, Mohanraj, FDA/CDER/OBP/DBRRIII; Verthelyi, Daniela, FDA/CDER/OBP/DBRRIII

Plain Language Synopsis: This study assesses the immune response to Sindbis virus during acute encephalitis and identifies biomarkers associated with improved survival. Understanding the effect of virus in the CNS will help assess and manage the relative risk of new and existing therapies for acute hemorrhagic fevers, particularly since they may need to be licensed or stockpiled based on the animal rule mechanism.

Abstract: Emerging class A pathogens such as Ebola, Lassa, Junin and Machupo are neurotropic viruses that cause a multi-system syndrome that includes vascular damage and encephalitis often resulting in death or long-lasting neurological sequelae. Developing an animal model to study viral encephalitis will aid in discerning the mechanisms that underlie the development of immunological and neurological sequelae and provide BSL-2 animal model to test and compare therapeutic approaches in terms of neurological sequelae. The CNS parenchyma is regularly devoid of lyM.P.H.ocytes, but astrocytes and microglia express pattern recognition receptors and upon viral infection rapidly produce interferons, pro-inflammatory cytokines and chemokines that lead to local inflammation, increased blood brain barrier permeability and infiltration by immune cells. Key to survival in patients with viral encephalitis is attaining a balance between effective virus elimination and detrimental inflammation. Here we use a lethal model of Sindbis virus (strain AR339) infection in neonatal mice, to assess whether direct modulation of the immune response can be used to increase viral clearance while reducing local inflammation to improve survival. We show that when mice are infected with Sindbis they mount a strong response characterized by increased mRNA expression of inflammatory chemokines and cytokines in the CNS. Treatment with immunomodulatory CpG oligodeoxynucleotides (ODN 1555, 50ug i.p) reduces viral titers in blood, spleen and brain, and achieves 100% survival. This protection is mediated solely by activation of the innate immune response as similar protection is seen in mice lacking T cells and B cells (CD3e KO or RAG1 KO). The improved clinical outcome is associated with significant reduction in the virus-mediated induction of IL-12, perforin, granzyme B, Interferon gamma, Cxcl10, Ccl5. Cxcl11, Stat1 and IL-1 indicating a possible role for NK cells in the CpG ODN mediated protection. Accordingly, mice defective in IFNg or M.D.A-5 could not be rescued by CpG ODN treatment although their viral titers were reduced compared with untreated mice. This work identifies a potential therapeutic path to improve survival in patients that develop viral encephalitis and identifies markers associated with viral clearance and improved outcome.

23. Long-term Stability Study of Prussian Blue: A Quality Assessment of Water Content and Cyanide Release

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Plain Language Synopsis: Prussian blue is the first FDA approved medical counter-measure to be used clinically in the event of a nuclear attack or radiological dirty bomb to reduce the radioactive body burden and in combination with antibiotics to treat acute radiation syndrome (ARS). However, elemental analysis and stoichiometric calculations have established that Prussian blue also known as ferric hexacyanoferrate, Fe4[Fe(CN)6]3 contains approximately 35-40% cyanide. The objective of this current study is to quantitatively determine the in vitro cyanide released from Prussian blue under physiological relevant pH conditions.

Abstract: Purpose: to assess the long-term stability of Prussian Blue (PB), drug products (DPs) and active pharmaceutical ingredients (APIs) by evaluating bound water content and cyanide release. PB or ferric hexacyanoferrate is an approved oral dosage form for the treatment of internal radioactive contamination of cesium or thallium. Of particular concern is cyanide which makes up 35-40% of PB's molecular composition, thus cyanide may be released during transit

through the digestive tract under physiological pH conditions. Methods: Test samples of API and DP were stored at ambient conditions for 10 years. Water loss from PB was measured using thermogravimetric analysis (TGA). An in vitro physiological pH model that brackets gastric exposure and GI transit was used for cyanide release. PB was incubated in situ at pH 1.0, 5.0 and 7.0 @ 37oC for 1-24 hours. Cyanide was measured using a validated colorimetric method by UV-VIS spectroscopy. Results: Although the water content of PB API and DP decreased by about 10.5% and 13.8%, respectively since 2003, the cyanide release remained comparable. At pH 7.0 for 24 hrs cyanide released from API-1 was 21.33 ± 1.76 mg/g in 2004, and 28.45 ± 3.15 mg/g in 2013; cyanide released from DP-1 was 21.89 ± 0.56 mg/g in 2004, and 27.31 ± 5.78 mg/g in 2013. At pH 5.0 for 24 hrs cyanide released from API-1 was 20.28 ± 0.72 mg/g in 2004, and 26.49 ± 6.86 mg/g in 2013; cyanide released from DP-1 was 20.71 ± 0.77 mg/g in 2004, and 20.01 ± 3.11 mg/g in 2013. At pH 1.0 for 24 hrs cyanide released from API-1 was 135.11 ± 5.19 mg/g in 2004, and 120.33 mg/g in 2013; cyanide released from DP-1 was also comparable in 2013. Conclusions: This is the first long-term stability study of PB that monitors product quality through the assessment of cyanide release and water loss. The 20% water loss had no significant impact on the amount and profile of cyanide released from PB at all GI relevant pH conditions. Therefore, the long-term stored PB does not present additional safety concerns for clinical use.

24. Effect of Soil, Material and Roughness on the Removal of Bacteria from Reusable Medical Device Materials

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Plain Language Synopsis: The presence of soil and the roughness of two polymers influenced cleanability of the coupons. There was little difference in cleaning bacteria and spores, between the two polymers. Water, ethanol, and bleach all effectively cleaned bacteria and soil from the coupons.

Abstract: Reusable medical device (RM.D.) surfaces are vulnerable to contamination by select agent pathogens (SAPs) either directly or through unintentional cross-contamination. We used wipes to clean materials commonly used to manufacture RM.D. surfaces after they were contaminated with surrogates of two pathogenic bacteria: methicillin-resistant Staphylococcus aureus (MRSA) and *Bacillus anthracis* spores. MRSA is a cause of nosocomial infection that is becoming more difficult to treat due to increasing antibiotic resistance. *B. anthracis*, the causative agent of anthrax, is classified as a CDC Category A organism because of its potential as a bioterrorism threat. We investigated whether the presence of soil or the material roughness affects the ability of different wipes to effectively remove bacteria from two materials: polypropylene (PPE) and ultra-high-molecular-weight polyethylene (UHMWPE). Artificial blood test soil, S. aureus, and B. atrophaeus spores were used as surrogates for blood, MRSA and *B. anthracis* spores, respectively. PPE and UHMWPE coupons, either smooth or rough, were spotted with bacteria and spores alone, blood test soil alone, or bacteria and spores with soil. After drying (1.5 hours), materials were cleaned with wipes soaked in water, 70% ethanol, or bleach (1:10), and analyzed for residual bacteria (CFU/ml) and for soil using a protein assay. Generally, water, ethanol, and bleach cleaned bacteria, spores and soil from both material types and both roughness values in comparison to the positive control. However, neither ethanol nor bleach consistently removed either bacteria or spores better than water. For PPE and UHMWPE smooth coupons, both bacteria and spores were more easily removed in the presence of soil than without soil. For rough coupons, the opposite was observed, bacteria were generally more difficult to remove in presence of soil. For PPE and UHMWPE smooth coupons, there were no differences in the removal of bacteria plus soil versus soil alone. For roughened coupons, soil was more difficult to remove in the presence of bacteria. Rough PPE coupons retained both more bacteria and soil than smooth PPE coupons. Rough UHMWPE coupons retained more protein than smooth coupons; however there was no clear trend in the retention of either bacteria or spores. Understanding how these variables affect the cleanability of RM.D.s will allow medical device manufacturers and FDA

reviewers to make scientifically-based decisions during RM.D. design and cleaning validation.

25. IFN-beta and IFN-lambda1 Induce Kinetically Distinct Patterns of Transcription Factor Interferon Stimulated Genes in Respiratory Epithelial Cells

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Plain Language Synopsis: Pathogens induce expression of interferons (IFN). Types I and III IFN initiate the downstream expression of interferon stimulated genes (ISG) that are transcription factors. Similar subsets of ISG are induced, but in distinct kinetic patterns which may be important for pathogen control or immune modulation.

Abstract: Viruses and other pathogenic stimuli induce expression of both types I and III interferons (IFN). Type I IFN includes IFN-beta and 12 subtypes of IFN-alpha and type III IFN include IFN-lambda1-4. Type I IFN receptor expression is ubiquitous, while type III IFN receptor expression is largely restricted to epithelial cells. Cooperative expression of types I and III IFN suggest unique roles for each, but their similar activation of STAT1/STAT2 heterodimers and interferon stimulated genes (ISG) suggest that types I and III IFN are redundant. We asked whether type I and III IFN initiate the expression of unique ISG subsets. We sought to determine unique or cooperative roles for types I and III IFN in cellular activation by stimulating BEAS-2B human respiratory epithelial cells with IFN-beta or IFN-lambda1 alone or together at multiple concentrations and time points. We measured expression of up to 23 transcription factor ISG (TF-ISG) and canonical ISG by qRT-PCR. While IFN-beta induces strong and early peak expression of TF-ISG, IFN-lambda1 induces a gradual, long-term induction of ISG expression. For a subset of TF-ISG (TRIM22, IRF7, STAT1) the response to IFN-beta is sustained, while for another ISG subset (BATF2, ETV7, TRIM25) the response wanes. At EC50 doses of IFN-beta and IFN-lambda1 together, peak expression of TF-ISG is additive, but their induction rapid and sustained. The types I and III IFN, IFN-beta and IFN-lambda1, respectively, each induce expression of TF-ISG in unique kinetic patterns. Together, these two IFN coordinate rapid, high, and sustained expression of ISG, including those that may affect subsequent cellular activity through their function as transcription factors.

26. Assessment of Medical Devices for Multiparameter Hemorrhage Detection Algorithms in a Conscious Sheep Model

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Plain Language Synopsis: Traditional methods of examining trauma patients may not provide early notification of significant hemorrhage to initiate life-saving interventions. Novel non-invasive patient monitoring approaches are being developed to monitor hemorrhage. We are using a conscious sheep model excluding possible hemodynamic modulation by anesthetics to better understand and evaluate these approaches.

Abstract: Traditional patient triage and monitoring for trauma during mass casualty incidents are conducted by examination of vital sign `snap-shots.' However, because of physiological compensatory mechanisms, vital signs can be late indicators of patient deterioration, such as from hemorrhage, and may not provide notification until the window of opportunity for critical life-saving interventions has passed. Emerging novel multi-variable monitoring algorithms aim to provide early notification of patient deterioration in such medical scenarios. Understanding physiological responses during hemorrhage is important to evaluate these technologies. Since experimentally inducing severe bleeding in conscious, non-anesthetized humans is not feasible, studying them in large mammalian models provides insights to extrapolate such knowledge to human hemodynamic responses. We are investigating hemodynamic responses in an experimental conscious sheep hemorrhage model as a development tool for hemorrhage monitors. Hemorrhage experiments are performed on conscious adult sheep (N=8). Each animal

undergoes two hemorrhage experiments at different hemorrhage rates adjusted for body weight (0.25 ml/kg per min and 1.25 ml/kg per min), each hemorrhage experiment is separated by 3+ days. Physiological monitoring data is collected for 1 hr of baseline, during hemorrhage (occurring until a drop in mean arterial pressure of >= 30mmHg), and during a post-hemorrhage period. Physiological monitoring includes both non-invasive-electrocardiogram, respiration rate, pulse-oximetry, skin temperature, regional oxygen saturation and invasive-intermittent blood gas analysis, blood pressure, heart rate, and cardiac output-procedures. Noninvasive physiological markers are compared with invasive measures during hemorrhage at two hemorrhage rates to fill knowledge gaps of how hemorrhage rate influences the physiology and detection of hemorrhage. This project will enhance the review of future products designed for medical countermeasures by informing the evaluation of efficacy for predictive algorithms and describing information available from novel noninvasive sensors during hemorrhage.

27. Interferon (IFN)-Beta Plays A Central Role in the Induction of IFN-Stimulated Gene Expression by LPS in Macrophages

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Plain Language Synopsis: Our studies demonstrate a major role for endogenous interferon (IFN)-beta expression in the induction of gene expression by lipopolysaccharide (LPS) in macrophages. Production of IFN-beta by macrophages may contribute substantially to the pathogenesis of septicemia induced by Gram-negative bacterial infections. Our findings suggest that blocking the activity of IFN-β could have therapeutic value in the treatment of bacterial endotoxemia.

Abstract: Toll-like receptor (TLR) agonists such as LPS and poly(I:C) induce expression of type I interferons (IFN) such as IFN-alpha and beta by macrophages. To examine the role of IFN-beta in the induction of IFN-stimulated genes (ISGs) by LPS, we compared the ability of LPS to induce ISGF3 activity and ISG expression in bone marrow–derived macrophages from wild-type (WT) and IFN-beta gene knockout (Ifnb1−/−) mice. We found that LPS treatment activated ISGF3 and induced expression of ISGs such as Oas1, Mx1, Ddx58 (RIG-I), and Ifih1 (M.D.A5) in WT macrophages but not in macrophages derived from Ifnb1−/− mice or IFN-alpha/beta receptor knockout (Ifnar1−/−) mice. The inability of LPS to induce activation of ISGF3 and ISG expression in Ifnb1−/− macrophages correlated with the failure of LPS to induce phosphorylation of STAT1 and STAT2 in these cells. Consistent with these findings, LPS treatment also failed to induce expression of ISGs in bone marrow–derived macrophages from STAT2 KO mice. Although activation of ISGF3 and induction of ISG expression by LPS was abrogated in Ifnb1−/− and Ifnar1−/− macrophages, activation of NF-kappa B and induction of NF-κB-responsive genes, such as Tnf (TNF-α) and Il1b (IL-1β) were not affected by deletion of either the IFN-beta or IFN-alpha/beta receptor genes. These findings demonstrate that induction of ISGF3 activity and ISG expression by LPS is critically dependent on intermediate production of IFN-beta and autocrine signaling through type I IFN receptors.

28. Rapid Detection of Thorium-232 in FDA Regulated Products

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Plain Language Synopsis: An analytical procedure was developed for rapid detection of Th-232 in FDA regulated products to ensure public safety and compliance with federal regulations, for example, NRC licensing regulations. Specific analytical procedures and activity results for Th-232 in various commercial products are presented.

Abstract: Thorium (Th)-232 is a naturally occurring, alpha-emitting metal found at low levels in soils and water and the Th-232 decay chain includes radionuclides that are gamma, beta and alpha-emitters. Due to the prevalence of gamma radiation monitoring systems, imported products flagged at Customs locations due to elevated gamma-ray activity are increasing. Often, this activity is shown to be concentrated levels of

naturally occurring radionuclides. One example is seen in sports tape, mineral patches and products touting positive health effects of "negative ion technology". It is difficult to intercede when these products have routes of exposure for which no health physics guidance has been established. Because health issues are associated with highly concentrated levels of Th_232 even when external radiation is assumed to be the only source of exposure, the Nuclear Regulatory Commission (NRC) has guidance for basing intervention action on the requirement for licensing to transfer and possess goods containing radioactive material such as Th-232 and its progenies. We validated a rapid method that can quantify Th_232 in flagged products as needed for regulatory decision making. Samples were analyzed using the "Determination of Thorium in Environmental and Workplace Materials by ICP-MS" by Holmes/Pilvio. Sample portions of less than one gram are spiked with Tl-205, which is used as a recovery standard, and microwave digested. The resultant digest is spiked with Bi-209 as an internal standard for immediate analysis of Th-232 on inductively-coupled plasma mass spectrometry (ICP-MS). The lab modified the procedure to enhance safety, speed and accuracy by changing the digestion process and adding a Tl-205 recovery standard. The modified procedure provides accuracy of 102%. The %RSD of the Tl-205 recoveries was 2.9%. The procedure is appropriate for activity levels above 1Bq/g - with a minimum detectable concentration (M.D.C) of 0.15Bq/g and a limitation of quantification (LOQ) of 0.3Bq/g. The procedure allows Th-232 results to be obtained within 24 hours as opposed to conventional chemical separation methods that require about 2 weeks. Activity results for Th-232 in various types of medical device and food matrices are presented.

29. Photoacoustic Tomography for Deep in vivo Imaging of Optical Biomarkers: System Design and Validation

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Plain Language Synopsis: Photoacoustic tomography (PAT) is an emerging imaging modality combining laser excitation with ultrasonic detection for applications such as vascular and molecular imaging for detecting infectious disease. We developed a custom ultrasound-PAT system as critical FDA research infrastructure for regulatory science and quantitatively characterized its performance using phantom-based test methods.

Abstract: Photoacoustic tomography (PAT) is a rapidly emerging imaging technology that combines laser excitation with ultrasonic detection to enable deep tissue imaging to 3-4 centimeters with contrast based on optical absorption. In addition to information on tissue morphology, PAT can provide noninvasive spectroscopy-based detection/assessment of blood oxygenation (including dyshemoglobin content, i.e. met- and carboxy-hemoglobin), targeted contrast agents such as nanoparticles, and biomarkers used for detection of infectious disease or toxic agent exposure. Other promising clinical applications include tissue oximetry, detection of breast and brain cancer, vascular imaging, and molecular imaging. Over the past decade, PAT research has increased rapidly in academia and industry, leading to clinical trials, commercially available research devices, and regulatory submissions. PAT system design can vary widely, including optical illumination, acoustic detection, and image processing techniques. Therefore, standardized test methods are needed to evaluate and compare system performance, thus facilitating technological development and regulatory decision-making. The goal of this project was to construct and quantitatively validate a custom high-performance, spectroscopic PAT system as critical FDA research infrastructure for regulatory science. Our bimodal system is capable of performing simultaneous, co-registered ultrasound (US) imaging and PAT. This system comprises a cart-based tunable near-infrared pulsed laser and a research-grade US acquisition system compatible with commercial transducers. We address design issues such as synchronization, cross-platform integration, and image reconstruction. Considerations such as energy variability, transducer selection, and spectral energy compensation, and their impact on measurements are presented. System performance was assessed by quantifying figures of merit, including spatial resolution, penetration depth, and contrast-to-noise ratio (CNR), using

phantoms made of a custom polymer designed to mimic both the optical and acoustic properties of human breast tissue. Phantoms contained fluid channels as deep as 3 cm which were injected with either hemoglobin or cyanine dye solutions. System axial and lateral resolutions were found to be 282 µm and 432 µm, respectively, while penetration down to 2.5 cm was achieved. A maximum CNR of 14.1 was achieved during hemoglobin imaging. These results provide evidence of system performance as well as insights into the factors that impact image quality and spectroscopic measurements in US-PAT systems.

30. Elucidation of Factors Influencing Performance of Portable Near-Infrared Spectroscopy (NIRS) Devices for Rapid, Field Detection of Intracranial Hematomas

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Plain Language Synopsis: This study is to develop a well-validated phantom-based method to evaluate emerging NIRS devices for intracranial hematomas detection. It includes developing an in vivo murine model of hematoma and an NIRS system. Factors influencing the performance of hematoma detection by NIRS system are studied, providing insights for phantom-based method development.

Abstract: Traumatic brain injury (TBI) leading to intracranial hematoma formation is a significant risk for military service members and civilian victims of terrorist bombings, auto accidents, violent crimes, falls, and sports injuries. Occurrence rates for TBI–induced hematoma are estimated at up to 100,000 in the US annually. Early identification of hematomas in TBI patients is fundamental to successful treatment. However, the diagnostic standard of care involves CT or MRI imaging systems that are unsuitable for rapid response. Near–Infrared Spectroscopy (NIRS) - a technique that is well established for providing real–time, non–invasive measurements of blood–related parameters in tissue - is rapidly emerging as a high–priority medical countermeasure. Two portable NIRS-based devices for hematoma detection have been cleared in recent years. However, there is currently a lack of well-validated, standard test methods for objective, quantitative, and consistent benchtop performance testing for NIRS device intercomparison and quality assurance. The purpose of this project is to alleviate this impediment to the regulatory process by developing a well-validated phantom-based test method. To facilitate understanding of relevant biological effects and light-tissue interactions, we are developing and validating an in vivo murine hematoma model. This model is capable of generating hematoma with controlled size and locations, induced by high-intensity focused ultrasound (HIFU) at calibrated positioning and dose. Morphological validation of the in vivo hematoma model based on micro-computed X-ray tomography is currently underway. A multi-wavelength NIRS system with a fiber-optic interface designed for noninvasive, transcranial hematoma detection in a murine model has also been developed. The NIRS system is capable of detecting changes in hemoglobin concentration and oxygen saturation using a non-negative linear least squares spectral decomposition approach, and has been validated with preliminary phantom measurements. The hematoma model and NIRS system represents a platform for in vivo investigations to elucidate the effect of probe design parameters such as source-detector separation distance, wavelength, tissue morphology/inhomogeneity and lesion size and depth on hematoma detectability. Insights gained from these studies will inform development of biologically relevant phantoms for full-scale NIRS systems. The final, validated test methods will benefit regulatory review, research, development, and postmarket surveillance of NIRS devices for hematoma detection, as a medical countermeasure to the threats of TBI caused by terrorist bombings, violent crimes, auto accidents, etc.

31. Identification and Characterization of *Bacillus anthracis* Gene Products in Production of Nitric Oxide

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Plain Language Synopsis: Evaluate pathogenic roles of *B. anthracis* generated nitric oxide.

Abstract: A *Bacillus anthracis* NOS-like protein (baNOS) was previously reported as the only

NO-producer and an essential virulence factor. Studies showed that the baNOS is able to bind L-arginine. However, due to the lack of a reductase domain, baNOS produces nitrite in vitro. The possible biological reductase for production of NO by *B. anthracis* in vivo remains to be identified. We recently identified *B. anthracis* genes that encode a putative nitrate reductase complex (NarGHJI) and nitrite reductases NirA and NirBD. We propose that the putative *B. anthracis* nitrate reductase complex and nitrite reductases may be involved in production of nitric oxide by using nitrate and/or nitrite as substrates. A set of *B. anthracis* denitrification deficient mutant strains were constructed and used in studies of reduction of nitrate and nitrite. Additionally, the *B. anthracis* nirA and nirB genes were cloned and expressed heterogeneously. Our data showed that *B. anthracis* wild-type cells were able to reduce nitrate and nitrite readily in vitro, indicating *B. anthracis* might consecutively express proteins functioning in reduction of nitrate and nitrite. The purified recombinant NirB demonstrated activities of reducing nitrite in vitro, and a nirB mutant strain of *B. anthracis* was unable to reduce nitrite, suggesting the *B. anthracis* nirB gene product may function in production of nitric oxide.

32. Assessment of Anti- Ebola Glycoprotein, GP 1,2 Responses in Mamu-transgenic-A01 mice

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Plain Language Synopsis: Ebola is a deadly disease. Ebola's surface glycoprotein (GP) can protect mice from the virus. Special mice (Mamu-A01) express the monkey immune system. We show that antibodies and hunter-killer cells can be followed in Mamu-A01 mice immunized with GP. The rapid diagnostics and mouse model may assist in screening vaccines.

Abstract: Background: Ebola induces a deadly hemorrhagic fever. Immunity to Ebola requires both cell mediated and humoral immunity. Importantly, the surface glycoprotein, GP12Fc, confers protection against Ebola challenge in mice. Rhesus macaques are used to assess Ebola vaccine responses. Recently, a murine transgenic model that expresses the Rhesus macaque MHC, Mamu A-01, has been developed. Study Rational: Given that the pivotal clinical trials will take place in monkeys, development of assays to follow both cell mediated and humoral immunity are needed. GP12Fc has been shown to confer protection against mouse adapted Ebola virus. The Mamu-A-01 transgenic model provides a means to rapidly screen vaccine candidates. Methods: A GP12 Zaire 15 mer/5 mer overlap mimeotope library was synthesized. Nine pools of 15 overlapping peptides were prepared. Flow cytometry was used to evaluate g-interferon and TNF-a synthesis using intracellular staining (ICS). GP12 Ebola Zaire sequence were analyzed by the Immune Epitope Database (IEDB) to identify immuno-dominant epitopes. Peptides were synthesized and screened using MHC stability assays. Two Tetramers were prepared by NIAID. Results: Mice were immunized with GP12Fc and the ICS response in splenocytes was assessed. Interestingly, the first pool induced the most robust response. In silico and MHC stability studies identified two Immunodominant epitopes, NTPVYKLDI, AA 390-398 (P20) and FTPQFLLQL, AA 248-256 (P21), Importantly, antigen specific T cells from GP 12 Fc immunized mice were clearly demonstrated using P20 and P21 Tetramers. Conclusions: Mamu-A01 mice have been generated and were immunized with the vaccine candidate GP12Fc. ICS revealed a robust response to GP12Fc. Further, IEDB analysis and MHC stability studies identified Immunodominant peptides, P20 and P21. Most importantly, tetramers against Ebola have been developed for Mamu-A01 and can detect GP12Fc specific immune cells. Future experiments will focus upon fully cataloging the immune response to vaccine candidates in transgenic mice that express Rhesus macaque Mamu-A01.

33. UPLC Method for Atropine Assay and Targeted Impurities

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Plain Language Synopsis: A novel UPLC method has been developed to reduce the analysis time of ATNAA (Antidote Treatment Nerve Agent Auto-injector) samples from 40-70 minutes to 8 minutes along with improved sensitivity and resolution.

Abstract: A gradient UPLC method has been developed for the analysis of ATNAA (Antidote

Treatment Nerve Agent Auto-injector) samples to analyze Atropine and its respective impurities. Two simultaneous UPLC runs (8 minutes per injection) were performed on two separate UPLC systems using the same mobile phase, reagents, and standards. In contrast, a HPLC run (40 minutes per injection) was also performed using the USP 37 method for Atropine Sulfate injection. The retention times of the 3 main Atropine degradants were significantly shorter for the UPLC method compared to the HPLC method (Tropic Acid 1.5min vs 18min, Apoatropine HCl 3min vs 26min, Atropic Acid 3min vs 72min). In the UPLC method, an Acquity UPLC BEH C18 1.7µm, 2.1 x 100mm column was used with a mobile phase consisting of a mixture of Solvent A (0.1% H3PO4) and Solvent B (0.1% H3PO4, 90%ACN, 10%H2O) as part of a gradient run. Correlation coefficients were calculated to be 0.99996 and 1.00000 for both UPLC systems with a LOQ of 3.942µg/mL for Atropine. Results for the UPLC method indicate that the following impurities can also be separated and analyzed using this proposed method: Noratropine, 4,4-Dihydroxydiphenyl ether, 2,4-Dihydroxydiphenyl ether, 4-Bromophenol, 4-Hydroxyatropine, Tropic Acid, Apoatropine HCl, Atropic Acid, Hydroquinone, Nitroethane, Phenol, and Catechol. The UPLC method demonstrated a far greater selectivity and shorter run time compared to the traditional USP 37 HPLC method.

34. Normal and Immune Deficient Mice Partially Reconstituted with T Cells are Protected from Lethal Challenge with IHD-J-Luc Vaccinia Virus by Post-Challenge Administration of Brincidofovir

Zaitseva, Marina, FDA/CBER/DVP; Thomas, Antonia, FDA/CBER/DVP; McCullough, Kevin, FDA/CDER/OBP; Trost, Lawrence, Chimerix, Inc.; Golding, Hana, FDA/CBER/DVP

Plain Language Synopsis: The effects of Brincidofovir on protection from lethal challenge with vaccinia virus were studied using bioluminescence imaging of live mice. Viral loads in infected mice were assessed using Area Under the Flux Curve analysis. The data showed that Brincidofovir reduced viral loads at the challenge site and in internal organs.

Abstract: Background: Mass vaccination continues to be a key part of the public health response in case of a smallpox outbreak. However, safe antiviral drugs are needed for prophylaxis of individuals with uncertain exposure status or for whom vaccination is contraindicated. Application of bioimaging to traditional model of lethal challenge with vaccinia virus (VACV) was used to gain insight on protection from lethality conferred by an orally bioavailable lipid conjugate of cidofovir, brincidofovir (BCV, CMX001) in normal and immune deficient (nu-/nu-) BALB/c mice infected with VACV. Methods: Whole body bioimaging was used to record total fluxes in the nasal cavity, lungs, spleen, and liver and to enumerate pox lesions on tails of live mice infected via the intranasal route with 105 pfu of recombinant IHD-J-Luc VACV expressing luciferase. Areas Under the flux Curve (AUC) were calculated for individual mice to assess viral loads and perform statistical analysis. Results: A three dose regimen of 20 mg/kg BCV administered every 48 hours starting on Day 1 or 2 post-challenge protected 100% of mice. Initiating treatment on Day 1 was more efficient in reducing viral loads and in protecting normal mice from pox lesions. Nude mice survived infection and showed reduced viral loads while on drug but succumbed ~10-20 days after treatment termination. Nude mice partially reconstituted with T cells before challenge and treated with BCV post-challenge survived the infection and cleared the virus from all organs. All BCV-treated mice that survived challenge were protected from re-challenge without additional treatment. Conclusion: BCV controls viral replication in the site of challenge and reduces viral dissemination to internal organs thus providing a shield for the developing adaptive immunity that clears the host of virus and builds virus-specific immunological memory.

35. Stability and Immunogenicity of Influenza Vaccine Seed Viruses Derived from Different Host Systems

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Plain Language Synopsis: MDCK and embryonic eggs representing mammalian and vertebrate systems respectively have different posttranslational modifications in proteins. This study is to investigate how different host

systems affect the genetic stability, antigenicity and immunogenicity of influenza vaccine seeds amplified in MDCK and embryonic eggs.

Abstract: Seasonal flu is a major public health concern, which causes flu-like illness in approximately 5-20% of US population and more than 200,000 people hospitalized annually. Timely supply of influenza vaccines is crucial for combating seasonal flu. Traditionally seasonal influenza vaccines are produced in embryonic hen eggs. However, generating vaccine seeds in eggs is not always a smooth process. In addition, studies have shown that egg grown influenza A H3N2 viruses are likely to acquire egg adapted mutations, some of which occur in the receptor binding site. These mutations not only change the binding specificity of H3N2 viruses, but also sometimes alter their antigenicity. This has resulted in a concern that egg grown influenza A viruses may not be suitable for seasonal influenza vaccine manufacturing. Thus Madin-Darby Canine Kidney epithelial cells (MDCK)-based cell matrix has been recommended as an alternative substrate for seasonal influenza vaccine production. MDCK and embryonic eggs are derived from mammalian and vertebrate systems respectively, and have different posttranslational modifications in proteins. The objective of this study is to investigate the genetic stability and immunogenicity of Influenza A vaccine seeds derived from different matrix systems. Recent seasonal H3N2 and pandemic H1N1 isolates were passaged into MDCK cells or embryonic eggs multiple times. The mutations in hemagglutinin (HA), a major surface glycoprotein for vaccination target were detected by full-length sequencing. The viruses adapted in different matrix systems were also tested for their receptor binding specificity, antigenicity and immunogenicity. Our preliminary results indicated that H3N2 viruses remained genetically stable in MDCK cells without antigenic or receptor binding specificity changes. However, pandemic H1N1 viruses were observed to have a relatively high tendency of acquiring mutations after multiple passages in MDCK cells. Most of these mutations occurred in antibody epitopes of HA or adjacent regions. As a result, these cell-adapted pandemic H1N1 viruses showed antigenic drift from the original isolates. In contrast, egg-adapted mutations acquired by pandemic H1N1 strains had no impact on the original antigenicity, but changed the pathogenicity in vivo. The results of our study would provide very useful information in support of seasonal influenza vaccine production.

Session 4: Implement a new Prevention-Focused Food Safety System to Protect Public Health

(Posters 36-66 are located in Section A, Posters 67-73 are located in Room 1406 and Posters 74-77 are located in Room 1408)

36. GenomeTrakr: A Pathogen Databases to Build a Global Genomic Network for Pathogen Traceback and Outbreak Detection

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Plain Language Synopsis: FDA has created and applied in real-time regulatory use, a U.S.-based open-source whole genome sequencing (WGS) integrated network of state, federal and commercial partners. The network, known as "GenomeTrakr," represents the first of its kind distributed genomic food shield for detecting and tracing foodborne outbreak pathogens back to their source.

Abstract: This study demonstrates how with the selection of proper data quality and use of data filtration techniques one can use desktop NGS sequencer data into a combined analysis. Multiple data analysis pipelines are tested to document the ability of some pipelines to combine draft genomes of bacterial data for phylogenetic clustering to provide leads in outbreak investigations of foodborne pathogens. This study outlines how these tools will be implemented to create a pathogen detection network where state and federal public health agencies can share data to build a publicly available and transparent reference data-base with data deposited into a public genomic database (NCBI). Herein we describe the components of the NGS pathogen network that includes studies and current integration among a pilot consisting of state public health laboratories (AK, AZ, FL, HI, M.D., MI, MN, NM, NY, VA, SD, WI and WA) as well as federal laboratories. Details of the successes and failures will be provided concerning communication, coordination, data acquisition, assembly, storage, and analysis. Several recent case studies will be reported on this initial pilot study. The hardware and software implemented allows us to compare and cluster complete genomes of thousands of taxa at a time, and the software outputs daily phylogenetic trees for source tracking of food and environmental isolates. Herein, we report enhanced molecular epidemiological insights gained by comparative analysis of Salmonella, E. coli, and Listeria genomes previously deemed indistinguishable by conventional subtyping methodologies. These results demonstrate an important investigative role for WGS tools within a regulatory environment while highlighting the novel additional insights provided to epidemiological investigations through comparison to a reference database. To read more about FDA's *Salmonella*, *E. coli*, and *Listeria* genomics efforts see: GenomeTrakr network where we have released >10,000 unpublished draft genomes for food safety into the SRA database. http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/default.htm

37. Risk Modeling to Inform Proposed Produce Rule – Raw Manure and Biological Soil Amendments

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Plain Language Synopsis: Risk models combine different big data sets to provide new insights into food safety issues. FDA, in collaboration with USDA, is developing a risk model to study approaches to setting a standard for safe use of raw manure and biological soil amendments under the FSMA Proposed Rule for Produce Safety.

Abstract: Risk models combine unique and different sources of big data sets to provide new insights into food safety issues. FDA, in collaboration with USDA, is developing a risk model to systematically study, analyze, and

evaluate approaches to setting a standard for safe use of raw manure and biological soil amendments under the FSMA Proposed Rule for Produce Safety. Our approach would first develop a predictive risk model for one pathogen and one commodity grown in fields amended with one type of raw manure, to address the complexity in pathogen survival dynamics in land-applied manure, the wide range of produce commodities, and highly variable on-farm practices and agro-ecological conditions. FDA will adapt two existing models and link them together to uniquely address this complex issue to model Salmonella in leafy greens grown in fields amended with poultry manure, FDA will use FDA-Produce Risk Modeling (FDA-PRM) tool and FDA-iRISK tool with a secondary survival module that characterizes the variability and uncertainty in Salmonella survival given different agro-ecological conditions (e.g., soil types, application methods and geographic locations/climatic factors) and time intervals between the application of raw manure and the harvesting of crops. The linked model will then be used to predict how changes in the type of pathogen, the type of commodity, and the type of manure impact the risk of illness. This poster presents a preliminary meta-analysis of the scientific literature on Salmonella contamination and survival in raw manure, the conceptual risk assessment model showing how FDA-PRM and FDA-iRISK will be linked together to create a means to quantitatively compare risks taking into account consumption, dose-response relationship, as well as contamination in the food supply system, from production to consumption. The risk assessment will identify research needed to fill data gaps, and to optionally inform the scientific basis needed for an appropriate standard for the use of raw manure.

38. Simultaneous Detection and Quantitation of Multiple Food Allergens using Multi-Analyte Profiling (xMAP®) Technology

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Plain Language Synopsis: The newly developed food-allergen detection method can simultaneously detect 14 different food allergens covering seven major food allergen groups, which is not possible with the currently used ELISA methods. The cross-reactivity profile generation can help to detect homologous proteins that might pose a health risk.

Abstract: Approximately 15 million Americans suffer from food allergies with about 5 million sensitive to more than one food allergen. Currently, FDA relies on ELISAs to meet the goals of the Food Allergen Labeling and Consumer Protection Act of 2004 and the Gluten-free regulation of 2013. However, ELISAs can only detect a single allergen, are expensive and time consuming, and cannot distinguish between cross-reactive homologous proteins. Using multi-analyte profiling (xMAP®) technology a commercial multiplex assay for the simultaneous detection of 14 food allergens (crustacean seafood, egg, gluten, milk, peanut, soy, and nine tree nuts including almond, Brazil nut, cashew, coconut, hazelnut, macadamia, pine nut, pistachio, and walnut) plus gluten was developed. The multiplex assay incorporates built-in confirmatory assays, performing the equivalent of 30 ELISAs using two wells of a 96-well microtiter plate. The incorporation of two different extraction protocols further increases the reliability of the results generated. The assay displayed limits of detection < 5 ng/mL for each analyte. Besides being able to quantify the targeted allergens, the 30 antibodies in the assay generate a cross-reactivity profile that can be used to detect homologous proteins that might pose a health risk.

39. A Loop-Mediated Isothermal Amplification Assay for the Rapid Detection of Salmonella in Animal Feed and Pet Food

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Plain Language Synopsis: Salmonella is a significant contaminant in animal feed and pet food. A molecular assay was developed and evaluated for the rapid detection of Salmonella in various animal feed and pet food matrices. Upon further evaluation, this method may be a useful tool in routine feed testing to improve laboratory efficiency and protect animal and public health.

Abstract: Salmonella is a significant contaminant in animal feed and pet food. Recent outbreaks and an updated FDA guidance underscore the

need for rapid and reliable detection methods in these diverse commodities. Loop-mediated isothermal amplification (LAMP) is a promising molecular method that gained wide application in food testing recently and could be applied in detecting Salmonella in animal feed and pet food. In this study, a modified LAMP assay was developed and evaluated for the rapid detection of Salmonella in various animal feed and pet food matrices. The assay specificity and sensitivity was determined using 186 bacterial strains (151 Salmonella and 35 non-Salmonella) and serially diluted Salmonella reference strains, respectively. Cattle feed, chicken feed, swine feed, dry cat food, and dry dog food were inoculated with serially diluted cultures (108 to 100 CFU per 25 g feed) of *Salmonella enterica* serovars Newport, Enteritidis, Choleraesuis, Typhimurium, and Infantis, respectively, and tested directly or after overnight cultural enrichment. A well-established qPCR assay was run as a comparison. The Salmonella LAMP assay was 100% specific among the 186 strains tested, without false positive or false negative results. The detection limits were approximately 3.6 cells per reaction in pure culture and 105 CFU per 25 g in spiked feed samples when tested directly. After overnight enrichment, all feed samples tested positive by LAMP at 100 CFU per test portion except for cattle feed, which was positive at 101 CFU per test portion. Comparable results were obtained with qPCR. Taken together, the Salmonella LAMP assay represents a rapid, specific, sensitive, and robust alternative to qPCR for routine screening of Salmonella in these commodities.

40. Low Concentrations of Erythromycin and Penicillin in Distiller's Grains Select for Resistant Bacteria

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Plain Language Synopsis: Distiller's grains, a co-product of the corn ethanol industry, are widely used in animal feed. We found that antibiotics including erythromycin and penicillin at levels similar to those detected in distiller's grains selected for resistance in Enterococcus and Campylobacter. This highlights the potential risk of using distiller's grains containing such antibiotic residues in animal feed.

Abstract: Distiller's grains, a co-product of the corn ethanol industry, are widely used in animal feed. Antibiotic residues including erythromycin and penicillin have been detected in these products, raising concerns of bacterial resistance development in the animal gut that could be passed down the food chain. In this study, we examined the selection of macrolide- or ß-lactam-resistant bacteria when exposed to very low concentrations (0.1, 0.25, and 0.5 µg/ml) of erythromycin or penicillin reflective of residue levels detected in distiller's grains. Campylobacter and Enterococcus were used as sentinel organisms for Gram-negative and Gram-positive bacteria, respectively. Ten strains belonging to each genus were tested against erythromycin and ten Enterococcus strains were tested against penicillin. All experiments were independently repeated twice and resistant mutants were confirmed by broth microdilution in vitro antimicrobial susceptibility testing. At 0.1 µg/ml of erythromycin, only one Enterococcus faecium strain developed resistant mutants, whereas the same E. faecium strain, plus two to three other strains, also developed resistance at 0.25 and 0.5 µg/ml of erythromycin. Among ten Campylobacter strains subjected to erythromycin selection, one C. coli strain consistently developed resistance at 0.1 and 0.25 µg/ml of erythromycin while two other C. coli strains developed resistance at 0.25 and 0.5 µg/ml of erythromycin. Two of the five C. jejuni strains also developed resistance under 0.25 and 0.5 µg/ml of erythromycin selection. For penicillin testing, three E. faecium strains consistently developed resistance at all three penicillin concentrations in both repeats. While preliminary and not yet tested in situ, these findings highlight the potential risk of stimulating bacterial resistance development as a result of using distiller's grains containing erythromycin or penicillin residues in animal feed.

41. Evaluation of RadEye Food Monitor to Screen Food for Gross Gamma Radioactivity

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Plain Language Synopsis: A method was developed to provide quick, in-situ, identification of radioactive contamination in foods using a portable radiation monitor. Rapid detection of radioactive contamination in foods is necessary and critical for the Food and Drug Administration to ensure food safety and protect public health in a nuclear or radiological emergency.

Abstract: With the implementation of the Food Safety Modernization Act (FSMA) and increasing public demand for ensuring food safety in the event of a large-scale nuclear or radiological emergency, there has been growing interest in developing rapid, in-situ, methods to screen for gamma radioactivity in foods. Field deployable methods are necessary and often critical for FDA to have prompt protective measures and maintain public confidence in food safety. Traditionally, FDA tests foods for radioactive contamination by collection and transport of samples to a laboratory specializing in radiation detection. This approach requires considerable time and manpower to acquire food samples and is particularly ineffective for testing large numbers of food samples during a radiological emergency response operation to overcome the limitation, a method was developed to provide quick, in the field, identification of radioactive contamination in foods using a portable radiation monitor. Such technology will enable FDA to: Assemble real-time analytics for prompt decision-making; ensure rapid inspection of fresh produce and perishable foods; gain greater analytical capacity and sample surge capacity; maintain a high level of food safety and public confidence; and Implement efficient and cost-effective food safety programs. Gamma radioactivity is detected with a sensitive high efficiency NaI(Tl) scintillation detector that is encased in modular shielding. Measured radioactivity in foods is attributed to a man-made source if it is above a natural radioactivity threshold and its health significance is evaluated by comparing it with a second threshold corresponding with an FDA Derived Intervention Level (DIL). A variety of natural and spiked foods were tested for method development and validation. In determining the viability of this device, foods were spiked with Co-60, Cs-134, Cs-137, and I-131. The study results demonstrated that the method has the ability to adequately detect man-made gamma radioactivity in foods and determine whether it is above FDA DILs without showing false positive or negative detection. In the event of a nuclear or radiological emergency, this simple user-friendly method will enable the use of an inexpensive portable monitor to provide rapid screening of food for gamma radioactivity in a laboratory setting or in the field.

42. Sequencing and Analysis of the Metagenomes from Oregano, White Pepper, and Cloves

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Plain Language Synopsis: Optimizing a protocol for shotgun metagenomic sequencing will assess its utility for spice research. Data revealed the presence of potential foodborne pathogens in the three spices analyzed; there were changes in the microorganisms detected in the different enrichment conditions. This can help FDA improve pathogen detection methods during outbreaks.

Abstract: Introduction: Recently, spices have been implicated in at least fourteen reported illness outbreaks worldwide, including the United States, resulting in 1946 human illnesses, 128 hospitalizations, and two deaths. It is necessary to identify potential foodborne pathogens associated with spices such as oregano, white pepper, and cloves and improve methods for mitigation and rapid outbreak response. Shotgun metagenomic sequencing is a useful tool for meeting these goals by: (1) simultaneously identifying pathogens of interest present in each spice, (2) evaluating the efficacy of enrichment methods and microbiome shifts throughout the process, and (3) establishing variations between the natural flora found in

spices. Spices are difficult to analyze because of their essential oils and chromophores; therefore, specific method optimization is often necessary. Purpose: The purpose of this study was to optimize a protocol for shotgun metagenomic sequencing and assess its utility to identify the microbiome of spices. Method: Oregano, white pepper and cloves were enriched following a modification of the BAM method using Tryptic Soy Broth (TSB) and modified Buffered Peptone Water (mBPW), with and without corn oil. Samples were collected throughout the enrichment process. DNA was extracted using the Qiacube and shotgun metagenomic sequencing was performed on the MiSeq using the Nextera kit with modifications. The data was analyzed using Metaphlan. Results: Analysis shows that the three spices tested have different metagenomic profiles; however, they contain many similar bacteria, including members of Salmonella, Cronobacer, Shigella, Yersinia, Escherichia coli and Bacillus cereus. Results also show that profiles shift throughout the enrichment process and differ depending on the growth media. Significance: Shotgun metagenomic sequencing revealed the microbiomes of oregano, white pepper, and cloves and the presence of potential foodborne pathogens. This and the ability to trace shifts in microbial communities during enrichment can help FDA improve our pathogen identification methods during outbreaks.

43. An Intercomparison Study on Gamma Spectrometry Methods Used by FDA Food Emergency Response Radiological Laboratory Network

Healey, Stephanie, FDA/ORA/WEAC; Lin, Zhichao, FDA/ORA/WEAC; Emanuele, Kathryn, FDA/ORA/WEAC; McKee, Lindsey, FDA/ORA/WEAC; Regan, Patrick, FDA/ORA/WEAC; Brooks, Susanne, FDA/ORA/ORS; Burr, Donald, FDA/ORA/ORS; Cunningham, William, FDA/CFSAN

Plain Language Synopsis: FDA's decision-making is based on large pools of data from diversified analytical methods. Ambiguous findings will inhibit FDA's ability to take prompt action on protecting food safety and public health. This intercomparison study was designed to strengthen method development, laboratory competence evaluation, and mutual data acceptance in FDA's Radiological Food Emergency Response Network.

Abstract: Proven analytical methods and a competent laboratory network are essential for FDA to implement food defense and safety measures under the Food Safety Modernization Act (FSMA). With growing risks imposed by global aging nuclear facilities and proliferation of radioactive materials, FDA faces increasing challenges in safeguarding the nation's food supply from radioactive contamination. To mitigate the imposing threats to food safety and public health, a radiological food emergency response network consisting of federal and state laboratories was established. This network serves to strengthen FDA's ability to respond to a radiological emergency. Measurement capability, data comparability, and an efficient data reporting mechanism are essential for emergency response when analytical data from cooperative laboratories are used for post-incident risk assessment and management to evaluate different gamma spectrometry methods currently used by member laboratories for food analysis, an intercomparison study was conducted using water samples containing mixed gamma radionuclides at different radioactivity levels. This presentation details the sample preparation and verification per ISO-43 and ILAC G13 guideline, insightful data analysis on evaluating method performance characteristics, and recommendations for developing harmonized methods for food analysis.

44. Evaluation of Sample6's Simple, Rapid, Sensitive, Bacteriophage-based Method for Live Listeria spp. Detection

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Plain Language Synopsis: The method is innovative in that it is the only method that is capable of detecting Listeria spp. on environmental surfaces without need for enrichment. Moreover, cultural isolates can be obtained by incubating the sample sponge/swab. This indicates that the method may be able to provide an answer within a single shift as to whether or not a sample is contaminated with a pathogen. This is revolutionary. There is no other product on the market capable of such a feat.

Abstract: Foodborne outbreaks are a major concern for consumers, food producers, and health organization. A critical step to prevent such outbreaks is the accurate and rapid detection of pathogen contamination of food products in the early stages. However, today's methods all require culture-based pathogen enrichments, often time-consuming, labor-intensive, expensive and require technically-trained personnel. Sample6 leveraged a patented bacteriophage technology developing a live Listeria spp. detection (DETECT/L), which requires minimal or no enrichment, and was awarded an AOAC certificate (AOAC-RI PTM). FDA is currently evaluating this technology. Preliminary results indicate the lower limit of detection (LLD) for ATCC19115 (*Listeria monocytogenes*) was 1-5 CFU/reaction. It can detect 10 cells very well and the signal can be detected in 1-3 hours in a pure culture. This technology can also detect ATCC 35967 (Listeria seeligeri), ATCC 19119 (Listeria ivanovii) and ATCC 19114 (*Listeria monocytogenes*), and ATCC 33090 (Listeria innocua) efficiently. It did not give false positive results for ATCC 25922 (E. coli), ATCC 13048 (Enterobacter aerogenes), ATCC 10721 (*Salmonella enterica*), ATCC25923 (Staphylococcus aureus), ATCC6939 (Rhodococcus equi), ATCC 9027 (Pseudomonas aeruginosa), ATCC19433 (Enterococcus faecalis), and ATCC13315 (Proteus hauseri). In a food sample (scallop) spiked with 30 cells of ATCC 33090 in the standard 250 ml scallop-blended BLEB medium, the listeria cells were detected very well after 9 hours of enrichment at 35ºC. Further extensive inclusivity/ exclusivity tests are underway.

45. Survey of Domestic Dark Chocolate Bars for Undeclared Milk Allergen

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Plain Language Synopsis: Consumers with food allergies rely on accurate food labels to avoid exposure to allergens. This study surveyed 100 domestic dark chocolate bars for the presence of undeclared milk. A large proportion of dark chocolate bars with no milk declaration on the label, inconsistent label statements and advisory statements contained milk.

Abstract: Food-allergic consumers rely on food labels to accurately disclose the presence of allergenic ingredients. The inadvertent introduction of allergens into a food can occur through cross-contact during manufacture, improper use of product rework, labeling errors and incomplete cleaning of equipment. From 2007-2012, 10% of allergen-related recalls were due to the presence of undeclared allergens in chocolates and other confections. This study surveyed 100 domestic dark chocolate bars for the presence of undeclared milk. Chocolate samples were divided into five categories based on label statements: 1) "contains milk", 2) precautionary statements, 3) "dairy-free" or "allergen-free" statements, 4) no statement regarding milk on label, and 5) inconsistent label statements (e.g. vegan claims with milk statements). Concentrations of milk in three samples of each chocolate bar were determined with a milk-specific ELISA kit (Neogen Veratox® for total milk). Confirmation of results for samples testing positive for milk was achieved with the Morinaga milk protein ELISA. Of the chocolates labelled "dairy-free", 12% (2/17) tested positive for milk; one sample had > 1000 µg/g (ppm) milk. Most (9/11) chocolate bars with inconsistent labels and a vegan statement contained > 100 ppm milk. Over 35% (5/13) of chocolates with no reference to milk on the label contained milk at concentrations of 60 - 3000 ppm milk. Samples with precautionary statements contained milk in 75% (39/52) of the chocolate bars, with levels ranging from 2.5 - 3000 ppm milk. Within this category, 6/11 chocolates with a "traces of milk" label statement had 3 - 1000 ppm milk. The results of this survey confirm that undeclared milk in dark chocolate is problematic and that a large proportion of dark chocolate bars with no milk declaration on the label, inconsistent labels and precautionary labels contain milk.

46. Evaluation of Cleaning Methods and Gluten Detection in a Pilot-Scale Brewing Line

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Plain Language Synopsis: Celiac disease is a disorder that afflicts nearly 1% of the U.S. population. Strict avoidance of gluten-containing grain is recommended. Food manufacturers who prepare gluten-free products on shared equipment with gluten-containing foods run the risk of cross-contact during manufacture. The risk of cross-contact can reduced through adequate equipment cleaning procedures.

Abstract: Celiac disease is an autoimmune disorder that afflicts nearly 1% of the U.S. population. There is no cure, and strict dietary avoidance of gluten-containing grains such as wheat, barley and rye is recommended. In 2013, FDA defined the term "gluten-free" as foods that contain less than 20 ppm gluten. However, manufacturers who prepare gluten-free products on shared equipment with gluten-containing foods run the risk of cross-contact during manufacture. This study investigated 1) the effectiveness of cleaning practices for removing gluten from a pilot-scale brewing line, 2) gluten cross-contact from an inadequately cleaned brewing line to a gluten-free (sorghum) beer, and 3) the ability of gluten-specific lateral flow devices to detect gluten residues on brewing equipment. Barley malt-containing beer was produced, and after each production run, the line was cleaned using a hot water rinse at 82°C, or a full cleaning treatment with heavy duty alkaline circulation cleaner followed by a hot water rinse. The effectiveness of cleaning treatments was assessed by swabbing four locations (mash tun, lauter tun, brew kettle and whirlpool) followed by analysis with five different gluten-specific lateral flow devices (LFDs). A cross-contact experiment measured the amount of gluten transferred to sorghum beer immediately after production of barley malt-containing beer. All experiments were done in triplicate. Statistical analysis of the LFD data from cleaning experiments was accomplished using a generalized mixed model, and significant differences ($p<0.05$) were found between the different LFDs, cleaning methods and equipment locations swabbed. The hot water cleaning rinse was less effective in removing gluten from the brewing equipment than the full cleaning protocol. Sorghum beer samples from cross-contact experiments were analyzed by ELISA, and up to 105.1 ± 9.3 ppm (µg/mL) gluten was detected. The results demonstrate the importance of validated cleaning procedures for preventing gluten cross-contact on shared brewing lines.

47. Evaluation of Modified Semisolid Rappaport-Vassiliadis motility agar (MSRV) as a Selective Enrichment Medium for the isolation of Salmonella from Sprouts with the Bacteriological Analytical Manual (BAM) Salmonella Culture Method

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Plain Language Synopsis: The objective of this study was to evaluate the effectiveness of Modified Semisolid Rappaport-Vassiliadis motility agar (MSRV) as a selective enrichment medium for use with the BAM Salmonella culture method for the anlaysis of mung bean, alfalfa, and clover sprouts. The effectiveness of Universal Preenrichment Broth as preenrichment media was also evaluated.

Abstract: Modified Semisolid Rappaport Vassiliadis (MSRV) motility agar was evaluated as a selective enrichment medium for the isolation of Salmonella from alfalfa, mung bean, and clover sprouts with the BAM Salmonella culture method. Fractional Positive Values (FPVs) for MSRV, Rappaport-Vassiliadis (RV), and tetrathionate media from analysis of 20 artificially contaminated test portions were compared to identify potential differences among the selective enrichment media ($p < 0.05$). Lactose broth (LB) and Universal Preenrichment Broth (UPB) were also evaluated as preenrichment media through statistical analysis of the FPVs ($p < 0.05$). FPVs of 7 for RV and 5 for TT (of 20) with UPB were higher than FPVs with LB (all 0) in a trial with alfalfa sprouts inoculated with S. Saintpaul , but no statistical differences were observed with the FPV of 3 for MSRV with UPB ($p > 0.05$). For the isolation of S. Newport from mung bean sprouts, the FPV of 6 for RV, with UPB, was higher than FPVs of 0 for MSRV, with UPB, and all of the FPVs for LB ($p < 0.05$). For the isolation of S. Stanley from clover sprouts, with UPB, the FPVs of 14 for RV and 20 for TT were higher than the FPV of 1 for MSRV, and the FPVs with LB of 0, 8,

and 0 for RV, TT, and MSRV, respectively ($p < 0.05$). UPB was also more effective than LB with RV and TT in a trial with clover sprouts inoculated with S. Oranienburg ($p < 0.05$). The FPVs for RV and TT were both 20 with UPB; with LB, the FPVs were 0 and 13 for RV and TT, respectively ($p < 0.05$). UPB is often more effective than LB when used as a preenrichment for sprouts, but MSRV affords no selective advantage over RV and TT for the isolation of Salmonella from the three different types of sprouts reported here.

48. Evaluation of Culture Methods for the Recovery of Salmonella from Cilantro

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Plain Language Synopsis: Decreased time to detect foodborne pathogens is a critical part of outbreak responses. High-throughput sequencing, in parallel with culturing of contaminated foods, can reveal inefficiencies during microbial enrichment processes that can be addressed by altering incubation conditions. Improving our detection methods will improve prevention and control measures of foodborne outbreaks.

Abstract: Salmonella enterica is a leading cause of foodborne illness in the United States. Salmonella detection from food involves incubations for 24 hours in nonselective broth, 24 hours in selective media, and 24 hours on plating media, according to FDA Bacteriological Analytical Manual (BAM). Previous work has shown cilantro incubated without shaking in modified buffered peptone water (mBPW) favored the growth of Gram-positive microaerophilic Firmicutes, suggesting that aeration via shaking may reduce these species and enhance recovery of aerobic enteric foodborne pathogens. This study evaluates alterations to the BAM for Salmonella enrichment in leafy greens using traditional microbiological analysis and 16S rRNA sequencing. Cilantro samples were spiked with S. enterica Newport at <10CFU/50g. Initial mBPW preenrichments were incubated without shaking for 5, 6, or 7 hours, then incubation continued with shaking at 165 rpm for 24 hours total. Aliquots from 5-7 hour static timepoints and 24 hour timepoints were transferred to Rappaport-Vassiliadis (RV) and Tetrathionate (TT) broths, then incubated overnight at 42.5°C and 150 rpm. All RV and TT cultures were plated on Xylose Lysine Tergitol-4 agar (XLT4). In parallel, genomic DNA was extracted from all sample time points. 16S rRNA amplicons were generated using primers specific to the V1-V3 regions and sequenced on an Illumina MiSeq for 600 cycles. All 24 and 48 hour enrichments that were culture positive for Salmonella on XLT4 were also diagnostically positive using 16S rRNA sequencing. Proportional abundance of Salmonella from TT enrichments increased significantly when 5-7 hour preenrichment aliquots were inoculated, as compared to 24 hour aliquots. Similarly, total Enterobacteriaceae increased with shaking. Some taxa, such as Pseudomonadeaceae and Moraxellaceae, decreased in proportional abundance following 24 hour shaking incubation. Conversely, some competitive taxa, such as Bacillaceae, increased. The population dynamics of the Clostridiaceae family depended heavily on length of shaking. In conclusion, aeration levels and incubation time can have pronounced effects on the microbial community, including Salmonella. Initial preenrichment incubation can be reduced, decreasing total time to detection for Salmonella by 24 hours in leafy greens.

49. Can Corn Oil Serve as an Additive to Help Increase the Recovery *Salmonella enterica* in Oregano?

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Plain Language Synopsis: The goal of this study was to evaluate the effects of adding corn oil in growth media as a compound to sequester the antimicrobial compounds found in spice, while allowing Salmonella to grow during pre-enrichment culture.

Abstract: Introduction: In recent years, spices have increasingly been associated with outbreaks of Salmonella, underscoring the need for enhanced

surveillance and an improved outbreak response. Spices, like vegetables, fruits, and medicinal herbs, are known to possess phenolic compounds that are associated with a variety of antioxidant and antimicrobial effects and properties. Subsequently, the presence of these antimicrobial compounds may impede the detection of Salmonella that persists in dried products. Purpose: The goal of this study was to evaluate the effects of adding corn oil in growth media as a compound to sequester the antimicrobial compounds found in spice, while allowing Salmonella to grow during pre-enrichment culture. Methods: Oregano samples were artificially contaminated with *Salmonella enterica*, pre-enriched in modified Buffered Peptone Water (mBPW) with and without 2% (vol/vol) corn oil and incubated overnight at 37oC. Samples were transferred to selective enrichment broth of Rappaport-Vassiliadis (RV) and tetrathionate (TT) and plated on Xylose-Lysine-Tergitol 4 (XLT-4) Agar and various other chromogenic agars. The plates were examined for the presence of typical Salmonella colonies as described in FDA Bacteriological Analytical Manual (BAM), and suspect colonies were cultured on Trypticase Soy Agar with 5% sheep blood agar (SBA) for confirmation of Salmonella using the colorimetric Gram Negative card and Vitek® 2 Compact software Version 5. In addition to the plating method, molecular approaches such as molecular serotyping PCR and shot-gun metagenomics were used to assess the increase in recovery of Salmonella. Results: From the 30 samples processed for each condition tested, an average 283 CFU/ml of Salmonella was recovered in samples artificially contaminated with Salmonella grown in mBPW with corn oil as compared to 17 CFU/ml without the corn oil. The results demonstrated that addition of corn oil increased the recovery of Salmonella by ≥ 50% in oregano samples. Significance: The addition of corn oil in the pre-enrichment broth may enhance the recovery of Salmonella from spices that contain antimicrobial compounds, a crucial step for enhancing detection of contaminants using both traditional culture and molecular methods.

50. *Listeria monocytogenes* Identification by MALDI-TOF MS Analysis

Nevins, Crystal, FDA/ORA/WEAC; Silverman, Matthew, FDA/ORA/WEAC; Wei, Cong, FDA/ORA/WEAC; Karbiwnyk, Christine, FDA/ORA/WEAC

Plain Language Synopsis: Rapid, sensitive, and accurate microorganism identification methods are crucial to reduce the incidence of foodborne illness. New technology to quickly identify and distinguish pathogenic *Listeria monocytogenes* from other Listeria species is under study. This technology could simplify and speed-up regulatory analysis establishing the presence of pathogenic bacteria.

Abstract: The Centers for Disease Control and Prevention (CDC) estimates that approximately 1600 illnesses and 260 deaths due to listeriosis occur annually in the United States. At least 90% of people who get Listeria infections are in a higher risk group (pregnant women, older adults, and people with weakened immune systems). The genus Listeria contains seven species: L. monocytogenes, L. ivanovii, L. seeligeri, L.innocua, L. welshimeri, L. martii, and L. grayi. Two species are pathogenic; L. monocytogenes is pathogenic to humans and animals and L. ivanovii primarily infects animals. Most human infections follow consumption of contaminated food, such as uncooked meats and vegetables, unpasteurized (raw) milk and cheeses, cooked or processed foods, certain soft cheeses, processed (or ready-to-eat) meats, and smoked seafood. *Listeria monocytogenes* is of particular concern for food manufacturers and processing plants as it can survive and replicate at refrigerated temperatures. Rapid, sensitive, and accurate microorganism identification methods are crucial to quickly assess the safety of foods to reduce the incidence of foodborne illness. Matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF MS) couples high sensitivity with accuracy and is ideal for the detection of high- and low-molecular-weight proteins enabling rapid identification of gram-negative and gram-positive bacteria. Isolated bacterial colonies are subjected to a short extraction procedure. The extracted cell proteins are deposited on a sample target, dried, overlaid with a saturated organic acid solution which forms the crystal matrix necessary for the ionization process, and analyzed by MALDI-TOF MS. Ionized proteins unique to each bacteria species reach the detector based on their size; yielding

profile spectra consisting of a series of peaks, a characteristic "fingerprint". Listeria species have nearly identical protein patterns; however, we found peaks unique to *Listeria monocytogenes* compared to Listeria innocua in MALDI-TOF mass spectra. These peak differences will be exploited to distinguish pathogenic L. monocytogenes from other Listeria species. This research specifically focuses on distinguishing pathogenic L. monocytogenes from other Listeria species by MALDI-TOF MS analysis. This technology could reduce the time needed to establish the presence of pathogenic organisms, therefore, simplifying and speeding regulatory analysis of food products.

51. Aptamer Based Molecular Tools for the Rapid Capture and Concentration of Foodborne Pathogens

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Plain Language Synopsis: Rapid, sensitive, and accurate microorganism identification methods are crucial to reduce the incidence of foodborne illness. New technology to quickly capture and concentrate pathogenic E. coli O157:H7 from food matrices is under study. This technology could simplify and speed-up regulatory analysis and protect the public from exposure to foodborne pathogens.

Abstract: Escherichia coli (E. coli) consists of a diverse group of bacteria. The Shiga toxin-producing E. coli (STEC) O157:H7 is most commonly associated with foodborne outbreaks. Around 5 -10% of those who are diagnosed with a STEC infection develop a potentially life-threatening complication known as hemolytic uremic syndrome. Current estimates indicate that STEC causes approximately 73,480 illnesses, 2,168 hospitalization and 61 deaths in the US each year. FDA Food Safety Modernization Act calls for the development of rapid technologies for the detection of foodborne pathogens before food distribution or ingestion to prevent illnesses. Rapid end point detection methods such as polymerase chain reaction and mass spectrometry are readily available however; effective techniques for the rapid capture and concentration of pathogens from complex sample matrices before detection are largely missing. Recently, an innovative technology based on 3D-structure forming single-stranded DNA oligonucleotides known as aptamers has emerged as a new molecular recognition element. Aptamers recognize and bind to specific molecules/proteins expressed on the surface of a given pathogen with specificity, selectivity and affinity comparable to antibodies. Unlike antibodies, aptamer chemical synthesis is inexpensive, quick and easily scalable; they are easy to chemically modify, have low batch to batch variability and are highly stable even in non-physiological conditions. This project strives to discover and develop aptamer based molecular tools for the rapid capture and concentration of foodborne pathogens using STEC O157:H7 serotype as a model organism. The results of this study will provide crucial information for the development of aptamer based molecular tools for the detection of foodborne pathogens in specific food matrices. Moreover, it is the goal of this project to identify highly specific STEC O157:H7 aptamers that can be used as an alternative to antibodies for a faster and more efficient capture and concentration of STEC O157:H7 directly from short-term enrichment cultures when applied to Enzyme Linked Aptamer Sorbent Assays, aptamer functionalized magnetic bead separation systems and a biosensor platform already in development. This project contributes to FDA's development of rapid technologies for the detection of foodborne pathogens before food distribution or ingestion to prevent illnesses.

52. Sweeteners Determination in Table Top Formulations Using Vibrational Spectrometric Methods and Chemometric Analysis

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Plain Language Synopsis: Non-nutritive sweeteners are used in foods as alternatives to sugar. Cyclamate is banned in the U.S. but has been found in table-top sweeteners, dried fruits, candies, and beverages. Screening methods have been developed for the rapid detection of sweeteners in raw materials and foods using vibrational spectroscopy and chemometrics.

Abstract: Non-nutritive sweeteners are commonly

used in the food industry as no- or low-calorie alternatives to sugar. While used in varying quantities and often in combination to limit undesirable tastes such as bitterness, the allowable sweeteners vary from country to country. As such, methods must be developed to rapidly assess these components as raw materials and in finished products to ensure compliance with U.S. regulations. Saccharin is one of the commonly used and one of the strongest sweeteners on the market with approximately 500 times the sweetening capacity of sugar. Cyclamate, an unapproved sweetener in the U.S., is commonly used in other countries as an alternative to saccharin and has been found in imported food commodities. Although a great variety of analytical tools have been applied to the analysis of these sweeteners in foods, rapid and non-destructive vibrational spectroscopic methods have not been extensively explored. Herein, rapid and non-destructive Raman and NIR methods are proposed for the quantification of saccharin and cyclamate in table top formulations. In Raman and NIR spectroscopy, analysis of the low percentage of sweeteners in these formulations is challenged by overlapping and/or broad spectral features which necessitates the use of multivariate statistical analysis for reliable quantification. The research presented here describes rapid and non-destructive vibrational spectroscopic methods with Partial Least Squares (PLS) data treatment for the evaluation of sweeteners in table top formulations.

53. A Rapid UHPLC-MS/MS Method for the Quantitation of Key Monosaccharides, Disaccharides and Polyols in Diverse Food Products

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Plain Language Synopsis: Atlanta Center for Nutrient Analysis (ACNA) is the only FDA field laboratory responsible for the analysis of nutrients in domestic and imported food products. The purpose of this project was to develop a liquid chromatography-mass spectrometry (UHPLC-MS/MS) method for the profiling of sugar and sugar alcohols in diverse food products.

Abstract: The increased awareness of correlation between obesity and the development of various chronic diseases has made control of caloric intake a primary public health issue. In this regard, reduction of the consumption of foods containing “added sugars” was eM.P.H.asized as a significant way to reduce obesity in the 2010 Dietary Guidelines for Americans. In response, the US Food and Drug Administration recently proposed modifications of the food label to include “added sugars” among others. The Code of Federal Regulations (21 CFR 101.9) state that `sugars shall be defined as the sum of all free mono- and disaccharides (such as glucose, fructose, sucrose, maltose and lactose). In addition, 21CFR 101.60 defines the conditions for the sugar content claims, such as “sugar free,” “free of sugar,” “no sugar,” “zero sugar,” “without sugar,” “sugarless,” “trivial source of sugar,” “negligible source of sugar,” or “dietarily insignificant source of sugar”. Use of such terms is allowed only when the product contains less than 0.5 g of sugars per labeled serving. Cheaper alternatives to natural and artificial sweeteners are often introduced by the manufacturers to meet these label claims, sometimes even with prohibited substances for human consumption. In addition, the sugar profiling analysis is the first step for the testing of authenticity in products such as honey (pure honey claim) and maple syrup. A new method, UHPLC-negative-ESI-MS/MS to separate and quantitate key mono- and disaccharides from polyols (xylitol, sorbitol, maltitol and lactitol) has been developed and successfully used in the analysis of regulatory samples with reduced or no sugar claims. These samples include honey, maple syrups, sauces, cookies and cake mixes, jams, candies, teas, and dietary supplement powders. This new method offers improved chromatographic separation of sugars and polyols, shortened time for the analysis, in addition to the confirmation by mass spectrometry (even in the presence of the isobaric compounds). The method is extended for use in the concurrent analysis of natural sweeteners including steviol glycosides, other oligosaccharides, and the soluble dietary fiber fractions (SDF) and some other artificial sweeteners.

54. Migration of Pressure Sensitive Adhesive Components to Isooctane and 50% Ethanol

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Plain Language Synopsis: Pressure sensitive labels are often pasted onto stretchable plastic food wraps containing meat, dairy products, and other food commodities. GC-MSD analyses indicate that the amounts of adhesive components migrating across plastic food wraps under typical conditions of uses are expected to be below the Threshold of Regulation.

Abstract: Pressure sensitive labels are often pasted onto stretchable plastic food wraps containing meat, dairy products, and other food commodities. They can also be applied directly onto many produce items such as fruits and vegetables. In spite of such diverse usage, the US FDA possesses little or no relevant migration data on components of pressure sensitive adhesives. The majority of modern pressure sensitive adhesives are prepared from the following compounds: vinyl acetate, ethylene, di-2-ethylhexyl maleate (DEHM) and/or fumarate (DEHF), acrylate and carboxylic acid. In this work, GC-MSD has been used for the determination of the migration of DEHM and DEHF from adhesive labels to two solvents, 50% ethanol and isooctane, which are can be used as food simulants for lower and high fat foods. The initial migration studies were performed at 40°C for 10 days and indicate that the highest combined amounts of DEHM and DEHF migrating from adhesive labels through a typical low-density polyethylene (LDPE) wrap is 18 ng/cm2 for 50% ethanol and 69 ng/cm2 for isooctane. Without the LDPE wrap, the highest amounts of DEHM and DEHF into 50% ethanol and isooctane are 50 and 8,000 ng/cm2, respectively. Because 50% ethanol is generally considered to be an acceptable food simulant for meat products, this migration data would provide fair estimates for the migration of DEHM and DEHF into meat products through an LDPE film. Since fruits and vegetables generally have low fat concentrations, data from the 50% ethanol migration study should conservatively estimate migration. This establishes that the expected combined migration of DEHM and DEHF is not expected exceed 50 ng/cm2 in meat, dairy, and produce through a LDPE film. In addition, due to low consumption factors for products using these adhesive labels, consumer exposure from adhesive components from pressure sensitive labels is expected to be below the threshold of regulation.

55. Rapid Screening of Radioactive Curium (Cm) in Foods by Solid Phase Extraction Liquid Scintillation Counting

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Plain Language Synopsis: Concerns over radioactive food contamination from nuclear and radiological activities rise considerably with aging nuclear power plants, continuing nuclear proliferation, and potential breach in safeguarding nuclear materials to ensure food safety in the event of an accidental or intentional release of radioactive materials, a high throughput liquid scintillation counting technique for detecting alpha radioactivity in foods is critically needed.

Abstract: A rapid radioanalytical method for screening radioactive curium in contaminated foods will be presented. Radioactive Curium was isolated from food matrix using DGA resin (N,N,N',N'-tetra-n-octyldiglycolamide) after digestion of sample in 8M nitric acid. The extracted Cm was reclaimed from the resin using 0.1M HCl -0.1M $H_2C_2O_4$ and alpha radioactivity of Cm was quantified by alpha/beta discriminative liquid scintillation counting. Two Cm isotopes of most concern, i.e., Cm-234 and Cm-244, were found to be selectively and quantitatively retained by DGA resin that effectively eliminates matrix and radiometric interferences. The method was developed and validated using meat, grain, vegetable, dairy, and composite food samples spiked with known amounts of Cm-234 and Cm-244. The method was capable of recovering >95% of Cm-234 and Cm-244 added to the food samples with 1 gram of DGA resin and 15 minutes of extraction. The study results showed that matrix and natural radiometric interferences can be adequately removed to meet the detection limit required for addressing radiological safety concerning Cm contamination of foods. The experimental results, instrument optimization, and

minimization of alpha/beta crossover interference will be discussed.

56. Identification of Irradiated Spices by Electron Paramagnetic Resonance Spectroscopy

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Plain Language Synopsis: Foods are treated with ionizing radiation to destroy pathogenic bacteria and delay ripening but currently there is no reliable methodology to ascertain if irradiation of the food has occurred. In this study, CFSAN explored the use of electron paramagnetic resonance spectroscopy to identify spices that have been treated with ionizing radiation. Once fully developed and validated, this method will ensure that foods that are purported or labeled as having been treated are indeed treated and allow the Agency to enforce current regulations and protect consumer health.

Abstract: The Food Safety Modernization Act requires food facilities conduct hazard assessments and if a hazard is reasonably likely to occur they must put in place preventive measures to control the hazard. Pathogens are reasonably likely to occur in some spices and manufactures may choose to irradiate their products to control this hazard. Currently, there are no reliable methods to determine if a product has been irradiated for verification of compliance with the Act. The use of Electron Paramagnetic Resonance (EPR) to detect irradiated spices was demonstrated in the early 1990's (EN 1787:2001 Detection of irradiated food containing cellulose by ESR spectroscopy). However, the stability of the radiation-induced radical and its spectral characteristics varied across the broad selection of spices and was considered a limitation to the use of this technique. We have recently reexamined this technique to determine EPR could be used to ensure that products that have been labeled as irradiated have indeed been irradiated. This paper will present data on the EPR spectra for over 30 spices. We have investigated the resultant spectra, including its stability with time. Most of the irradiated spices analyzed display an EPR Spectrum that is assigned to a relatively cellulosic radical. Several spices demonstrated a more complex radical spectrum, such as garlic powder and onion powder, which appears to originate from other components. The stable radical found in garlic or onion powder, the radical is stable for over 6 months. For black pepper, the spectrum is multi-component with complexities at early times (day one vs day seven). For most of the spices that were analyzed it was easy to differentiate the irradiated spice from the non-irradiated spice up to 90 days post irradiation treatment.

57. Detection of Cyclospora cayetanensis on Fresh Produce: Assessment of Improved Produce Wash, DNA Extraction, and Molecular Detection Procedures

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Plain Language Synopsis: Development and validation of improved methods for surveillance, compliance, and outbreak investigations of foodborne Cyclospora cayetanensis are critical to the safety of our food supply. In this study we evaluate modifications to FDA's analytical detection method that enhance and streamline detection of Cyclospora cayetanensis on produce.

Abstract: Cyclospora cayetanensis is a coccidian parasite causing foodborne diarrheal disease linked to consumption of contaminated fresh produce such as leafy greens and raspberries. During the U.S. outbreaks of 2013 and 2014 a total of 935 cases of cyclosporiasis were confirmed in several states. In both outbreaks, some illnesses were linked to the consumption of imported fresh cilantro. Detection of Cyclospora cayetanensis on produce relies heavily on efficient oocyst recovery and sensitive molecular methods since this organism cannot be enriched or cultured. The objective of this study was to evaluate new methods of detection for Cyclospora cayetanensis on produce for future implementation into FDA regulatory analyses. Samples of cilantro (25 gram) and raspberries (50 gram) were seeded with oocysts. Six or more replicates (n) at each seeding level were tested. Oocysts were recovered from seeded samples using a detergent wash solution and DNA was extracted using a commercial kit. Molecular detection was performed using a conventional nested PCR assay currently used in FDA labs and a new optimized qPCR assay. Nested PCR detected C. cayetanensis DNA in 100%,

100%, 42%, and 25% of cilantro replicates seeded with 200 (n=11), 10 (n=6), 5 (n=12), and 2 (n=8) oocysts, respectively. Using qPCR, detection rates were 100%, 100%, 75%, and 63%, respectively. Detection rates using nested PCR for raspberries seeded with 200 (n=12), 10 (n=10), 5 (n=8), and 2 (n=6) oocysts were 100%, 50%, 38%, and 33%, respectively, and 100%, 80%, 38%, and 33%, respectively, using qPCR. The low detection limit for C. cayetanensis was 10 or fewer oocysts on two commodities, which is the presumed infective dose for this organism. In addition, qPCR provides a robust, streamlined, and faster alternative to nested PCR. These results support public health and FDA's mission by providing improved detection methods for the foodborne parasite, Cyclospora cayetanensis.

58. Development of an LC-MS-MS Method for the Determination of Hypertension Drugs in Dietary Supplements

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Plain Language Synopsis: Currently, we are interested in dietary supplements marketed for hypertension treatment, which can be harmful to the consumer. We have developed a UPLC/MS/MS method to determine hypertension drugs in dietary supplements. This way the consumer will have more information about the content of the products that might be purchase.

Abstract: Background: Dietary supplements are sold as natural composed mixtures for improving patient's health. However, manufacturers tend to adulterated them by adding drugs that should be regulated by FDA. The purpose of this work is to develop and validate an UHPLC/MS/MS method for the determination of hypertension drugs in dietary supplements. Methods: The method was developed for four types of drugs in which capsules were mixed and approximately 200 mg was weighed, placed in a centrifuge tube containing 10 mL of solvent, vortexed for 20 seconds, sonicated for 10 minutes and centrifuge at 8,000 rpm for 10 minutes. The supernatant was transfer to an HPLC vial and injected into the UHPLC/MS/MS instrument. The system consisted of an Agilent 1200 with a Zorbax SBC18, 1.8µm, (50x2.1) mm, coupled with a Thermo Scientific LTQ Velos ion trap MS. Method conditions consist of a gradient using 0.1% formic acid in water and 0.1% formic acid in acetonitrile at 0.23 mL/min flow, an injection volume of 1µL, oven and autosampler temperature of 40°C and 15°C, respectively and run time of 10 minutes. Compounds were monitored using multiple reaction monitoring with a collision induced dissociation energy of 30 in a scan range of 100-500m/z. Qualifying ions for each compound are (M+H, Transition ion) m/z: Terazosin (388.3-290.00), Prazosin (452.30-344.00), for Doxazosin (384.3-247.0) and Finasteride (373.3-305.00). The method was validated for precision, linearity, limit of quantification (LOQ), limit of detection (LOD), specificity, and matrix effect. Results: Interferences were observed from the matrices that impact the detection of the drugs. LOD and LOQ for each drug were determinate as 0.0025 ppm and 0.0125 ppm, respectively. Linearity was evaluated in a concentration range of 0.0125 to 0.60 ppm having correlation coefficients in a range of 0.995 to 1.00 for samples. Average recoveries ranged from 26% to 112%, with relative standard deviations ranging from 6.0% to 9.8%. Conclusions: The results for these drugs met the acceptance criteria of FDA over the required range. This method has better detection limits, reduces sample preparation time and provides more specificity in comparison with other methods found in the literature.

59. Airborne Transmission may be a Factor in the Contamination of Fresh Produce by Bacterial Pathogens

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Plain Language Synopsis: Using new technologies such as metagenomics, we study microbiological risks to consumers associated with the farm to fork continuum. Data presented here suggest that surveillance of airborne pressures to crops will also be necessary to understand contamination events and accurately predict risks to consumers.

Abstract: We have launched a research project designed to better understand the role of

airborne transmission of bacterial pathogens for fresh produce fields that may be near livestock operations. We have examined agricultural waters, soils, pesticides and other agricultural components and practices that may contribute to contamination events, including the baseline microbial ecology of important food plants. Previous work has shown that airborne transmission may play a bigger role in the establishment of microflora on the surfaces of crops than either pesticides or water. A trend that is emerging in many studies is the significance of currently uncharacterized environmental pressures, such as airborne transmission. We have devised a plastic plant surrogate to study the significance of airborne transmission on produce microbiology. We compared plastic plant surfaces co-located in the field with live plants at multiple time-points to get a better understanding of the microflora that may be delivered to the plant from airborne sources. We compared the flora found on plastic plants with that of living plants to identify microflora that may be mediated by host plant biology. The data showed that 92 to 100% of all bacterial genera seen on living plants were also present on the plastic surrogates. This suggests that air is playing a significant role in seeding bacteria onto the surfaces of plants and that further study of aerobiology in agricultural environments will be necessary to fully understand the most important risks factors for contamination of fresh produce.

60. Challenges to Develop a Detection Method for Foodborne Virus Contamination in a Complex Food Containing Berries

Papafragkou, Efstathia, FDA/CFSAN; Hida, Kaoru, FDA/CFSAN; Cromeans, Theresa, CDC/Division of Viral Diseases; Kulka, Michael, FDA/CFSAN

Plain Language Synopsis: Research in the area of development of analytical methods for foodborne virus isolation and identification in foods will greatly enhance the agency's overall knowledge of these agents and will further enhance the agency's capabilities for a timely response during inspections, surveillance, and disease outbreak management.

Abstract: Introduction: The number of reported outbreaks caused by foodborne viruses, primarily human noroviruses and hepatitis a (HAV), has been on the rise. However, even though the means of transmission can be identified as foodborne, the virus cannot always be isolated from the suspected food vehicle. For HAV in particular, although its overall frequency has declined in the developed countries and has more sporadic occurrence, it still remains a public health hazard featured in highly publicized outbreaks. Berries have frequently been associated with HAV outbreaks. In some cases it is hard to separate the fruit from the rest of the matrix, especially in multi-component food items. A study was undertaken to detect HAV from artificially contaminated strawberries in a cake frosting mix. Methods: HAV was used as inoculum from either purified cell culture lysate or clinical samples to seed a 30g of strawberries-frosting mix sample. HAV was eluted with a 0.1 M Tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.2 (TGBE) containing 2% Polyvinyl Pyrrolidone (PVP). After a brief spin, the eluate was combined with chloroform to separate the fats from the frosting, and after a second re-extraction was precipitated with 10% polyethylene glycol overnight. The next day the pellet was washed with chloroform-butanol, the virus was eluted with TGBE buffer and concentrated with a second PEG precipitation. Finally, virus RNA was isolated from the pellet with a commercial kit and detected with a real-time RT-PCR. Results: HAV could be detected at a level of seeding of at least 10^4 PFU of purified cell culture lysate per 30g of sample. When the frosting-strawberries mix was inoculated with a clinical isolate of HAV, recovery was achieved at a low contamination level of less than 200 RNA copies per 30g sample. : Currently, there are very few methods available for sensitive and timely extraction and detection of HAV from such complex commodities. Having developed such a methodology can provide insight into useful steps for reducing the inhibitory effect of polyphenolic and fat substances often present in produce and produce-related food items and eventually provide a tool for critical response during disease outbreaks.

61. Development of a Sensitive and Rapid HPLC Method for Quantitative Measurement of Ginkgolic Acids in Dietary Supplements

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Plain Language Synopsis: Dietary supplement containing extracts of Ginkgo biloba are one of the most widely used supplements. Studies indicate ginkgolic acids, a group of structurally-related alkyl phenols, are responsible for negative health effects. An HPLC-UV method was developed to quantify three forms of ginkgolic acids in dietary supplements.

Abstract: Dietary supplements containing extracts of Ginkgo biloba are one of the most widely used supplements. These products are used for their purported benefit to improve cognitive functions, blood circulation and in management of neurodegenerative conditions. The beneficial effects of extracts are primarily attributed to flavonoids and triterpene lactones. Ginkgolic acids, a group of structurally-related alkyl phenols, have been shown to have some negative health effects. The European Union currently limits the amount of ginkgolic acid in supplements to a level of 5 parts-per-million (ppm) or less. Recently, the National Toxicology Program reported that G. biloba extracts caused cancers of thyroid and liver in rats and mice models. In the light of this report, we intend to measure ginkgolic acids content of Ginkgo dietary supplements in US market. An HPLC-UV method using a biphenyl column was developed for the separation of three forms of ginkgolic acids (13:0, 15:1, 17:1). The samples were extracted by the QuEChERS method, in which the ginkgolic acids were partitioned in the acetonitrile layer, followed by HPLC-UV analysis. The developed method was validated for linearity, repeatability, accuracy, limits of detection, limits of quantitation and spike recovery. The method successfully quantified total ginkgolic acid amounts below 5 ppm levels. Quantitative data on the ginkgolic acid content of the dietary supplements will be presented.

62. Proficiency Test for Determination of Gluten in Breakfast Cereal

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Plain Language Synopsis: A proficiency testing program was conducted to evaluate laboratory performance for quantitation of gluten in 3 blind-coded breakfast cereal samples using the RIDASCREEN® and MIoBS ELISA methods, with 6 participating laboratories and 16 analysts. Results were statistically analyzed following the ISO13528:2005 protocols to evaluate laboratory and test kits performance.

Abstract: Celiac disease affects an estimated 3 million people in the United States, a condition that can be managed by consuming a gluten-free diet. FDA in 2013 issued a final rule that established a regulatory definition of gluten-free. Verification of compliance necessitates use of validated methods by proficient analysts and laboratories. Proficiency testing (PT) is a systematic way to evaluate analytical performance of analysts and laboratories. A PT round was conducted to evaluate laboratory performance for quantitation of gluten in breakfast cereal samples. Twenty one breakfast cereal samples purchased from local grocery stores were screened for gluten using the R5 RIDASCREEN® ELISA test kit and the gluten levels in positive samples were quantified using the RIDASCREEN® and Morinaga (MIoBS) ELISAs. Based on the gluten concentrations, three corn-based cereal samples containing three levels of gluten were used to prepare the PT samples. Cereal samples were ground and homogenized with a Hobart blender. The resulting homogenate was subdivided into three blind coded test samples (A-01, A-02, and A-03) and tested for homogeneity before shipment to participating laboratories. A total of 16 analysts submitted results. The results were statistically analyzed according to the internationally harmonized protocol ISO 13528:2005 to evaluate the laboratory and test kits performance. The mean values of gluten obtained using the RIDASCREEN® ELISA (n=16) for the two positive samples (A-01 and A-02) were 797 ± 211 and 56 ± 14 ppm compared to the mean values of 379 ± 72 and 30 ± 4 ppm obtained using

the MIoBS ELISA (n=16). All laboratories identified the negative control sample (A-03) and no false positive results were reported. Fourteen (87.5%) analysts had z scores below 2 for all the two positive samples using both methods, and two (12.5%) analysts had z scores greater than 2 for one of the two positive sample using either ELISA. Interestingly, the gluten concentrations obtained using the MIoBS ELISA were 50% lower compared with those obtained using the RIDASCREEN® ELISA. This difference could represent the presence of barley in the products, which the MIoBS ELISA displays poorer cross-reactivity. Overall, all analysts displayed excellent proficiency.

63. Accuracy and Precision Data of Analytical Methods Derived from Proficiency Testing: Case Studies from United States and Germany

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Plain Language Synopsis: Proficiency testing (PT) is used to evaluate the performance of food safety laboratories, while method validation is used to evaluate performance of analytical methods for food product testing. Using PT case studies from the United States and Germany, examples showing how PT results can validate methods will be given.

Abstract: Proficiency testing (PT) is used to evaluate performance of laboratories for specific tests. Method Validation (MV) is an investigation to determine if the analytical purpose of a method is achieved. Use of validated methods by proficient analysts and laboratories is essential to the Food Safety Modernization Act (FSMA). It is critical that FDA incorporate MV into its scientific programs and develop criteria equivalent to international data standards. Integrating MV into the existing PT programs is logical due to the many similarities that exist between these two types of multi-laboratory studies. In both PT and MV, homogenous samples are prepared, multiple laboratories independently analyze samples under repeatability/reproducibility conditions, and resultant data can be used to measure accuracy and precision of methods. Conducting PT or MV multi-laboratory studies requires an enormous amount of time and resources. Therefore, a strong scientific and financial need for an integrated PT/MV program exists. By utilizing experimental design principals and advanced statistical techniques, the data generated by PT or MV studies could be used inter-changeably; establishing a "perform once, use many times" mantra to efficiently use FDA resources. While the purpose of PT (assessing laboratories) and MV (assessing methods) differs, properly designed PT studies could be used to simultaneously conduct MV studies. Proper design of PT studies includes determining sample numbers, preparing blind duplicates, assuring participation of >10 laboratories, etc. Because several test methods are often used in PT studies, proper design allows PT organizers to analyze data on different methods according to standard method comparison techniques and robust statistics. For example, in FDA's milk PT study, several analysts in one laboratory analyze the same samples. Therefore, repeatability, reproducibility, and additional precision figures can be obtained according to ISO 5725-3 for reference and alternative methods. Similarly, PT studies for heavy metals and pesticide analysis in various matrices by Germany's Federal Office of Consumer Protection and Food Safety (BVL) demonstrate the use of PT data to derive method precision data. Using FDA and BVL PT case studies, examples of utilizing PT data to determine measurement error and assessment of method equivalency will be presented.

NOTE: Numbers 64, 65, and 66 are skipped intentionally

67. Evaluating Canine Fanconi Syndrome Occurrence in Association with Jerky Pet Treat Ingestion

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Veterinary Medicine, Section of Medical Genetics

Plain Language Synopsis: Since 2007, FDA received about 5,000 reports on animals ill from eating jerky pet treats. About 4-5% of reports indicate Fanconi Syndrome, a rare kidney disorder in small animal pets. Vet-LIRN collects urine samples from sick dogs to test. The results are discussed.

Abstract: Since 2007, FDA has received approximately 5,000 reports of pet illnesses potentially associated with the consumption of jerky pet treats (JPT). A specific cause of the reported illnesses has not been identified. Approximately 4-5% of all reports indicated Fanconi Syndrome (FS), a broad defect in proximal renal tubular reabsorption. Affected animals show nonspecific signs of illness and are frequently recognized due to the presence of glucosuria despite normoglycemia. FS in dogs can be genetic in origin (primarily the Basenji breed) or acquired (any breed, potentially due to toxin exposure). Because acquired FS is rare in the small animal pet population, in 2012, FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) began collaborating with veterinarians across the country to collect urine samples from dogs with a variety of illness types following JPT consumption, not only those reported to FDA as having glucosuria/suspected FS. As of December 1, 2014, Vet-LIRN, in collaboration with the University of Pennsylvania's PennGen Metabolic Genetics Laboratory, tested 190 dogs from a variety of breeds with a urinary Fanconi screen. A total of 125 dogs tested positive for markers of FS, including glucosuria, aminoaciduria, ketonuria, and lactic aciduria. Eighty-seven positive dogs were tested a second time, approximately 1-2 months after the first test, and 74.7% continued to test positive for FS. Vet-LIRN continues to monitor Fanconi positive dogs to see how long markers of FS remain in the urine after JPT are withdrawn. This information is being used by FDA to better characterize the clinical manifestations of FS and to inform veterinary practitioners on how best to recognize and diagnose illness that may be associated with JPT consumption. By performing Fanconi screening of urine, Vet-LIRN is enabling FDA to focus product testing resources on product samples associated with confirmed FS cases. Vet-LIRN's ability to leverage the resources of network veterinary diagnostic laboratories to investigate consumer adverse event reports provides data previously unavailable to FDA, thereby increasing the government's ability to identify emerging threats.

68. Developing Field Screening Methods Using Surface-Enhanced Raman Spectroscopy

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Plain Language Synopsis: FDA is tasked with managing a global food and pharmaceutical supply with limited resources. Ideally, field investigators could screen samples; forwarding positive samples to laboratories. The objective of our research is to develop methods to prevent economic adulteration of milk-products often consumed by some of the most vulnerable populations.

Abstract: Currently FDA's analytical repertoire is concentrated in laboratories; whereby inspectors collect and send samples for analysis. Synergistically merging advances in risk based triage and field screening would enable FDA to proactively monitor the United States food and drug supply. Traditionally, Raman spectroscopy was overlooked for other spectroscopic methods due to lack of sensitivity. However, advances in plasmonics and lithography have yielded uniform, low-cost gold nanoparticle sensors enabling rapid, sensitive analyte detection using surface-enhanced Raman spectroscopy (SERS). We are developing methods enabling non-experts to quickly and efficiently assay foods, excipients, and drugs for contaminants in the field. Elegant and detailed analyses may be accomplished in dedicated laboratories; however, for field screening, sample throughput and simplicity are paramount. Our initial method development is focusing on milk, milk products, lactose and bulk protein products to detect the presence of compounds potentially used in economic adulteration; whereby, the protein content, as determined via nitrogen amount, is artificially enhanced by the addition of nitrogen rich, low molecular weight compound(s). Recent events, including the use of melamine for adulteration of Chinese infant formula and milk in 2008, highlight the need for screening a multitude of consumer products. Continued vigilance is necessary due to the international community relying on the Kjeldahl method for determining

nitrogen concentration, and then correlating percent nitrogen to protein content. Our initial focus described above is the first application; we envision building SERS sensor methods pragmatically. Future method development activities will broaden to include additional FDA regulated products. Nitrogen containing, small molecules are ubiquitous and can be found in/as pesticides, drugs, and commodity chemicals. Furthermore, SERS is not limited to nitrogen compounds and products, and we envision using this method for multiple future applications.

69. Rapid Detection of Processed Uranium in Food

Abdur-Rafay Shareef, FDA/ORA/WEAC; Lin, Zhichao, FDA/ORA/WEAC; Cunningham, William, FDA/CFSAN/ORS

Plain Language Synopsis: The US FDA is tasked with protecting and promoting public health; therefore, maintains the capability to respond to emergencies: chemical, biological, and radiological. The research goal is to develop rapid inductive coupled plasma mass spectrometry (ICP-MS) methods for the measurement of alpha- and beta-emitting radionuclides in foods.

Abstract: FDA has excellent capability for monitoring food for radionuclide contamination. However, the current methods available for alpha- and beta-emitting radionuclides are limited because they are time consuming with multi-day procedures often required to isolate them from sample matrix and interfering radionuclides. In the aftermath of an accident involving nuclear-energy or -weapon materials, the US FDA could be tasked with monitoring the food supply for this type of contamination. Our research goal is to develop rapid and high-throughput methods for these radionuclides in foods. Our initial application will be the detection of processed uranium in foods using isotope ratio mass spectrometry (IR-MS). Although uranium is a naturally-occurring radionuclide, processed uranium has nuclear weapons and energy applications whereby its natural isotopic ratio of 238U/235U has been altered for its intended uses. The natural 238U/235U ratio is 137.82 ± 0.21 (1s) but this ratio is much greater for processed uranium. For example energy grade uranium contains 3 - 5% of 235U, and weapons grade uranium contains >90% of 235U. The IR-MS method is capable of revealing uranium source term via accurate and precise measurement of 238U/235U ratio. This project is intended to further improve FDA's radioanalytical capacity for monitoring radioactive food contamination.

70. Advances in Food Safety: Applications of Liquid Chromatography-High Resolution Mass Spectrometry to the Detection of Chemical Contaminants in Food

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Plain Language Synopsis: A modern challenge to food safety is the ability to rapidly and confidently detect known and unknown chemical contaminants in the global food supply. The advent of modern LC-MS technologies has greatly improved the screening capacity and quantitation of chemical contaminants in foods, enhancing food safety and protecting public health.

Abstract: Sensitive and unambiguous identification of chemical analytes and contaminants in foods is a challenge for food safety and control. The advent of modern LC-MS technologies has greatly improved the screening capacity and quantitation of chemical contaminants in foods. Novel qualitative and quantitative methods using ultrahigh performance liquid chromatography-quadrupole-orbital ion trap mass spectrometry (UHPLC -Q-Orbital ion trap MS) for the analysis of pesticides and mycotoxins in food will be presented. The performance characteristics of these studies using UHPLC -Q-Orbital ion trap MS will be presented. The results demonstrate that HRMS in full (70,000 FWHM) scan mode provides the accurate mass of the precursor ion used in quantitation. Data dependent MS-MS (dd MS -MS) acquisition in product (17,500 FWHM) scan modes can provide better determination of product ions. This technology has also been applied to the qualitative screening (with identification) of pesticides in teas using QuEChERS cleanup followed by analysis using UHPLC -Q-Orbital trap-dd MS -MS and a pesticide library database of over 600 pesticide spectra. Additionally, data independent acquisition techniques and ways to

facilitate the sharing and harmonizing of methods and screening libraries among government laboratories around the world will be discussed. The goal of this work is helping laboratories and regulatory agencies to become better prepared for and to respond more quickly to potential chemical contaminant alerts in the global food supply.

71. Distribution of Residues in Various Muscles of Cattle Following Intramuscular Administration of Hormones

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Plain Language Synopsis: This study was conducted to determine whether hormonal drugs are distributed evenly between various bovine muscles. Results showed that residue levels were highest in the diaphragm and consistent in the limb muscles for most drugs. These findings suggest that the diaphragm is the preferred muscle when monitoring for hormone residues.

Abstract: Hormones are used in animal agriculture to increase feed efficiency, promote muscle development, and improve meat quality. Previously, our laboratory developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method suitable for the simultaneous detection of sixteen hormonal drugs in bovine muscle. Recently, we applied the method to determine if hormones are distributed evenly among different muscle regions. Five animals were dosed intramuscularly with hormone mixtures consisting of one to four drugs. Muscles from five different anatomic regions (right front limb, left front limb, right hind limb, left hind limb, and the diaphragm) of each animal were collected for the study. The hormones of interest included testosterone, progesterone, 19ß-nortestosterone, 17α-methyltestosterone, melengestrol acetate, megestrol, 17ß-estradiol, ethynyl estradiol, zeranol, estriol, and dienestrol. Results showed that residue levels were highest in the diaphragm of dosed animals for all drugs except testosterone. Drug levels in the forelimb and hind limb muscles were relatively consistent among all drugs except megestrol, which had levels twice as high in the hind limbs as in the front limbs. These findings suggest that the diaphragm is the preferred muscle when monitoring for hormone residues.

74. Need Validated Study Outcome Measures for Docosahexanoic and Arachidonic Acids (DHA & ARA) Supplemented Infant Formula Trials: Call to Tackle Non-Validated Endpoint Issues

Sun, Haihao, FDA/OC/OPT; Como, Peter, FDA/CDRH; Smith, P. Brian, Duke University; Rodriguez, William, FDA/OC/OPT; Murphy, Dianne, FDA/OC/OPT

Plain Language Synopsis: Since 2002, fatty acid DHA/ARA has been added to infant formula with statements that they enhance growth and impact brain & eye development. While leading to increased prices for infant formula, evidence of DHA/ARA benefit lacks as most trials use varieties of assessment measures that affect DHA/ARA supplementation study outcomes.

Abstract: Background: Primary endpoint selection considerably influences the reliability and interpretability of clinical trials. Since 2002, DHA/ARA has been added to infant formula with statements that they enhance growth and impact brain & eye development. While leading to an increased price for infant formula, evidence of DHA/ARA benefit is lacking as most trials use varieties of study endpoints. Objectives: Systematically review the endpoints used in clinical trials assessing neurodevelopmental outcomes ascribed to DHA and ARA supplementation of infant formula; assess the impact of these endpoints on trial outcomes. Methods: to identify studies, we searched PubMed/Medline using the phrase “infant formula/ AND (DHA or ARA or AA or LCPUFA or LCFA)”. We reviewed all neurodevelopmental endpoint measures in all randomized, controlled trials with DHA/ARA supplements in infants. We evaluated the validity of endpoint measures based on the criteria for studying infant formula additives recommended by the Institute of Medicine (IOM). The outcome of each study was examined to assess the impact of the endpoint measures. Results: We reviewed 21articles that met the selection criteria. The endpoints used were: Bayley Scale of Infant Development (BSID) I and II (n=12), Brunet-Lezine test (n=2), videotape infant’s movements (n=1), record time to milestones (n=1), Stanford Binet IQ test (n=1), problem solving test (n=2), brainstem auditory evoked potential (n=1), and Touwen examination (n=1). None of these measures were

designed to determine long-term predictive value but rather to yield a measure of development or cognitive ability at a specific point in time due to the variability of normal human development. Based on the IOM recommendations, BSID is age appropriate, adequately sensitive, and has documented brain-behavior links, but requires well trained examiners. Compared to standard infant formula, beneficial effects of DHA/ARA supplementation on neurodevelopment were reported in 2/12 studies using BSID vs. 5/9 studies using other endpoint measures. Conclusion: The choice of endpoint measures affects the outcomes of DHA/ARA supplemented infant formula trials. BSID is a validated endpoint for the measurement of neurodevelopment outcome at the time of assessment. Available data are currently inadequate to conclude that DHA/ ARA supplementation has a clinically meaningful benefit effect upon neurological development.

75. Impact of Supplementing Infant Formula with Long Chain Polyunsaturated Fatty Acids: A Systematic Review

Sun, Haihao, FDA/OC/OPT; Rodriguez, William, FDA/OC/OPT; Smith, P. Brian, Duke University; Chambers, Wiley, FDA/CDER; Downey, L. Corbin, Duke University; Murphy, Dianne, FDA/OC/OPT

Plain Language Synopsis: Since 2002, fatty acids (DHA/ARA) have been added to infant formula and have resulted in price increases (5–30%). However, available data are currently inadequate to conclude that DHA/ARA supplementation has a clinically meaningful beneficial effect upon neurodevelopment, growth or visual development, nor to recommend supplementation with these fatty acids.

Abstract: Background: Since 2002, docosahexanoic and arachidonic acids (DHA & ARA) have been added to infant formula with statements that they enhance growth, and impact development of the brain and eye. This has resulted in an increase in the cost (5 – 30%) of infant formula. Objectives: to systematically analyze literature reports of growth, neurologic and visual developmental outcomes ascribed to DHA and ARA supplementation of infant formula. Design/Methods: Studies were identified by searching 8 electronic databases (PubMed/ Medline, ISI web of Knowledge, Agricola, Pascal, Foodline, Food science & technology, Biosis previews, and Embase) using phrases such as "infant formula/ AND (DHA or ARA or AA or LCPUFA or LCFA)". No language and time restrictions were applied during the search. The last search was conducted on September 28, 2011. The reference lists of initially retrieved literature were also reviewed to identify articles that were missed by the database search. We reviewed all randomized, controlled human trials using DHA and ARA supplements with clinical endpoints addressing neurodevelopment, growth and visual development. Data identifying trial design, endpoints, supplement source, and study population were organized by outcomes of interest-neurologic, growth and visual development. An assessment of the evidence for each outcome was then determined. Results: We identified 471 relevant articles for our analysis. 67 studies met the selection criteria and were reviewed. 35 studies used standard assessment methods and were well conducted thus were included in final assessment. Table-1 summarizes numbers of studies showing beneficial effects of DHA/ARA supplementation on growth, neurologic and visual development when compared to the standard infant formula. Conclusion: Available data are inadequate at the present time to conclude that DHA/ARA supplementation, for either term or preterm infants, has a clinically meaningful beneficial effect upon neurological development, growth or visual development. There is an inadequate evidence base to recommend supplementation with these fatty acids.

76. Shiga Toxin Production by Different Escherichia Coli Serotypes Grown under High Osmolarity Conditions

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Plain Language Synopsis: Shiga toxin-producing E. coli (STEC) include serotypes with different levels of pathogenicity to humans. Salt is widely used as food preservative in meat processing and other foods. The purpose of this study is to compare the effect of high osmolarity growth conditions on the survival and toxin production by STEC.

Abstract: Shiga toxin-producing E. coli (STEC) include serotypes with different levels of pathogenicity to humans. The toxins produced by STEC (Shiga toxin 1 and 2), and their subtypes,

are one of their major pathogenic attributes. Ultraviolet light, certain antibiotics, and low iron levels have been shown to affect toxin production, but little is known about how other environmental factors affect growth and toxin production among STEC. The purpose of this study is to compare the effect of high osmolarity growth conditions on the survival and toxin production by STEC. We used the Phenotypic Array (PM) developed by Biolog and focused on the PM-9 plate to determine the effect of 96 different osmolyte/ionic conditions, on the growth and toxin production among STEC. PM-9 plates were inoculated following manufactures instructions, and data was analyzed using Biolog software to determine growth kinetics. The presence of Shiga toxin from PM-9 filtrates was determined by ELISA. Our preliminary results show a significant variability on the survival of different STEC to salt (NaCl). The 2011 German outbreak strain E. coli O104:H4 is highly osmotolerant and survives well even in the present of 6% NaCl (W/V). However, E. coli O157:H7 strain EDL933 cannot grow optimally above 3%, and EC4045 (O157:H7) from the spinach outbreak, grows well up to 4%. We also observed differences on the Shiga toxin produced under these stress conditions among the different serotypes. Shiga toxin was detected from E. coli O104:H4 cultures grown on media supplemented with 6% NaCl. Salt is widely used as food preservative in meat processing and other foods, alone or in combination with an organic acid. Although the effect of NaCl in the survival and toxin production of E. coli O157:H7 is known, little work has been done on other STEC serotypes that can also present an important threat to public health.

77. Evaluating Innovative Instrumentation and Chemometrics for Rapid, Non-targeted Screening of Adulterants in Milk Powder

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Plain Language Synopsis: FDA needs non-targeted screening methods for determining if foods contain contaminants, such as melamine illegally added to milk powder. We are developing spectroscopic and chemometric techniques to be employed by field laboratories and investigators. These portable instruments and data analyses should provide rapid and reliable detection of chemical adulterants.

Abstract: FDA has placed a priority on developing screening methods that employ portable, rapid, high-throughput instrumentation. This goal is evident in the call from FDA's 2011 Strategic Plan for Advancing Regulatory Science in further developing a prevention-focused food safety system. Additionally, the FDA OFVM Strategic Plan 2012-2016 Program Goal 6.2 focuses on investigation and adaptation of innovative technologies. By evaluating and advancing research in portable devices that could be used in the field, FDA will be better able to preventatively protect our Nation's food supply. As such, our research is focused on developing non-targeted screening methods based on Raman and near-infrared (NIR) spectroscopies. One commodity that is vulnerable to attack and that is consumed by some of the most vulnerable populations (e.g., infants and persons on restricted diets) is milk powder (MP). The recent economic adulteration of MP with melamine in 2008 highlights the needs for innovative methods to prevent contamination. As a first step, a large (70+) collection of MPs are being used to establish libraries of authentic spectra that include the natural variations in this product (e.g., origin, processing temperature, fat and protein content, etc.). Initial research has shown that advanced data processing methods (i.e., chemometrics) are necessary to fully fingerprint a sample/contaminant and create successful models for verifying authenticity. To evaluate the universal models and determine the ability to screen out potential contamination, melamine was chosen as the initial contaminant of interest. The chemometric models have shown success at identifying melamine-contaminated MP as not being authentic MP samples, thus allowing for a potential rapid, first-line of defense that could be used to screen a large number of MP samples. Work is underway to assess the false-positive/-negative rates of identification as well as the benefits/drawbacks to each method. Future studies to expand the testing to other potential adulterants are underway. As the methods and instrumentation are further developed, we envision a system where these analytical tools can be employed by non-experts to proactively protect the food supply and consumers.

Session 5: Support New Approaches to Improve Product Manufacturing and Quality

(Posters 1-52 are located in Section A)

1. Human Nasal Cavity Model for In vitro Characterization of Nasal Spray Products

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Plain Language Synopsis: An in vitro human nasal cavity model is developed and tested as a more realistic experimental tool to study nasopharyngeal deposition of nasal spray particles. Following further validation, this model – in combination with other characterization tests – holds the promise of a clinically relevant pharmaceutical development tool for nasal spray products.

Abstract: Several locally acting nasal spray products are available to treat allergic rhinitis and/or nasal congestion for which deposition pattern within the nasal cavity can markedly influence clinical performance. In this regard, the guidance for industry titled Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action (2003) recommends characterization of droplet size distribution since it affects deposition pattern in the nose. This study aims to develop an alternative more realistic characterization tool using human nasal cavity model to investigate deposition pattern of nasal spray products within nasopharyngeal region. The model uses human geometries of the nasal cavity and mouth-throat regions with dimensions that are consistent with adult population means. The hollow model with lower, middle and upper nasal passages was developed using plastic resin. Spray deposition pattern was investigated with varying insertion angle of 30, 40 or 50 degrees from the horizontal, keeping the nasal spray product (Nasonex®) vertical. Two spraying conditions were applied with varying actuation force, hold time and force fall time. Nasal deposition of sprayed particles was studied in the absence and presence (slow and fast) of inhalation flow. Results demonstrate that around 75% drug was deposited in the anterior chamber while 16% and 10% were deposited in the middle passage and nostril, respectively. An increase in deposition on the nasal spray was observed with corresponding decrease in deposition in the anterior and middle passage as the angle was increased from 30 to 50 degrees. Incorporation of inhalation profile resulted in a significant increase in the deposition at the middle passage compared to that without inhalation profile. Moreover, particle deposition profile in the nasal cavity model closely resembles that obtained with a computational fluid dynamics (CFD) model. Following further validation, this model - in combination with other characterization tests - holds the promise of a clinically relevant pharmaceutical development tool for nasal spray products.

2. Process Parameter Screening Utilizing a Plackett-Burman Design for a Model Monoclonal Antibody and Exploring the Linkage Between Cell Culture and Downstream Processing

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Plain Language Synopsis: Protein drugs such as enzyme therapies and monoclonal antibodies are largely produced via cell culture in bioreactors and require multiple manufacturing steps to create a safe and effective drug product. This laboratory based case study used novel statistical, analytical, and processing techniques to advance protein drug manufacturing understanding and quality.

Abstract: Consistent high quality yield of antibody biopharmaceuticals is the goal for bioprocess cell culture. This endpoint, a key aim of the Quality by Design initiative, typically is achieved in commercial settings through product and process engineering of bioreactor parameters during development. However, in a poorly understood process, the yield may not be the optimal goal; small changes in composition and process may yield shifts in quality attributes and a less desirable final product a potential barrier for follow-on biosimilar development. Recently,

Design of Experiments (DoE) based approaches have been explored to rapidly and efficiently achieve this goal of optimized yield with a good understanding of product and process variables that affect a product and its critical quality attributes (CQAs) to potentially allow better design of biosimilars. In addition, linkage of upstream cell culture with downstream processing is crucial for economically producing high-quality biopharmaceutical proteins. A balance must be met between product yield in the upstream phase and its quality and consistency as an input material for capture and downstream chromatography. Here, we present a lab-scale model culture where we apply a Plackett-Burman screening design to parallel cultures to study the main effects of eleven process variables, such as agitation, sparge rate, feeding regimens, temperature and pH shifts. Emerging analytical technologies, such as Mass Spectrometry, Advanced protein A biosensors, circular dichroism, and Ultra High Performance Liquid Chromatography were incorporated into the study to characterize both in process and purified materials to understand the impacts bioreactor parameters have on product quality, not just yield. We found engineering changes relating to culture temperature and non-essential amino acid supplementation significantly impacted glycan profiles associated with key glycan attributes that can impact in vivo product function or pharmacokinetics like fucosylation, ß-galactosylation, and sialylation. This study also found linkage between cell culture process parameters and downstream capture chromatography efficiency, bulk drug substance stability, and selected CQAs such as aggregate formation.

3. Development of a Protein Marker Panel for Characterization of Human Induced Pluripotent Stem Cells (hiPSCs) Using Global Quantitative Proteome Analysis

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Plain Language Synopsis: Differentiated induced pluripotent stem cells (iPSC) have a potential to become a significant source of autologous cells for therapeutic treatments. Currently, there is no QC method for iPSC. Using mass spectrometry and immunochemistry we selected a panel of protein markers that could facilitate the QC assays and clinical application of hiPSC.

Abstract: The emergence of new methods for reprogramming of adult somatic cells into induced pluripotent stem cells (iPSC) led to the development of new approaches in drug discovery and regenerative medicine. Investigation of the molecular mechanisms underlying the self-renewal, expansion and differentiation of human iPSC (hiPSC) should lead to improvements in the manufacture of safe and reliable cell therapy products. The goal of our study was qualitative and quantitative proteomic characterization of hiPSC by means of electrospray ionization (ESI)-MSe and MALDI-TOF/TOF mass spectrometry (MS). Proteomes of hiPSCs of different somatic origin: fibroblasts and peripheral blood CD34+ cells, reprogrammed by the same technique, were compared with the original somatic cells and hESC. Quantitative proteomic comparison revealed approximately 220 proteins commonly up-regulated in all three pluripotent stem cell lines compared to the primary cells. Expression of 21 proteins previously reported as pluripotency markers was up-regulated in both hiPSCs (8 were confirmed by Western blot). A number of novel candidate marker proteins with the highest fold-change difference between hiPSCs/hESC and somatic cells discovered by MS were confirmed by Western blot. A panel of 22 candidate marker proteins of hiPSC was developed and expression of these proteins was confirmed in 8 additional hiPSC lines.

4. Measurement Techniques for Conductive Polymer Membrane Based Biosensors

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Plain Language Synopsis: Conductive polymer membranes are an attractive technology to be incorporated into a biosensor for real time environmental monitoring of foodborne pathogens. Several electrical measurement techniques have been evaluated to transduce the signal from the membrane to a field deployable device.

Abstract: The conformal thin-film deposition of conducting polymer (CP) via vapor deposition onto a fiber mat is an attractive platform to base an electrochemical biosensor (Bhattacharyya, 2011). The monomer 3-thiopheneethanol (3-TE), with an accessible hydroxyl group, has been successfully co-polymerized with 3,4-ethylenedioxythiophene (EDOT) onto melt-spun fibers utilizing oxidative vapor deposition. The functional groups of the co-polymers are used to covalently attach antibodies to the surface of the platform for binding to targets such as E. coli O157:H7. We compare and contrast electrochemical detection methods utilizing resistance and potentiostat for environmental monitoring. An open-source potentiostat (Rowe, 2011) was constructed for voltammetric techniques to assess the functionalized conducting polymer as the working electrode of a standard three electrode system. Though the electrochemistry of antibody functionalized conducting polymers binding to target antigen does not involve a redox reaction typically encountered in voltammetry, interactions between antibody and antigen induce a capacitance change of the conducting polymer leading to a polarization of the membrane and a detectable signal (Sargent, 1999). We have also explored the traditional redox approach applied to our system. Potassium ferricyanide and ascorbic acid, both commonly used in oxidation-reduction experiments, have been tested in our sensor system. With an increase in the number of bacteria bound to the surface of the membrane, the electrons of the redox couple are inhibited from transfer to the membrane surface, thereby decreasing the peak observed in voltammetry. Resistance based methods (McGraw, 2012) with two point probes have also been shown to be promising detection signals and were evaluated for sensitivity. In addition, a four point probe was evaluated to minimize the effect of contact resistance. However, the four point probe applied to a porous membrane leads to poor measurement reproducibility and is used only as a screening tool for choosing a membrane to incorporate into a biosensor. Both resistive and voltammetric techniques offer sensitive and (near) real time monitoring for environmental assaying of O157:H7. A head to head comparison of both techniques and their ability to be incorporated into a field deployable device was investigated to determine a final configuration.

5. Predictive Gene Markers of Multipotent Stromal Cell Proliferation

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Plain Language Synopsis: This work correlates gene markers with several proliferation assays using multipotent stromal cells in an effort to identify quality markers.

Abstract: Multipotent stromal cells (MSCs) are known for their distinctive ability to differentiate into multiple cell lineages such as adipocytes, chondrocytes and osteocytes. They can be isolated from numerous tissue sources including bone marrow. Because of their differential potential and secretion of many growth factor(s), MSCs are thought to exhibit inherent qualities of regeneration and immune suppression. Based on these characteristics, MSCs are seen as advantageous for the field of regenerative medicine in treating a variety of injuries and disorders, in addition to graft versus host disease. Since the percentage of MSCs derived from bone marrow is low, MSCs must be cultured for several cell passages to obtain sufficient cell numbers for a desired cell-based therapy. However, after several rounds of passaging, we have shown previously that the quality of these cells declines as demonstrated by decreases in cell proliferation, increases in cell size, reduced multipotent differentiation potential and differences in gene expression. In this study, we wish to identify molecular markers of proliferation of MSCs that can predict the cell population quality. Human MSCs derived from the bone marrow of 8 different donors were grown under identical conditions and total RNA was harvested at cell passages 3, 5, and 7. The proliferative potential was measured for each donor/passage using two different assays, percent confluency at 96 hours and percentage of EdU positive cells after 6 hours in culture. Total RNA was hybridized on a two color microarray for each donor/passage and gene expression data was correlated with both cell proliferation assays. Using a regression model and a multiplicity adjustment, 28 genes were identified as statistically significant and highly correlated ($0.72 \leq r \leq -0.73$) with cell

proliferation. When the significant gene lists were analyzed by Ingenuity Pathway Analysis software, these genes were involved in the top scoring networks ($p<0.05$) of cellular growth and proliferation, cellular development, and cell cycle. Thus, we have identified novel gene markers that are indicative of MSC proliferative quality and may be used to rapidly assess a population of MSCs.

6. A Novel Approach to the Method Validation of a Rapid SE-HPLC Method for the Molecular Size Distribution Analysis of Immunoglobulins in IGIV Products

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Plain Language Synopsis: We report a novel approach for the validation of an internally developed size exclusion HPLC method, which provides relative quantitation of actives (monomer and dimers) and product-related impurities (aggregates and fragments) simultaneously in IGIV products. The experimental design and results are discussed in light of the ICH Q2(R1) guidance.

Abstract: Size exclusion chromatography (SEC) is widely used for the molecular size distribution (MSD) analysis of therapeutic proteins, including immune globulins, monoclonal antibodies, and coagulation factors to measure relative percentages of different molecular forms, such as aggregates, oligomers, dimer(s), monomer, and fragments. The monomer and sometimes also the dimer(s) (e.g., IgG) constitute active forms of proteins because they contribute to the therapeutic benefits, while aggregates, oligomers and fragments constitute impurities. The aggregates and oligomers are associated with mild to severe pathological conditions, including immunogenic reactions, hypotension, and anaphylaxis. The fragments often have modified or no biological activities. Our SE-HPLC method provides relative quantitation of the active and product-related impurities simultaneously. Thus, validation of the method requires two separate approaches, as a quantitative test for the active(s) (assay) and a quantitative test for impurities. During our review of BLA submissions, we often find that the validation reports are not satisfactory probably because the available guidances on HPLC method validation have been written primarily for the determination of concentrations of analytes. We present a new and unique approach for the method validation of an internally developed SE-HPLC method for MSD analysis of IgG in products as an example, including experimental design and results to demonstrate specificity, linearity, accuracy, precision, range and limit of quantitation (LOQ). The conventional approaches to method validation, described in ICH Q2(R1), can be applied to this method only to a limited extent for three reasons. The reportable results from this method are not analyte concentrations but peak area percentages of different molecular forms. Secondly, dilution of the sample does not change the peak percentages. Thirdly, the method does not use a standard and no authentic standard is available. The approach is particularly novel for the assessment of specificity for all molecular forms, and linearity and LOQ of aggregates, oligomers and fragments. The results are discussed in light of the ICH Q2(R1) guidance.

7. A Rapid SE-HPLC Method for the Molecular Size Distribution Analyses of Immunoglobulins and Coagulation Factors: Effect of Perchlorate, a Chaotropic Agent

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Plain Language Synopsis: We report development of a rapid size exclusion chromatography method for molecular size distribution analyses of immunoglobulin G and coagulation factors (therapeutic proteins), which quantitates actives and critical impurities in products in one chromatography. The method reduces analysis time considerably and show significantly improved data quality compared to reported methods.

Abstract: Aggregation of proteins is a major problem in their use as drugs because aggregated proteins can produce a variety of mild to severe pathological conditions, including immunogenic reactions, hypotension, and anaphylaxis. Thus, control of aggregates and fragments are critical

for safety and quality of therapeutic proteins. However, methods reported in the literature, including those in European Pharmacopeia (EP), typically require long run time and show significant interaction between the analyte and the stationary phase. Such interactions could lead to erroneous results because the aggregates are preferentially retained by the column due to stronger interaction with the stationary phase. We report the development of an SE-HPLC method for MSD analyses of IgG and coagulation factors (FVIII, FIX and FXIII), which requires significantly less time (<15 min), by including 0.4 M sodium perchlorate, a chaotropic agent, in the eluent (pH 6.8). The results show that sodium perchlorate did not affect size and relative distribution of the molecular forms of the proteins but did eliminate or significantly reduce interactions between the protein and the stationary phase. Results obtained with IgG products show essentially a symmetric peak with no tailing (Tf ~1.1) for the aggregate peak, while the EP method shows significant tailing. The chromatograms also show peaks for an oligomer and two dimers, in addition to the monomer and fragment peaks. Molecular weight determinations by on-line MALS detection suggest that the oligomer peaks consists of 4-10 IgG molecules, and one of the dimers consists of two intact IgG molecules, while the other consists of an intact monomer and fragments. For a rFVIII product, the reported SE-HPLC method required ~140 injections to pre-condition the column before actual run, showed significant run-to-run carry-over, and the absorbance did not return to the base-line even after 80 minutes elution. However, our method required only 12 minutes to complete, required only 3-4 pre-conditioning injections, and showed no carry-over and symmetrical peak (Tf < 1.5) for the monomer. The chromatograms of rFIX and rFXIII are similar, except that our method requires about half the run times and no column pre-conditioning compared to the respective reported methods.

8. Development and Partial Validation of a Thrombin Generation Assay Method

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Plain Language Synopsis: Factor XIa has been identified as the causative factor for thrombotic events associated with administration of immune globulin intravenous (IGIV). We report development of a robust and reliable method based on the thrombin generation assay, which measures FXIa activity in IGIV products using International Reference Material as the standard.

Abstract: In 2010, an increased number of thrombotic events was associated with the administration of immune globulin intravenous (IGIV) products. Investigations identified plasma-derived coagulation factor XIa (FXIa) as the causative factor. It was recognized that there is an urgent need to develop and standardize an assay method for the detection of procoagulant activity in IGIV products. Availability of International Reference Material (IRM) from NIBSC has made it feasible to directly compare different methods. We report development and partial validation of a robust method for the quantitation of FXIa in IGIV products using the IRM as the standard. The method is based on the thrombin generation assay which measures FXIa activity from thrombin peak height, Lag Time for onset of the reaction and Time to Peak (TTP) by monitoring the fluorescence generated by the cleavage of a fluorogenic substrate upon activation of the coagulation cascade by a tissue factor in the presence of negatively charged phospholipids in factor XI deficient plasma. The results of FXIa activity show low variability (RSD < 15%) in the assay range, 0.3 - 5.0 mU/mL. The LOQ of the assay, 0.3 mU/mL, is well below the approved specifications limits for IGIV products. Multiple lots of two IGIV products, Octagam and Bivigam, were assayed. In all cases, the results obtained by us and the respective manufacturer were below the approved specification limits. Furthermore, boarder-line OOS samples, generated by spiking IRM to samples of Octagam and Bivigam, show results that are above the specification limits. The results indicate reliability and suitability of the method. Data with additional IGIV products show comparable

results between this method and an orthogonal commercially available chromogenic FXIa activity assay method.

9. Optimizing Bioassays for Assessing the Quality of Anticancer Drug Products

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Plain Language Synopsis: This presentation describes the development of an innovative platform that can quantify the bioactivity of anticancer drugs in killing cancer cells.

Abstract: Bioassays are essential for assessing the quality of biologics and other complex drug products. When adequately designed, bioassay(s) help to ensure product quality and manufacturing consistency. To achieve this goal, bioassay(s) should be designed to reflect as much as possible the mechanism of action of a drug product. For anticancer drugs, a bioassay should be the measure of a drug's ability in killing cancer cells. Funded by Critical Path initiative fund, we have created a cell-based biosensor by stably transfecting a fluorescence resonance energy transfer (FRET) probe into a human cancer cell line. The cellular biosensor has been proven to be suitable for quantifying the activity of several cancer drugs in inducing apoptotic cell death. Upon qualification or validation, this assay can be adapted as a characterization or release test for relevant drug products. Our approach is advantageous over most traditional anticancer bioassays in the ability to distinguish between cell growth arrest and true apoptotic cell death. This work will help FDA reviewers in making more informed regulatory decisions regarding product quality, manufacturing consistency, and interpretation of clinical efficacy and adverse events associated with specific products.

10. Regulatory Considerations for Controlling Intermediates in Type-II Drug Master Files for the Manufacture of Generic Drug Substances

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Plain Language Synopsis: The active ingredients of drugs are manufactured in multiple steps. Controlling the quality attributes of each step of the process increases the quality of the final active ingredient. The principles used to control the quality at the intermediate stages will be summarized and commonly used strategies will be highlighted.

Abstract: The manufacture of active pharmaceutical ingredients (APIs), also referred to as drug substances, often proceeds through several chemical intermediates. These chemical intermediates can be isolated or non-isolated, depending on numerous factors including their reactivity and stability, and the efficiency of the synthetic processes used to generate them. Guidelines available from regulatory agencies and within pharmaceutical compendias focus on the characterization and quality control of the final drug substances and drug products. However, the adequate control of intermediates contributes substantially to the quality of drug substance and drug product manufacturing. The principles used to control final drug substances and products are generally applicable to manufacturing process intermediates as well. This poster illustrates these principles and outlines a parallel strategy to characterize chemical intermediates and control the impurities present in intermediate stages of the manufacturing process of the APIs. A broad survey of hundreds of generic drug substance Master Files, the regulatory pathway usually employed to submit such information to FDA, has been undertaken. We will present our findings identifying the most common problems associated with the characterization of and impurity control in intermediates in generic drug substance submissions.

11. Systematic Evaluation of Formulation Factors on Aerosolization Performance of Metered Dose Inhalers

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Inc., Raleigh, NC; Hickey, Anthony, Cirrus Pharmaceuticals, Inc., Durham, NC; Lee, Sau, FDA/CDER/OPQ; Saluja, Bhawana, FDA/CDER/OGD/ORS

Plain Language Synopsis: The understanding about the effects of drug-to-excipient interactions on M.D.I product performance is challenging. Using a systematic approach, we found that extreme excipients amounts and drug particle size distributions greatly affect the M.D.I aerosol characteristics. This work allows evaluate the extent to which the formulation factors govern M.D.I product performance.

Abstract: Introduction: Metered dose inhaler (M.D.I) product performance depends on a multitude of factors including, but not limited to, the physicochemical properties of drug(s), device geometries, nature and amount of inactive ingredients.1 Although much is known about effect of device geometry on M.D.I aerosolization performance,2 there is a lack of systematic understanding of effects of drug-to-excipient interactions on the M.D.I product performance. Therefore, the purpose of this work is to investigate the influence of concentration of excipients and drug particle size distribution (PSD) on aerosolization performance of M.D.Is. Methods: A marketed M.D.I product was chosen as initial model system - a suspension formulation consisting of albuterol sulfate (drug), ethanol (co-solvent), oleic acid (surfactant), and HFA-134a (propellant). The product was reverse engineered, and its aerosolization performance characterized by determination of aerodynamic particle size distribution (APSD) and delivered dose (DD) using an Alberta Idealized throat (AIT). A statistical design of experiments (DOE) approach was used to vary excipient levels outside the Q2 limit,3 and drug PSD (D50). M.D.I batches were manufactured and their aerosolization performance characterized (3 canisters per batch). Results: No statistically significant effect of any formulation factor on the mean DD was observed. However, the ethanol factor had statistically significant effect on DD at beginning lifestage and DD trend (doses from beginning to end lifestages determinations). The D50 and ethanol formulation factors showed a number of statistically significant effects on APSD. As the amount of ethanol and D50 increased, the fine particle dose less than 5mcm decreased by 35-55%. The oleic acid formulation factor had no statistically significant effect on APSD. The results were consistent between beginning and end lifestages determinations. Conclusion: Extreme ranges of excipients concentrations outside the Q2 limit, along with drug PSD, have significant effect on aerosolization performance of suspension-based M.D.I products. The outcomes of this study allowed defining design spaces for DD and APSD parameters according to the levels of formulation factors. This systematic approach is being expanded to other types of formulations and will help evaluate the extent to which the formulation factors govern the performance of M.D.I products.

1 Smyth, H. D. C. Advanced Drug Delivery Reviews, 2003. 55(7): p. 807-828.

2 Stein, S.W., et al. AAPS PharmSciTech, 2013. 15(2): p. 326-338.

3 Q2 (quantitative sameness) means that concentrations/amounts of the inactive ingredient(s) used in the test product are within ± 5% of those used in the reference product. FDA also recommends a formulation to be Q1 (qualitative sameness), meaning that the test product uses the same inactive ingredient(s) as the reference product.

12. Comparison of Visual Assessment Techniques for Taper Wear/Corrosion in Modular Hip Replacement Systems

Di Prima, Matthew, FDA/CDRH/OSEL/DAM; Vesnovsky, Oleg, FDA/CDRH/OSEL/DAM; Topoleski, Tim, University of Maryland Baltimore County, FDA/CDRH/OSEL/DAM

Plain Language Synopsis: Plain language Synopsis: Analysis of wear and corrosion on medical implants that have been removed from a patient is often complicated by complex geometry of the implant. We have developed an imaging approach for modular hip components that allows for direct wear/corrosion measurements and trends the same way as current qualitative techniques.

Abstract: Wear and corrosion at a modular connection (e.g. taper and trunnion) has been associated with revisions of total hips. However, the factors leading to taper wear/corrosion, as well as the clinical effects of wear/corrosion, are not well understood. Analyses of both explanted and in vitro tested hip replacement systems typically include a visual assessment of the amount of wear

and corrosion based on a subjective grading scale. We have developed an approach that images the entire taper/trunnion surface which allows for a more objective quantification of wear and corrosion of the contacting surfaces which we call the digital mosaic method (DMM) to compare the results of this new methodology to the Goldberg method (the current visual inspection standard) and Anderson method (an expanded version of the Goldberg method), we examined the modular surfaces of 10 retrieved Metal-on-Metal hip replacement systems. Each set of taper and trunnion surfaces were imaged and stitched into a composite image of the entire taper/trunnion surface. Three independent observers measured the surface area fraction of damage (wear and/or corrosion) for each surface, which was in turn compared with the visual scoring methods for the same surfaces to determine a measure of the intra-observer repeatability of the new method, a single reviewer measured the surface fraction of damage for the same set of explanted surfaces across different days, blinded to any previous specimen identification, with the image order randomized. The results of this study show that the DMM damage quantification gives similar scores to the current visual scoring approaches (Goldberg and Anderson). There are additional advantages to using this approach, including better damage quantification, allowing for spatial analysis, identifying matching damage locations on the two surfaces, and higher resolution of the contact area.

13. Effect of Rotary Bend Fatigue on the Corrosion Resistance of Common Medical Alloys

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Plain Language Synopsis: Cardiovascular devices experience fatigue when implanted in the body, which was thought to reduce the device's corrosion resistance. We investigated the effect of fatigue on the corrosion resistance of 5 different types of alloys used in cardiovascular devices and found that fatigue does not adversely affect corrosion performance.

Abstract: The effects of fatigue have been thought to be detrimental to corrosion resistance of cardiovascular devices by damaging the surface oxide and through crack initiation. For this study 316LVM stainless steel, MP35N cobalt-chromium alloy, and nitinol wires were obtained. Given the sensitivity of nitinol behavior to surface finish, electro-polished, mechanically polished, and black oxide wires were obtained resulting in a total of five different wire materials/finishes. Before corrosion testing, wires were split into subgroups and subjected to either (1) high strain fatigue for several minutes; (2) phosphate buffered saline (PBS) soak for several minutes; (3) low strain fatigue for eight days; (4) PBS soak for eight days; or (5) nothing, i.e. as received. All wire fatigue testing was conducted with guided bend rotary bend fatigue testers in PBS at 37°C and at a test speed of 60 Hz; none of the fatigue samples fractured before corrosion testing. Potentiodynamic polarization testing was performed to ASTM F2129 for all wires and a post-test visual inspection was performed on all samples. Overall, any difference in the corrosion behavior could be accounted for by PBS soak time with minimal effect from fatigue. With the exception of the black oxide nitinol, the breakdown potential was not affected by any of the fatigue or soak conditions. While all materials had differing rest potential in the as received state, increasing PBS soak time showed a convergence of the rest potential to 0 mV for all materials. This led all materials except for the black oxide nitinol to have a normalized breakdown potential (difference in breakdown and rest potential) to converge to the vertex potential of the test, 1000 mV. Based on the materials and conditions tested, it appears that conducting fatigue testing on samples before corrosion testing does not greatly affect corrosion behavior.

14. Unexpected Peaks in Tandem Mass Spectra of Drugs and Drug Metabolites Due to Reaction of Product Ions with Residual Water in Mass Spectrometer Collision Cells

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Plain Language Synopsis: New discovery and analysis of some drugs and drug metabolites reaction with residual water in the collision cell of electrospray ionization tandem mass spectrometry. This reaction often leads to the formation of ions that cannot be formed directly

from the precursor ions, and this complicates the mass spectra and may distort MRM (multiple reaction monitoring) results.

Abstract: Rationale: Certain product ions in electrospray ionization tandem mass spectrometry are found to react with residual water in the collision cell. This reaction often leads to the formation of ions that cannot be formed directly from the precursor ions, and this complicates the mass spectra and may distort MRM (multiple reaction monitoring) results. Methods: Various drugs, pesticides, metabolites, and other compounds were dissolved in acetonitrile/water/formic acid and studied by electrospray ionization mass spectrometry to record their MS2 and MSn spectra in several mass spectrometers (QqQ, QTOF, IT, and Orbitrap HCD). Certain product ions were found to react with residual water in collision cells. The reaction was confirmed by MSn studies and the rate of reaction was determined in the IT instrument using zero collision energy and variable activation times. Results: Examples of product ions reacting with water include phenyl and certain substituted phenyl cations, benzoyl-type cations formed from protonated folic acid and similar compounds by loss of the glutamate moiety, product ions formed from protonated cyclic siloxanes by loss of methane, product ions formed from organic phosphates, and certain negative ions. The reactions of product ions with residual water varied greatly in their rate constant and in the extent of reaction (due to isomerization). Conclusions: Various types of product ions react with residual water in mass spectrometer collision cells. As a result, tandem mass spectra may contain unexplained peaks and MRM results may be distorted by the occurrence of such reactions. These often unavoidable reactions must be taken into account when annotating peaks in tandem mass spectra and when interpreting MRM results. Published in 2014. This article is a U.S. Government work and is in the public domain in the USA.

15. 3D-Printed Realistic Optical Phantoms for Hyperspectral Reflectance Imaging Assessment

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Plain Language Synopsis: Three-dimensional (3D) printing is a rapidly emerging technique to fabricate biologically relevant objects. We designed and fabricated biomimetic vasculature phantoms using a photopolymerization-based 3D printer. Vascular channels were filled with hemoglobin solutions at controlled oxygenation levels. Phantoms were imaged with a hyperspectral reflectance imaging system to demonstrate their potential use in evaluating device performance.

Abstract: Three-dimensional (3D) printing is a rapidly emerging technique to fabricate biologically relevant objects. 3D-printed objects can be used for different medical purposes such as training, surgery planning, patient-specific implants, and fabrication of realistic phantoms for device testing. Previously, we printed cylindrical channel phantoms with different diameters and depths to explore the geometrical quality of 3D printers based on fused deposition molding and photopolymerization. Furthermore, optical properties of different 3D-printing materials were measured and compared to biological values. These results indicate that 3D printed phantoms for optical imaging have the potential to enable more realistic assessment of device performance under realistic conditions. Advancing the use of 3D-printed phantoms for studies of light-tissue interactions and regulatory evaluation of biophotonics devices is the main purpose of this research. Specifically, this work focuses on hyperspectral reflectance imaging technology which has been cleared for tissue oximetry measurements. To develop a phantom that incorporated realistic, image-defined vascular morphology, we used a human retina image captured by a commercial fundus camera. The image was converted to a segmented vessel network using Photoshop software. Several segmented maps were stacked and modified slightly to establish channels with circular cross sections. Phantoms with one or two layers of vascular arrays were fabricated using a photopolymerization-based 3D printer. Optical properties of the phantoms were verified by integrating sphere spectrophotometry with inverse

adding doubling software. Phantom morphology was verified with μ-CT imaging. Channels were filled with hemoglobin solutions at controlled oxygenation levels, and the phantoms were imaged by a near-infrared HRI system. Volume fractions of chromophores such as oxyhemoglobin (HbO2)bHH, deoxyhemoglobin (Hb), and water were estimated by applying a non-negative least squares minimization technique on raw HRI spectral data. Oxygen saturation (SO2) was then calculated using the solved HbO2 and Hb volume fractions. We found that the intensity of chromophore volume fraction maps is affected by vessel density. Also, SO2 level (especially for avascular regions of phantom) is highly affected by neighboring vascular channels. Furthermore, experiments on two-layer phantom showed that calculated SO2 value was roughly twice as sensitive to the top superficial vascular layer as the bottom layer. In general, results of this study showed 3D-printed phantoms have the potential to improve the evaluation of device performance; however, more realistic phantoms could provide even better clinically-relevant understandings from device performance.

16. Cell Based Method to Detect Innate Immune Response Modulating Impurities in Therapeutic Proteins

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Plain Language Synopsis: Biologics may contain process-related immune-activating impurities that modify the risk of the product inducing an immune response in the patients. Currently routine impurity monitoring is hindered by lack of sensitive methods. We developed a sensitive assay that detects trace levels of multiple impurities. This may contribute to assessing risk following manufacturing changes.

Abstract: Therapeutic proteins can contain multiple impurities, some of which are variants of the product, while others are derived from the cell substrate and the manufacturing process. Such impurities, even when present at trace levels, have the potential to activate innate immune cells in peripheral blood or embedded in tissues causing expression of cytokines and chemokines, increasing antigen uptake, processing and presentation by antigen presenting cells and fostering product immunogenicity. Currently, products are tested for host cell protein content, assays to control innate immune response modulating impurities (IIRMIs) in products are focused mainly on endotoxin and nucleic acids, however, depending on the cell substrate and the manufacturing process, numerous other IIRMI could be present. In these studies, we tested two approaches that allow for the detection of a broader subset of IIRMIs. In the first, we used commercial cell lines transfected with Toll like receptors (TLR) to detect receptor-specific agonists. This method is sensitive to trace levels of IIRMI and provides information of the type of IIRMIs present but is limited by the availability of transfected cell lines and requires pre-existing knowledge of the IIRMIs likely to be present in the product. Alternatively, the use of a combination of macrophage cell lines of human and mouse origin allowed for the detection of a broader spectrum of impurities, but did not identify the source of the activation. Importantly, for either system the lower limit of detection (LLOD) of impurities was similar to that of PBMC and it was not modified by the therapeutic protein tested, even in settings where the product had inherent immune modulatory properties. Together these data indicate that a cell-based assay approach could be used to screen products for the presence of IIRMIs and inform immunogenicity risk assessments, particularly in the context of comparability exercises.

17. Identifying Species-Specific DNA Signatures for Rapid Detection of Six Bacterial Pathogens Using PCR Assays

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Plain Language Synopsis: Establishing a computational workflow to effectively identify species-specific DNA sequences from bacterial genomes for rapid development of real-time PCR assays that detect the target bacteria with high specificity and sensitivity.

Abstract: About 2 million tissues are recovered from cadavers and processed by the industries

to be used as grafts in surgeries every year in the US. To minimize risks of transmitting communicable diseases in tissue transplantation, the establishments need to examine the tissues retrieved for presence of harmful microbes using standard microbial culture system. According to the Standard of American Association and Tissue Banking, presence of certain "high-risk" bacteria necessitates discard for the tissues. Specifically, Streptococcus pyogenes, Clostridium sp. need to be excluded in cardiac and vascular tissues. In addition to all the bacteria listed above, Staphylococcus aureus, Enterococcus sp. and Gram negative bacillus also need to be excluded in skin grafts. In comparison with detection of microbes using the standard laboratory culture system, the nucleic acid-based methods have many clear advantages. However, the crucial challenge of developing such highly sensitive and specific molecular assays for rapid detection of the target pathogenic microbes very much depends on the effective identification of truly "species-specific" DNA signatures that can be used as primers for PCR. Using Streptococcus pyogenes as a model, we previously established a stepwise computational workflow to analyzewhole genome sequences to effectively identify bacteria species-specific DNA signatures. In this study, we have extended the computational workflow for rapid development of PCR assays targeting 6 different "high-risk" bacteria, Enterococcus faecalis, E. faecium, Clostridium perfringens, C. difficile, C. tetani and Staphylococcus aureus. We examined a total of 43 DNA signatures selected from the workflow for PCR primers design targeting these 6 bacteria and experimentally tested them in conventional and/or real-time PCR assays. Finally, we selected 21 primer sets that had a limit of detection of 5-50 fg of target bacteria DNA (approximately 1- 10 genome copies) with the highest specificity in real-time PCR when tested against a panel of 5 ng DNA of 42 non-target species examined as final candidates for assembly into a PCR array in the future. The current results further support that the workflow could be applicable for rapidly identifying species-specific DNA signatures in designing PCR-based assays for the rapid detection of target pathogenic bacteria with various safety concern.

18. Generation of Interleukin-13 Receptor alpha2 Antigen Expressing Modified Vaccinia Ankara Recombinant Virus For Potential Cancer Immunotherapy

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Plain Language Synopsis: Modified Vaccinia Ankara (MVA) virus is derived from vaccinia virus (VV) strain. VV has a proven safety profile for human use, as it has been administered to >120K people as a smallpox vaccine. MVA is highly immunogenic with large packaging capacity for insertion of multiple foreign genes. Recombinant MVA expressing IL-13Ra2 antigen is expected to induce robust immune response in tumor-bearing animal models and could be a potential cancer vaccine for treatment of patients with cancer.

Abstract: Genetically modified recombinant poxviruses have shown promise in preclinical models of cancer immunotherapy due to their ability to induce effective cell-mediated immunity against target tumor-associated antigens (TAA). One such vector, recombinant Modified Vaccinia Ankara (MVA), is capable of expressing foreign genes in infected host cells. MVA is replication restricted in most mammalian cells exemplifying a unique safety profile. We have demonstrated that the interleukin-13 receptor a2 (IL-13Ra2) is selectively expressed in various solid tumors but not in normal tissues making it a promising TAA. Prophylactic and therapeutic vaccination with a plasmid vector expressing IL-13Ra2 caused only partial regression of established tumors, suggesting that host immune responses against IL-13Ra2 needed further enhancement. Thus, we constructed a recombinant MVA (rMVA-IL13Ra2) expressing both IL-13Ra2 and a green fluorescent protein (GFP) reporter gene. Purified virus titration by immunostaining using anti-vaccinia antibody and anti-IL-13Ra2 antibody confirmed the identity and purity of the recombinant MVA. Western Blot analysis showed the presence of IL-13Ra2 protein (65 kDa). Flow cytometric analysis of IL-13Ra2 negative T98G glioma cells infected with rMVA-IL13Ra2 virus (T98G-IL13Ra2) demonstrated surface expression of IL-13Ra2, indicating

the infectivity potential of the recombinant virus. Incubation of T98G-IL13Ra2 cells with varying concentrations (0-100 ng/ml) of IL13-PE (interleukin-13 fused to truncated Pseudomonas exotoxin resulted in depletion of GFP+ T98G-IL13Ra2 cells in a concentration-dependent manner. Higher concentrations of IL13-PE (10-1000 ng/ml) also inhibited the protein synthesis in T98G-IL13Ra2 compared to cells infected with control pLW44-MVA. We further observed that IL13-PE treatment of rMVA-IL13Ra2 infected chicken fibroblast, DF-1 cells led to a reduction in virus titer compared to untreated cells. These results indicate that rMVA-IL13Ra2 virus can successfully infect mammalian cells and express IL-13Ra2 in a biologically active form on the cell surface. The immunization studies of rMVA-IL13Ra2 are ongoing in a syngeneic mouse model of metastatic breast carcinoma. Based on in vitro results, we expect the rMVA-IL13Ra2 to be a useful agent in tumor immunotherapy as a vaccine alone and in combination with other therapeutic agents to eradicate metastatic tumors.

19. Characterization of Chondrocyte Adhesion on Protein-Coated 2D and 3D Substrates

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Plain Language Synopsis: Chondrocytes cultured in vitro undergo morphological and phenotype changes that can be seen by measuring their adhesive characteristics. This study evaluated how the inclusion of matrix proteins in both 2D monolayer and 3D culture systems affects the chondrocyte phenotype. A 3D culture system was shown to better retain chondrocyte phenotype.

Abstract: Articular cartilage defects can be repaired with chondrocytes that are isolated and cultured in vitro in 2D monolayer culture to achieve adequate cell numbers. However, chondrocytes propagated in monolayer undergo phenotypic changes, including changes in cell morphology and matrix production. Our goal is to explore how the presence of the extracellular matrix proteins, fibronectin and hyaluronic acid, in both 2D and 3D cultures, impact the maintenance of the chondrocyte phenotype and cell adhesion. We hypothesize that the inclusion of proteins in both 2D and 3D culture systems will better mimic the native chondrocyte environment and result in adhesive changes that contribute to phenotype maintenance. Articular chondrocytes were isolated from the tibiofemoral joints of young calves, and cultured for 8 days in 2D monolayer culture or encapsulated within a 3D scaffold. Chondrocytes were removed using EDTA and assessed at three timepoints, more specifically day 0, 4 and 7, using quantitative real-time PCR, immunohistochemistry, and a cell adhesion centrifugation assay. For the centrifugation assay cells were incubated for 20 minutes in multi-well plates that were pre-treated with concentrations of fibronectin and hyaluronic acid. Results indicate that 2D culture, regardless of protein inclusion, shows increased adhesion in comparison to all 3D groups, with increased adhesion levels up to 20% greater than their 3D counterparts across all timepoints. This increased adhesion was accompanied by biochemical changes indicating the loss of the chondrocyte phenotype. Current studies to investigate the role of the α5β1 integrin and CD44 receptors, the adhesion molecules for fibronectin and hyaluronic acid, respectively, in impacting adhesive events and phenotypic changes are ongoing. Through characterization of how environmental factors impact adhesive events and phenotype, material characteristics may be optimized to promote proliferation with phenotype maintenance. Using a combination of methodologies described in this abstract is expected to better characterize conditions on how to propagate chondrocytes which may be used to regenerate cartilage.

20. Importance of Additional Bioequivalence Studies in Evaluating the Impact of Product Quality on In vivo Performance of Generic Delayed-Release Drug Products

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Plain Language Synopsis: The additional BE studies data is an important tool to gain better understanding of the product quality on in vivo performance of the generic drug products. This data provide valuable information whether BE studies failed due to inadequate study design or poor drug product quality.

Abstract: Purpose: FDA's "bioequivalence (BE) data rule requiring submission of all BE studies" was published in the Federal Register in January 2009. With the publication of this rule, we evaluated the impact of data from additional BE studies in assessing BE and identified the reasons for failed BE studies for generic delayed-release (DR) drug products. Methods: The Agency database from January 2009 - March 2014 was searched to identify Abbreviated New Drug Applications (ANDAs) submitted for DR drug products containing additional BE studies. Per FDA's Guidance for Industry: Submission of Summary Bioequivalence Data for ANDAs (May 2011), "the percentage (%) differences for non-release and release controlling excipients between the to-be-marketed (TBM) formulation (R) and experimental formulation (T)" were calculated (R-T/R x 100) to determine whether or not the formulations used in the submitted studies were considered the same as the TBM formulation. Results: Seventeen ANDAs contained 39 additional BE studies (1-2 formulations/study), which were categorized as either pilot or pivotal studies. Twenty of 22 BE studies conducted on the TBM formulation did not meet the BE criteria either due to (i) insufficient power (17 studies), or (2) inadequate blood sampling schedule (3 studies). Seventeen BE studies were conducted on 15 formulations different from the TBM formulation. These experimental formulations primarily differed in the ratio of components of the enteric coating layer and/or amount (i.e., %w/w) of enteric coating layer with respect to their core or seal coated pellet/tablet. It should be noted that some of the experimental formulations identified as "same drug product formulation" by the ANDA applicants are actually "not considered to be the same" based on FDA's Guidance (May 2011) mentioned above. Conclusions: The data from these additional BE studies indicate that inadequate BE study design can lead to failure of the BE on the same formulation. Also, the additional BE studies on formulations different from the TBM formulation help us link the formulation design to the product performance in vivo. The additional BE studies data is an important tool to gain better understanding of the product quality on in vivo performance of the generic drug products.

21. Exploration of Model-Based Bioequivalence (BE) Evaluation as an Alternative Approach for the Sparse Pharmacokinetic (PK) Sampling Design

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Plain Language Synopsis: We assessed the model-based bioequivalence evaluation method as an alternative approach using sparse concentration data obtained from eyes after drug administration in rabbits. Partially consistent findings from different methods suggest that the model-based BE analysis needs further evaluation to be used as an alternative method.

Abstract: Purpose: to evaluate the model-based BE evaluation as an alternative method to the bootstrap-based evaluation method recommended for ophthalmic products with sparse PK sampling scheme. Background: Traditional BE analysis is not feasible for studies with a sparse PK sampling design because each subject contributes a limited number of samples to the analysis. The current draft guidance on ophthalmic products recommends bootstrapping concentrations to attain the mean concentrations at each time point t to derive the mean profile for each product. However, the bootstrap based BE evaluation method may be influenced by outliers and does not use all available information to accurately derive Cmax. Therefore, an alternative approach for analysis of studies utilizing the sparse sampling design was explored. Methods: Three formulations (A, C and D) of budesonide suspension were dosed topically to New Zealand white rabbits (n=73), in a 30 µL eye drop, placed in the cul-del-sac of both eyes. Each animal was euthanized at one of six times points and the aqueous humor was removed from each eye and stored frozen until analysis. Bioequivalence was evaluated using three approaches: Method 1 - the currently recommended BE evaluation approach (using arithmetic means), Method 2 - same as Method 1 but with geometric means and Method 3 - a model-based BE evaluation with simulated data from a population pharmacokinetic model. Results: Method 1 produced the following ratios

of AUC0-6hr: 0.981 [0.420 1.542] (C vs A, point estimate [90% confidence intervals]), 0.644 [0.255 1.034] (D vs. A), 0.663 [0.457 0.870] (D vs. C). The ratios of Cmax were 1.099 [0.569 1.629 (C vs. A), 1.484 [0.080 2.888] (D vs. A), 1.347 [0.304 2.391] (D vs. C). Method 2 produced consistent results as Method 1; however, Method 3 yielded partially consistent results (D was found to be BE to A). Conclusions: None of the three suspensions were found to be bioequivalent to each other using Methods 1 and 2. Method 1 and 2 may have been underpowered while model-based method may give higher power. However, the model-based approach needs further assessment to serve as an alternative for studies utilizing a sparse sampling design.

22. Application of NIR Chemometric Methods for Quantification of the Crystalline Fraction of Warfarin Sodium in Drug Product

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Plain Language Synopsis: NIR chemometric models were developed for quantification of the solid forms of warfarin sodium in drug products with different API content (4.4% and 10% API content) and formulation excipients.

Abstract: Monitoring of the physical state of warfarin sodium (WS) in products is essential for minimizing product quality variability to ensure consistent clinical performance. This study reports the development of chemometric models for quantifying the crystalline and amorphous fractions of WS in commercial drug products using NIR spectroscopy. Formulations based on commercially available products with different API to excipient ratio were used for the study. For each level of drug, two formulations containing either lactose monohydrate or lactose anhydrous as the predominant formulation excipient were prepared. Two formulations containing either 100% amorphous WS or crystalline WS were prepared and mixed in various ratios to obtain sample matrices containing amorphous/crystalline WS 0 -100%. The uniformity of the samples was confirmed by near infrared chemical imaging. Data was mathematically pretreated by multiplicative signal correction and Savitzky-Golay second derivative. Principal component regression and partial least square regression models were developed from mathematically treated data. All the models showed linear trend for amorphous and crystalline fractions of the WS as indicated by correlation and R2 >0.99 and >0.98, respectively. The models demonstrated good performance parameters with a low root mean squared error, standard error and bias. The model predicted crystalline and amorphous WS contents were in very close agreement with the actual values. The study indicated the utility of NIR chemometric methods in quantification of the crystalline and/or amorphous fraction of WS in its products

23. A Software Safety Assurance Case Template for Infusion Pumps

Li, Catherine, FDA/CDRH/ODE; Chapman, Rick, FDA/CDRH/ODE

Plain Language Synopsis: This study created a Software Safety Assurance Case Template for Infusion Pumps to provide manufacturers a common framework for justifying the safety of their infusion pump software, and for regulatory reviewers to assess the software safety. The results of this effort can be used to streamline regulatory software reviews.

Abstract: The safety of infusion pumps used in health care has been a serious issue, receiving considerable attention from FDA, manufacturers, and healthcare providers. Considerable effort and resources have been spent to improve the safety of infusion pumps, including FDA's 2010 infusion pump improvement initiative. However, the safety of infusion pump software remains an issue. Infusion pump software controls drug delivery, monitors sensors, generates alarms, handles security, processes drug and patient data, and many other aspects of pump functions. A priori, if the software (system) isn't safe, neither is the infusion pump. FDA published infusion pump guidance in 2014, which recommends the use of Assurance Cases (AC) as a means for the manufacturer to justify the safety of their device. The safety assurance case establishes claims, arguments, and evidence that is fundamental for a safety argument. In this study, we have created a Software Safety Assurance Case Template for infusion pumps. The study focuses on improving

the consistency and adequacy of safety assurance cases included in pre-market submissions, which can help to streamline the pre-market review process. It relies on software engineering best practices to demonstrate aspects of an infusion pump software safety assurance case. It makes use of ISO 14971 which establishes the basis for safety in medical devices and IEC 62304 which establishes a software life cycle development process for medical devices. We believe that the proposed study will serve to accomplish two milestones: a) provide manufacturers a common framework for justifying the safety of their infusion pump software, and b) provide regulatory reviewers a common framework to assess the manufacturer's arguments. We believe the results of this effort can ultimately be used to streamline regulatory software reviews, not only for infusion pumps, but other devices as well; reducing time and costs for all parties involved.

24. Differentiation of Heparin and Heparin-like Glycosaminoglycans via Peroxide-based Digestion with LC-MS and CE-UV Detection

Li, Hongli, FDA/CDRH/OSEL/DBCMS; Wickramasekara, Samanthi, FDA/CDRH/OSEL/DBCMS; Kaushal, Rahul, University of Maryland; Nemes, Peter, George Washington University

Plain Language Synopsis: Heparin is used in various medical products but the contaminants present in heparin can cause adverse effects including deaths. Here we developed a fast and inexpensive peroxide-based digestion method to assess the purity of heparin which is simple enough to be broadly adopted for the quality control of heparin.

Abstract: Heparin is an anticoagulant polysaccharide widely used in medical devices and drug formulations since the 1930s. Contamination of heparin with over sulfated chondroitin sulfates (OSCSs) resulted in hundreds of adverse reactions including deaths worldwide between 2007 and 2008. Development of novel analytical methodologies capable of assessing the purity of heparin is critical to protecting the public health. A simplified hydrogen peroxide H2O2 digestion protocol was developed to transform structurally complex glycosaminoglycans (GAGs) into small oligosaccharides before measurements by liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis ultraviolet (CE-UV) detection. GAG standards (0.25 mg/mL) were spiked with 12.4% H2O2, and the mixture was heated at 125oC for 18 min to promote radical digestion of the polysaccharide chains. The resulting digestion products were separated on a C18 column using hexylamine ion pairing chromatography and detected by MS/MS. As a number of the digestion products were characteristic of the parent GAG, multivariate analysis of the LC-MS data successfully differentiated between heparin, OSCS, and other GAGs (dermatan sulfate and chondroitin sulfate A) with high confidence. Additionally, CE-UV detection revealed that the digestion efficiency was >90%, fostering a lower detection limit of 10 µg/mL for OSCSs and 50 µg/mL for other GAGs. Furthermore, using disaccharide standards, it was possible to quantify the extent of OSCS contamination in heparin. The H2O2 digestion protocol is simple and fast enough to be broadly adoptable for the quality control of heparin, specifically for the screening of OSCSs and GAG contaminants in heparin.

25. Targeted lentiviral Vector Integration at "safe harbor" Genomic loci

Li, Pingjuan, FDA/CBER/DCGT; Marino, Michael, FDA/CBER/DCGT; Reiser, Jakob, FDA/CBER/DCGT

Plain Language Synopsis: We describe a novel procedure to introduce foreign gene sequences into desired regions of the human genome

Abstract: Lentiviral vectors provide powerful tools for therapeutic gene delivery in vitro, ex vivo and in vivo because of their ability to mediate long-term transgene expression. However, there are safety concerns since these vectors integrate nonspecifically into actively transcribed genes. Our goal is to engineer lentiviral vectors so that their integration preference is shifted to genomic "safe harbor" sites. The AAVS1 locus on chromosome 19 constitutes such a genomic "safe harbor". We generated integrase-defective lentiviral vectors (IDLVs) capable of integrating at the AAVS1 locus through homologous recombination (HR). To improve the efficiency of this approach, an engineered AAV2 protein (Rep78) was used because it preferentially nicks the DNA at the AAVS1 locus. To assess the efficiency of HR induced by Rep78 in HEK 293 cells, we co-delivered, by IDLVs, the coding region for Rep78, and a donor

vector bearing a puromycin resistance gene (PuroR), flanked by homology arms corresponding to the AAVS1 site. Puromycin-resistant cell clones were counted and characterized by PCR. The number of puromycin-resistant clones obtained from cells transduced with Rep78 and the PuroR containing donor vector was up to 5 fold higher compared to cells transduced by the donor vector only. PCR results showed that in up to 41.7% of the puromycin-resistant clones the PuroR transgene had specifically integrated at the AAVS1 locus. These results indicate that Rep78 can significantly increase the efficiency of HR at the AAVS1 site. We expect this gene editing approach to be useful for incorporating other transgene sequences at the AAVS1 locus.

26. Method Development for the Characterization of Physiochemical Properties of Ag Nanomaterials in Consumer Products

Lim, Jin-Hee, FDA/ORA/ARL; Howard, Paul C., FDA/NCTR/OSC; Linder, Sean W., FDA/ORA/ARL

Plain Language Synopsis: Ag nanoparticles become the most common materials found in nano-related consumer products. In this study, the chemical composition, size, morphology, and optical properties of Ag nanomaterials in consumer products (i.e., dietary supplements, sanitary napkins, soaps, deodorant, and other personal care products) were characterized using various analytical techniques.

Abstract: Silver (Ag) is a well-known material used as an antimicrobial agent for several decades. With the development of nanotechnology, Ag nanoparticles become the most common materials found in nano-related consumer products in USA, for example, textiles, wound dressing, catheters, food packaging materials, dietary supplements, and personal care products. The predominant reason for the use of Ag nanoparticles in consumer products is that nanoparticles with a large surface area can continuously release a low level of silver ions to provide antimicrobial activities. With the increased claims of Ag nanoparticle use in such consumer products, and to perform science-based risk assessments, the development of methodologies to detect and quantify the presence of nanomaterials in various consumer products is essential. In this study, dietary supplements, sanitary napkins, soaps, deodorant, and other personal care products claiming to contain Ag nanoparticles were purchased from various retailers. The chemical composition, size, morphology, and optical properties of Ag nanomaterials were detected and characterized using techniques such as inductively coupled plasma-mass spectroscopy (ICP-MS), dynamic light scattering (DLS), disc centrifuge system (DCS), field emission scanning electron microscope (FESEM), transmission electron microscope (TEM), energy dispersive x-ray spectrometry (EDS), and UV-Vis spectroscopy. We demonstrate the methods required for quantitative assessment of Ag, and describe the utility of confirmatory methods for detecting the nanoparticles within commercial products.

27. Advancing Public Health needs through Innovative Formulations for the President's Emergency Plan for AIDS Relief (PEPFAR)

Lunn, George, FDA/CDER/ONDQA; Shanmugam, Balajee, FDA/CDER/ONDQA; Nwokike, Jude, FDA/OGROP/OSPA

Plain Language Synopsis: PEPFAR supports millions of patient on life-saving antiretroviral treatment. FDA has played a critical role in that effort by approving quality and safe drugs for use in resource-limited countries. Many of which are innovative formulations that improve adherence to treatment, reduce risk of developing resistance, and simplify supply chain.

Abstract: As of September 2014 PEPFAR has supported 7.7 million people on treatment in resource poor countries around the world. Since 2004, FDA's support has been the foundation of PEPFAR's success in making high quality antiretroviral medications (ARVs) from low-cost manufacturers available. FDA review ensures that the safety, efficacy, and manufacturing quality standards for these ARVs are identical to the standards that apply to any product approved for marketing in the U.S. Products for which the patents have expired are given full approval and products for which patent or other market protection exists in the U.S. are given Tentative Approval. Between 2005 and 2013 the proportion of FDA-approved drugs used by PEPFAR increased from 16% to 98% driving down treatment cost per patient from $1,053 in 2005 to $315 in 2013 and resulting in more patients placed on treatment.

FDA's program has 181 ARVs as of 1/15/2015. These products include fixed-dose combinations that improve adherence to treatment, reduce the risk of developing resistance, and simplify supply chain. Of these 109 were reviewed under section 505(j) of the FD&C Act and 72 NDAs under section 505(b)(2). FDA's guidance on Fixed Dose Combinations, Co-Packaged Drug Products, and Single-Entity Versions of Previously Approved Antiretrovirals for the Treatment of HIV provides guidance to industry. FDA has participated in 6 PEPFAR-related international outreach initiatives. These meetings provided critical guidance for the successful submission of ARV original applications and process change submissions. Additionally, these meetings provided opportunities for manufacturers to directly interact with FDA and ask questions concerning innovative formulations and submissions to meet FDA's standards. Interactions mainly focus on specifications, analytical methods, stability, and control of starting materials and intermediates. Through these interactions with FDA, industry can develop innovative formulations, patients have increased access to simplified ARV drugs, and the PEPFAR program is able to extend its reach. Examples of innovative formulations include chewable and dispersible tablets for pediatric use.

28. Extended Control to Pharmaceutical Quality: Assessing Aggregation of Therapeutic Monoclonal Antibodies in Human Plasma

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Plain Language Synopsis: Therapeutic monoclonal antibodies (mAbs), when administrated with an inappropriate diluent, can form aggregates in plasma. The aggregation process appears to be triggered by the low pH of product formation and abundant plasma proteins. These data demonstrate a strong need of extended quality control throughout the clinical administration procedures of mAbs.

Abstract: Aggregation of therapeutic monoclonal antibodies (mAbs) has the potential to adversely impact product safety and efficacy. Therefore, the level of aggregates in the final product must be tightly controlled during formulation, storage, and clinical administration. Clinically, protein products are usually administrated via intravenous infusion after being mixed with a diluent (e.g., 5% dextrose). Such a clinical protocol may introduce complex matrix interactions with the product, which increases the likelihood of product aggregation. However, the potential of product aggregation in clinical settings is not always rigorously characterized in the product quality control program. This project aims at identifying common factors that induce aggregation of therapeutic mAbs under conditions mimicking clinical administration settings, which involve the mAb protein itself, formulation excipients, diluent, and plasma components. We have investigated the aggregation properties of representatives of FDA approved mAbs. We found distinct aggregation patterns for two mAbs, which rapidly formed aggregates when mixed with 5% dextrose and human plasma. Using mass spectrometry, we demonstrated for the first time that the aggregates contain not only the mAb itself but also several plasma proteins (e.g., complement proteins). By varying formulation conditions, we found that the pH value of the formulated product is a critical determinant of aggregation process. At low pH (6.0-6.2) of the product formulation, excipients appear to synergize with dextrose in triggering destabilization/aggregation of plasma proteins, and subsequently co-precipitation with mAb. The acquired knowledge will help the manufacturers develop control strategies (e.g., optimizing formulation and diluent for infusion) to mitigate product aggregation in human blood, and importantly help FDA reviewers in making more informed regulatory decisions regarding the safety and efficacy of therapeutic mAb products.

29. Sesquiterpenoid Tropolone Glycosides from Liriosma ovate

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Plain Language Synopsis: Two new sesquiterpenoid

tropolone glycosides, liriosmasides A and B, along with two known compounds, secoxyloganin and oplopanpheside C, were isolated and identified from the roots of Liriosma ovata. These compounds will be used as marker compounds to monitor the authenticity of dietary supplements labeled as containing Liriosma ovata.

Abstract: Two new sesquiterpenoid tropolone glycosides, liriosmasides A (1) and B (2), along with two known compounds, secoxyloganin (3) and oplopanpheside C (4), were isolated from a methanol extract of the roots of Liriosma ovata. The structures of 1 and 2 were elucidated by spectroscopic methods including 1D and 2D NMR and by high-resolution mass spectrometry involving an ultra-high performance liquid chromatography -quadrupole-orbital ion trap mass spectrometric (UHPLC -Q-orbitrap MS) method. Compound 1 showed weak inhibitory activity against HIV RNase H. These compounds will be used as marker compounds to monitor the authenticity of dietary supplements labeled as containing Liriosma ovata.

30. High Content Imaging of Early Morphological Profiles Demonstrates High Correlation with Long Term Mineralization Capacity of Osteogenically-induced Human MSCs as Revealed by High Dimensional Principal Component Analysis

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Plain Language Synopsis: High correlation of early morphological profiles of human mesenchymal stem cells (MSCs) with long term mineralization was demonstrated using high content imaging combined with principal component analysis. MSCs with low mineralization capacity exhibited a more dramatic morphological response to osteogenic stimuli compared to MSCs with high mineralization capacity.

Abstract: Human bone marrow-derived multipotent mesenchymal stromal cells, otherwise known as mesenchymal stem cells (MSC), represent a highly attractive cell source for a multitude of tissue engineering and regenerative medicine applications due to their widely documented potential for multi-lineage differentiation, immunomodulation, and paracrine factor secretion. A major complication for current MSC-based therapies is the lack of well-defined characterization methods that can robustly predict how they will perform in a particular in vitro or in vivo setting. Significant advances have been made with identifying molecular markers of MSC quality and potency using multivariate genomic and proteomic approaches, and more recently with advanced techniques incorporating high content imaging to assess high-dimensional single cell morphological data. We sought to expand upon current methods of high dimensional morphological analysis by investigating whether short term cell and nuclear morphological profiles of MSCs from 8 different donors (at multiple passages) correlated with long term mineralization upon osteogenic induction. Using the combined power of automated high content imaging followed by automated image analysis, we were able to demonstrate that MSC morphology after 72 hours was more highly correlated with 35 day mineralization compared to more traditional methods of MSC osteogenesis assessment (gene expression and alkaline phosphatase activity). Principal component analysis (PCA) was employed to permit high dimensional morphological profile data analysis using both unsupervised and supervised techniques. Conserved differences in 72 hour morphology between MSCs cultured in growth and osteogenic medium were observed for all donors/passages, irrespective of mineralization capacity, with more drastic changes in morphology observed for donors with lower mineralization capacity.

31. Rhizopus Oryzae Infection in an Infant: Response Activities and Prevention Strategies

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ORA/NYK-DO; Palo, Matthew, FDA/ORA/NYK-DO; Ruiz, Melinda, FDA/ORA/NYK-DO; Langello, Kimberly, FDA/ORA/NWE-DO; Maushart, Todd, FDA/ORA/NWE-DO; DeBeck, Heidi, FDA/ORA/MIN-DO; Paterson, Judith, FDA/ORA/PHI-DO

Plain Language Synopsis: An infant death was associated with consumption of a dietary supplement at a Connecticut hospital. The suspected open and intact packages of the supplement lots and clinical samples tested positive for Mucor species. FDA investigators collected finished dietary supplement samples from the hospital that tested positive for Rhizopus oryzae.

Abstract: Food and Drug Administration (FDA) Office of Foods and Veterinary Medicine's Coordinated Outbreak Response and Evaluation Network (CORE) was notified of an infant death which occurred on October 11, 2014 at a Connecticut hospital. The death was associated with consumption via a gastric tube, of an ABC Dophilus dietary supplement, (Solgar Inc., Leonia, NJ). This was the only reported adverse event associated with the supplement. The hospital collected and tested suspect lots of open and intact samples of the supplement, and the infant's clinical samples. All samples tested positive forRhizopus oryzae. FDA District Offices including New England, New Jersey, New York and Minnesota inspected and collected finished dietary supplement samples from the hospital, warehouses, packing facilities, and headquarters. The supplement samples were analyzed by FDA's Denver laboratory and Pacific Region South West laboratory (PRL-SW). Environmental samples were collected at a repacker and analyzed by PRL-SW. The intact dietary supplement samples collected by FDA from the hospital tested positive for Rhizopus oryzae. As a result, Solgar, Inc. recalled three ABC Dophilus lots on November 13, 2014. FDA and the Centers for Disease Control and Prevention coordinated release of an Epidemic Information Exchange (Epi-X) alert, a Health Alert Network (HAN) posting, a World Health Organization (WHO) International Network of Food Safety Authorities (INFOSAN) alert, and a Dear Health Care Provider Letter to increase awareness. This incident highlighted two novel issues outside CORE's foodborne illness outbreak response activities: 1) physician's use of probiotic supplements in sensitive populations and 2) a need to establish regulatory mold limits and speciation requirements in supplements. CORE, Office of Regulatory Affairs (ORA), Center for Biologics Evaluation and Research (CBER), Center for Foods Safety and Applied Nutrition (CFSAN), and Center for Drug Evaluation and Research (CDER) continue to discuss probiotic supplement regulations and potential additional prevention measures by industry and FDA to prevent future injuries. In addition, CORE and ORA's Office of Regulatory Science will hold discussions with the firm to establish mold threshold and speciation standards in products marketed to sensitive populations.

32. Development of a Versatile Bioassay Platform for the Quantification of Potency and Thrombogenicity in Products for Bleeding Disorders

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Plain Language Synopsis: Drugs used to treat life-threatening bleeding disorders can have complications that include uncontrollable bleeding or undesirable blood clots due to incorrect dosing to improve the quality of these therapeutic regimens, we developed a versatile clotting and clot dissolution method to better characterize the effects of the treatment.

Abstract: CBER regulates products indicated for the treatment of life-threatening bleeding disorders. These products include plasma-derived and recombinant coagulation factors, stored plasma and platelets, which may contain anti-coagulant or thrombogenic impurities that can affect product efficacy and safety. Functional evaluation and manufacturing control of these impurities are difficult because traditional assay methods are not sufficiently sensitive to the balance between pro- and anti-thrombotic activities. In addition, it is difficult to assign an acceptably safe range for impurities because their impact on patients may vary greatly depending on patient condition or treatment regimen. Our laboratory uses a global hemostasis assay paradigm to develop in vitro methods for characterization of product potency

and thrombogenicity. In a global hemostasis assay, blood plasma samples are tested under conditions representative of the balance between coagulation factors and plasma inhibitors. We developed a high- throughput and robust global hemostasis assay system allowing simultaneous detection of the three primary coagulation events -- thrombin generation (TG), fibrin clot generation (FG) and fibrinolysis (FL) -- in human plasma spiked with different doses of procoagulant drugs. Analytical parameters of this assay system can be optimized to selectively measure the activities of either the main pharmaceutical ingredients or the identified thrombogenic impurities. In the case studies, we successfully tailored the TG/FG/FL assay for potency evaluation of concentrates of factor (F) VIII, FIX, FVIIa or platelets; for thrombogenicity testing of FIXa impurity in FIX products; and for FXIa impurity in immune globulin products. In each case, a sufficiently wide linear range of assay responses was established to allow the quantification of product potency or impurity activities in units traceable to publicly available reference standards. The TG/FG/FL assay performed favorably in comparison with the traditional methods in several collaborative studies for international standards of various coagulation factors. In most cases, the TG/FG/FL assay demonstrated comparable robustness and lower limits of detection when compared to the traditional methods. Our findings suggest that application of physiologically relevant analytical methods can facilitate the evaluation of investigational drugs; stimulate the development of safer treatments; and assist in the comparison of the effectiveness of existing products.

33. Quantification of Cold Flow in Marketed Estradiol Transdermal Drug Delivery Systems

Yellela, Krishnaiah, FDA/CDER; Pavurala, Naresh, FDA/CDER; Yang, Yang, FDA/CDER; Hunt, Robert, FDA/CDER; Khan, Mansoor A., FDA/CDER

Plain Language Synopsis: Cold Flow (CF) in Marketed Estradiol Transdermal Drug Delivery Systems is quantified using stereomicroscopic imaging. The effect of product formulation and temperature on CF is also studied. The results suggested that the degree of CF in estradiol TDDS products is formulation-specific and temperature-dependent.

Abstract: Purpose: Excessive cold flow (CF) is one of the product quality defects associated with drug-in-adhesive type of transdermal drug delivery systems (DIA-TDDS). The dimensional change and drug loss due to CF may impact the performance as well as safety of DIA-TDDS. The objective was to quantify CF in terms of dimensional change and drug loss in CF region of three marketed estradiol DIA-TDDS (products-A, B and C). Methods: Circular samples of estradiol DIA-TDDS were punched out and induced with CF at 25°C/60%RH and 32°C/60%RH by applying 1-kg force for 3 days. At the end of study period (i) dimensional change in the area of samples due to CF was measured using stereomicroscopic imaging, and (ii) estradiol concentration in CF region was determined by HPLC. Results: Percent dimensional change due to CF measured by stereomicroscopic imaging was ranging from 13.8±2.8 to 59.6±4.2 depending on the temperature of testing conditions and product composition. A similar trend (6.5±0.8 to 51.2±3.1) was observed with respect to CF measured by HPLC in terms of percent drug loss. Estradiol TDDS product-C exhibited higher tendency of cold flow when compared to products-A and B at accelerated test conditions simulating to shelf-storage and skin temperature. Conclusion: The results suggested that the degree of CF in estradiol TDDS products is formulation-specific and temperature-dependent.

34. Seamless Insertion of Real Pulmonary Nodules into Chest CT Exams: Validation and Application to CAD Training

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Plain Language Synopsis: Collection of data and establishment of ground truth for medical images are both costly and difficult. We are developing an image blending tool that allows users to modify or supplement existing datasets by seamlessly inserting a real lesion extracted from a source image into a different location on a target image to assist with CAD training.

Abstract: The availability of large medical image datasets is critical in many applications such as training and testing of computer aided diagnosis

(CAD) systems, evaluation of segmentation algorithms, and conducting perceptual studies. However, collection of data and establishment of ground truth for medical images are both costly and difficult. To address this problem, we are developing an image blending tool that allows users to modify or supplement existing datasets by seamlessly inserting a real lesion extracted from a source image into a different location on a target image. We minimize the impact of user skill on the perceived quality of the blended image by using powerful image processing algorithms that limit user involvement to two simple steps: the user first draws a casual boundary around the nodule of interest in the source image, and then selects the center of desired insertion area in the target image. In addition to ease of use, our techniques provide the following capabilities: 1) the ability to insert lesions into areas with complex background, 2) the ability to insert non-isolated nodules, and 3) the ability to properly transfer the noise properties. We investigated the viability of our insertion techniques for chest CT by comparing the noise power spectrum (NPS) of blended nodules versus that of native nodules in simulated phantoms, and by conducting a reader study involving clinical examples. To compare the performance of a CAD system without and with the use of our image composition tool, we trained the system on three sets of data. The first training set was obtained from original CT cases, the second set consisted of the first set plus nodules from the first set inserted into new locations, and the third set consisted of the first set plus inserted nodules transformed in shape and contrast before insertion. We then compared the performance of the three CAD systems in differentiating nodules from normal areas by testing them against a fixed dataset of real nodules, and using the area under the ROC curve (AUC) as the figure of merit. The performances of the systems trained with the augmented datasets were found to be significantly better than that trained with the original data under several training scenarios.

35. Increased Concerns for the Lack of Therapeutic Interventions against Histomoniasis (Blackhead Disease) in Turkeys

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Plain Language Synopsis: Therapeutic options for the control and prevention of Histomoniasis (Blackhead disease) in turkeys are limited. FDA is interested in exploring with colleagues in academia, the pharmaceutical industry, the poultry industry and others, possible therapeutic interventions against histomoniasis in turkeys, leading to an approved new animal drug to fill this important therapeutic need.

Abstract: Histomoniasis (Blackhead disease) is a serious concern for the turkey industry in the United States (US). At least 50 cases of histomoniasis were reported each year since 2007 in the US (2013 USAHA Annual Meeting Proceedings) and mortality in the infected turkey flock can reach 100%. Therapeutic options for the control and prevention of the disease are limited. There is a need for research into the development of new animal drugs and other possible interventions, including vaccines and management techniques for the control and prevention of histomoniasis in turkeys. The purpose of this presentation is to highlight FDA's interest in exploring with colleagues in academia, the pharmaceutical industry, the poultry industry and others, possible therapeutic interventions against histomoniasis in turkeys, leading to an approved new animal drug to fill this important therapeutic need.

36. To Evaluate the Performance of Generic Lansoprazole Delayed-release Capsules through Nasogastric Tube via in vitro Testing

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Plain Language Synopsis: FDA had previously received adverse events reports indicating that the delayed-release orally ODT clogged and blocked the feeding tube. The OGD is concerned about issue and tried to develop in vitro NG tube testing to evaluate performance profile of generic drug instead in vivo testing. We collected the in vitro NG tube data from five approved ANDAs

for Lansoprazole DR Capsules. The results of sedimentation testing, particle size distribution, recovery, and acid resistance testing demonstrated that the delivery through the NG tube did not impact the integrity of the enteric coating for generic drug products.

Abstract: Purpose: Lansoprazole Delayed-release (DR) Capsules can be administered via nasogastric (NG) tube per the product labeling. FDA had previously received adverse events reports indicating that the delayed-release orally disintegrating tablets (ODT) clogged and blocked the tube. In some cases, patients have had to seek emergency medical assistance to unclog or replace their feeding tubes. Therefore, it is critical that generic products have a similar performance through the NG tube as that of the reference product. The purpose of this study was to compare the performance of generic and reference listed drug lansoprazole granules through the NG tube. Methods: Based on FDA's lab study results and extensive regulatory and literature research, FDA developed in vitro NG tube study recommendations including sedimentation testing, particle size distribution study, recovery testing and comparative acid resistance stability testing. FDA requested Abbreviated New Drug Application (ANDA) applicants to conduct in vitro NG tube studies comparing the transit of lansoprazole granules through 16 French NG tube of the test product to that of the reference product. Results: We retrospectively collected the in vitro NG tube data from five approved ANDAs for Lansoprazole DR Capsules. The results of sedimentation testing showed that sedimentation volume was similar between the test and reference. In addition, the sedimentation testing provided the initial visual observation for granule performance in the dilution solvent. Differences in particle size distribution between the test and reference in all five applications were not statistically significant with a few exceptions in D-span and D10. The recovery was greater than 90% recovery in all five studies and the generic drug products showed comparable recovery to the reference product. It should be noted that no clogging or obstruction was observed. The results of acid resistance testing showed that the % release for both test and reference was much less than 10% in all five applications. Conclusion: The results of in vitro NG tube studies demonstrated that the process of dispersing lansoprazole granules and delivery through the combination of oral syringe and NG tube did not impact the integrity of the enteric coating for generic drug products and that the generic products had a similar performance profile as that of the reference product during NG tube administration.

37. Dynamics of Histone Modifications at the Cytokine-inducible Promoters for the Immunomodulatory Genes IDO1 and TSG6 in Human Bone Marrow-derived MSCs

Rovira Gonzalez, Yazmin, FDA/CBER; Lynch, Patrick, FDA/CBER; Stultz, Brian, FDA/CBER; Hursh, Deborah, FDA/CBER

Plain Language Synopsis: Mesenchymal stem cells (MSCs) are being investigated clinically due to their ability to migrate to sites of injury, reduce inflammation, and lessen tissue damage, after exposure to specific cytokines. Here, we evaluated the relationship between epigenetic chromatin modifications and expression of genes responsible for suppressing the immune system in MSCs.

Abstract: Bone marrow-derived multipotent stromal cells (BM-MSCs) are attractive candidates for cell-based therapies due to their capacity to modulate immune system cells. The immunosuppressive potential of BM-MSCs depends in part on their ability to activate expression of factors that dampen the immune response, which can vary between donors and cell culture conditions. In this study, we investigated whether specialized chromatin structures contribute to regulating the expression of critical immunomodulatory genes in BM-MSCs. Using chromatin immunoprecipitation (ChIP) coupled with quantitative real-time PCR, we assayed the levels of transcriptionally permissive and repressive histone modifications surrounding the transcriptional start sites for indoleamine 2,3 dioxygenase 1 (IDO1) and tumor necrosis factor, alpha-induced protein 6 (TSG-6), which were selected for their roles in immunomodulation and sensitivity to pro-inflammatory cytokines. Consistent with the lack of IDO1 mRNA transcripts in resting BM-MSCs, the IDO1 promoter was enriched by repressive methylation at histone H3 lysine 9 (H3K9me3) in resting BM-MSCs. Forty-eight hour treatment with cytokines IFN-γ and

TNF-a resulted in loss of H3K9me3 concomitant with a gain in permissive acetylation of the same lysine residue (acH3K9) and activation of IDO1 mRNA. Residual levels of H3K9me3 were still observed above background in at least one of the BM-MSC donors following exposure to cytokines. Cytokine treatment of fibroblasts for the same amount of time resulted in complete loss of H3K9me3 from IDO1. Similar to IDO1, gene expression of TSG-6 was up-regulated during cytokine treatment. In contrast to IDO1, we observed no H3K9me3 enrichment at TSG-6. Acetylated H3K9 associated with TSG-6 at similar levels before and after cytokine treatment. In conclusion, our results demonstrate that activation of IDO1, but not TSG-6, is regulated in part through repressive chromatin structures that are removed upon exposure to pro-inflammatory cytokines. Furthermore, our data indicate that the efficiency of repressive chromatin turnover at the IDO1 promoter may contribute to heterogeneity of gene expression with the potential to affect immune plasticity.

38. Development of New Analytical Method for Evaluation of Product-Related Impurities in Blood Coagulation Factor VIII Products Used for Treatment of Hemophilia A

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Plain Language Synopsis: A new analytical method based on affinity chromatography for testing FVIII products for product-related impurities was developed. Using this method, we showed that several FVIII products contain various amounts of structurally different FVIII. We hypothesize that these forms of FVIII contribute to immunogenicity of FVIII products.

Abstract: Background: Deficiency in blood coagulation factor VIII (FVIII) results in a bleeding disorder (Hemophilia A), which is treated by FVIII products regulated by FDA. About 30% of such patients develop anti-FVIII antibodies (inhibitors) making the treatment ineffective. Recent data suggest that the brand of FVIII product may contribute to its immunogenicity, however, not all relevant attributes of the products are known yet. A relatively wide range of FVIII specific activity in FVIII products indicates that some of them contain significant amount of inactive FVIII. It was reported that FVIII products contain a fraction not capable to associate with a physiological FVIII ligand, von Willebrand factor (VWF), which protects FVIII from uptake by antigen presenting cells. We propose that this fraction of FVIII (FVIII*) represents an inactive structurally compromised FVIII (i. e. impurity), which contributes to immunogenicity of the product. Objective & Relevance to FDA Mission: to develop a method to evaluate FVIII* as a product-related impurity in FVIII products. This will improve the quality of FVIII products and, respectively, treatment of Hemophilia A. Methods: A FVIII-affinity sorbent was prepared by covalent immobilization of vWF on sepharose. Commercial products with recombinant FVIII were subjected to chromatography on the vWF-sepharose followed by analysis of the fractions by PAGE-SDS and Western-blot. Results: All three tested FVIII products were found to contain a fraction not able to bind with vWF; this fraction (FVIII*) varied between the products from 7 to 20% of the total protein. In all these FVIII products, the fraction of FVIII* contained was presented by isolated fragments of FVIII, known as the heavy chain and light chain (low amount), and FVIII aggregates. Conclusions and Future Plans: We developed a method to analyze FVIII products for FVIII fraction unable to bind vWF. In several FVIII products, we demonstrated a presence of considerable amount of isolated heavy chain of FVIII. Our future plans involve testing of additional FVIII products and characterize the FVIII* form, as impurity, for immunogenicity using in vitro and in vivo models.

39. Ingredients of Personal Lubricants on Condoms: Polyurethane vs. Natural Rubber Latex

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Plain Language Synopsis: Synthetic and natural rubber condom materials may absorb ingredients of personal lubricants and change in size or elastic modulus, which would affect the seal integrity of the condom. Herein we observe the changes in characteristics of condom materials in the presence of various ingredients of commercial

personal lubricants.

Abstract: Personal lubricants are composed of various chemicals; however, the effects of individual ingredients of lubricants have never been studied on either natural rubber latex (NRL) or polyurethane (PU) condoms, which were introduced to the US market as an alternative to NRL condoms. An improper combination of lubricants and condoms may swell the condom material leading to diminished integrity of the prophylactic device and compromising its usability. The research objective is to determine the effects, if any, of ingredients of commercial PLs on condoms. This work compares the effects of major ingredients (composing > 10%) in over the counter lubricants, on PU and NRL condoms from one of a few commonly used US condom brands. Experiments were performed to identify changes in PU and in NRL condom properties, including swelling, elastic modulus and stress reduction, in the presence of various lubricant bases, e.g., water, silicones, and glycols, which are predominantly found in over-the-counter lubricants. Mineral oil and glycerin were used as positive and negative controls respectively. Experiments were performed before and after fluid application. Both methodologies were used to amass longitudinal and cross-sectional effects and observations. In the swelling study, the percent area change of condom samples was calculated through an optical analysis using ImageJ [NIH] before fluid application and again at predetermined intervals afterwards. A dynamic mechanical analyzer [Model RSA3, TA Instruments, Wilmington, DE] captured the changes in the induced stress within condom samples during elastic modulus and stress reduction testing. Following an initial load of 2.0 g, elastic modulus was measured up to 50% extension at various strain rates, and stress reduction was studied under a constant 20% strain and at 37°C. Baseline tests were conducted free of any ingredient. NRL swelled significantly due to cyclic and low viscosity silicones, while petroleum jelly and hydroxylterminated dimethicones caused severe swelling in PU. A few glycols and the hydroxyl-terminated dimethyl caused substantial stress reduction in PU. Change in elastic modulus was observed only at a very low strain rate. Preliminary results suggest a need for further testing on a wider range of condoms and lubricants.

40. Current Perspectives on Initial Completeness Assessment for Type II API DMFs under GDUFA

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Plain Language Synopsis: A metrics analysis was performed to evaluate the factors that affect the review process based on over 2500 Drug Master File (DMF) submissions, as the Initial Completeness Assessment of Type II Active Pharmaceutical Ingredient (API) DMFs became mandatory under Generic Drug User Fee Amendments of 2012 (GDUFA).

Abstract: The Generic Drug User Fee Amendments of 2012, commonly referred as GDUFA, requires that Type II Active Pharmaceutical Ingredient (API) Drug Master Files (DMFs) must undergo an FDA initial Completeness Assessment (CA). This new approach aims to improve product manufacturing and quality by including a 62-item checklist for submission completeness at the initial stage. During an 18-month period (from October 1st, 2012 to Aril 30th, 2014), over 2500 Type II API DMFs have been subjected to Completeness Assessment review. Based on the collected data, a metrics analysis was performed to evaluate the factors that relate to the review process in terms of submission types (paper vs electronic), monthly assignments, review durations, incomplete item distribution, and first-cycle completion rate, etc. The discussion on the performance metrics as well as common issues the DMF holders tend to neglect or misunderstand would facilitate the holders to submit Type II API DMFs of high quality.

41. Analytical Methodologies to Isolate and Quantify Free and Liposomal Bound Doxorubicin from Biological Samples Using LC-HRMS and LC-QQQ-MS

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Plain Language Synopsis: Liposomal nanoparticles are being used in an increasing number of drug products. To evaluate the quality and toxicology of these products, methods to quantitatively

describe nanoparticle stability and distribution during treatment is essential. Here we developed two methods to determine the amount of free and encapsulated doxorubicin in biological samples.

Abstract: In the medical field, nanotechnology has sparked a rapidly growing interest as it has the potential to solve a number of issues associated with conventional therapeutics. Over the past several decades, remarkable progress has been made in the development and application of engineered nanoparticles to treat cancer more effectively. One of these key nanomaterials is the liposome, which is currently being used to encapsulate various chemotherapeutic agents. This encapsulation is engineered to increase the efficacy of the chemotherapeutic agent by protecting it against degradation, prolonging circulation time, improving cellular uptake, and reducing side effects that limit dosage amounts. As the commercialization of liposomes has increased, regulatory-based analytical methodologies to investigate nanoparticle biodistribution have not been well documented. As such, to ensure the safety and quality of liposomal products, the development of analytical methodologies to quantitatively describe nanoparticle biodistribution is essential. In this work, we have focused on the development two analytical methods, rooted in the use of liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) or triple quadrupole mass spectrometry (QQQ-MS), to isolate and quantify doxorubicin from tissues and plasma samples obtained from xenograft mice. The first method was developed to isolate and quantify both free doxorubicin and liposomal bound doxorubicin in blood plasma. We based this separation upon the selective retention of free doxorubicin on a hydrophobic reversed-phase solid phase extraction (SPE) cartridge. The liposomal fraction exhibited no retention, while the free doxorubicin was retained on the column and eluted with acidified methanol. The liposomal fraction was collected, treated with acetonitrile to extract the doxorubicin, and rerun on SPE. After extraction the samples were run on LC-QQQ-MS to quantify the amount of doxorubicin present in each fraction. The second method was developed to extract and quantify total doxorubicin from tissues without the potential for contamination. In this method we extract the doxorubicin from homogenized tissue made by disrupting biological samples through high-speed shaking in plastic tubes with stainless steel beads. The total doxorubicin is quantified using LC-HRMS. The development of these advanced analytical methods will enable FDA to begin to address future regulatory issues related to liposomal drug products.

42. Testing of Imported Eyeglass and Sunglass Lenses at the Port of Entry

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Plain Language Synopsis: This CDRH OSEL/ORA WEAC collaboration will expand FDA field testing capabilities, providing investigators with a new field exam protocol and field lens impact resistance test kit. This involves development of a portable lens impact testing apparatus with field guidance for testing lenses at their point of entry.

Abstract: Winchester Engineering Analytical Center (WEAC) along with Center for Devices and Radiological Health (CDRH) Office of Compliance (OC) and Office of Regulatory Affairs (ORA) Division of Import Operations (DIO) have developed several product evaluation protocols for ORA investigator use in assessing various medical devices and radiation-emitting products in the field. Field exam results are used to detain or release products or ascertain whether entry samples are collected for submission to WEAC for analyses. A new field exam protocol and “test kit” for ORA investigator use is being developed for field lens impact resistance testing. A portable lens impact testing apparatus based upon the laboratory impact resistance test apparatus is being developed, providing ORA investigators the capability to quickly, accurately and repeatedly perform field lens impact testing. In addition, field-specific protocols, operation, calibration, sampling procedures and guidance are also under development, incorporating input from Office of Science and Engineering Laboratories (OSEL), WEAC, CDRH OC, ORA DIO and District staff. The test requirements are described in 21CFR801.410 and the instruments used for testing are commercially available. However, this commercial equipment cannot be used in the field environment since the design is for use in

the laboratory. More importantly, an additional requirement to test eyeglass and sunglass lenses in their frames made the existing apparatus completely unusable. A new field transportable test kit is being designed and developed which meets these new requirements. The kit is enclosed in a rigid plastic wheeled case (20" x 16.5' x 12.5") with a telescoping handle allowing for assembly of the test apparatus on site. The test apparatus when fully assembled incorporates the case as part of the structure, and includes adjustable legs, the test chamber, an integral level, and leveling adjustments. This design provides a simple portable apparatus for field testing imported eyeglass and sunglass lenses.

43. Development of Preclinical Standard Techniques to Evaluate Critical Optical Properties of Novel Intraocular Lens (IOL) Devices

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Plain Language Synopsis: Here we address concerns about quantitatively evaluating the optical properties of intraocular lenses. The OTMN lab has worked towards being able to successfully evaluate optical property concerns at the non-clinical evaluation stages (e.g. image quality, focusing power, and scattering of light).

Abstract: FDA/CDRH Optical Therapeutics and Medical Nanophotonics Laboratory (OTMN Lab) works on developing preclinical techniques to quantitatively evaluate critical optical properties associated with intraocular lens (IOLs) devices. The OTMN Lab has developed the confocal laser method (CLM), scanning light scatter profiler (SLSP), and US Air Force (USAF) resolution chart optical bench to address specific optical property concerns associated with novel IOL designs. The CLM approach was original developed in 2008 to precisely measure the dioptric power of monofocal IOLs. Since its inception, the CLM capabilities have expanded to other IOL classes (e.g. multifocal, toric, and extended-depth-of-focus), additional optical properties measurements (e.g. cylinder power, image quality, and depth of focus), and the impacts of various optical characteristics (e.g. wavelength of light and beam profile) can impact these optical properties. Here, we present the current state of the CLM approach and how it can be used to address real non-clinical optical property concerns. Glare, glistenings, optical imperfections, dysphotopsia, and poor image quality are known imperfections of intraocular lenses (IOLs) and all of these optical phenomena may be related to excessive scattered light. However, the specific direction of the scattered light can be a critical difference between debilitating glare and an unnoticeable decrease in image quality. As a result, measuring the quantity of scattered light and its direction is essential to appropriately evaluate the safety and efficacy of IOLs. At the OTMN Lab we introduce the scanning light scatter profiler (SLSP) as a novel approach capable of quantitatively evaluating the scattering of light with a nearly 360˚ view. Employing the SLSP test methodology, we are aiming to simulate real situations by controlling the parameters of the light source as well as its angles of incidence. By doing so, the SLSP method can lead to a more effective preclinical approach for the evaluation of scattering light as it passes through IOLs.The CLM and SLSP techniques are two unique approaches to preclinically evaluate IOL optical properties. As a non-clinical approach, these techniques can potentially be used to evaluate IOLs before implantation, resulting in fewer negative patient reports and ultimately improve overall public health.

44. Comprehensive Preclinical Evaluation of Mechanical Properties of Transvenous Cardiac Leads

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Plain Language Synopsis: We are working to identify the major factors responsible for lead perforation and lead conductor fracture, as part of the development of a standard test method that more accurately reflects the true service conditions of cardiac leads.

Abstract: Over 300,000 pacemaker and implantable cardioverter defibrillator (ICD) procedures are performed in the US each year, including implantation of a transvenous lead in the heart to treat various cardiac arrhythmias. Dangerous and sometimes fatal failures can occur with leads, including perforation of the heart

by the lead tip, fracture of the conductor wires that transmit current to and from the heart, and abrasion of the lead insulation materials. It is generally acknowledged within the cardiac leads industry that present standards for mechanical bench testing of leads do not adequately address these potential modes of failure. For example, in 2007 a marketed lead was recalled due to the risk of conductor fractures even though the lead had been assessed using methods similar to those drafted in industry-recognized standards before approval. We are currently collaborating with industry, academia, and the Association for the Advancement of Medical Instrumentation (AAMI) to develop improved preclinical test methods to distinguish cardiac lead designs that have the potential for life threatening failures from those that are safe and effective. Specifically, we are working to identify the major factors responsible for lead perforation and lead conductor fracture. To do this, we are developing new test methods that include conditions that may more closely simulate the in vivo mechanical environment experienced by the leads. We are investigating whether those test conditions affect the data generated during our leads testing. We will share our latest results on the effect of simulated anatomical constraints on the loads at the distal lead tip under static and dynamic loading conditions, using a tissue substitute and commercially available leads. Our experiments show that factors such as including a constraint to simulate the effect of right ventricular contraction during systole, and even the way the leads are held in the test set-up, will affect the data. We will also share the results of screening studies with AAMI on tissue substitute properties (related to perforation) and lead body stiffness testing (related to fatigue fracture of the lead conductors).

45. Comparison of Methods for Quantitative Evaluation of Endoscopic Distortion

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Plain Language Synopsis: Endoscopes are commonly used throughout the medical field to either assist in performing surgeries or screen for various diseases. However, the industry lacks regulations to evaluate an endoscope's optical performance characteristics, such as geometric distortion. We evaluated existing methods of distortion evaluation and developed a new method. This study will help to improve upon, evaluate, and compare current technologies, and to ensure the quality of all endoscopes in clinical use.

Abstract: Endoscopy is a well-established paradigm in medical imaging, and emerging endoscopic technologies such as high resolution, capsule and disposable endoscopes promise significant improvements in effectiveness, as well as patient safety and acceptance of endoscopy. However, the field lacks practical standardized test methods to evaluate key optical performance characteristics (OPCs), in particular the geometric distortion caused by fisheye lens effects in clinical endoscopic systems. As a result, it has been difficult to compare an endoscope's image quality or assess its changes over time. The goal of this work was to identify optimal techniques for objective, quantitative characterization of distortion that are effective and not burdensome. Specifically, we compared distortion measurements from a commercially available distortion evaluation/correction software package with a custom algorithm based on a local magnification (ML) approach. Measurements were performed using both a clinical gastroscope and a capsule endoscope to image square grid targets. Recorded images were analyzed with the ML approach and the commercial software where the results were used to obtain corrected images. Corrected images based on the ML approach and the software were compared qualitatively by visualization and quantitatively by comparing them with undistorted images. The effects of target grid size and distance were evaluated as well. The study showed that the ML method can assess distortion patterns more accurately than the commercial software. Overall, the development of standardized test methods for characterizing distortion and other OPCs will facilitate development, clinical translation, manufacturing quality and assurance of performance during clinical use of endoscopic technologies.

46. Development of an In vitro Release Testing Method for Predicting In vivo Performance of Risperidone Microspheres

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Plain Language Synopsis: Microsphere-based drug delivery systems are novel complex dosage forms, whose in vivo performance is sensitive to manufacturing changes. We developed an in vitro release method to identify the effect of process variables on critical quality attributes of qualitatively and quantitatively equivalent microspheres and to predict their in vivo performance.

Abstract: Introduction and Regulatory Science Importance: Microsphere drug delivery systems are complex dosage forms, designed to deliver drugs at a controlled rate to optimize efficacy. However, due to their complex formulation and manufacturing process, the critical physicochemcial characteristics of complex parenteral microsphere drug products are sensitive to even minor manufacturing changes (e.g., manufacturing site or instrumentation changes). Consequently, formulations which are qualitatively (Q1) and quantitatively (Q2) the same in composition may not be bioequivalent. Accordingly, it is crucial to understand and develop appropriate in vitro performance testing methods that can identify the effect of process variables on critical quality attributes of Q1/Q2 equivalent microsphere formulations and predict their in vivo performance. This in vitro-in vivo correlation (IVIVC) could be useful for sponsors and the Agency to: 1) ensure product quality when post approval manufacturing changes occur, and 2) facilitate the development of generic microsphere products. The objective of the current study is to develop an IVIVC for Q1/Q2 equivalent risperidone-loaded microspheres that are manufactured differently. Methods, Results and Conclusion: PLGA with similar molecular weight as that of the commercial product, Risperdal Consta, was used to prepare Q1/Q2 equivalent risperidone microspheres with manufacturing differences. Critial physicochemcial properties of the prepared Q1/Q2 equivalent microspheres were determined. Three prepared risperidone-loaded microsphere formulations had similar drug loading (~37%). Formulations 1 and 2 had similar particle size, which was larger than that of Formulation 3. Morphology and porosity studies revealed that Formulation 1 was less porous compared to Formulations 2 and 3 despite that Formulation 1 and 2 had similar particle sizes. These results indicated that the critical physicochemical properties of risperidone-loaded microspheres with Q1/Q2 equivalence were very sensitive to manufacturing differences. The prepared microspheres were administered in rabbits to obtain in vivo release data. A Level A IVIVC was established by testing in vitro release using USP apparatus 4 and simulating in vivo release using a deconvolution approach. Results demonstrated that the developed in vitro release testing method using USP apparatus 4 can differentiate Q1/Q2 equivalent risperidone microspheres with distinct physicochemical properties and most importantly, predict the in vivo performance of these microspheres.

47. Developing Standardized Methods for Determining the Flexural Rigidity of Surgical Meshes

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Plain Language Synopsis: Our objective is to develop standardized methodology to determine both the stiffness of surgical mesh and factors that may affect it. Defined methodology may assist manufacturers in evaluation of their devices and facilitate pre-market submission reviews through streamlined inter-device comparisons, thereby benefitting public health by improving access to new devices.

Abstract: Over 300,000 Americans each year receive surgical intervention to treat conditions like urinary incontinence (UI, in males & females) and pelvic organ prolapse (POP, in females). Both conditions have considerable public health impact, including diminished sexual, urinary, and defecatory function in women and poor self-rated health and quality of life, social isolation, and depressive symptoms in both men and

women. Surgical intervention for UI and POP can include implantation of surgical mesh. There has been a dramatic increase in reported adverse events for surgical mesh indicated for UI and POP, with mesh erosion being the most common mesh-specific event. We believe mesh stiffness influences erosion through tissue; and therefore, development of improved methods to evaluate stiffness is crucial. Currently, premarket applications for surgical mesh devices to treat UI and POP contain test methods and values that vary greatly between devices, making device comparisons difficult. There is also a general lack of understanding of the effects of stiffness and mesh characteristics on device functionality. The overall goal of this project is to develop standardized test methods for characterizing surgical mesh mechanical behavior and to understand how different mesh characteristics (e.g., pore size, density, and filament diameter) affect the mechanical characteristics. Representative polypropylene knit mesh samples were acquired over a range of filament diameters, pore sizes, and knit patterns. Samples were tested using a modified version of ASTM D1388 to determine the flexural rigidity (i.e., bending stiffness) of the meshes along different material directions (machine, cross-machine, and 45° to machine). Maximum stiffness measurements correlated well with filament diameter and mesh density with most example mesh exhibiting anisotropic behavior, that is, different apparent stiffness values depending on the material direction. Interestingly, stiffness values were also found to vary depending on which surface (i.e., top vs. bottom) was facing upward during testing with some values being up to 6 times larger compared to the corresponding opposite surface. The methods developed in this project will provide a simple and standardized test to determine the flexural rigidity of surgical mesh for UI and POP and provide a means to compare devices in subsequent submissions.

48. Comparison of Two Sampling Practices for Phenytoin Sodium Content Assessment in Powder Blends: Sampling Errors and Potential Solutions

Wu, Huiquan, FDA/CDER/OPQ; Sowrirajan, Koushik, FDA/CDER/OPQ; Faustino, Patrick, FDA/CDER/OPQ; Khan, Mansoor, FDA/CDER/OPQ

Plain Language Synopsis: Pharmaceutical Powder Blending Process Assessment; Sampling practice; Sampling error; Potential solutions.

Abstract: Purpose: Phenytoin Sodium (PS) is a Narrow Therapeutic Index drug. It is an ideal model drug for powder blending uniformity and content uniformity study due to its cohesive nature. However, the accuracy of drug content assessment of powder blends can be complicated by particle size, sampling method, and wet assay method employed, etc. The objective of this study is to examine the effect of sampling practices on the accuracy of PS content assessment. Methods: A model system of PS-MCC-Lactose with fixed compositions and fixed PS particle size range was used. The particle size distribution of PS and excipients was characterized by a Sympatec Rodos/L system. The model system with three different particle size ranges of MCC and Lactose (specified by a full factorial design) was executed in a KG-5 high shear mixer at 200 RPM for 60 minutes. The final powder blends were sampled by thief sampler and spatula separately and subjected to HPLC analysis which was in-house validated for PS content. Results: The HPLC method was validated according to USP 1225 category I requirements and met all acceptance criteria. The method demonstrated acceptable analytical repeatability (less than 0.4%). However, for the same blends, significant differences in PS content results were observed between the thief sampling method and spatula sampling method: the averaged deviation from the expected PS content across all blends was 30% for samples collected by thief sampler, and was 1.3% for samples collected by spatula. The mean residual standard deviation of the PS content among the triplicates of blends was much larger for thief sampler (11.64%) than spatula sampler (3.44%). The particle size ranges for PS, MCC, and lactose was correlated to the observed measurement bias. Conclusions: This study suggests that the thief sampler has a sampling bias towards larger excipient particles, hence neglecting the smaller PS particles. Therefore, thief sampler provides significantly lower accuracy of PS content assessment than spatula sampling. This study highlights the critical importance of selecting an appropriate sampling method for accurately assaying drug content in powder blends and thus evaluating

associated powder blending homogeneity during pharmaceutical development and manufacturing.

49. A Quality-by-Design (QbD) Approach to Assess the Effect of Wet Granulation Process Variables on the Granule's Quality and its Yield for a Model Formulation

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Plain Language Synopsis: Pharmaceutical wet granulation process; Effect of wet granulation process variables; Granule quality attributes; Wet granulation process yield.

Abstract: Purpose: Granulation is the key unit operation required to improve the flowability and tableting of drug. Among the granulation methods, wet granulation is the most widely used approach. Depending on the physicochemical properties of drug and the formulation system, wet granulation process needs to achieve a balance between the requirements of both the yield and the granule's quality. In this study, the effects of wet granulation process variables on the granule yield and the granule quality were investigated systematically by using Quality-by-Design (QbD) approach. Methods: Ibuprofen (IBU), three excipients (microcrystalline cellulose (MCC), lactose monohydrate and polyvinyl pyrolidone (PVP) and water as granulating fluid were selected as the model formulation components further mixed and granulated in high shear granulator. For better understanding of process variables, a three factor two level full factorial design (impeller speed X1, chopper speed X2 and granulation time X3) with three center points has been executed. The response variables include mean particle size, yield (mass ratio of granules smaller than 1.2 mm), lump ratio (mass ratio of granules bigger than 1.2 mm), and hold-up ratio (mass ratio of wet powder attached to granulator wall and/or bottom which is hard to remove), sphericity (determined by SYPATEC imager), flow property and true density. ANOVA was performed to determine statistically significant variables for each response variables. Results: This study showed that impeller speed, granulation time, and their interaction have positive significant effect on mean particle size of granules. The impeller speed has significant positive effect on yield, and negative effect on hold-up and lump ratio. Chopper and impeller speed interaction and chopper speed-time interaction have positive and negative effect on lump ratio, respectively; impeller speed -time interaction has significant negative effect on hold-up ratio. In addition, analysis of these interactions revealed that for a specific set of wet granulation process parameters, the chopper orientation is critical. Conclusion: Statistically significant wet granulation process variables and their interactions have been identified for multiple response variables using a QbD approach. A critical issue of chopper orientation pertinent to the high shear granulator was identified and explained.

50. Applying PAT for Pharmaceutical Powder Blending Process Assessment: Challenges and Opportunities

Wu, Huiquan, FDA/CDER/OPQ

Plain Language Synopsis: Pharmaceutical Powder Blending Process Assessment; Process Analytical Technology (PAT); Real-time Process Monitoring and Control; Challenges and opportunities.

Abstract: Pharmaceutical powder blending presents challenges for high risk category products such as highly potent drugs, low dose drugs, drugs with narrow therapeutic indices (NTI), or drugs that have strong segregation or cohesion tendencies. Depending on the goals, applying PAT for pharmaceutical powder blending can help to achieve different levels of process understanding, such as real-time process monitoring and fault detection, process control strategies to ensure final product quality, or multivariate calibration model development and verification for real time testing and release, etc. When applying PAT for pharmaceutical powder blending, certain unique challenges are to be addressed from a multi-disciplinary or integrated approach. For example, i) materials aspect: inherent heterogeneity of pure components or mixture; ii) sampling aspect: sampling strategy, sample size, and scale of scrutiny; iii) measurement aspect: experimental errors associated with sampling practice, dynamic process environment, dynamic measurement environment; and iv) analytical aspect: appropriate reference method establishment. Unprecedented opportunities exist for innovation and process understanding. For example, particles with inherent heterogeneity (due to for example morphology difference) or

strong segregation tendency (due to for example particle size difference) may call for particle engineering strategies (such as milling), novel formulation strategies, or innovative equipment/process design strategies such as continuous processing. In this presentation, case studies based on FDA research results will be presented to highlight several technical issues, such as dynamic measurement errors, sampling strategy (sample location and sample size), sampling bias, analytical verification, general linear modeling for process design space development, and scale-up effect. The effect of process variables on the dynamic measurement errors, design space, and scale-up will be discussed. Finally, a science- and risk-based approach for pharmaceutical powder blending will be discussed from a regulatory science perspective.

51. Advancing Quality Measurement of Therapeutic Glycoproteins

Zhang, Lei, FDA/CDER/OBP/DBRRIV; Luo, Shen, FDA/CDER/OBP/DBRRIV; Bozza, William, FDA/CDER/OBP/DBRRIV; Zhang, Baolin, FDA/CDER/OBP/DBRRIV

Plain Language Synopsis: Glycan moieties of therapeutic glycoproteins are critical product attributes that must be adequately analyzed and controlled to ensure product quality and manufacturing consistency. The current methods (e.g., MS, HPLC) detect the released glycan(s) from a glycoprotein which lack the required accuracy and robustness. We are developing a lectin-based microarray platform for direct glycan profiling of intact glycoproteins.

Abstract: Glycan moieties of therapeutic glycoproteins are a critical product attribute that can affect protein folding, bioactivity, pharmacokinetics, and immunogenicity. Therefore, glycan variants attached to a protein must be adequately analyzed and controlled to ensure product quality and manufacturing consistency. The current methods rely on mass spectrometry (MS) and high-performance liquid chromatography (HPLC) to detect the released glycan(s) from a glycoprotein. However, these methods involve a lengthy process of enzymatic digestion followed by separately analyzing the resulting glycans and proteins. Importantly, it has been difficult to achieve 100% release of glycans from a glycoprotein, leaving the uncleaved glycans unaccounted. This project aims at developing a lectin-based microarray assay to directly measure glycan profiles onto intact glycoprotein products. Using commercial lectin chips, we have evaluated the suitability of the lectin-based microarray in determining glycan profiles of 12 FDA approved glycoproteins. We demonstrated distinct glycan profiles for individual products, which are generally consistent with the known glycan patterns as determined by MS/HPLC methods. This presentation will describe the lectin-based technology in glycan analysis with eM.P.H.asis on its potential use in characterizing glycan variants of therapeutic glycoproteins. By including additional products, the data accumulated could lead to glycan "fingerprints" for relevant therapeutic glycoproteins. This work has the potential to deliver an innovative platform for a rapid, robust, and sensitive measurement of glycan variants onto an intact glycoprotein without the need of clipping glycans. Such a method could be adapted for use as characterization or lot release assays of glycoprotein products. The ability of directly measuring glycan variants will help FDA reviewers in making more informed regulatory decisions regarding lot-to-lot consistency and similarity or difference in glycosylation patterns between a biosimilar candidate and its corresponding reference product.

52. Physiologically-Based Pharmacokinetic (PBPK) Modeling of Liposomal Doxorubicin

Li, Min, FDA/CDER/OPQ/IO/SRS; Zou, Peng, FDA/CDER/OPQ/IO/SRS; Tyner, Katherine, FDA/CDER/OPQ/IO/SRS; Lee, Sau, FDA/CDER/OPQ/IO/SRS

Plain Language Synopsis: Physiological-Based Pharmacokinetic (PBPK) modeling was used for predicting pharmacokinetics and biodistribution of liposomal doxorubicin in mice following IV administration and then extrapolated to humans by interspecies scaling. The developed model provides a tool for optimizing liposomal doxorubicin therapy. The PBPK model can be potentially applied to other nanoparticle products.

Abstract: This project investigated the pharmacokinetics of liposomal doxorubicin in mice following IV administration using a Physiological-Based Pharmacokinetic modeling (PBPK) framework and then extrapolating the

results to predict the drug's pharmacokinetics and biodistribution in humans. The whole-body PBPK model for mice, which can predict pharmacokinetics and biodistribution of released and encapsulated doxorubicin, was constructed with 10 organ compartments, with each compartment divided into vascular and extravascular sub-compartments. Different sub-compartment models were used to represent the specific transport mechanisms in individual organs. For tissues with mononuclear phagocyte system (MPS) uptake of liposomes (liver, spleen, lung and bone marrow), an uptake clearance (CLup) was used to represent the unidirectional active uptake of liposomes. A tumor-specific passive partition coefficient and a permeability-surface area coefficient were assigned to illustrate the enhanced permeability and retention effect in the tumor tissues. For the other tissues, a passive permeability mechanism of liposome transport is assumed between the vascular and extravascular sub-compartments. The release rate constants of doxorubicin were set to be tissue-specific, which reflected various drug release mechanisms in individual tissues. Subsequently, this mouse model was successfully extrapolated to humans by interspecies scaling of model parameters. In general, the results showed that the PBPK models could accurately predict the plasma and tissue concentrations both in mice and humans. The human pharmacokinetics of liposomal doxorubicin calculated from simulated data, e.g. plasma PK parameters of AUC, t1/2, CLtot, Vss, were highly consistent with the independent experimental data reported in the literature after i.v. administration of DOXIL® to human subjects. To ensure that the models reflect the transport mechanisms of liposomes and different drug release kinetics in various tissues, parameter sensitivity analysis was performed, which revealed that the uptake clearance of liposomes in liver (CLup,li) andspleen (CLup,sp), as well as the release rate constant in blood and normal tissues (K1) are critical for the pharmacokinetics of liposomal doxorubicin. The developed PBPK model can be used for optimizing liposomal doxorubicin therapy and assessing safety risks in humans for different formulations of liposomes. In addition, the PBPK model can be modified and potentially applied to other nanoparticle products.

Session 6: Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Posters 53-66 are located in Section A, Posters 67-73 are located in Room 1406 and Posters 74-77 are located in Room 1408)

53. Embodiment, Performance, and Preference: Patient-centered Regulatory Science for Brain-controlled Prosthetics

Benz, Heather, FDA/CDRH; Kilpatrick, Elizabeth, FDA/CDRH; Civillico, Eugene, FDA/CDRH

Plain Language Synopsis: We are establishing quantitative, objective measures to aid regulatory decision-making about novel upper-limb prosthesis technology. These measures will help to compare functional outcomes between novel prostheses, existing prostheses, and intact limbs; understand amputee needs, preferences, and risk tolerance; and assess upper limb amputees' sense of prosthesis ownership.

Abstract: Upper limb amputees' needs are not being met by existing prostheses, as evidenced by prosthesis rejection, non-wear, and user reports of restricted activities. Emerging technologies such as novel robotic limbs, implantable electrodes for improved control, and electrical stimulation for sensory feedback may confer significant improvements, but also pose risks. Evaluation of these technologies to maximize public health requires articulation of novel benefits in a quantitative and patient-centered way. We are developing tools for this quantitative, patient-centered evaluation along three main axes of research: (1) advancing functional outcome measures of arm and hand performance, (2) assessing benefit and risk preferences in a candidate user population, and (3) understanding the sensory conditions that lead to prosthesis acceptance. Here we provide an update on these early-stage efforts. Some outcome measures have been validated in amputee populations, but benchmarks in individuals with functioning, intact limbs are lacking. We aim to establish variability and reproducibility of existing and novel outcome measures in able-bodied subjects and in amputees. These results will improve the design of clinical trials for upper limb prostheses, facilitate device review, and inform patient and clinician prosthesis decisions. We are developing an open-ended interview and survey to assess patient preference and risk tolerance for upper limb prostheses. This work extends approaches developed within CDRH to assess patient preferences for weight-loss devices, and incorporates advances in patient-centered benefit-risk assessment led by the Medical Device Innovation Consortium. Amputees' sense of prosthesis ownership, or embodiment, may be linked to prosthesis acceptance and effectiveness. Research indicates that embodiment may be a quantifiable cognitive phenomenon; this opens the possibility of systematically assaying it for the development and evaluation of restorative devices. To investigate such an assay, we characterize subject responses to convergent sensory inputs using electroencephalography, self-report of limb location and perception, and changes in limb temperature. By varying stimulus properties and observing the resulting physiology and behavior, we aim to elucidate "cognitive windows" for incorporating an artificial limb into the body image. These three research directions share a common goal: the advancement of medical device development tools in preparation for the most innovative devices.

54. Long-term Skin Morphological Changes After a Single Sunburn-inducing UV Exposure Underscores Importance of Avoiding Sunburns for Skin Cancer Prevention

Coelho, Sergio G., FDA/CDER/OND/ODEIV/DNDP; Miller, Sharon A., FDA/CDRH/OIR/DRH; Hearing, Vincent J., NIH/NCI; Michele, Theresa M., FDA/CDER/OND/ODEIV/DNDP

Plain Language Synopsis: Sunburn exposures may induce long-term changes to human skin which are sustained, and sufficient to produce long-term skin color differences. These modifications have similar characteristics to age spots, which are known risk factors for precancerous lesions. These results support reduction of UV overexposure in skin cancer prevention public health messages.

Abstract: Human skin functions as a dynamic external barrier continuously responding to stimuli while maintaining homeostasis. Not surprisingly, as diverse as the skin color gradient is among

people, so is their response to ultraviolet radiation (UV) either from the sun and/or from artificial UV sources such as sunlamps. Of particular concern in the last 15 years, has been the increased incidence rate of melanoma in fair-skinned women ages 20-49 compared to their male counterparts of the same age group. Although UV-induced pigmentation fades over time, a portion of the population develops a persistent form of pigmentation termed long-lasting pigmentation (LLP) that lasts for months to years. It is unclear whether this long-term effect may or may not be involved in carcinogenic progression in skin malignancies. Since no long-term evaluation of single UV exposures had ever been done, the goal was to investigate whether there were any UV-induced long-term changes in terms of melanogenesis, cell proliferation, signaling and/or skin morphology. Six individuals (3 LLP+ and 3 LLP-) were evaluated more than 9 months after a single sunburn-inducing UV exposure. Our results suggest that the hyperpigmented basal layer, increased proliferation by a subset of keratinocytes and increased rete ridge formation are a function of skin cells trying to maintain normal skin homeostasis due to impaired pigment removal. In addition, the increases in interdigitation at the epidermal-dermal junction are a function of basement membrane plasticity through decreased hemidesmosome density and attenuation of hemidesmosomal partners (integrin a6b4 and plectin). The fact that a single sunburn-inducing UV dose led to increased long-term visual pigmentation along with similar histopathological features to other forms of UV-induced hyperpigmentation, i.e. age spots or solar lentigos, which are known risk factors for precancerous lesions, merits further inquiry. Our results further validate public health messages advising the use of multiple methods of practical prevention against UV overexposure.

55. Endothelial Biocompatibility of Biodegradable Polymers

Hancock, Galen, The George Washington University; Dutta, Debargh, FDA/CDRH; Wood, Steven, FDA/CDRH; Young, Megan, FDA/CDRH; Jamison, Joshua, FDA/CDRH; Tesfamariam, Belay, FDA/CDER

Plain Language Synopsis: The aims of this study were to develop a system of assays suitable for characterizing biocompatibility of biodegradable polymers and drugs using measures of cell death, cell stress, and proinflammatory mediators in endothelial cells.

Abstract: Background: Biodegradable polymer-based scaffolds have emerged as an alternative to the permanent metallic device implants to treat a transient vascular healing problem. The development of a suitable polymer has been challenging because it must exhibit vascular biocompatibility while allowing time- and dose-controlled antiproliferative drug release such that complete healing is achieved. The aims of this study were to characterize intimal biocompatibility of biodegradable polymers and drugs using screening assays of apoptosis, oxidative stress, proinflammatory mediators, adhesion molecules, prothrombotic and antithrombotic mediators in endothelial cells. Method: Endothelial cell line EAHy926 were cultured in chamber slides coated with poly-DL-lactide (PDLA) and paciltaxel for seven days. Flow cytometric analysis was used to measure cytotoxicity, apoptotosis (annexin V and 7-amino-actinomycin D staining, 7-AAD), nitrotyrosine expression, thrombomodulin, tissue factor, cell adhesion molecules (CD31/PECAM-1, CD62E/P-selectin and CD162/PSGL-1), activated protein C receptor (CD201) and co-stimulation modulators (CD154/CD40L, CD27/TNF receptor). Results: Treatment of endothelial cells with PDLA and paclitaxel induced increase in annexin V expression to 4.43% compared to 0.33% in control, but not 7-AAD stained dead cells, indicating enhanced apoptosis. Paciltaxel alone and in combination with PDLA showed upregulation of tissue factor (38.8%, control of 7.37%) and downregulation of thrombomodulin (86.8%, control of 98.5%). Endothelial cells incubated in PDLA and paciltaxel showed a marked increase in nitrotyrosine expression (61.8%, 10.1% in control) and exhibited an apparent cell death compared to the negative control. Paciltaxel alone or in combination with PDLA showed upregulation of microtubule-associated protein 1A/1B-light chain 3 and p62 protein (56.4%, 7.21% in control) indicating stimulation of autophagy. PDLA and paciltaxel had no significant effect on the expression of cell adhesion molecules, activated protein C receptor, or co-stimulation modulators.

Conclusion: The results indicate that PDLA and paciltaxel induce nitrative oxidative stress, stimulate autophagy, promote prothrombotic mediators, and cytotoxicity in endothelial cells. The study eM.P.H.asizes endothelial biocompatibility screening of newly evolving biodegradable polymers as drug carriers and bioresorbable vascular scaffolds.

56. Improving Proarrhythmic Risk Assessment of Drugs: Combined Retrospective and Prospective Clinical Studies of 42 Drugs

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Plain Language Synopsis: Disruption of the electrical activity of the heart, measured by an increased QT interval on the electrocardiogram, determines if a drug increases the risk for a fatal heart rhythm. Because multiple drugs increase QT without increased risk, we developed a better biomarker through retrospective analysis and two prospective clinical studies.

Abstract: Background: Fourteen drugs have been removed from the market because of risk for torsade de pointes, a potentially fatal ventricular arrhythmia. Almost all drugs that cause torsade block the human ether-a-go-go-related (hERG) potassium channel and prolong the QT interval in the electrocardiogram (ECG). Some drugs, however, block the hERG potassium channel but exhibit minimal QT prolongation and have a low risk for torsade due to additional inward current block: late sodium (amiodarone, ranolazine) or calcium (verapamil). Therefore detection of the presence of additional inward block using the ECG has the potential to improve proarrhythmic risk assessment of drugs. Methods: A retrospective analysis of 34 thorough QT studies was conducted (17 QT prolongers and 17 drugs with no effect or QT shortening) and combined with ECG simulations. The retrospective analysis consisted of analyzing different ECG sub-intervals and correlating them with preclinical information for each drug. In addition to the retrospective analysis we also conducted two prospective clinical studies with the goal of evaluating either the sub-intervals identified in the retrospective analysis (study 1 and 2) and strategies for drug-induced QT prolongation mitigation (study 2). These prospective studies included a total of 8 drugs and 3 drug combinations. Results: The retrospective study showed that drugs which block inward currents (calcium or late sodium) preferentially shorten early repolarization, while hERG potassium channel block alone prolongs both early and late repolarization, consistent with simulations and both prospective clinical studies. The second prospective study also showed that co-administration of a late sodium current blocker (mexiletine or lidocaine) could shorten hERG block (dofetilide) induced QT prolongation by 20 ms due to shortening of early repolarization. Conclusions: We showed in a retrospective analysis that additional inward current block can be detected by evaluating the relationship between early and late repolarization measured on the ECG, which we confirmed in two prospective clinical studies. These findings demonstrate that the ECG can detect additional inward current block of relevance for torsade risk and that drug-induced QT prolongation can be mitigated by co-administration of a late sodium current blocker.

57. Identifying Predictive Biomarkers for Molecularly Targeted Cancer Therapies

Kim, Su-Ryun, FDA/CDER/OPQ/OBP; Zhao, Liqun, FDA/CDER/OPQ/OBP; Twomey, Julianne FDA/CDER/OPQ/OBP; Zhang, Baolin, FDA/CDER/OPQ/OBP

Plain Language Synopsis: Our data identify potential biomarkers for predicting tumor response to the death receptor targeted cancer therapies, thereby facilitating personalized cancer treatment towards better clinical outcomes.

Abstract: Death receptors (DRs), including TNF receptor 1 (TNFR1), Fas, DR4 and DR5, are attractive targets for cancer treatment. These receptors are characterized by a death domain that mediates activation of a caspase cascade and ultimately apoptosis in the target cells. DRs can be activated by agnostic antibodies or their cognate

ligands such as TNF, Fas ligand or TNF-related apoptosis inducing ligand (TRAIL). Currently, multiple clinical trials are underway to evaluate the antitumor activity of recombinant human TNF, TRAIL, and monoclonal antibodies against DR4 or DR5. These products have shown a tolerated safety profile in early clinical studies; however, their therapeutic potential is possibly limited by the emergency of drug resistance in tumor cells. This presentation will highlight our recent findings on molecular mechanisms rendering cancer resistance to the DR-targeted therapies. We provide evidence that DRs undergo constitutive endocytosis or become trapped in intracellular compartments (e.g., autophagosomes), resulting in an absence of DRs on plasma membrane. Notably, lack of surface DRs is sufficient to render cancer cell resistance to the DR-targeted therapies. Using a genomic approach, we demonstrated a correlation between the expression of oncogenic H-Ras GTPase and DR surface deficiency. Further, biochemical studies reveal a critical role of H-Ras in the regulation of spatial expression of DRs. These findings not only advance our scientific knowledge of cancer drug resistance but also provide a rational of biomarkers that help identify subpopulation patients that are likely responsive or resistant to the DR-targeted therapies. The acquired information also has implications in helping FDA reviewers in making more informed regulatory decisions regarding specific drug combination strategies and interpretation of clinical safety and efficacy data.

58. Evaluation of Hypothalamic-Pituitary-Adrenal Axis Suppression from Use of Prescription Topical Corticosteroid Products: the Need for Innovative Approach to Estimation of Risk

Ko, Hon-Sum, FDA/OMPT/CDER/OND/ODE3/DDDP; Temeck, Jean, FDA/OMPT/OSMP/OPT

Plain Language Synopsis: We reviewed topical corticosteroid labels for data on hypothalamic-pituitary-adrenal axis suppression. Earlier approved products relied on basal hormone tests. More recently approved products usually had stimulation testing with ACTH. There are challenges in the interpretation of risk from these study data, which calls for newer approaches in assessing this risk.

Abstract: Background and Objective: Topical corticosteroids have been available for the relief of the manifestations of inflammatory skin conditions for over half a century. Systemic absorption resulting in suppression of the hypothalamic-pituitary-adrenal (HPA) axis is a major concern, particularly in pediatric patients. Studies evaluating HPA axis function is an important part of the clinical development of topical corticosteroid products. We attempt to investigate the data pertaining to the effects on HPA axis function from the labeling of prescription topical corticosteroids. Materials and Methods: Of 152 new drug applications on topical products containing corticosteroid as active ingredient approved since 1957, there are 62 products currently not withdrawn or discontinued. Their labeling was reviewed for information on evaluation of HPA axis suppression. Results: Although the labeling for topical corticosteroid products routinely provide warning/precaution on the risk of HPA axis suppression from their use, specific evaluation information is rarely present in older labels, some of which may give urine hydroxycorticosteroid or plasma cortisol levels without details such as urine collection completeness, time of blood withdrawal, extent of corticosteroid use, or age range of the subjects studied. The labels of more recently approved products (~70%) usually contain a description of HPA axis suppression study/studies using ACTH stimulation (cosyntropin test) under pharmacodynamics, precautions, or pediatric use sections or subsections, and provide an estimate of risk, based on the population studied. Data were interpreted as indicative of HPA axis suppression by the product in almost all ACTH stimulation studies. However, there are challenges on data interpretation due to imprecision of sample collection, lack of adjustment of stimulation dose of ACTH with age, disagreements in defining normal response especially with respect to pediatric patients, and inconsistencies in follow-up to full recovery in patients showing “suppression.” These may lead to over- or under-estimates of the true risk. Innovative approaches for more accurate assessment especially in pediatric patients would be useful. Conclusions: Review of topical corticosteroid labels has revealed challenges in the interpretation of HPA axis suppression data which may affect risk estimation. This calls for the need to develop newer approaches in the assessment of this risk.

59. Quantitative Imaging Biomarkers: application to lung nodule volumetry

Li, Qin, FDA/CDRH; Gavrielides, Marios, FDA/CDRH; Zeng, Rongping, FDA/CDRH; Myers, Kyle, FDA/CDRH; Sahiner, Berkman, FDA/CDRH; Liu, Songtao, FDA/CDRH; Gong, QI, FDA/CDRH; Petrick, Nicholas, FDA/CDRH

Plain Language Synopsis: We performed statistical analyses of CT lung nodule volumetry based on data from a large phantom study. We ranked factors that contribute to measurement error and validated a subgroup that achieved a clinically relevant performance claim. Our results contribute to standardization of data acquisition and validation of quantitative imaging biomarkers.

Abstract: There has been great interest in quantitative imaging biomarkers (QIB) over the last decade, defined as "the extraction of quantifiable features from medical images for the assessment of normal or the severity, degree of change, or status of a disease, injury, or chronic condition relative to normal", according to Radiological Society of North America. The use of volumetric CT as a QIB has been examined over the last decade, with the hope that it will provide a more accurate and consistent clinical metric for assessing lung nodule size and change in size. However, a number of factors can affect measurement uncertainty for this task, including nodule characteristics, imaging conditions and the selected estimation tools/software. A number of studies have been conducted to examine the effects of these factors on the associated measurement error. However, the limited size of any individual study and the difference in analyses among the various studies make it difficult to consolidate results across the studies and to use the reported results in the design of a larger, more definitive study. As part of our Quantitative Imaging project, we have performed a series of well-controlled systematic phantom studies investigating the impact of the aforementioned factors on nodule volumetry. Phantom studies provide a framework with reference standard available regarding the true size of nodules, thus allowing for the analysis of accuracy in addition to precision. In this poster, we present statistical analysis results based on about 40,000 measurements obtained from our phantom studies. The analysis methods follow the Quantitative Imaging Biomarker Alliance (QIBA) recommendation for technical assessment of a QIB. The outcomes of this comprehensive study are (1) we quantified and ranked the sources of measurement error for volumetric CT and (2) we validated performance on a subgroup meeting the QIBA Profile conditions (document defining conditions under which a specific QIB performance claim is achievable). The results of this work contribute to the standardization of imaging protocols for minimizing the measurement error of CT lung nodule volumetry. More generally, they provide a paradigm for assessing QIBs and facilitate the transformation of radiology into a more quantitative science.

60. Evaluation of the Therapeutic Index of Sirolimus

Lin, Ho-Pi, FDA/CDER; Zhang, Xinyuan, FDA/CDER; Kinjo, Minori, FDA/CDER; Fang, Lanyan, FDA/CDER; Jiang, Wenlei, FDA/CDER; Lionberger, Robert, FDA/CDER

Plain Language Synopsis: Providing affordable medications through approval of generic products to assure the quality of public health in United States is an important mission of FDA. Generic drugs with narrow therapeutic index (NTI) properties require more stringent regulation. This presentation summarizes our efforts to evaluate sirolimus against critical characteristics of NTI.

Abstract: Objective: The purpose of this work is to evaluate sirolimus against the critical characteristics of narrow therapeutic index (NTI) drugs. Background: NTI drugs are those drugs where small differences in dose or blood concentration may lead to serious therapeutic failures and/or adverse drug reactions that are life-threatening or result in persistent or significant disability or incapacity. Currently, European Medicines Agency and Health Canada classify sirolimus as an NTI drug, and apply tighter BE standards. Methods: We reviewed data from the drug product label, literature, and as well as relevant new drug applications (NDA) and abbreviated new drug applications (ANDA) to evaluate the following characteristics of NTI drugs: 1) there is little separation between therapeutic and toxic blood concentrations; 2) sub-optimal concentrations or doses lead to serious therapeutic failures or severe adverse

events; 3) therapeutic drug monitoring is recommended; 4) the within-subject variability is low-to-moderate (i.e. CV is less than 30%); and 5) in clinical practice, dose is often adjusted in very small increments in the maintenance stage (such as less than 20%). Results: The target sirolimus trough concentrations are recommended to be 16 to 24 ng/mL for the first year following transplantation, and 12 to 20 ng/mL thereafter, for patients at low-to moderate-immunologic risk following cyclosporine withdrawal. In one clinical trial targeting high-immunologic risk patients, the target concentrations were adjusted to be within 10-15 ng/ml. Sub-optimal concentrations of sirolimus lead to serious therapeutic failures, i.e. acute rejection, and adverse drug reactions, such as thrombocytopenia and hyperlipidemia. Therapeutic drug monitoring is recommended for all patients taking sirolimus. The mean within-subject variability estimated from the residual coefficient of variation (%CV) in two-way crossover studies for Cmax and AUC0-72 are 18.4 (range 10.0%-25.9%) and 14.2% (range 10.6%-20.2%), respectively (Table 1). Clinical practice data collection regarding dose adjustment in the maintenance stage is still ongoing. Conclusion: Based on the currently available data, we conclude that sirolimus satisfies the first 4 criteria as listed above, but there is limited data on sirolimus dose adjustments in the maintenance stage.

NOTE: Numbers 61-66 are skipped intentionally

67. Enabling Integrated Analyses of Hypoglycemic Agents through Standards and Technology

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Plain Language Synopsis: Combining trial data allows an assessment of outcomes by subgroups. Standardized data and computational resources enables these analyses.

Abstract: Section 907 of FDASIA directed FDA to report an integrated summary of safety and efficacy by demographic subgroups (age, gender, and race). However, the limited representation of subgroups in submitted new drug applications (NDAs) is often insufficient to enable required safety and efficacy analyses by subgroup. One possible way to overcome this limitation is to pool data from clinical trials across an NDA or across multiple NDAs. The Office of Translational Sciences in the Center for Drug Evaluation and Research (CDER) at FDA converted legacy data from 51 pivotal clinical diabetes trials submitted in support of NDAs into the Analysis Data Model (ADaM). We selected and aggregated specific clinical trials from these converted NDAs and presented safety and efficacy analyses by demographic and treatment subgroups.

68. Comparison of Two Standalone Computer-Aided Detection Systems at Multiple Operating Points

Sahiner, Berkman, FDA/CDRH/OSEL/DIDSR; Chen, Weijie, FDA/CDRH/OSEL/DIDSR; Pezeshk, Aria, FDA/CDRH/OSEL/DIDSR; Petrick, Nicholas, FDA/CDRH/OSEL/DIDSR

Plain Language Synopsis: We have investigated a new statistical method for performance comparison between two computer-aided diagnosis (CADe) devices. The method compares devices at multiple operating points, a feature introduced in some newly-designed CADe systems. Our simulation results indicate that the new method has a substantial advantage over more traditional methods.

Abstract: Computer-aided detection (CADe) devices aim at improving the performance of clinicians in abnormality detection by providing an objective and consistent second opinion regarding the presence of abnormalities in medical images. A typical CADe device automatically detects regions of interest, and then calculates a suspiciousness score for each detected region. The device displays a mark on the location of the candidate abnormality to alert the clinician when the level of suspiciousness is above a fixed threshold. In the assessment as well as design of CADe systems, comparison of standalone performance of two systems is essential. Comparisons typically rely on sensitivity (rate of correctly-detected abnormalities) and the number of false-positives

(false marks) per image. One approach for comparison is to select the parameters of the two systems to yield a target false-positive rate that defines an operating point, and to compare the sensitivities at that operating point. Increasingly, CADe developers offer multiple operating points, which implies that multiple comparisons need to be carried out between two CADe systems. To manage the Type I error, multiple-comparison correction is needed for keeping the family-wise error rate (FWER) under control. An important aspect of controlling FWER is to account for the correlations that may exist among test statistics. The sensitivities of a single modality at different operating points are correlated. In addition, the sensitivities of the two modalities at the same or different operating points are also likely to be correlated. When test statistics are correlated, well-known methods for controlling the FWER are conservative. In this study, we compared the FWER and power of three methods, namely the Bonferroni, step up, and adjusted step up methods in comparing the sensitivities of two CADe systems at multiple operating points. Previous investigations had evaluated the adjusted step-up method when the correlations are deterministically informed by the study design. A novel aspect of our study is that the correlations are estimated from the data. Our results indicate that the adjusted step-up method has a substantial advantage over the Bonferroni and step-up methods both in terms of the FWER and power, and that for moderate to large sample sizes, the FWER of the adjusted step-up method is very close to the target FWER.

69. Evidentiary Considerations for Qualification of Biomarkers at CDER: Lessons Learned

Sanyal, Sarmistha, FDA/CDER/OTS; Amur, Shashi, FDA/CDER/OTS; Noone, Marianne, FDA/CDER/OTS; McCune, Susan, FDA/CDER/OTS; Buckman-Garner, ShaAvhrée, FDA/CDER/OTS

Plain Language Synopsis: The Biomarker Qualification Program (BQP) at CDER was established as a drug development tool to qualify biomarkers to use them across multiple drug development platforms. The thoughts on general evidentiary considerations regarding qualification process will be presented.

Abstract: Qualification is a conclusion that within the stated Context of Use (COU), the results of assessment with a drug development tools (DDT) can be relied upon to have a specific interpretation and application in drug development and regulatory review. The Biomarker Qualification Program (BQP) at CDER is one of three currently available DDT qualification programs dedicated to providing a framework for the evaluation of biomarkers to be used to enhance the drug development paradigm. According to the DDT Qualification Guidance finalized in 2014, the qualification process includes three stages: initiation, consultation and advice and review of the full qualification package. To date, four sets of biomarkers have been qualified by BQP, with the first clinical biomarker qualified in 2014. The qualified biomarkers are encouraged to be used across multiple drug development platforms. One challenging aspect of the biomarker qualification process is the need for clear evidentiary standards for qualification of biomarkers. Some considerations are predicated on the evaluation of benefit and risk of using the biomarker in drug development. We describe lessons learned through qualification and thoughts on potential evidentiary considerations.

70. Can Drug-drug Interactions be Used to Inform Gene-drug Interactions and Vice Versa?

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Plain Language Synopsis: It is currently unclear under which circumstances drug-drug interactions (DDIs) can be used to reliably predict gene-drug interactions (GDIs) for polymorphic CYP enzymes. Our results show that for CYP2D6, DDIs generally predict GDIs and vice versa. However, CYP2C9 and 2C19 show discrepancies, which seem to be substrate, inhibitor or study related.

Abstract: Every year Adverse Drug Reactions (ADRs) are responsible for more than 100,000 cases of death, making it the 4th leading cause of death in the US. About 60% of drugs associated with ADRs are metabolized by polymorphic phase

I enzymes, which makes them susceptible to drug-drug interactions (DDIs) and/or gene-drug interactions (GDIs). It is a significant burden for industry to conduct both. The objective of this study was to delineate the circumstances under which DDIs can be used to reliably predict GDIs, and vice versa, of prototypical victim drugs and thereby reduce the regulatory burden on companies without jeopardizing drug safety for the American public. “Victim” drugs (preferably fraction metabolized > 0.8) were selected based on their clearance by the most common polymorphic cytochrome (CYP) enzymes 2D6 (atomoxetine, venlafaxine) and 2C9 (celecoxib). Strong inhibitors were selected as “perpetrator” drugs (CYP2D6: paroxetine, quinidine; CYP2C9: fluconazole). Drug-specific parameters were obtained from the literature and integrated into the commercially available physiologically-based pharmacokinetic (PBPK) software platforms PK-Sim® and Simcyp®. Developed PBPK models were externally qualified using clinical data in in extensive (EMs) and/or poor (PMs) metabolizers from the literature. Once developed and qualified, these models were used in simulations to determine if DDIs can be predicted based on GDIs and vice versa. Congruence between DDIs and GDIs was concluded if the observed clinical data was contained within the 90% model-predicted confidence interval of 100 DDI trial simulations. There was a congruence between GDIs and DDIs for the CYP2D6 victim drugs atomoxetine and venlafaxine using the strong CYP2D6 inhibitors paroxetine and quinidine, respectively. However, the situation was less clear cut for the CYP2C9 as DDI-GDI convergence was only achieved at 400mg but not 200mg fluconazole doses. Inhibition of CYP2D6 by strong inhibitors resulted in complete phenotypic conversion of EMs to PMs and respective DDIs were found predictive of GDIs. For CYP2C9, DDI-GDI convergence was not achieved even though DDIs used so-called strong inhibitors; convergence is dependent on the specific dose of the inhibitor and the sensitivity of the substrate drug. Future work will be conducted to evaluate the DDI-GDI convergence for CYP2C19 substrates.

71. Identifying Predictive Cancer Biomarkers to Promote Personalized Medicine

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Plain Language Synopsis: This project aims at characterizing circulating tumor cells (CTCs) as potential biomarkers for predicting tumor metastasis and therapeutic response.

Abstract: The recently launched Precision Medicine Initiative highlights the unmet need of predictive cancer biomarkers to identify patient populations that likely respond to a specific therapy. The main challenge in cancer biomarker discovery lies in the inherent heterogeneity of tumor cells between tumor types, individuals with the same tumor type, and within one tumor of a patient at any given time. The current approaches usually rely on limited samples of achieved primary specimens, such as single needle biopsy or surgical excision. Such specimens are unlikely to accurately capture the complete molecular landscape of a patient’s cancer. Moreover, quantitative biomarkers may be misleading, as they are based on the average expression across a heterogeneous tumor. This project aims at evaluating the biomarker potential of “Liquid tumors” - Circulating Tumor Cells (CTCs). CTCs are cancer cells which dissociate from the primary tumor and circulate within the peripheral blood. The total number of CTCs in the blood of individuals has been associated with poor prognosis of breast, colon and prostate cancers. However, little is known about the molecule characteristics of CTCs linked to tumor initiation and metastasis. CTCs exhibit a shifted phenotype from the parental tumor, developing the ability to survive in non-adherent conditions. We attempt to identify a distinct subpopulation of CTC cells with a stem-like phenotype. To this end, breast cancer cell lines are cultured in non-adherent culture conditions to mimic and differentiate cells towards a CTC phenotype and are then characterized for surface markers against CD44+/CD24-, CD44+/CD133+, ALDHbr, and EpCam+. The cells which exhibit the CTC cancer stem cell (CSC) phenotype are then tested for chemotherapy resistance in comparison to the parental cell line as well as the non-adherent CTC-like population that do not exhibit stem-like capabilities. Developing the ability to test chemotherapies against cells which exhibit the parental cancer type, a circulating tumor phenotype as well as the CSC

phenotype is expected to more accurately predict tumor response as well as the ability to reduce metastases. Development of predictive biomarkers will enhance the tailoring of oncology drugs to the appropriate patient population as well as provide new targets for drug development.

72. Comprehensive Analysis of Novel Electrocardiographic Device Algorithms for Differentiating Arrhythmic Risk

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Plain Language Synopsis: Electrocardiographic signatures of drug-induced ion channel block closely parallel the electrocardiographic signatures seen with congenital long QT syndromes. A combined approach of electrocardiographic device algorithms (ECG subintervals and morphology) along with multiple ion channel assessment may provide the greatest insight into drugs' proarrhythmic risk.

Abstract: Background: FDA recently cleared electrocardiographic (ECG) device algorithms that measure ECG morphology (e.g., T wave flatness, asymmetry and notching). These ECG morphology biomarkers were developed to identify abnormal T waves present on the ECG of congenital long QT syndrome type 2 patients (abnormal hERG potassium channel). Similar T waves have been observed with a limited number of hERG blocking drugs. Long QT and hERG block are associated with torsade de pointes, a potentially fatal arrhythmia. This study assesses nine ECG morphology biomarkers induced by hERG blocking drugs with high (dofetilide, quinidine) and low (ranolazine, verapamil) torsade risk. Methods: In a double-blind, randomized, placebo-controlled five-period crossover clinical study, twenty-two healthy subjects received a single dose of a pure hERG blocker (dofetilide) and three drugs that also block calcium or sodium (quinidine, ranolazine and verapamil) and placebo. At pre-dose and 15 time points post-dose, ECGs and plasma drug concentration were assessed. T wave flatness, asymmetry and notching were automatically assessed with QT-Guard+ (GE Healthcare), and five additional ECG morphology biomarkers were assessed by custom software. In addition, ion channel patch clamp experiments were performed to assess block of hERG, calcium (L-type) and late sodium currents for each drug. Results: Pure hERG block (dofetilide) and strong hERG block with lesser calcium and late sodium block (quinidine) caused substantial ECG morphology changes ($p<0.001$). Strong late sodium current and hERG block (ranolazine) still caused ECG morphology changes ($p<0.01$). Strong calcium and hERG block (verapamil) did not cause ECG morphology changes. At equivalent QT prolongation, multichannel blockers (quinidine and ranolazine) caused equal or greater ECG morphology changes compared to pure hERG block (dofetilide). Discussion/Future Work: ECG morphology changes are directly related to amount of hERG block, and multichannel block (quinidine and ranolazine) did not prevent T wave morphology changes. We are currently investigating whether pure late sodium or calcium blockers mitigate the ECG morphology changes induced by pure hERG blocking drugs. A combined approach of electrocardiographic device algorithms (ECG subintervals and morphology) along with multiple ion channels assessment may provide the greatest insight into drug-ion channel interactions and torsade de pointes risk.

74. Improving Transfusion Safety by Development of Comprehensive DNA Reference Panels for Blood Group Genotyping.

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Plain Language Synopsis: We are working on developing a genomic DNA reference panel for molecular blood group genotyping. The panel will be an invaluable tool for use in clinical genotyping as well as in biotech industry for assays manufacturing and quality assurance.

Abstract: Transfusion of allogenic red blood cells (RBC) required for treatment of some diseases can lead to hemolytic transfusion reactions in recipients due to alloimmunization. Extended

RBC matching is an effective strategy to reduce alloimmunization, but screening large numbers of donors for all potential antigens is impractical using traditional serological methods, and additional matching for rare antigens is limited. Molecular blood group genotyping has been gaining popularity as a more reliable method allowing high-throughput testing and typing of blood groups for which reagents for classical serological testing are not available. However, very limited reference materials currently exist for blood group molecular genotyping, which increases the probability of mistyping. Since a genotyping test is often done only once in a person's lifetime, consequences of such a mistake can be dire. In this project, we aim to develop genomic DNA reference panels that can be used in genotyping laboratories to improve accuracy and assure quality of their assays. We established EBV immortalized cell lines from leukocytes from selected blood donors recruited based on their historical phenotype and genotype. These cell lines will serve as an unlimited source for genomic DNA isolation and panel renewal. We performed genetic characterization on each sample by testing for each genetic polymorphism using a TaqMan assay, allele-specific PCR and Sanger sequencing so that the results can be independently confirmed. To date, we have tested 53 blood donors for 39 single-nucleotide polymorphisms with TaqMan assays. Genetic sequencing of 16 blood donors covering 39 polymorphisms has also been performed. The proposed panel will be formulated in steps; we have already selected 25 cell lines for DNA extraction and lyophilization that will cover genetic polymorphisms associated with 41 target alleles in 18 blood group systems. We have also identified collaborating laboratories willing to perform external characterization of the prototype panels to establish their suitability as reference materials.

75. UGT1A1 Metabolism Assay with Phenotyped Microsomes Assay Optimization and Analytical Validation

Volpe, Donna, FDA/CDER/OTS/OCP/DARS; Avaritt, Brittany, FDA/CDER/OTS/OCP/DARS; Hartman, Neil, FDA/CDER/OTS/OCP/DARS

Plain Language Synopsis: Increased blood levels of bilirubin has been observed in patients treated with a new class of anticancer agents called tyrosine kinase inhibitors. This project seeks to understand whether there is a relationship between inhibition of a liver enzyme that facilitates the elimination of bilirubin with observed bilirubin elevation in patients.

Abstract: Hyperbilirubinemia has been observed in patients treated with tyrosine kinase inhibitor (TKI) drugs. It would be beneficial to understand whether this adverse event is a class effect for these drugs and whether there is a causal relationship between inhibition of uridine 5'-diphospho-glucuronosyltransferase (UGT) 1A1 with observed bilirubin elevation in patients, with or without Gilbert's syndrome. UGT1A1 glucuronidates bilirubin allowing its excretion into the bile. To examine this issue, an in vitro assay of glucuronidation is used to determine the inhibitory effect of TKI drugs with phenotyped human liver microsomes (HLM) representing normal and reduced levels of UGT1A1. To initiate the project, metabolism and analytical methods were developed for the inhibition assays: optimization of metabolism assay using ß-estradiol (ßE2) glucuronidation to estradiol-3-glucuronide (E3G) in the HLM as a surrogate for predicting bilirubin glucuronidation; validation of a fluorescent HPLC assay for the detection of ßE2 and E3G; and enzyme kinetics for the genotyped HLM representing wild type (UGT1A1*1/*1), medium (UGT1A1*1/*28), and low (UGT1A1*28/*28, Gilbert's syndrome) metabolic activity. A reversed phase HPLC analytical assay was developed to measure E3G in experimental samples with ethinylestradiol-17-glucuronide (EE17G) as the internal standard. The assay had good selectivity, accuracy and linearity. For E3G, day-to-day variation was no greater than within day variation up to 7 days. Long term stability was tested with 3 sets of samples from a pilot study that were re-analyzed 4 weeks later. The results for E3G taken at both times were indistinguishable and within the standard deviation of the measurements for any given day. The HLM assay was optimized for ice incubation time with HLM and alamethicin, alamethicin concentration, ßE2 and HLM concentration, and stop solution composition. The different polymorph's Km and Vmax values were determined for estradiol glucuronidation with different HLM concentrations

and incubation times showing differences in ßE2 metabolism. This UGT1A1 assay can be used to determine whether in vitro inhibition of genotyped HLM are able to predict clinical hyperbilirubinemia in patients expressing wild-type and Gilbert's syndrome glucuronidation activity. The next step is to evaluate and compare the inhibitory effects of several TKI drugs on UGT1A1 glucuronidation utilizing the genotyped HLM.

76. Adaptive Enrichment with Subpopulation Selection at Interim

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Plain Language Synopsis: Enriching patient population with possible sample size increase in an ongoing trial that is pre-specified and employs valid statistical methods can address a clinical question of whether an experimental treatment is effective in all-comers or in a subset of patients in the context of personalized medicine from controlled clinical trials.

Abstract: There seems to be a growing interest in patient subpopulation enrichment, whether pursued at study baseline or in an ongoing clinical trial, for its potential to achieve the goal of personalized medicine. Adaptive enrichment has been proposed in multiple disease areas in drug development programs. Some use exploratory adaptive enrichment and others aim at confirmatory adaptive enrichment. According to FDA draft guidance on enrichment strategies for drugs and biologics, in principle, enrichment can help to reduce patient heterogeneity, select a high risk patient subset, or identify a subgroup of patients who are more likely to respond to treatment. In this poster presentation, we will give a brief overview on adaptive enrichment and the methodologies that are growing in the statistical literature. We will discuss prognostic enrichment versus predictive enrichment. An approved neurologic drug development case example, which was planned to adapt two design elements, patient population adaptation and statistical information adaptation, will be shared. We will articulate the challenges in the implementation of a confirmatory adaptive enrichment trial and in identification of the population to which the treatment effect applies. We will also assess the consistency of treatment effect before and after statistical information adaptation.

77. Systems Biology for LyM.P.H.oma Subtyping and Biomarker Identification

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Plain Language Synopsis: With this lyM.P.H.oma study, we demonstrated how to use combination of NGS, microarray and a wide variety of bioinformatics tools to understand basic tumor biology, transcription based regulation network, functional genes and pathways and ultimately lead to discovery of Ibrutinib as special therapeutic agent for a subgroup of DLBCL patient.

Abstract: Rapid advancement in sequencing technology has enhanced throughput and sample processing with affordable cost. It makes variety of applications readily available for research community, and enables the large cohort study for various diseases to be conducted. The examples include NCI's the Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and Cancer Genome Characterization Initiative (CGCI) project that study of somatic mutations in dozens of human cancers. These projects offer an unbiased analysis of genome-wide genetic alteration of tumors. The discoveries from these projects and many other alike have changed the traditional classification of disease. Patients thought to belong to the same disease group are actually separated into many subgroups based on their genetic mutation markers. The molecularly stratified patient groups can be linked to different clinic outcomes and responsiveness to therapeutics agents, which is the foundation for precision medicine. Using lyM.P.H.oma study as a showcase, we demonstrated how to use combination of NGS, microarray and a wide variety of bioinformatics tools to understand basic tumor biology, transcription based regulation network, functional genes and pathways. Briefly, via barcode-based shRNA screening, we identified a set of genes and their associated pathways that are critical for the growth of cancer cells. By interrogating

a set of transcription factors using ChIP-Seq, we established a transcription regulation sub-network within one subtype of lyM.P.H.oma. Furthermore, with combination of RNA-Seq, DNA-Seq and SNP microarray, we identified genetic landmarks to stratify tumor into subgroups which ultimately would lead to specific therapeutic intervention based on patient characteristics. To the end, we discovered that Ibrutinib (market name as Imbruvica), a BTK inhibitor that blocks BCR mediated NF-κB pathway would selectively kill ABC DLBCL cell lines. Our lyM.P.H.oma study presented a good example for utilizing bioinformatics tools to integrate results from NGS and microarray and to identify biomarkers and actionable genes for therapeutics. Knowledge accumulated from this line of research would also help to develop guidance and protocol for drug development and clinical trial that could lead to an enhanced approach towards precision and personalized medicine.

Session 7: Modernize Toxicology to Enhance Product Safety

(Posters 1-26 are located in Section A)

1. Inactivation of Bacteriophages and Feline Calicivirus-Surrogates of Enteric Viruses with Silver Nanoparticles

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Plain Language Synopsis: Silver nanoparticles are increasingly used in various products that pose a risk to the gastrointestinal system and homeostasis of the resident bacteria and viruses. This study showed, the virus inactivating properties of AgNP against feline calicivirus (FCV) and bacteriophage species (PP7, MS2, phiX174) that represented a model for enteric viruses.

Abstract: Following the increased commercial application of nanotechnology lots of consumer products have been introduced to the market. Due to their antimicrobial properties Silver nanoparticles (AgNP) are one of the most widely used nanoparticles in consumer products. This widespread use of AgNP may ultimately result in increased human exposure and a public health concern. This is particularly true for the gastrointestinal system and homeostasis of the resident bacteria and viruses that are essential to host health. In this study, the virus inactivating properties of AgNP of variable sizes (10 nm, 75nm, 110 nm) and doses (25 µg/ml, 50 µg/ml, and 100 µg/ml) were evaluated on feline calicivirus (FCV) and bacteriophage species (PP7, MS2, phiX174) that represented a model for enteric viruses. The antiviral activities of the AgNP were evaluated by comparing the FCV TCID50, appearance of cytopathic effects (CPE), detection of viral capsid protein by western blotting, and the bacteriophages plaque forming units (PFU) ratio of viral suspension treated with AgNP to the untreated controls. The results showed that the 10 nm AgNP at 50 µg/ml and 100 µg/ml levels were able to completely inactivate FCV in a dose-dependent manner within two to four hours of exposure resulting in loss of up to 6 log10 TCID50 viral titer, absence of viral CPE in Crandell-Rees Feline Kidney cells (CRFK) and significant reduction in viral capsid protein. Similar trends were observed for inactivation of bacteriophages where the 10 nm AgNP resulted in modest reduction of PFU that ranged from 1 log10 to 3 log10 PFU. However, for both the FCV and the bacteriophages, no significant antiviral effect was seen for the 75 nm and 110 nm AgNP. Taken together, these results indicate that antiviral effect of AgNP is size dependent. The small size AgNP could inactivate resident gut viruses and may lead to perturbations of the overall gut microbial ecosystem. Further studies are in progress to evaluate such effects of silver nanoparticles on the overall resident gut viruses that play a beneficial role to host health.

2. A Beating Heart Cell Model to Predict Cardiotoxicity: Effects of the Dietary Supplement Ingredients Higenamine, Phenylethylamine, Ephedrine and Caffeine

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Plain Language Synopsis: We studied dietary supplement ingredients in beating human heart cells in culture. We used realistic levels of ephedrine, caffeine, higenamine and PEA. Low to moderate levels of PEA or higenamine increased beating rate. Ephedrine increased beating, while caffeine had no effect except at toxic levels. Our results resembled human/animal reports.

Abstract: Some dietary supplements may contain cardiac stimulants and potential cardiotoxins. In vitro studies may identify ingredients of concern. A beating human cardiomyocyte cell line was used to evaluate cellular effects following phenylethylamine (PEA), higenamine, ephedrine or caffeine treatment. PEA and higenamine exposure levels simulated published blood levels in humans or animals after intravenous administration. Ephedrine and caffeine levels approximated published blood levels following human oral intake. At low or midrange levels, each chemical was examined plus or minus 50 uM caffeine, simulating human blood levels reported after consumption of caffeine-enriched dietary supplements. To measure beats per minute (BPM), peak width, etc., rhythmic rise and fall in intracellular calcium levels following 30 min of treatment was examined.

Higenamine 31.3 ng/ml or 313 ng/ml significantly increased BPM in an escalating manner. PEA increased BPM at 0.8 and 8 ug/ml, while 80 ug/ml PEA reduced BPM and widened peaks. Ephedrine produced a significant BPM dose response from 0.5 to 5.0 uM. Caffeine increased BPM only at a toxic level of 250 uM. Adding caffeine to PEA or higenamine but not ephedrine further increased BPM. These results illustrate the usefulness of a beating heart cell model to increase product safety using more advanced toxicological methods. Potentially unsafe products can be readily detected for further testing. These in vitro results suggest that additional in vivo testing of higenamine and PEA may be warranted to further evaluate their cardiac effects.

3. Measurement of Mucin Secretion for Potential Evaluation of the Toxicity of Tobacco Products in Human Air-Liquid-Interface Airway Models

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Plain Language Synopsis: An in vitro human air-liquid-interface airway model possesses many of the structural and physiological characteristics of the human bronchial epithelium. The use of such models as biological platforms for toxicity testing represents a major opportunity for improving the predictive accuracy of preclinical safety assessment.

Abstract: Evaluating cigarette smoke toxicity using traditional assays (e.g., assessing cytotoxicity and oxidative stress in monolayer cell cultures) has proven insufficient for predicting the adverse health impacts of tobacco use. It is increasingly recognized that in vitro human air-liquid-interface (ALI) airway cultures enable the measurement of endpoints relevant to tobacco smoke toxicity that have the potential for differentiating between the toxicities produced by different tobacco products. We have developed an airway ALI model that may be useful in evaluating tobacco smoke toxicity. As an initial test of this model, we assessed the mucin producing ability of two whole smoke solutions (WSSs) prepared by smoking 60 Marlboro Red (R60) or Marlboro Silver (S60) cigarettes. This pilot study was conducted using WSSs prepared with the ISO and the Health Canada Intense (HCI) smoking-machine protocols. Exposing the ALI model to ISO WSS concentrations equivalent to the smoke produced by 0.00006 to 0.0006 cigarettes caused an exposure- and time-dependent increases in mucin 5AC and 5B secretion, with R60 generally having a lower effect dose for the induction of both mucins. When these experiments were repeated with WSSs prepared using the HCI protocol, similar time- and exposure-dependent increases in mucins also were detected, although the differences in mucus hypersecretion between the two WSSs at the same doses were diminished. Our findings suggest that human ALI airway models show promise for evaluating tobacco smoke toxicity relevant for COPD.

4. How Adsorbed Fibrinogen Conformation Affects Platelet Adhesion: Novel Screening Method to Determine Thrombogenicity of Materials

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Plain Language Synopsis: Although fibrinogen is a primary component of the coagulation/hemostatic process and is vital to prevent blood loss upon injury, the molecule has also been implicated in life-threatening vascular thrombosis. This research focuses on how fibrinogen adsorbs to surfaces and how this adsorption influences thrombosis formation.

Abstract: Coagulation is a complex process essential to wound hemostasis. It is initiated through a series of reactions that lead to thrombin activation, fibrinogen conversion to fibrin, and the formation of a fibrin fiber network replete with bound/activated platelets. Fibrinogen plays a primary role in the body's coagulation process and its ability to seal vascular wounds and prevent blood loss. Although fibrinogen is vital to prevent blood loss upon vascular insult, the molecule has also been implicated in the formation of life-threatening vascular thrombosis. This research focuses on understanding the adsorption behavior of fibrinogen on a variety of surfaces and how this adsorption affects platelet interaction/adhesion and eventual thrombosis formation. Fibrinogen

fiber networks were formed on the various spun cast films by incubation with fibrinogen solutions (0.1 mg/ml and 4 mg/ml) at room temperature for different times (10 min, 20 min, 2 h, 12 h and 24 h). Atomic Force Microscopy was used to characterize the morphology of fibers on these surfaces and the Pierce BCA protein assay was used to measure the amount of protein adsorbed. Platelet rich plasma (PRP) and isolated platelets within a Trizma buffered saline solution (TBS) were obtained from fresh human blood. The surfaces were then incubated with the PRP or isolated platelets, either statically or dynamically (flow loop). Platelet adhesion and activation was then monitored using Scanning Electron Microscopy and fluorescent imaging (fluorescently labeled-CD41 antibody). The data indicates that the conformation of fibrinogen can drastically affect the adhesion of platelets, with increased platelet adhesion being observed with fibrinogen fibers compared to fibrinogen lawn (individual molecules adsorbed on surface in non-fiber form). It is hypothesized that there is an increased exposure of the platelet binding domain (D domain) on the fibrinogen molecule when in fiber form resulting enhanced platelet adsorption.

5. The Use of Time-Lapse Optical Coherence Tomography to Image the Effects of Microapplied Toxins on the Retina

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Plain Language Synopsis: Detection of drug neurotoxicity is a major concern for new drug development and evaluation. We have developed a non-destructive real-time imaging device to study the effects of neurotoxic drugs micro-applied to small regions of the live retina using an optically transparent drug applicator.

Abstract: Purpose: We developed a novel technique for accelerated drug screening and retinotoxin characterization using time-lapse optical coherence tomography (OCT) and a drug microapplication device. Methods: Using an ex vivo rabbit eyecup preparation, we studied retinotoxin effects in realtime by microperfusing small retinal areas under a transparent fluoropolymer tube. Known retinotoxic agents were applied to the retina for 5-minute periods, while changes in retinal structure, thickness, and reflectance were monitored with OCT. The OCT images of two agents with dissimilar mechanisms, cyanide and kainic acid, were compared to their structural changes seen histologically. Results: We found the actions of retinotoxic agents tested could be classified broadly into two distinct types: (1) agents that induce neuronal depolarization, such as kainic acid, causing increases in OCT reflectivity or thickness of the inner plexiform and nuclear layers, and decreased reflectivity of the outer retina; and (2) agents that disrupt mitochondrial function, such as cyanide, causing outer retinal structural changes as evidenced by a reduction in the OCT reflectivity of the photoreceptor outer segment and pigment epithelium layers. Conclusions: Retinotoxin-induced changes in retinal layer reflectivity and thickness under the microperfusion tube in OCT images closely matched the histological evidence of retinal injury. Time-lapse OCT imaging of the microperfused local retina has the potential to accelerate drug retinotoxicological screening and expand the use of OCT as an evaluation tool for preclinical animal testing.

6. Early Molecular Markers of Cardiac Tissue Injury in Mice Chronically Treated with Doxorubicin

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Plain Language Synopsis: Clinical use of the potent anti-cancer drug, doxorubicin, is limited by the serious side effect of cardiotoxicity. We have identified predictive biomarkers of cardiotoxicity in hearts of mice chronically exposed to doxorubicin. These biomarkers may prove valuable in early diagnosis of cardiotoxicity induced by doxorubicin or other potentially cardiotoxic drugs.

Abstract: Currently used cardiac-specific injury markers, troponins, are released in plasma only

after tissue damage has occurred. Identification of early biomarkers of myocardial injury is therefore crucial in preventing heart damage from cardiotoxic drugs, such as the anti-cancer drug, doxorubicin (DOX). To address this, a systems biology approach was used. Male B6C3F1 mice were given 3 mg/kg DOX or saline (i.v.) once a week for 2, 3, 4, 6, and 8 weeks, resulting in 6, 9, 12, 18, and 24 mg/kg cumulative DOX doses, respectively. Following euthanization a week after the last dose, myocardial injury as indicated by elevated plasma troponin T levels was observed in mice exposed to 18 and 24 mg/kg cumulative doses, whereas microscopic examination of hearts showed cytoplasmic vacuolization only at 24 mg/kg DOX. Mouse hearts also showed 24 differentially expressed miRNAs with expression of 1, 1, 2, 8, and 21 miRNAs being altered at 6, 9, 12, 18, and 24 mg/kg cumulative doses, respectively. The pro-apoptotic miR-34a was the only miRNA that was up-regulated at all cumulative doses with a significant dose-related response. At 12 mg/kg DOX, before cardiac injury was observed, up-regulation of miR-34a was associated with down-regulation of pro-hypertrophy miR-150. DOX-induced early expression changes were also observed before tissue injury and included genes related to energy metabolism, apoptosis, DNA damage-repair, and cell death and survival. Corroborating early genomics findings were changes in levels of proteins involved in energy metabolism. Genomics and proteomics analyses also revealed molecular changes associated with cardiac hypertrophy before myocardial injury. Metabolomics analysis indicated a significant increase in levels of citrulline and ornithine of the urea cycle and several amino acids in heart and plasma at 6 mg/kg cumulative dose. Conversely, many acylcarnitines were significantly decreased in heart, suggesting mitochondrial injury and altered beta-oxidation of fatty acid. Altogether, identification of molecular changes before myocardial injury may aid in discovery of predictive biomarkers of cardiotoxicity that may prove valuable in monitoring the risk of developing cardiotoxicity by DOX, other anthracyclines or other potentially cardiotoxic drugs.

7. Updating Bacterial Mutagenicity Assays for Medical Device Safety Assessment

Kabir, Farah, FDA/CDRH/OSEL/DBCMS; Balsam, Joshua, FDA/CDRH/OIR/DTDC/DDDB; Elespuru, Rosalie, FDA/CDRH/OSEL/DBCMS

Plain Language Synopsis: The bacterial mutagenicity (Ames) assay is an FDA standard for screening medical devices, drugs, and other FDA-regulated products for potential cancer risk. We present our work on updating this assay so that it requires smaller amounts of test sample and allows the easy test of many samples at one time.

Abstract: The reverse bacterial mutagenicity (Ames) assay is an FDA standard for screening candidate materials and devices (as well as drugs, food additives and veterinary products) for potential cancer risk. The standard plate assay with Salmonella typhimurium developed 40 years ago has not been updated in any major way that retains the sensitivity of the standard method while enhancing, e.g. high through-put. Besides the lack of a high through-put method, there are several problems in particular for medical devices, including the need for as many as hundreds of small devices (e.g. cardiovascular stents) to meet the material standards for the test. This is a burdensome aspect of the assay that we are attempting to alleviate. We have developed a "hybrid" method that retains the agar plate for measuring mutant yield, while using a pre-incubation step in microtiter dishes that miniaturizes the reaction between test article and bacteria, allowing the use of substantially less material in the test; it is also potentially amenable to high through-put. We have compared the hybrid method with the standard method for mutagens acting via different mechanisms. Luminescent versions of the standard strains obtained through an MTA have been used for development of a high through-put microtiter assay (still in progress). In addition, we used the luminescent bacteria to track colony development on agar plates by time-lapse photography of luminescence. We expected the population of colonies to demonstrate a relatively uniform development of a DNA sequence change as the revertants progressed from wild-type (normal) to mutant (positive in the assay). However a diversity of kinetic profiles was seen that was greater than the number of different

single base pair changes that were possible. On re-plating of individual colonies, a uniform profile was seen. This indicates that the pathway to sequence change is diverse in a population of bacteria, but once the sequence is altered, the bacteria behave uniformly. It may be possible to capitalize on these results to better use and understand this standard assay. We will demonstrate the progress made in modernization of the Ames bacterial mutagenicity assay.

8. Untargeted Global Metabolomics for Identification of Biomarkers of Liver Toxicity and Liver-Generated Metabolites of Food-Related Chemicals

Flynn, Thomas, FDA/CFSAN/OARSA/DOT; Liu, Yitong, FDA/CFSAN/OARSA/DOT; Pugh-Bishop, Shelia, FDA/CFSAN/OARSA/DOT and ORISE

Plain Language Synopsis: FDA frequently receives reports of liver toxicity associated with the use of herbal dietary supplements. The goal of this project is to develop rapid screening methods that can identify the potential for liver toxicity in regulated products and guide regulatory decisions that will minimize risks to public health.

Abstract: Norcoclaurine (aka higenamine) is a tetrahydroisoquinoline phytochemical that is found in some products marketed as dietary supplements. There is only limited data on the in vivo metabolism of norcoclaurine, but a presumptive metabolic pathway is through methylation by catechol-O-methyltransferase (COMT) to coclaurine. This is reported to be a detoxification reaction. LC/MS-based metabolomics studies were conducted using HuH-7 human hepatoma cells, a metabolically competent cell line, treated with norcoclaurine or coclaurine at 100 µg/mL for 48 hours. Examination of the supernatant culture medium, which is regarded as an in vitro surrogate for blood plasma, identified twelve putative metabolites of norcoclaurine. About 60% of the norcoclaurine was metabolized by the HuH-7 cells. The major metabolite, accounting for over 90% of the identified metabolites, was coclaurine confirming COMT as the major metabolic pathway for norcoclaurine. In addition to coclaurine, eleven additional metabolites were putatively identified. Additional reactions included desaturation, hydroxylation, and both sulfate and glucuronide conjugation. A similar series of metabolizing reactions was observed for coclaurine regardless of whether it was the primary substrate or derived from enzymatic methylation of norcoclaurine. Significant ($p < 0.05$, fold change > 1.5) metabolomic changes in the supernatant medium from either norcoclaurine or coclaurine treatment included increased use of glucose (↑5-fold) and glutamine (↑3-fold) suggesting increased flux through the TCA cycle. Similarly, levels of markers of oxidative stress such as methionine sulfoxide (↑2.5-fold) were increased in the medium following treatment with either compound. Finally, markers of mitochondrial function such as 3-methylglutaconic acid (↓2.6-fold) and hydroxymethyglutaric acid (↓2.6-fold) were decreased in the medium following treatment with either compound. These findings suggest that norcoclaurine is extensively metabolized by HuH-7 cells and that norcoclaurine and/or its metabolites can stimulate flux through energy use pathways, decrease mitochondrial function, and increase generation of reactive oxygen species. Further study is needed to determine whether these metabolic changes are pathologic or adaptive and to assess the relevance of the tested concentration to real life exposures.

9. FSH Increases VCAM-1 in Endothelial Cells Exposed to Metal-on-Metal (MoM) Particles

Jamison, Joshua, FDA/CDRH/OSEL/DBCMS; Wood, Steven, FDA/CDRH/OSEL/DBCMS

Plain Language Synopsis: Some of the biological factors that cause failure of metal-on-metal (MoM) hip prostheses are inflammation and blood vessel formation that support an immune reaction/ pseudo tumor formation, especially in women. The hormone FSH, increased in post-menopausal women, was found to support blood vessel formation and an immune reaction.

Abstract: Second-generation metal-on-metal (MoM) hip prostheses are widely used for hip replacement, consisting of cobalt -chromium metal alloys. However, the devices fail through pathological inflammation, osteolysis, and/ or pseudotumor formation. Inflammation also results in increased angiogenesis which augments the pathological conditions of further inflammation and pseudotumors. Furthermore, hip implants fail more in women. Given that follicle stimulating hormone (FSH) is elevated

in women receiving hip implants, FSH has been shown to promote increased angiogenesis. We are currently examining how angiogenesis and the inflammatory response may be the result of untoward interactions of FSH with endothelial cells exposed to MoM wear particles. To determine the direct effect of FSH on endothelial cells exposed to MoM particles, angiogenesis was investigated with Matrigel tube formation assays. We found increased total cord length and decreased width in presence of MoM particles observed during cord formation. FSH promoted increased cord length and THP-1 monocyte recruitment to endothelial cords in the presence of MoM particles. Endothelial cells were further investigated for VCAM-1, which has been previously shown to regulate vessel integrity through integrin interaction and promote inflammatory response through monocyte recruitment. Increased VCAM-1 protein expression and mRNA was observed in endothelial cells exposed to FSH. Furthermore, inhibition of FSHR though siRNA inhibited VCAM-1 mRNA expression and protein expression. Increased VCAM-1 localized aggregation was further observed in immunostained endothelial cells exposed to FSH. In sum, up-regulated FSHR induced VCAM-1 further promotes increased cohesion of endothelial cells required for vessel integrity during inflammation. Most importantly, monocyte recruitment subsequently drives increased inflammation in response to MoM particles. Inflammation driven by this mechanism may be one of the factors leading to the failure of MoM hip implants in women.

10. Development of Gastrointestinal Toxicity Models to Assess the Effect of Silver Nanoparticles on Microbiome

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Plain Language Synopsis: This study demonstrated the responses of silver nanoparticles on intestinal health, including the effect on the commensal microbiota and host immune functions using in vitro, in vivo and ex vivo models.

Abstract: Silver nanoparticles (AgNP) are widely used for their antibacterial properties; however the effects of such exposure on the gastrointestinal system are mostly unknown. To maintain the gastrointestinal homeostasis, the microbial communities within gastrointestinal tract should be in balance. A shift in the subpopulation of two major phyla (Bacteroidetes and Firmicutes) may results in several metabolic diseases. Recently we have shown that AgNP are among several other existing toxicants that can disturb the ratio of Bacteroidetes and Firmicutes. The present study was focused to assess the effect of AgNP on the overall ecological balance of the microbial communities. Ileal mucosal samples were taken from the Sprague-Dawley rats (both male and female) that were gavaged orally with discrete sizes of AgNP (10, 75 and 110 nm) and doses (9 mg/ml, 18 mg/ml and 36 mg/ml) for 13 weeks. DNA was extracted and subjected to 16S rRNA gene sequence-based analysis to examine the characteristic bacterial communities in the ileum. The Weighted UniFrac dissimilarity was used to calculate taxon, genus and family abundance in different samples; whereas UniFrac method was used to assess only the presence or absence of taxa. The Adonis test is used for finding significant whole microbiome differences among discrete categorical or continuous variables. No separation of the microbiome was observed between male and female in the two dimensional plot of UniFrac dissimilarity values. In general, bacterial community richness varied with the treatment. A dominance of phylum Firmicutes was observed in all the samples. When comparing the microbiome of size effect (10nm of AgNP), 38 Operation Taxonomic Units (OTUs), with significance in their abundance were detected. Principal component analysis showed a separation of microbiome between samples from 18 mg/ml and all other doses. A difference in presence/absence of OTUs at phylum-level was observed for Planctomycetes. A significant difference in OTU abundance at phylum-level was observed for Proteobacteria. To our knowledge this is the first study to systematically characterize bacterial communities in vivo during AgNP exposure. Furthermore, we have confirmed the cross talk between the bacterial species and host functional properties using the in vivo, in vitro and ex vivo model.

11. FDA/CDER (Q)SAR Computational Toxicology Consultation Service

Stavitskaya, Lidiya, FDA/CDER/OTS/OCP; Minnier, Barbara, FDA/CDER/OTS/OCP; Powley, Mark, FDA/CDER/OPQ/OND; Hartman, Neil, FDA/CDER/OTS/OCP; Kruhlak, Naomi, FDA/CDER/OTS/OCP

Plain Language Synopsis: The Computational Toxicology Consultation Service serves as a CDER-wide resource providing (Q)SAR model predictions, chemical structure-based searching of in-house and publicly available databases, and expertise in the interpretation of (Q)SAR data submitted to FDA by pharmaceutical manufacturers. These capabilities are used to fill data gaps for endpoints such as genotoxicity, carcinogenicity, and drug-induced liver injury, and are most often requested by CDER reviewers for the safety assessment drug impurities under the ICH M7 guideline.

Abstract: Chemical and biological data have been extracted from FDA archives and public sources and, after careful curation, have been used to create and validate (quantitative) structure-activity relationship ((Q)SAR) models. These computational models work in conjunction with three commercial (Q)SAR software programs provided to FDA/CDER by Research Collaboration Agreement partners to rapidly predict potential toxicities and adverse human clinical effects of drugs and components of drug products to provide decision support information during the drug safety review process. At FDA/CDER, (Q)SAR models are most frequently used to support the qualification of drug impurities under the recently finalized International Conference on Harmonisation (ICH) M7 (Step 4) guideline, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. This international guideline clearly outlines the process for the use of (Q)SAR predictions of bacterial mutagenicity as a replacement for empirical testing to qualify drug impurities for mutagenic potential. Other applications of (Q)SAR supporting regulatory decision-making include providing supplemental information to resolve inadequate or equivocal experimental results, or hypothesis generation for the interrogation of unanticipated post-market safety signals. Predictions are made available to CDER reviewers upon request through the CDER Computational Toxicology Consultation Service (CCTCS). Requests are submitted electronically through a website on FDA's intranet and a report of the results is typically returned within a week. The software programs applied through the CCTCS use complementary prediction methodologies, making it possible for superior predictive performance to be obtained by integrating the results from more than one model for a given endpoint. The same (Q)SAR models developed and applied at CDER are now routinely used by the pharmaceutical industry.

12. Development of Improved (Q)SAR Models for Predicting the Outcome of the in vivo Micronucleus Genetic Toxicity Assay

Yoo, Jae Wook, FDA/CDER/OTS/OCP; Minnier, Barbara, FDA/CDER/OTS/OCP; Kruhlak, Naomi, FDA/CDER/OTS/OCP; Stavitskaya, Lidiya, FDA/CDER/OTS/OCP

Plain Language Synopsis: This presentation describes the enhancement of in-house databases and (Q)SAR models for predicting the outcome of the in vivo micronucleus assay, a component of the ICH S2 genotoxicity test battery required for all drugs under review. These models and structure-linked databases will be used to support regulatory decision-making by providing genetic toxicity predictions for components of drug products that have limited safety data, and will be made available through the CDER Computational Toxicology Consultation Service.

Abstract: All drugs entering clinical trials are expected to undergo a series of in vitro and in vivo genotoxicity tests as part of their safety assessment. These tests, described in the International Conference on Harmonization (ICH) S2 Guideline, enable the identification of compounds that induce damage to DNA to assess their potential as a human carcinogen. Among the standard battery of genotoxicity tests used for pharmaceuticals, the in vivo micronucleus assay, which measures the frequency of micronucleated cells mostly from blood or bone marrow, is recommended for detecting clastogens and aneuploidy inducers. (Quantitative) structure-activity relationship [(Q)SAR] models may be used as early screening tools to assess genetic toxicity risk during drug development or to support regulatory safety decisions for drugs and drug products when experimental data are insufficient

or absent. We used commercially available modeling software to construct (Q)SAR models for in vivo micronucleus induction from an in-house database of non-proprietary study findings in mice. The database was recently enhanced with new data harvested from publicly available FDA approval packages and the published literature. An earlier generation in vivo micronucleus (Q) SAR model built at CDER featured high specificity (90%) but low sensitivity (45%); however, the new models constructed here were tuned for higher sensitivity while maintaining a balance of other predictive characteristics, consistent with FDA's mandate to protect patient safety. Cross-validated performance statistics for the new models showed sensitivity of up to 75% and negative predictivity of up to 86% based on a training data set of 996 compounds. In addition, the models demonstrated cross-validated specificity of up to 74% and coverage of up to 94%. These new models will provide more reliable predictions and offer enhancement in the quality of drug safety assessment with regards to identifying potentially genotoxic compounds.

13. Construction and Application of (Q) SAR Models to Predict In vitro Chromosome Aberrations

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Plain Language Synopsis: This presentation describes the enhancement of in-house databases and (Q)SAR models for predicting the outcome of the in vitro chromosome aberrations assay, a component of the ICH S2 genotoxicity test battery required for all drugs under review. These models and structure-linked databases will be used to support regulatory decision-making by providing genetic toxicity predictions for components of drug products that have limited safety data, and will be made available through the CDER Computational Toxicology Consultation Service.

Abstract: In drug development, genetic toxicology studies are conducted using a battery of in vitro and in vivo tests to identify potential chemical -induced gene mutations and clastogenic damage, as described in the ICH S2 guideline. Varying numbers and types of studies are required for active ingredients, major metabolites, impurities, and degradants of drug substances. In some instances, (quantitative) structure-activity relationship ((Q)SAR) model predictions may be used to supplement or replace the use of experimental testing, in particular when empirical data are limited or lacking. In the current study, two commercially available (Q)SAR platforms were used to build models for predicting chromosomal aberrations in Chinese Hamster Lung (CHL) and Chinese Hamster Ovary (CHO) cell lines. The criteria set by OECD 473 were used to govern the data selected for model construction. Datasets were well-balanced, with 53% and 45% positives, respectively, and contained both re-evaluated legacy as well as new data to expand the chemical space of our previous models. Cross-validated (Q) SAR model performance of the CHL models built using 876 training compounds showed sensitivity of up to 79% and negative predictivity of up to 75%, an improvement over previous CHL models constructed from 749 compounds, which had cross-validated sensitivity up to 49% and negative predictivity up to 66%. For the CHO cell line, cross-validated performance of the new (Q)SAR models showed sensitivity up to 63% and negative predictivity up to 72% based on a training data set of 821 compounds, in contrast to the earlier models based on 688 compounds with sensitivity up to 47% and negative predictivity up to 70%. These two models more effectively predict in vitro chromosome aberration assay outcomes and cover a broader range of chemical structural attributes and functionality than earlier models, providing a state-of-the-art approach to genetic toxicity screening for components of drug products.

14. Enhanced QSAR Models for Predicting Rodent Carcinogenicity

Guo, Dongyu, FDA/CDER/OTS/OCP; Stavitskaya, Lidiya, FDA/CDER/OTS/OCP; Minnier, Barbara, FDA/CDER/OTS/OCP; Kruhlak, Naomi, FDA/CDER/OTS/OCP

Plain Language Synopsis: This presentation describes the enhancement of in-house databases and (Q)SAR models for predicting the outcome of the rodent carcinogenicity bioassay, a required component for the non-clinical safety assessment of new pharmaceuticals. These models and structure-linked databases will be used to

support regulatory decision-making by providing carcinogenicity predictions for components of drug products that have inadequate toxicity data, and will be made available through the CDER Computational Toxicology Consultation Service.

Abstract: Two-year rodent carcinogenicity studies are the most costly of the required toxicology studies for new drugs in both time and the number of animals used, and the outcome of these studies can significantly impact the marketability of a drug product. Through an FDA/PhRMA partnership, an initiative is currently underway to assess the ability of a battery of shorter-duration tests to replace the two-year rodent bioassay. In silico screening of new pharmaceuticals for carcinogenic potential using structure-based methodologies may provide addition information to support this effort, potentially benefiting both industry and regulatory audiences. After developing a large, high-quality rodent carcinogenesis database (n=1682) covering a large number of structural alerts and characteristics of both genotoxic and non-genotoxic carcinogens, we constructed, optimized and validated a battery of quantitative structure-activity relationship (QSAR) models based on the non-proprietary portion (n=1518) to the predict rodent carcinogenicity of untested chemicals. These models were constructed using a partial least-squares regression algorithm in conjunction with fragment-based descriptors derived from medicinal chemistry building blocks within a commercially available cheminformatics platform. Models were developed to predict four rodent carcinogenicity study groups: male rat, female rat, male mouse, and female mouse carcinogenicity. In external validation experiments using an additional set of 722 test compounds (49% positive) derived from the public sources, the models exhibited negative predictivity ranging from 82% to 83% and sensitivity from 70% to 82%, which are statistical parameters considered important for protecting patient safety. In addition to possessing higher underlying data quality, these models demonstrated good coverage, ranging from 85% to 88% for pharmaceutical and industrial chemical structures. These newer, higher-quality training datasets and models can provide a high-throughput assessment of carcinogenic potential for new drugs and components of drug products.

15. A Mechanistic and Model-Based Approach to Evaluating Reports of Bioinequivalence

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Plain Language Synopsis: The goal of this research is to develop a tool that would enable the dissection of reported adverse events related to generic drug substitution. It is computer-based and uses actual clinical events reported to FDA through their adverse event reporting system. The tools is mechanistic so it provides an explanation as to why a given adverse event would occur. The tool will allow FDA to separate adverse events that are plausible from those that are due to reasons other than the respective generic drug.

Abstract: We have developed a model-based step-wise approach to systematically evaluate potential mechanisms and risk factors associated with purported adverse events or loss of efficacy that may have been reported for generic drugs. The goal of this research is to provide FDA CDER Office of Generic Drugs with a scientific tool which they can use to conduct their own investigation of such reports. The tool consists of integrated mathematical and pharmacological platforms that include physiologically-based pharmacokinetic (PBPK) models, exposure-response (E/R, PKIPD) models and molecular target and pathway (systems pharmacology) models. Together, these platforms address challenges related to the nature of data collected such as incomplete pharmacokinetic information and incomplete clinical data surrounding adverse event reports that one might find in FDA's Adverse Event Reporting System (FAERS). We will use several hypothetical examples in this presentation to illustrate the step-wise approach. We selected anti-epileptic drugs (AEDs) because there have been reports of bioinequivalence among genetic drugs used for seizure control. The first example is a case involving levetiracetam. First, data mining was conducted in FAERS to identify AEs (2004-2014) and the platform MASE (molecular analysis of side effects) to dissect the molecular targets and pathways associated with the drug. Second,

PBPK models for immediate- and extended-release formulations were built and qualified so that a sensitivity analysis could be performed to evaluate changes in exposure that may be associated drug- and formulation-related properties (e.g., solubility, permeability and dissolution), and/or system properties such as pH. Third, PKIPD models were developed to estimate the probability of a given response as a function of changes in exposure. The second example is a case of thrombocytopenia reported for levetiracetam after switching from Kepra to generic levetiracetam. First, we identified comparative frequency of adverse events for all anti-epileptics when used for epilepsy using a software called AdverseEvents. Second, we looked at the most common adverse events (AE) in FAERS for levetiracetam in terms of absolute numbers and proportional reporting rations of each AE. The PRR associated with thrombocytopenia was 4.7 indicating that these AE is almost 5 times more common with levetiracetam compared to the frequency at which thrombocytopenia occurs in patients taking other drugs. Third, we identified the top 10 other drugs linked to thrombocytopenia along with their respective PRRs. Fourth, the top 20 molecular targets (CYP enzymes, transporters and pharmacological receptors) were identified for levetiracetam to link them mechanistically to thrombocytopenia. In summary, prostaglandin g/h synthase 1 was the target most closely linked to the cardiovascular AE with a modest PRR of 1.21 demonstrating that the AE was pharmacologically plausible.

16. Pt Nanoparticles: An Efficient And Stable Catechol Oxidase Mimetic

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Plain Language Synopsis: Pt nanoparticles (NPs) exhibit catechol oxidase-like activity effectively to oxidize polyphenols into the corresponding o-quinones. Since polyphenol oxidase plays important roles in the production of melanin or other pigments, which results in fruit and vegetable browning or dermal darkening, our results could provide insights to the potential usage of Pt NPs in cosmetics, food packaging, or dietary supplements.

Abstract: Polyphenols, as potent antioxidants and related bioactive substances, have been closely linked to many beneficial actions, including antiaging, prevention of cancer, cardiovascular disease, etc. However, the oxidation of polyphenols also attract much attention because their oxidized products may accumulate in foods and human body, which cause harm to human health. In this study, we report that Pt nanoparticles (NPs) exhibit catechol oxidase-like activity effectively to oxidize polyphenols into the corresponding o-quinones. Four unique different approaches are employed to demonstrate the catechol oxidase-like activity exerted by Pt NPs. First, UV-vis spectroscopy is used to monitor the oxidation process of polyphenols catalyzed by Pt NPs. Second, the oxidized products of polyphenols are identified by HPLC separation followed by mass spectrometric. Third, electron spin resonance (ESR) oximetry techniques is used to confirm the oxygen consumption during the oxidation reaction. Fourth, the intermediate products of semiquinone radicals during the oxidation of polyphenols are determined by ESR using spin stabilization. The overall results indicate Pt NPs possess the catechol oxidase-like activity, which can destroy the protective role of antioxidants, potentially lead serious adverse health effects to humans.

17.Evaluation of 'Dream Herb,' Calea zacatechichi, for Nephrotoxicity Using Human Kidney Proximal Tubule Cells

Mossoba, Miriam, FDA/CFSAN; Flynn, Thomas, FDA/CFSAN; Vohra, Sanah, FDA/CFSAN; Wiesenfeld, Paddy, FDA/CFSAN; Sprando, Robert, FDA/CFSAN

Plain Language Synopsis: Safety testing of 'dream herb', Calea zacatechichi, is lacking. We investigated its safety profile using an in vitro kidney cell model. We found that in vitro exposure of human kidney cells to Calea zacatechichi is potentially cytotoxic and kidney-specific through mechanisms of reactive oxygen species production and mitochondrial damage.

Abstract: A recent surge in the use of dietary supplements, including herbal remedies, necessitates investigations into their safety profiles. 'Dream herb,' Calea zacatechichi, has long been used in traditional folk medicine for a variety of purposes and is currently being marketed in

the U.S. as a treatment for several conditions, including diabetes. Despite the inherent vulnerability of the renal system to xenobiotic toxicity, there is a lack of safety studies on the nephrotoxic potential of this herb. Additionally, the high frequency of diabetes-associated kidney disease makes safety testing of C. zacatechichi especially important. We exposed human proximal tubule HK-2 cells to increasing doses of this herb alongside known toxicant and protectant control compounds (cisplatin and valproic acid, respectively) to uncover potential toxicity effects of C. zacatechichi relative to these controls. We evaluated both cellular and mitochondrial functional changes related to toxicity of this dietary supplement and found that even at low treatment doses (37 ug/ml for 24 hrs), evidence of cellular and mitochondrial toxicity was significant. We also found that reactive oxygen species (ROS) production from cells treated with up to 333 ug/ml of C. zacatechichi were intermediary between that from the positive- and negative-control treated cells. At the 1000 ug/ml dose, however, C. zacatechichi induced ROS levels that surpassed those in cisplatin-treated cells. Moreover, these findings correlated with significantly elevated levels of FDA-qualified biomarkers of nephrotoxicity: KIM-1, Albumin, Cystatin C, and Beta-2-Microglobulin. Overall, our findings lend further support for the need to scrutinize the safety of this herbal dietary supplement.

18. 'Snake Root Plant,' Rauwolfia serpentina, Induces Adverse Effects on Human Kidney Cells

Mossoba, Miriam, FDA/CFSAN; Flynn, Thomas, FDA/CFSAN; Vohra, Sanah, FDA/CFSAN; Wiesenfeld, Paddy, FDA/CFSAN; Sprando, Robert, FDA/CFSAN

Plain Language Synopsis: Snake root plant, or Rauwolfia serpentina, is used in dietary supplements for maintaining healthy blood pressure, but its safety testing is lacking. We used an in vitro model of the human kidney to test for potential kidney-damaging effects. We found evidence of strong adverse effects on kidney cells.

Abstract: Rauwolfia serpentina (or Snake root plant) is a botanical dietary supplement marketed in the U.S. for maintaining blood pressure. Very few studies, however, have addressed the safety of this herb, despite its wide availability to consumers. Its reported pleiotropic effects underscore the necessity for evaluating its safety. We used a human kidney cell line (HK-2) to investigate the possible negative effects of R. serpentina on the renal system in vitro, with a specific focus on the renal proximal tubules, which are common targets of nephrotoxicity. We evaluated cellular and mitochondrial toxicity, along with a variety of other kidney-specific toxicology biomarkers. We found that R. serpentina was capable of producing highly detrimental effects in our in vitro renal cell system. Specifically, our renal cell model displayed dose-dependent cytotoxicity and attained near-complete cell death at the treatment concentrations 200 ug/ml and above, which was similar to the effects of our positive control, cisplatin. This effect was paralleled by clear evidence of dose-dependent mitochondrial depolarization, suggesting the induction of mitochondrial injury as a potential mechanism of action by R. serpentina. We also uncovered significant elevations of FDA-qualified nephrotoxicity biomarkers: Albumin, B2M, Cystatin C, and KIM-1. These results suggest more studies are needed to investigate the safety of this dietary supplement in both kidney and other target organ systems.

19. Direct Exposure of Adhatoda zeylanica to Human Renal Cells Lacks Toxicity

Mossoba, Miriam, FDA/CFSAN; Flynn, Thomas, FDA/CFSAN; Vohra, Sanah, FDA/CFSAN; Wiesenfeld, Paddy, FDA/CFSAN; Sprando, Robert, FDA/CFSAN

Plain Language Synopsis: Adhatoda zeylanica is used in dietary supplements to support weight loss, respiratory abilities, and immune regulation. We investigated its safety using an in vitro cellular model of the human kidney. In our study, it lacked severe toxicity, but more studies are needed to better understand its potential toxicity towards kidneys.

Abstract: Adhatoda zeylanica is an ingredient present in several types of dietary supplements, including weight loss, respiratory relief, and immune regulating products. Due to its reported wide range of uses in traditional medicine, we hypothesized that its potential ability to target multiple organs could lead to a range of toxicity features. To begin an evaluation of the safety of

this herbal ingredient, we sought to investigate its effects on the kidney, with eM.P.H.asis on proximal tubule cells. These cells are a common target of toxicity owing to their ability to concentrate solutes from its glomerular filtrate, thus exposing them to potentially high toxin concentrations. We employed a variety of in vitro techniques to screen for potential nephro-toxicological effects of A. zeylanica, using HK-2 cells as a cellular model. Based on our cell viability testing, however, our results show that A. zeylanica is not significantly toxic under conditions of direct exposure for 24 hours, unless the treatment dose reaches very high values of around 1000 ug/ml. The mechanism of this action may be explained by the surge in reactive oxygen species (ROS) production that was observed exclusively at this high dose. Interestingly, we also detected general mitochondrial damage that was present at levels directly proportional to the treatment dose. Finally, we checked for early signs of proximal tubule damage to uncover a possible nephron-specific toxicity signature for A. zeylanica using the biomarkers Albumin, B2M, Cystatin C, and KIM-1, which have been qualified for nephrotoxicity testing by FDA. Consistent with our cell viability data, all four tested biomarkers revealed that A. zeylanica was as innocuous as our negative control, valproic acid, towards HK-2 cells at the treatment doses of 111 ug/ml and 333 ug/ml. Taken together, our screening study exonerates A. zeylanica from being a highly acute toxic herb under the experimental conditions that our investigation models, but that more testing would be required before it can be considered safe for consumption, especially when it is consumed with other food, supplement, or drug ingredients.

20. Comparison of Induced Pluripotent Stem Cell Derived- Human Cardiomyocytes from Two Major Suppliers

Pang, Li, FDA/NCTR/DBT; Lyn-Cook, Beverly, FDA/NCTR/DBT; Yang, Xi, FDA/NCTR/DSB; Word, Beverly, FDA/NCTR/DBT; Norman Stockbridge, FDA/CDER/OND/DCRP

Plain Language Synopsis: This study demonstrates the importance of characterizing induced pluripotent stem cell derived- human cardiomyocytes model for drug safety assessment.

Abstract: Drug-induced proarrhythmia is a major safety issue in drug development. Sensitive in vitro assays that can predict drug-induced cardiotoxicity in humans have been the focus of toxicology research for the past two decades. Recently, induced pluripotent stem cell derived-human cardiomyocytes (iPSC-hCMs) have become a popular model because they largely resemble the electrophysiological behavior of human ventricular myocytes. However, iPSC-hCMs are derived from individuals with diverse genetic backgrounds, and different laboratories/suppliers may use different differentiation processes and various conditions to culture these cells. Therefore, the responses of different iPSC-hCMs to cardiotoxic drugs may vary. In this study, we compared iPSC-hCMs from two major suppliers: Cellular Dynamics International (CDI) and Axiogenesis. We found that the two cell lines had different sensitivities to the hERG channel blocker dofetilide: the lowest concentration of dofetilide that could induce irregular beats in iCells from CDI was 3- 10-fold lower than what was needed with Cor.4U cells from Axiogenesis. Moreover, the expression levels of cardiac ion channel genes were different between the two cell lines: iCells had higher expression levels of SCN5A, CACNA1C, and KCNJ2, while Cor.4U cells had more transcripts from KCNE1 and KCNIP2 genes. These results are consistent with the observation using microelectrode arrays that iCells have a bigger sodium spike, lower firing rate, and longer field potential duration than the same parameters recorded with Cor.4U cells. Mutations in SCN5A, CACNA1C, KCNJ2, and KCNE1 genes are linked with Long QT syndrome. KCNIP2 has been found to increase A-type potassium currents (Kv4 channels) and alter channel kinetics. Therefore, the different expression profiles of cardiac ion channel genes between the two cell lines provide valuable information in understanding the molecular basis of the differences in cardiac electrophysiology and the data interpretation for utilizing these cells for drug safety assessment.

21. Genotoxicity of Nanosilver in Human Liver Hepg2 and Colon Caco2 Cells Evaluated by the Cytokinesis-Blocked Micronucleus Assay

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FDA/CFSAN/OARSA

Plain Language Synopsis: Our study demonstrates that two different sizes of nanosilver, having the same shape, composition and purchased from the same source, show different genotoxic potential in the same cell culture systems. The smaller nanosilver is genotoxic, but the larger nanoparticle is not. Therefore, nanoparticle size is a critical factor for its genotoxic potential.

Abstract: Nanosilver is used in food- and cosmetics-related consumer products. Extensive human exposure to these products is of public concern. Genotoxicity is an important endpoint for safety assessment of regulated products. The in vitro cytokinesis-blocked micronucleus (CBMN) assay is a very useful test for predictive genotoxicity assessment as suggested by the OECD Guidelines. This study was undertaken to test the hypothesis that nanoparticle size and cell types were critical determinants of its genotoxicity. Following the OECD Guidelines we used the same in vitro cell models, experimental conditions and genotoxic endpoint to evaluate genotoxicity of two different sizes (20 nm and 50 nm) of nanosilver having the same shape, composition and purchased from the same source. They were characterized by FDA's White Oak Nanotechnology Core Facility using TEM, DLS and ICP-MS. TEM images of both nanoparticles showed that they were stable in our cell culture medium without noticeable aggregation. Average sizes of 20 nm silver determined by TEM and DLS were 20.4 nm and 21.4 nm, respectively, consistent with the manufacturer provided value of 20.0 nm. Average sizes of 50 nm nanosilver determined by TEM and DLS were 44.7 nm and 54.9 nm, respectively, close to the manufacturer provided value of 50.0 nm. We determined cytotoxicity of nanoparticles using the resazurin reduction and cellular double-stranded DNA content assay; CBMN assay as the endpoint of genotoxicity; and the same in vitro models, human liver HepG2 and colon Caco2 cells, representing the liver and gastrointestinal tract, respectively. Results of our study show that up to the concentrations tested, the smaller (20 nm) nanoparticle induces dose- and time-dependent increases in micronucleus formation in both cell types, but the larger (50 nm) nanoparticle does not. The HepG2 and Caco2 cells appear to be useful in vitro models for genotoxicity evaluation of nanomaterials. In conclusion, our results demonstrate that two different sizes of nanoparticles having the same shape, composition and purchased from the same source can have different genotoxic potential in the same test system. We found the smaller 20 nm nanosilver is genotoxic, but the larger 60 nm nanoparticle is not. Therefore, nanoparticle size is a critical factor for its genotoxic potential.

22. Investigation of Cytochrome P450-mediated Toxicity of Extracts of Polygonum multiflorum and Chelidonium majus

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Plain Language Synopsis: Chelidonium majus is used in gastric conditions whereas Polygonum multiflorum is used as an anti-aging ingredient. Several cases of hepatotoxicity are reported following use of dietary supplements containing P. multiflorum and C. majus. Toxicity of the extracts and pure compounds on liver cells was investigated in an in vitro assay.

Abstract: Botanical dietary supplements are commonly used throughout the world, and adverse hepatic reactions have been reported following their intake. In several cases, hepatic toxicity of botanical dietary supplements is the result of cytochrome P450 (CYP450) - mediated mechanisms leading to the formation of reactive metabolites. These reactive metabolites covalently bind to cellular macromolecules such as DNA and protein, leading to toxicity via multiple mechanisms. Our study is focused on investigating the metabolism-mediated toxicity of extracts of Polygonum multiflorum and Chelidonium majus. Recently, several incidences of liver injury due to the use of P. multiflorum and C. majus preparations are reported. In our study, human hepatocarcinoma (HepG2) cells were incubated with plant extracts in the presence and absence of an external metabolizing system (rat liver S9 fraction and NADPH) for 6 h and the cytotoxicity was assessed as lowered mitochondrial activity (reduction of MTT). These extracts were further fractionated to identify the most toxic fractions. Selected compounds from each plant were also subjected to the in vitro toxicity assay to study their toxicity profile. Sanguinarine was also

found to be the most toxic compound in C majus followed by chelerythrine. Further, it was observed that toxicity of the fractions and pure compound of C. majus and P. multiflorum was diminished following incubation with metabolic activation system.

23. Biodistribution and Pharmacokinetics of Squalene-containing Adjuvants in Mice Following Intramuscular Injection of Adjuvanted H5N1 influenza vaccine

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Plain Language Synopsis: We evaluated distribution and time course of squalene, a component of immune stimulating agents in a certain influenza vaccine, in mechanistically and toxicologically relevant target tissues. Major proportion of squalene was found in injection site muscles, local fats, and draining lyM.P.H. nodes. Systemic exposure to squalene was minimal.

Abstract: Squalene is a component of oil-in-water emulsion adjuvants used in certain influenza vaccines marketed outside the United States. The pharmacokinetic data for squalene-containing adjuvant after intramuscular injection of vaccine is quite limited. We evaluated the biodistribution and pharmacokinetics of squalene in mice after intramuscular injection of the emulsion adjuvant (AddaVaxTM) alone or with H5N1 antigen. The biodistribution of radiolabelled squalene was assessed in mechanistically and toxicologically relevant target tissues up to 14 days following injection into quadriceps muscle. Blood concentration versus time data were analyzed using a population pharmacokinetic (PK) model fitting approach. The disposition of squalene in quadriceps muscle, inguinal fat, and draining lyM.P.H. nodes was not statistically different between AddaVax versus AddaVax + H5N1 groups; hence data were pooled for further analysis(n=160 mice). The decay of squalene from the quadriceps muscle was described by a one-phase exponential curve with a half-life of 12.8 hr (8.3-27.4; 95% CI). Twenty-four hour after the injection, 5.1±1.9% of the dose was found in inguinal fat and 1±0.5% in draining lyM.P.H. nodes. Systemic biodistribution of squalene based on the peak tissue concentration for kidney, brain, bone marrow, and spleen was less than 1% of the injected dose and the presence of the H5N1 antigen did not significantly alter the distribution of the adjuvant. The liver concentration peaked at 3 hr for AddaVax injected group (3.4±0.3%) and at 6 hr for AddaVax+H5N1 (2.2±0.1%). The area-under-blood-concentration curve (AUC) for the AddaVax injected group (142.2±6.3%xhr) was significantly different from that of AddaVax+H5N1 (177.5±7.9%xhr). A one-compartment PK model, with first order absorption and elimination, described the blood concentration versus time data. The population PK model will be used for further statistical analysis and estimation of PK parameters. The results of this study are relevant for exposure assessment of squalene-containing emulsion vaccine adjuvants, as well as in informing regulatory benefit-risk analyses.

24. Infant Toxicology: State of the Science and Considerations in Evaluation of Safety of Infant Food Packaging

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Plain Language Synopsis: Infants experience a different exposure scenario to migrants from food packaging than adults. This time period is also characterized by important developmental changes. Safety assessment for infants should consider both exposure parameters as well as life-stage specific potential sensitivities.

Abstract: CFSAN convened a Food Advisory Committee meeting aimed at examining risk assessment for potentially-susceptible subpopulations (Dec, 2014). The FAC meeting identified that subpopulation risk assessment may be appropriate in following scenarios: 1) There is a subpopulation that appears vulnerable based upon critical windows of toxicokinetic immaturity or toxicodynamic sensitivity, 2) The subgroup will receive a disproportionately higher exposure to a food or product that contains

toxicants of particular concern. Infants (≤12 months) represent a subpopulation of interest in the context of food packaging based on exposure and biological factors, fulfilling these criteria. Infants are predominantly sole-source consumers of breast milk and/or infant formula for at least the first 6 months after birth, potentially increasing their exposure to any one packaging type. Infants consume more food per kg body weight than adults, further raising exposure to chemicals migrating from food packaging. Finally, infants undergo rapid biological maturation and exhibit different physiology from adults which may impact their potential sensitivity to chemical exposures (Neal-Kluever et al., 2014). Previously, FDA's safety review of migrants from food packaging was based on the assumption that the dietary exposure and toxicological concerns for infants would be captured under the safety assessment for the general population. FDA now considers key biological and exposure elements and their impact on infant safety for food contact materials. In the context of infant safety assessment, additional considerations in test protocol selection and study design may enhance the utility of toxicology data. Extended 1 generation or 2 generation developmental and reproductive toxicity assays may capture the greatest number of relevant endpoints. OFAS data from 31 generational studies (29 chemicals) presented a low toxicity profile, with roughly 20% of the studies eliciting no adverse effects on either generation. Of the studies that elicited adverse effects, the majority of these had similar effects on both generations. Systemic endpoints were more sensitive than developmental or reproductive endpoints in the majority of cases. Further work such as comparing these generational developmental toxicity studies with other juvenile toxicity study paradigms may assist the optimization of protocols to be used in the context of infant product safety assessment.

25. Determination of Gold Nanoparticle Penetration into Vaginal Mucosa using a Rat Model

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Plain Language Synopsis: We established a rat model to test whether engineered nanoparticles penetrate the vaginal mucosa, and found that intravaginally infused gold nanoparticles penetrated the mucus and tissue in a size dependent manner. The nanomaterials were detected using advanced methodology including transmission electron microscopy, hyperspectral dark-field microscopy and inductively-coupled plasma mass spectroscopy.

Abstract: Nanotechnology is the manipulation of matter at the atomic level. Nanomaterials are being included in FDA-regulated products, and it is incumbent on FDA to have the appropriate methods available to detect and quantify nanomaterials in FDA regulated products. Many commercial feminine hygiene products claim to contain nanoscale materials, potentially penetrating into the vaginal mucosa and presenting an unknown potential hazard to the consumer. There are limited data on (1) the nanomateiral content of feminine hygiene products and (2) nanomaterial penetration into the vaginal mucosal tissue. We describe the second part of this project, that is, establishing an in vivo rodent model for vaginal penetration of nanomaterials and evaluated penetration of gold (Au) nanoparticles (NPs) into rat vagina mucosa. Physicochemical properties of Au-NPs (30 nm, 100 nm) coated with polyvinylpyrrolidone (PVP) or polyethyleneglycol (PEG) were characterized using transmission electron microscopy (TEM), particle size analysis, UV/Vis spectroscopy, inductively-coupled plasma mass spectroscopy (ICP-MS) and other methodologies. The sexually mature female rats were injected with 100 mg/kg of medroxyprogesterone to induce a synchronized estrous cycle before vaginal lavage of Au-NPs at a dose of 100 µg/kg of body weight. Vaginal cytology examination indicated that all animals were induced an extended di-estrus status. No consistent elemental gold was detected in liver, lyM.P.H. nodes, kidney, spleen and blood using ICP-MS at 24 hours post-Au-NPs application. However, gold from Au-NPs were detected by ICP-MS in vagina tissue but not in ovary or uterus tissues in the reproductive tract. The 100 nm Au-NPs appear to form aggregates associated with mucinous materials in lumen of the vagina, as evaluated by light microscopy. Furthermore,

both 30 nm PEG-Au-NPs and 30 nm PVP-Au-NPs were found to pass through the vagina mucosa, as evaluated using dark-field microscopy and TEM. In contrast, 100 nm PEG- and PVP-Au-NPs did not penetrate the vaginal mucus barrier. These results suggest that the size of the nanoparticles play an important role in the diffusion through mucus and subsequent vaginal penetration. In this study we established the rat model for determining vaginal mucosal penetration of nanomaterials as a screen for possible human exposure and risk.

26. Developing in vitro and in vivo Models to Predict Drug-Induced Acute Allergic Adverse Reactions

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Plain Language Synopsis: All medications have the potential to produce adverse events (AEs) and such adverse events can lead to significant morbidity and mortality. We have developed several in vitro and in vivo models that can quickly identify the potential causes of drug-induced acute allergic adverse reactions, help inform marketing decisions and post-market surveillance.

Abstract: All medications have the potential to produce adverse events (AEs) and such adverse events can lead to significant morbidity and mortality. Premarket clinical studies for drugs involve hundreds to thousands of patients. International Council on Harmonization guidance suggests 1500 patients should be exposed to new products in clinical trials. Thus adverse events with a frequency of less than 1 to 2 per thousand are unlikely to be detected in advance of marketing. However lower frequency serious adverse events, particularly severe life-threatening anaphylaxis that can occur within minutes after drug administration, have been considered significant enough to take drugs off the market. Thus, ideally, premarket studies based on sensitive models would predict rare serious adverse events and could inform marketing decisions and post-market surveillance. From the study of 2008 heparin adverse events which caused hundreds of acute hypersensitivity-like symptoms and death to the more recent recall of Omontys (peginesatide) which caused anaphylaxis, our research group has established several in vitro and in vivo models to rapidly evaluate potential causes of an acute anaphylactic reaction. These methods are based upon current understanding of the mechanisms of clinical anaphylaxis, and include: 1) flow cytometry- based drug-specific IgE/IgG screening and in vitro Type 1 sensitization model (using drug-binding antibodies and mast cell degranulation); 2) direct mast cell degranulation; 3) assays for a cytokine storm (using activated T cells and macrophages from PBMC or whole blood culture); 4) complement- activation generated anaphylatoxins assays (C3a, C4a and C5a); and 5) contact system (kinin/kallikrein) activation assays. In addition, we are also developing a C1 inhibitor deficient mouse model to enhance the prediction of contact system- associated acute allergy in vivo. These in vitro and in vivo models will not only help the agency quickly respond to and identify the potential causes of drug-induced acute allergic adverse reactions, but could also facilitate development of safer products by improving the understanding and mitigation of such risks.

Session 8: Harness Diverse Data through Information Sciences

(Posters 27-44 are located in Section A)

27. JSpecies Tetra Analysis of *Listeria monocytogenes* Whole Genome Sequencing Data to Rapidly Assess the Genetic Relatedness of Isolates

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Plain Language Synopsis: L. monocytogenes is one of the leading causes of death caused by foodborne illnesses in the USA. This project evaluates JSpecies Tetra, a genome analysis tool that can rapidly link isolates, allowing a faster removal of contaminated foods from the market and improving food safety and public health.

Abstract: As part of our participation in FDA's efforts to build a comprehensive genomic approach to the identification of related isolates and to further understand trends in listerial evolution that may be critical for food safety response, we have been evaluating tetranucleotide frequency analysis via the JSpecies program to rapidly analyze more than 100 hundred Lm genomes as a QC step and to provide a cursory analysis of strain relatedness. Tetranucleotide (tetra) analysis plots standardized (z-score) tetramer word frequencies of two strains against each other and uses linear regression analysis to determine similarity. This tool was able to validate the close relationships between outbreak related strains with known common sources from four different outbreaks and identified a close relationship with two isolates that were previously unlinked to our knowledge. We also analyzed Listeria strains isolated during the recent caramel apple outbreak, and stone fruit incident in 2014. We identified that many of these isolates shared a common serotype, 4bV, using a qPCR tool developed in our lab. The 4bV serotype is characterized by the presence of a 6.3 Kb DNA normally found in serotype 1/2a strains and not found in serotype 4 or 1/2b strains. Based on these findings, we decided to compare these strains at a genomic level using the JSpecies Tetra tool. We used this tool to compare several 4bV, 4b and 1/2a isolates and identified a high level of similarity between the stone fruit and apple 4bV strains, but not the 4b strains co-identified in the caramel apple outbreak. Additionally, these fruit-derived 4bV strains didn't show any significant relationship with other 4bV strains in our collection. The identification of these potential links shows that Tetra analysis, in addition to MLST and whole genome SNP analyses, can be a useful tool in rapidly assessing relatedness of Lm isolates during outbreak investigations, and comparing historical isolates. Additionally, these results lead to the identification of two separate incidents involving highly related strains which suggests the possibility of a new epidemic clone that may be better adapted for certain foods and/or environment.

28. Sex Subgroup Analyses in Pivotal Clinical Trials of New Drug and Biologic Products Approved by FDA Center for Drug Evaluation and Research in 2014

Chen, Alice, FDA/CDER/OTS; Itana, Hawi, FDA/OC/OWH; Xia, Guanjun, FDA/CDER/OTS; Pepe, Salvatore, FDA/CDER/OND; Pariser, Anne, FDA/CDER/OTS

Plain Language Synopsis: Per FDASIA requirements for reporting on demographic subgroup representation in clinical trials, a retrospective review of sex subgroup analyses was conducted on a subset of 2014 new drugs approved by CDER. Data were reviewed in various ways to explore the extent of female participation and sex subgroup analyses by reviewers.

Abstract: Background: Beginning in the 1980s and '90s, concerns were raised about underrepresentation of women in clinical trials. In 1992, US Government policy was changed with the intention of addressing these inequities, and recent FDASIA legislation directed FDA to report on the extent to which demographic subgroups were included in marketing applications submitted to FDA and to improve public availability of such data. Objectives: to assess the participation of women in pivotal trials and the evaluation of safety and efficacy in sex subgroups for New Molecular Entities (NMEs) and Original Biologics License Applications (BLAs) approved by FDA CDER in January-August 2014 ("new drugs"). Methods: A retrospective review of sex subgroups in pivotal trials for new drugs approved by FDA CDER from January-August 2014 was conducted.

Demographics were assessed from clinical and statistical reviews, product labeling, and other regulatory information. Results: From January-August 2014, CDER approved 19 NMEs and 7 BLAs for 30 indications. Overall, women accounted for 35-40% of subjects in pooled pivotal efficacy trials and safety analysis populations. By therapeutic area, female participation ranged from 21% (Dermatology) to 71% (Neurology). For the 19 indications receiving priority review and the 14 orphan drug indications, female participation was 41% and ~40%, respectively. Drug sponsors provided demographic data in 97% of their marketing applications. FDA reviewers assessed demographic data in 100% of reviews, and provided sex subgroup analyses for efficacy and safety in 88% of reviews. 7% of the time, sex subgroup analysis resulted in an actionable result, such as dosing recommendations, in the product labeling. Conclusions: Female participation in clinical trials varies greatly depending on the therapeutic area. Pooling across disease indications can be misleading, since disease prevalence by sex may vary substantially by indication. Overall female participation was slightly higher in priority review applications and in rare disease applications, but the reasons for this are unknown. Demographic data are provided by sponsors and sex subgroup analyses are performed by FDA reviewers for almost all applications; however, sex-based information (e.g., dosing recommendations) was included in labeling for only 7% of applications. Assessment of new drugs for the remainder of 2014 is ongoing.

29. The Development of Liver Toxicity Knowledge Base (LTKB) for Research and Review of Drug-Induced Liver Injury

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Plain Language Synopsis: Drug-induced liver injury (DILI) presents a significant challenge to drug development and regulatory application. The Liver Toxicity Knowledge Base (LTKB) aims to provide literature data and regulatory information about DILI to support research and review of drug safety through providing a reference database and a panel of predictive models.

Abstract: Drug-induced liver injury (DILI) presents a significant challenge to drug development and regulatory application. The Liver Toxicity Knowledge Base (LTKB) aims to provide literature data and regulatory information about DILI to support research and review of drug safety. The LTKB contains ~3000 unique prescription drugs, including ~1400 drugs approved by FDA, ~1300 drugs approved by other agencies like EMA, and 210 drugs withdrawn from the worldwide market. The following data are available for most of drugs in the LTKB: chemical structure, therapeutic use, PD/PK, DILI types and severity, DILI mechanisms, histopathology, drug targets, side effects, etc. The LTKB can serve as 1) a reference database when drug/DILI-related data need to be queried; 2) an assessment tool of DILI risk in humans for new chemical entities in the review process; and (3) a tool to support biomarker studies using emerging technologies (e.g., genomics, in vitro studies).

30. Comparative Pathogenomics of Clostridium tetani

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Plain Language Synopsis: We have performed next-generation sequencing in parallel with strain characterization on several strains of Clostridium tetani to better understand the contribution of mobile genomic elements in vaccine production for tetanus toxoid and glycoconjugate biology.

Abstract: Clostridium tetani (C. tetani), the bacterium that produces tetanus toxin, is found throughout the environment in soil and the digestive tracts of animals and humans. Tetanus intoxication, in the absence of vaccination, can lead to generalized rigidity and skeletal muscle spasms and, ultimately death. Vaccination against tetanus with a formalin-inactivated toxin (or toxoid) is extremely effective and has remained essentially unchanged for more than 90 years. Furthermore, the cultivation of specific vaccine strains and preparation of toxin has not advanced significantly since its introduction. As part of the

strategic plan for modernizing regulatory science, we performed next-generation sequencing and comparative pathogenomics of several strains of C. tetani including the Harvard vaccine strain (C2) to identify specific genomic changes that may benefit the production of tetanus as a toxoid and immunoreactive glycoconjugate in vaccine research. We have observed that the C2 vaccine strain has undergone a number of phenotypic changes as a result of selection for growth and increased toxin expression such as loss of sporulation and reduced swarming motility. We find that the most significant genomic changes are in mobile genomic elements (the mobilome), including prophage genomes, CRISPR arrays, and polysaccharide processing modules. The single plasmid, harboring the tetanus toxin gene, has undergone insertions and deletions as well; in some wild strains, loss of toxigenicity. This is in contrast to the closely related organisms, C. botulinum where the toxin genes (botulinum neurotoxin) may be encoded on plasmid, phage, or chromosome. Further analysis of the genomes of wild C. tetani strains may help better characterize the contribution of the mobilome to pathogenicity and ultimately the prevention of disease through more modern vaccines.

31. Comprehensive Evaluation of microRNA and mRNA Signatures of Platelets during Storage

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Plain Language Synopsis: During storage under standard blood bank conditions, platelets normally start to lose their viability with time due to several morphological and physiological changes, collectively known as platelet storage lesion (PSL). We are examining stored platelet to find microRNAs which can serve as biomarkers for the determining the quality of stored platelets.

Abstract: MicroRNAs (miRNAs) are small noncoding RNAs and posttranslational regulators of cellular mRNA expression. We have envisioned that since these small RNAs regulate cellular events, evaluation of miRNA:mRNA interactions at different time points during platelet storage would help us identifying affected physiological pathways and in the process, we may also identify miRNAs that could serve as surrogate markers of platelet quality during storage. In this study, RNA was isolated from day 0, day 5 and day 9 stored human leukocyte depleted platelets and subjected to differential miRNA and mRNA profiling and bioinformatics analyses. The analyses identified 222 differentially expressed miRNAs over time in storage. The Ingenuity Pathway Analysis (IPA) identified 81 differentially expressed miRNAs potentially targeting 24 known apoptosis signaling mRNAs and a set of 53 mRNAs implicated in platelet activation, adhesion, aggregation, binding, hemostasis and coagulation. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and IPA both identified Phosphoinositide-3-kinase (PI3 K)/Protein Kinase B (AKT) signaling pathway as potentially involved in platelet storage. Overall, our results reported here support the idea that miRNA:mRNA interactions are consequential to the cellular physiological and biochemical perturbations in stored platelets, collectively termed platelet storage lesions (PSL). Further, expression of miR-103 members (e.g. miR-103b) was previously reported to be correlated with cellular glucose levels and in our study here, miR-103b demonstrated increased expression pattern for up to day 5 and then started to decrease by day 9. Based on previous reports and our observation in this report together, suggest that high levels of miR-103b in platelets may improve its glucose levels and hence quality during storage, which warrants further experiments in this direction.

32. Considerations of Environmental Effects of Smokeless Tobacco

Edwards, Ronald, FDA/CTP/OS

Plain Language Synopsis: This poster presents the major issues FDA considered in its first programmatic environmental assessment (PEA) before the marketing authorization, through the SE pathway, of several new smokeless tobacco products manufactured in Denmark. The PEA addressed the environmental effects of the manufacturing, use, and disposal of the products and their packaging.

Abstract: Objective: Through the substantial equivalence pathway (SE), FDA has authorized tobacco products to be marketed in the US. In doing so, FDA considered the environmental effects due to its actions. This poster presents the major issues FDA considered in its first programmatic environmental assessment (PEA) before the marketing authorization, through the SE pathway, of several new smokeless tobacco products manufactured in Denmark. The PEA addressed the environmental effects of the manufacturing, use, and disposal of the products and their packaging. Methods: The evaluation of the environmental impact of FDA's action was established based on import data, market volume projections, and mass of the products. Results: Smokeless tobacco products include chewing tobacco, snus, snuff, and dissolvable products. In 2013, Denmark exported 45.5 tons of tobacco products to the US, 13.2 tons of which were chewing tobacco and snuff. The individual and cumulative projected market volumes for the new products are less than 1.5% of these imports. While US sales of smokeless tobacco products have increased by 57% since 2009, smokeless tobacco imports from Denmark have declined. Therefore, the emissions and material mass anticipated to be released into the environment as a result of manufacturing due to the proposed action are not substantial compared to that of all tobacco product imports from Denmark. The environmental introduction as a result of use is negligible or does not exist. The environmental introduction as a result of disposal following use by US consumers is negligible compared to that of all tobacco products. The environmental introduction as a result of disposal of the packaging materials following use by US consumers is negligible compared to total municipal solid waste generated in the US. Conclusions: FDA carefully considered the potential environmental impact of the action of issuing an order, through the SE pathway, allowing the introduction of the new tobacco products into interstate commerce and concluded that these actions will not have a significant impact on the quality of the human environment. Therefore, an environmental impact statement was not required.

33. Demographics by Sex and Race of Study Participants in Clinical Trials for Oncology Products Approved by FDA in 2013

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Plain Language Synopsis: The purpose of this study is to assess the participation of women and racial/ethnic minorities in clinical trials in support of New Drug Applications (NDAs) and Biologics License Applications (BLA) for oncology products approved by FDA in 2013.

Abstract: Background: According to the American Cancer Society, there are approximately 1.6 million people living with cancer in the United States, 51.3% of which are men and 48.7% of which are women. African-Americans have the highest incidence rate of cancer (508.3 per 100,000), followed by Caucasians (483.6 per 100,000), and then American Indian/ Alaskan Natives (391.8 per 100,000). The US Food and Drug Administration (FDA) has implemented several regulations and guidances for adequate representation of women and ethnic/racial minorities in clinical drug trials. Objectives: The purpose of this study is to assess the participation of women and racial/ethnic minorities in clinical trials in support of New Drug Applications (NDAs) and Biologics License Applications (BLA) for oncology products approved by FDA in 2013. Methods: The sex and race of subjects in all cancer drug clinical trials submitted to FDA in New Drug Applications (NDAs) and Biologics License Applications (BLAs) approved in 2013 were examined for this study. Demographics were assessed from final clinical study reports submitted by the product's sponsor to FDA. Results: Eight (including 1 application each for prostate (Xofigo) and breast cancer (Kadcyla)) oncology products were approved during the period studied (6 NDAs and 2 BLAs). A total of 83 clinical trials classified as phase 1, 2, or 3 studies were submitted in the NDAs/BLAs. Pooled demographic analysis indicated that the mean participation of women in these trials was 48% and the majority of the trial subjects were Caucasians (74%). When analyzed by phase of the trials, mean female participation was 40%, 43% and 53% for phases 1, 2, and 3 respectively. Overall, racial/

ethnic minority participation was 2.3% (Black), 17.5% (Asian), 1.3% (other) and 4.0% (unspecified). Conclusion: Overall, the participation of women in clinical trials is similar to proportion of women in the US cancer patient population. In Phase 3 trials, during which much of the safety and efficacy are assessed, female participation is higher. However, there is lower representation of women in Phase 1 trials, during which the preliminary safety of new drugs and biologics are evaluated. The participation of African Americans in all phases is considerably lower than would be expected based on the US cancer patient population prevalence by race.

34. FDALabel Database: Enabling Insights from Product Labeling to Accelerate Advancement of Regulatory Sciences

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Plain Language Synopsis: FDALabel Database is a web application allowing users to search ~ 70,000 FDA Structured Product Labeling (SPL). FDALabel provides a user-friendly interface with customized searches against the entire text of product labeling. It provides FDA reviewers and scientists an effective and efficient means of accessing rich information in SPL.

Abstract: FDA Structured Product Labeling (SPL) provides rich product information including product indications, target populations, and adverse events (AEs) collected from manufacturer data and post-marketing surveillance. Recently, the amount of information captured in product labeling has grown rapidly due to the finalization of US FDA labeling regulations and laws. This rapid pace of change, along with the breadth and depth of information contained in the labeling, highlights the need for a powerful search tool. FDALabel is a comprehensive product labeling database with powerful web-based full-text search capabilities. The database contains the full set of approximately 70,000 FDA SPLs. The products include human drugs and biologic products, animal drugs, human devices and human vaccines. The simple search options in FDALabel database include full-text searches as well as searches within only the product name. The advanced search options allow querying text based on any combination of specific sections, document types, market categories, market date and other information. To demonstrate FDALabel database's utility, we have selected study cases including a pharmacogenomics biomarker study and also an AE study that uses Medical Dictionary for Regulatory Activities (MedDRA) standard terminologies. FDALabel provides regulators, researchers, manufacturers, and physicians an effective and efficient means of accessing the rich amount of information in product labeling for product safety and effectiveness. It also opens up the possibility for novel uses of product labeling in supporting FDA's goals of advancing regulatory sciences, e.g., to identify trends in AEs that are associated with increased risks to public health.

35. Systematic Risk Assessment of Human Exposure to Tobacco Products using Computational Models

Erkkila, Brian, FDA/CTP; Yeager Phil, FDA/CTP; Chemerynski, Susan, FDA/CTP; Weil, Roxana, FDA/CTP; Nguyen, Luc, FDA/CTP; Jackson, Habibah, FDA/CTP; Sholtes, Deborah, FDA/CTP; Kitani, Takashi, PointCross, Inc.; Tran Ho, Lam Thuy Vi, PointCross, Inc.; Madhavan, Suresh, PointCross, Inc.; Nath, Shree, PointCross, Inc.; Sadashivaiah, Prasanna, PointCross, Inc.

Plain Language Synopsis: CTP has initiated the development of a new Risk Modeling and Simulation Tool (RMST) to continue to develop and meet performance standards for the timeliness of tobacco product reviews while maintaining the scientific rigor necessary for the protection of public health. RMST incorporates diverse data streams into computational models, resulting in advanced visualizations to aid in the systematic assessment of human health risks associated with human exposure to tobacco products.

Abstract: A strategic priority for CTP is to continue to establish and meet performance standards for tobacco product reviews. There are several refinements that could assist in meeting these performance standards while maintaining the scientific rigor necessary for the protection of public health, including improving regulatory

reporting, standardizing Harmful and Potentially Harmful Constituent (HPHC) listings , and developing more efficient systems for evaluating submissions. To meet these important regulatory and scientific needs, CTP engaged with PointCross, Inc. through a Broad Agency Announcement (BAA) initiative in 2014 to collaboratively build a Risk Modeling and Simulation Tool (RMST). The RMST allows for systematic assessment of health risks associated with human exposure to tobacco products through the use of computational models and advanced visualization. Within the tool, CTP Toxicologists may define, edit, store, and specify parameters for multiple tobacco cancer risk models. These parameters include: a) Smoker characteristics, b) HPHC properties, c) HPHC concentrations, and d) Cancer risk algorithms. In addition, regulatory toxicologists may access pre-defined models to run multiple cancer risk assessments of tobacco products and dynamically assess visual representation of the data. Further, comparisons may be made between different types of tobacco products and different types of predictive models. The tool enables visual analysis of the executed model, supplemented with tabulated data summaries, and generates reports for regulatory reporting purposes. Future development of RMST will support larger sets of variables to describe a range of carcinogenicity risks using stochastic simulations. Since such simulations will generate large data volumes that must be stored, post-processed and summarized for CTP use, the tool is being built using the Hadoop platform, which provides the scalability and robustness necessary to meet CTP's long-term needs. CTP scientists have reviewed the initial version of the RMST. Based on this initial review and ongoing development, it is expected that in the future, the tool will contribute to CTP's strategic priority to meet performance standards for tobacco product reviews while maintaining the scientific rigor necessary for the protection of public health.

36. How did We Complete Individual PK Study Analyses from an Entire NDA in 3 Days?

Lee, ID Peter, FDA/CDER/OTS/OCP; Porta Martin-Moreno, Eduard, FDA/CDER/OTS/OCP

Plain Language Synopsis: Clinical pharmacology studies are pivotal for determining the appropriate dosing instructions in drug labels. A typical NDA submission could include up to 20 or more studies, each designed differently. A review tool, PKView, is developed to automate the PK analyses of all clinical pharmacology studies in a NDA submission.

Abstract: Clinical pharmacology study results are pivotal for determining the appropriate dosing regimens for patient populations across various intrinsic and extrinsic factors, such as hepatic and renal disease, demographic and concurrent drug administration. Several FDA guidance's address the sciences and regulatory utilities of pharmacokinetics data as the bridging information from typical patient to special populations and extrinsic factors. Typically for each new molecular entity (NME) submission, multiple clinical pharmacology studies, up to 20 or more, are conducted to support the labeling claims. Each study is designed differently for the corresponding study objectives, ranging from crossover, parallel, sequential, cohort, to nested, with most studies examining multiple arms of patients. In addition, pharmacokinetic observations can span from single dose to steady state, including parent drug and metabolites blood concentrations. PKView is designed to automate the PK analyses of all clinical pharmacology studies in a NDA/BLA submission. The objective is to efficiently identify study reports that show unique issues related to PK analyses and might warrant further detailed reviews. During the test phase of PKView, PK studies from more than 40 NDA's were analyzed. Many of the studies showed some degree of discrepancy between sponsor's analyses and PKView base analyses. Preliminary reviews of sponsor's reports and Clinical Pharmacology reviews provided hints to the causes for these discrepancies. While the causes of discrepancy might have been identified, the justification for sponsor's analyses and the impacts of the discrepancy on drug labels should be evaluated with the overall knowledge of the submissions, and the understanding of the risk/benefit factors of individual drugs. Please note that the PKView base analysis does not necessarily represent the final analysis of a study but serves as a tool to identify any special data handling, unique analyses, study design features, study conduct issues, or potential reporting errors. The method of base analysis adopted in the PKView is described in the User Guide.

37. Transfusion-Related Acute Lung Injury (TRALI) Occurrence among Inpatient Medicaid Beneficiaries, under 65 Years of Age, as Recorded by Large Administrative Databases during 2007-2010

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Plain Language Synopsis: Our retrospective claims-based study used large Medicaid databases to assess occurrence of Transfusion-Related Acute Lung Injury (TRALI), a serious and rare transfusion complication, and to ascertain potential risk factors among inpatient Medicaid beneficiaries under 65 years of age, during 2007-2010.

Abstract: Objectives: Transfusion-Related Acute Lung Injury (TRALI) is a serious transfusion complication resulting in pulmonary edema and respiratory failure. The study objectives were to assess TRALI occurrence and potential risk factors among inpatient Medicaid beneficiaries under 65 years of age, during 2007-2010. Methods: This retrospective claims-based study used large Medicaid databases. Transfusions were identified by recorded procedure and revenue center codes, while TRALI was ascertained via ICD-9-CM diagnosis code. Revenue center units were used to quantify blood use. Study evaluated TRALI rates (per 100,000 transfusion stays) among Medicaid beneficiaries, overall and by year, age, sex, race, number of units, and blood components transfused. Results: Of 1,122,907 inpatient transfusion stays for Medicaid beneficiaries during 2007-2010, 162 had TRALI diagnosis recorded, an overall rate of 14.43 per 100,000 stays. Annual TRALI rates were 12.70, 10.67, 16.61, and 18.14, respectively. TRALI rates for ages 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, and 60-64 were 5.15, 13.41, 17.23, 19.60, 19.45, 13.66, and 10.54. Rates for females and males were 12.78 and 17.28, whereas for whites and non-whites were 15.55 and 13.45. TRALI rates by number of units were: 9.56 for 1 unit, 7.72 for 2-4 units, 17.70 for 5-9 units, and 62.12 for >9 units. Rates by blood component groups were: 13.86 for RBCs only, 4.93 for plasma only, 23.22 for platelets only, 37.37 for platelets and plasma, 56.02 for RBCs and plasma, 43.12 for RBCs and platelets, and 74.75 for RBCs, plasma, and platelets. Conclusions: This is the first and largest-to-date claims-based TRALI study among Medicaid beneficiaries. The results show a possible trend of increasing TRALI occurrence over time. The findings also suggest that TRALI rates vary by age, sex, race, number of units, and blood components transfused, with highest rates for stays with >9 units transfused and for stays with RBCs transfused in combination with plasma and platelets. Overall, our study shows usefulness of large administrative databases in assessment of rare adverse events, and thus, will help to harness diverse data to enhance blood safety surveillance, facilitate development of prevention strategies, and improve patient outcomes in the United States.

38. Steps on a Journey: Re-Use of Analysis Scripts and Standardized Tuberculosis Trial Data

Li, Xianbin, FDA/CDER/OTS; Witczak, Bobbie, FDA/CDER/OTS; Li, Joy, FDA/CDER/OTS; Rosario, Lilliam, FDA/CDER/OTS; Navarro, Eileen, FDA/CDER/OTS

Plain Language Synopsis: The development of clinical trial data standards for tuberculosis enabled the combining of trial data, contributed to the a technical guide for submission of standard trial datasets, contributed to the assessment of a new trial endpoint and could allow the reuse of an analysis tool across new drug submissions for tuberculosis.

Abstract: Standardizing clinical trial data elements, terminologies, and data structures have proven useful in enabling the automation of safety analyses. Our experience in the testing and research implementation of the Tuberculosis data standard illustrates some of these potential benefits for efficacy analyses. The poster describes lessons learned from testing the utility of data standards in three use cases conducted from 2008 - 2014. Initial testing of the TB data standard contributed to the development of the Technical Conformance Guidance. Standardized data enabled aggregation of legacy converted datasets to inform utility of a new efficacy endpoint and finally, the reuse of the analysis script for the new endpoint was assessed against 3 new drug applications in tuberculosis. Adherence of analysis datasets to

the Analysis Data Reviewer Guide enables reuse of analysis scripts and could provide additional efficiency to review. A time to event analysis script can be readily deployed for a new trial submission with standard variable names, standard variable definitions and adequate censoring information. Further, the inclusion of analysis population flags (Y/N) (ITTFL, mITTFL, PPFL), and covariates used in regression analyses in the ADTTE would bring us one procedure closer to efficient analysis.

39. Leveraging FDA's Archived Text Documents: How to Improve Access to Key Data through Semantically Enhanced Search

Porta, Eduard, FDA/CDER/OTS/OCP; Lee, Peter, FDA/CDER/OTS/OCP

Plain Language Synopsis: A wealth of text documents is archived at FDA. Reviewers often want to resort to exploring these archives, but the amount of available textual data is overwhelming. We propose a search tool that strives to provide meaningful answers, by leveraging text analysis techniques.

Abstract: A wealth of text documents is archived at FDA, with historical cases that may have certain characteristics similar to an ongoing NDA in terms of clinical outcomes or regulatory scenarios. Reviewers often find themselves in a situation where, they may want to go back and resort to exploring these archives. The amount of available textual data is overwhelming, yet keyword based search often delivers a great number of documents that may contain the search terms, but are far from providing information relevant to the case. We propose a search engine that strives to provide useful and meaningful answers, by combining the simplicity of text search with the accuracy of text-mining techniques. To do this, our query processor performs a first pass identifying text sections with keyword based matches, and subsequently analyzes the syntactical relationships among the matched words to determine a relevancy score. The proposed system can search for numerical ranges and categorical variables, with an approach that avoids the use of complicated syntax or cluttered user interfaces. It also provides the option to specify multiple search queries to be matched in different sections of the document. The user interface is designed to be simple and intuitive. It can present the search results in a tabulated format, summarizing numerical and text information in statistical metrics, which has proven valuable for regulatory research projects.

40. PCR Free Full Genome Characterization of Highly Diverse HIV Strains by Nextgen Sequencing

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Plain Language Synopsis: Accurate characterization of HIV-1 is important for understanding the dynamics of the pandemic at the population level. HIV-1 genetic diversity and recombination pose difficulty in successful PCR amplification of viral genome. Here, we show a PCR free full genome characterization of HIV-1 by RNA seq approach with the use of novel bioinformatics.

Abstract: Background: HIV-1 genotyping is an important tool for clinical and epidemiological studies. Recombination and mutations pose difficulty in successful PCR's and accurate characterization. In addition novel emerging subtypes may not be detected with standard overlapping PCR amplicon sequencing. Here we report a novel PCR free multiplex method for full length (~9.7kb) HIV-1 characterization by Nextgen RNA seq approach. Methods: A total of 38 diverse HIV viruses representing subtype A-G, CRFs, URFs and Group O were obtained from Duke-EQAPOL and represented isolates from their Global HIV panel. Viral RNA was extracted, normalized, fragmented, reverse transcribed and sequenced in Miseq as described in illumina Truseq RNA Kit. Reads are quality checked, filtered and reference mapped using CLC genomic work bench software v6.0.4. Consensus sequences were phylogenetically characterized for neighbor-joining tree based on the Kimura two-parameter substitution model and recombinations revealed from Simplot analysis. Drug resistance was inferred from Stanford HIV drug resistance program and viral tropism was determined from g2p 10% FPR. Results: Multiplex RNA sequencing approach yielded >10000x coverage for each of the viral genomes and discriminated through bio-

informatics. After filtering reads specific for HIV, each viral nucleotide had >1000x coverage. This approach enabled to reconstruct whole genome HIV haplotypes accurately including flanking LTRs. Analysis of full genome using Simplot has identified mosaic structures of CRFs and URFs. All these HIV subtypes identified were comparable to Sanger sequencing. In addition to subtyping, this approach revealed NNRTI, integrase and protease drug specific minor variants and point mutations with >1000x coverage. HIV-1 viral tropism predicted 89% of isolates as R5 tropic and the remaining were X4 tropic viruses. Conclusions: Our findings provide a reliable approach to characterize novel recombinants and distantly related strains of HIV for panel development. Multiplexing and bio-informatic discrimination approaches save cost and labor. In addition HIV-1 characterization through the PCR free approach described here reduces errors and artificial recombination.

41. Sex Subgroup Analysis from US and International Sites for Pivotal Clinical Trials of New Drugs and Biologic Products Approved by FDA in January-July 2014

Guanjun Xia, FDA/CDER/OTS; Alice Chen, FDA/CDER/OTS; Itana, Hawi, FDA/OC/OWH; Salvatore Pepe, FDA/CDER/OND; Anne Pariser, FDA/CDER/OTS

Plain Language Synopsis: Per FDASIA requirements for reporting on demographic subgroup representation in clinical trials, a retrospective review of sex subgroup analyses was conducted on a subset of 2014 new drugs approved by CDER. Data were reviewed in various ways to explore the extent of female participation and sex subgroup analyses.

Abstract: Background: Since the 1980s, concerns have been raised about whether US women are being adequately represented in premarket drug trials. Increasingly, drug development relies upon data from international sites, raising additional concerns about generalizability to the US population. Objectives: The purpose of this study is to assess the participation of women from US and international sites in pivotal trials for new drugs approved by FDA CDER in January-July 2014. Methods: A retrospective review of sex subgroup analysis in pivotal trials for New Molecular Entity (NME) and Original Biologic License Application (BLA) marketing applications approved by CDER from January-July 2014 was conducted. Results: CDER approved 16 NMEs and 7 BLAs in this time period. Overall, women accounted for 32.3% of subjects in pooled pivotal efficacy trials, 34.4% at US and 31.3% at international sites. Percent female participation was generally similar at US and international sites for most disease indications. One example of an exception was a COPD drug program, where female participation was higher in the US (43.1%) than internationally (18.8%). Conclusions: Female participation in clinical trials varies depending on the therapeutic area. Generally, female participation for US vs. international sites was similar. The difference in the COPD program was likely due to differences in disease prevalence by the region; however, efficacy by sex in the US trial was still able to be assessed.

42. Innovative Analytics for Big Data: Utilizing National and International Registries to Enhance Medical Product Evaluation

Yue, Lilly, FDA/CDRH/OSB; Campbell, Gregory, FDA/CDRH/OSB; Lu, Nelson, FDA/CDRH/OSB; Xu, Yunling, FDA/CDRH/OSB; Zuckerman, Bram, FDA/CDRH/ODE

Plain Language Synopsis: National and international registries, which reflect real world evidence, can play important roles in regulatory decision making in pre-market medical product evaluations. This presentation focuses on developing innovative analytics to use such data - prospectively design clinical studies with propensity score methodology and perform appropriate objective statistical inference.

Abstract: Regulatory decisions are made based on the assessment of risks and benefits of the medical products, including drugs, biologics and medical devices, at the time of pre-market approval, and subsequently, when post-market risk-benefit balance needs re-evaluation. Such assessments depend on scientific evidence obtained from pre-market studies, post-approval studies, post-market surveillance studies, and relevant registries. FDA has been making efforts on harnessing diverse data to improve health outcomes through, for example, national and international registries. Such registries provide real world evidence and are playing more and more important roles in enhancing the safety and effectiveness evaluations

of medical products. While these registries provide a huge amount of data reflecting real world practice and potentially reduce the cost of clinical trials, challenges arise concerning how to use the data to draw reliable statistical inferences. This presentation will focus on developing innovative analytics - prospectively design pre-market clinical studies utilizing national and international registries with propensity score methodology and preform appropriate outcome analysis for objective statistical inference. Statistical and regulatory challenges and opportunities will be presented with examples encountered in medical device regulatory reviews.

43. A Meta-Analysis of the Safety Profile of Inactivated Trivalent Influenza Vaccines and Quadrivalent Influenza Vaccines in Children and Adults

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Plain Language Synopsis: Using meta-analysis method to analyze the trends of adverse events (AEs) of quadrivalent influenza vaccines (QIVs) vs. trivalent influenza vaccines (TIVs), we found that there was no difference in the frequency of fever or any unsolicited AEs between QIVs and TIVs groups in either children or adults.

Abstract: Influenza vaccines are critically important in preventing morbidity and mortality due to influenza illness. Seasonal inactivated trivalent influenza vaccines (TIVs) include three influenza viruses (A/H3N2, A/H1N1 and B [either Yamagata or Victoria lineage]). However, two influenza B lineages have co-circulated since 2001, often leading to mismatch between the predominant circulating B lineage and the B lineage contained in the vaccine. To address this issue, the World Health Organization issued a recommendation to include both B lineages in annual influenza vaccines in 2012, leading to the development and licensure of several inactivated quadrivalent influenza vaccines (QIVs). Several publications have compared the immunogenicity and safety of QIVs to TIVs in single clinical trials, but to date, no comprehensive and systematic analysis to compare the safety profile of QIVs with TIVs has been published. We therefore performed a meta-analysis using clinical trial data obtained from the Food and Drug Administration's database of approved QIVs. We analyzed adverse events (AEs) reported using the Medical Dictionary for Regulatory Activities preferred terms from nine randomized clinical trials conducted in children and adults comparing QIVs with TIVs. The nine trials included 9880 subjects (5047 children and 4833 adults) receiving QIVs and 6870 subjects (4719 children and 2151 adults) receiving TIVs. The frequencies of specific solicited AEs (erythema, swelling and fever) as well as unsolicited AEs grouped by System Organ Class were compared. When possible, an odds ratio was calculated from each study and a common odds ratio was computed along with a 95% confidence interval. Some trials had no events in either the QIVs or TIVs groups and in such cases, computing an odds ratio was not possible; however, a correction was used that allowed us to include these trials in our analyses. In general, the safety profile was similar between QIVs and TIVs. In children, the frequencies of two injection site-related adverse reactions, erythema (QIVs/TIVs common odds ratio: 2.14, 95%CI: 1.48-3.22) and swelling (QIVs/TIVs common odds ratio: 2.10, 95%CI: 1.47-3.16) were greater in the QIVs group, but no difference was identified in adults. There was no difference in the frequency of fever or any unsolicited AEs between QIVs and TIVs groups in either children or adults. There were no reports of Guillain-Barré Syndrome in these nine clinical trials. Our analyses support the similarity in the safety profile of QIVs and TIVs, and suggest that such meta-analyses may provide information that is useful to further characterize the safety of this class of vaccines.

44. Cardiac Resynchronization Therapy in Women: Meta-Analysis of Clinical Trials and Post-Market Comparative Effectiveness Studies

Zusterzeel, Robbert, FDA/CDRH; O'Callaghan, Kathryn, FDA/CDRH; Sanders, William, FDA/CDRH; Selzman, Kimberly, FDA/CDRH; Pina, Ileana, FDA/CDRH; Masoudi, Frederick, University of Colorado; Spatz, Erica, Yale University; Bao, Haikun, Yale University; Curtis, Jeptha, Yale University; Varosy, Paul, university of Colorado; Ponirakis, Angelo, ACC; Canos, Daniel, FDA/CDRH; Strauss, David, FDA/CDRH

Plain Language Synopsis: Not including enough women in clinical trials leads to an information gap in the safety and effectiveness of medical

devices in female patients. This is highlighted in a recent FDASIA Section 907 Action Plan. Performing research such as this one demonstrates that medical devices can perform differently among women and men and highlights the importance of including more women in medical device clinical trials.

Abstract: Background: Women have been historically underrepresented in clinical trials. Therefore, an information gap sometimes exists on the safety and effectiveness of medical devices in women, highlighted in Section 907 of the recent Food and Drug Administration Safety and Innovation Act (FDASIA). One area where women are particularly underrepresented is in trials of heart failure devices, such as cardiac resynchronization therapy (CRT). CRT is a pacemaker therapy for selected patients with chronic heart failure and has been shown to reduce heart failure hospitalizations and mortality while improving quality of life. We assessed the effectiveness of CRT separately in women and men using pre-market and post-market data sources. Methods: In 3 separate studies (1 pre-market meta-analysis and 2 post-market registry studies) we assessed long-term mortality outcomes after CRT in subgroups by sex and electrocardiographic (ECG) characteristics (i.e. QRS morphology and duration). In the meta-analysis of pre-market clinical trials we combined individual-patient data from 3 clinical CRT-defibrillator (CRT-D) trials (MADIT-CRT, RAFT, and REVERSE; >4,000 patients total) submitted to FDA as part of pre-market approval applications. In the 2 post-market studies we used data from the National Cardiovascular Data Registry (NCDR) Implantable Cardioverter Defibrillator Registry (~32,000 and >75,000 patients respectively). Results: The pre-market meta-analysis showed that women had a 55% relative mortality reduction with CRT-D (compared with standard implantable defibrillator [ICD] alone) while men had a 15% reduction. With left bundle branch block (LBBB, a block in the left-sided conduction system of the heart), women benefited even more (61% relative reduction) while men benefited to a lesser extent than women (32% reduction in mortality). Without LBBB, neither women nor men benefited from CRT-D. In the first post-market study of ~32,000 CRT-D patients, we observed that women had an 18% lower mortality risk than men, while with LBBB this difference was even greater (21% lower death risk in women than in men). Among patients without LBBB, CRT-D was not associated with a mortality differential by sex. In the second post-market study of >75,000 patients, women had a 23% lower mortality risk with CRT-D than those with an ICD while in men this difference was 12%. Both women and men with a LBBB had lower mortality risks but again this was more pronounced among women (26% lower mortality risk in women and 16% lower risk in men). Without LBBB, women and men only had slightly lower mortality risks. Conclusions: In both pre-market and post-market studies, CRT-D was associated with a significantly lower mortality in women than in men particularly in the presence of LBBB. This sex difference highlights the importance of including more women in clinical trials, and conducting sex-specific analyses such as these of combined datasets and large post-market registries.

FDA Highlights

Session 1: Ensure FDA Readiness to Evaluate Innovative Emerging Technologies

Scientific breakthroughs are driving major changes in the development of medical treatments and diagnostics. Discoveries in complex chemistry and advances in biosynthesis have the potential to yield new drug candidates. Recent breakthroughs in electronics, nanotechnology, and materials science have revolutionized medical devices. And emerging fields such as gene therapy, cell therapy, tissue engineering, optogenetics, high intensity focused ultrasound, and information technology are also driving innovative ways to improve our health.

These novel and increasingly complex approaches to health, foods, and medical products present growing challenges to FDA's readiness to evaluate new products. Our regulatory science must include the necessary tools and methods to reliably assess their safety and efficacy. As part of the agency's efforts to ensure its regulatory science resources can meet this challenge, we seek to form collaborative partnerships with researchers from other agencies and institutions.

Our own research includes powerful tools and methods that are already helping us to prepare for the new medical products we will be evaluating in the future.

Next Generation Sequencing

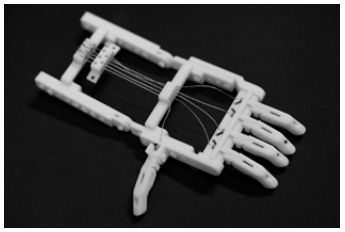
We use next generation sequencing (NGS) and related data analytics capability to advance medical product development. Since NGS data sets are large (typically terabytes), they pose challenges to efficient, cost-effective data storage, data transfer, and data analytics. Therefore, FDA uses a powerful computer technology called High-Performance Integrated Virtual Environment (HIVE) to digest, analyze, and manage all this data.

FDA uses NGS in a variety of applications, from whole genome microbial sequencing for food-borne bacteria detection and trace-back investigations, to developing new diagnostics and therapeutic interventions to achieve the promise of personalized medicine.

Screening Methods for Field Laboratories

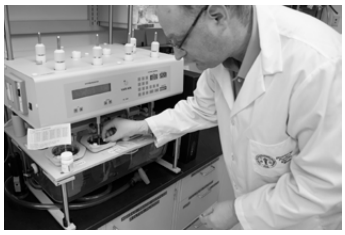
FDA is developing screening methods for field laboratories to improve the ability to detect chemical contaminants. We are evaluating new, state-of-the-art instrumentation for chemical measurement in food, including mass spectrometry, NMR, infra-red, and Raman spectroscopy. In addition, we are establishing methods to detect new, emerging, and unidentified chemical contaminants in FDA regulated products.

Materials Science and 3D Printing



FDA is studying improved materials and processing for emergency production of medical devices; we are also studying the use of 3D printing for tissue engineering and bone and tissue repair scaffolds

Advanced Drug Analysis



FDA is studying analytical approaches to evaluating complex therapeutics, including biosimilars and both innovator and generic drugs.

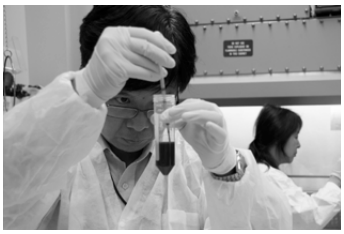
- Advanced analytical technologies, such as high-field, multidimensional NMR, high-resolution mass spectrometry, and modern spectroscopy to fully characterize complex therapeutics. This work will enable us to determine the level of 'sameness' for complex generic drugs and the similarity of biosimilars to previously approved products. The work requires us to develop new analytical methods and standards to ensure the quality of products from global sources.
- Methodologies for in vitro analysis of complex innovator and generic dosage forms, such as extended release formulations, inhaled drugs, liposomes, and targeted therapies. We will couple this information with clinical performance data to better understand which quality attributes are most critically linked to clinical performance.

Traumatic Brain Injury

FDA is investigating methods for developing electrodes used for brain-computer interfaces and other neurological implants to assess electrophysiological outcome measures (EEG, evoked potentials) in the evaluation of traumatic brain injury.

Quality by Design and Advanced Manufacturing Techniques

- FDA is incorporating concepts of Quality by Design (QbD) and applying Process Analytical Technologies (PAT) into laboratory-scale manufacturing. This work will help the agency to better understand and control critical quality attributes through process parameters and to evaluate advanced manufacturing technologies, such as 3D printing and continuous manufacturing.
- Most biological products are not amenable to terminal sterilization or even robust pathogen removal methods. Therefore, FDA is exploring novel approaches to pathogen inactivation for certain blood products for their feasibility and their ability to inactivate pathogens without negatively impacting the blood product.



Session 2: Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products

FDA uses various strategies to protect the public from harm and promote public health; one way is by ensuring easy public access to accurate information that consumers can use to make informed decisions about choosing and using regulated medical products, foods, and tobacco products.

To achieve this goal, FDA uses a science-based approach to develop its communication strategy: 1) developing messages for the public; 2) testing public understanding of specific, drug-related information; 3) ensuring optimal delivery to relevant populations; 4) assessing the impact of the information on public understanding, attitudes, and behaviors. A major challenge is adapting FDA's communications to rapidly evolving technologies that are driving major shifts in how consumers choose to receive and share information. These studies will greatly benefit from collaborations with other agencies as well as with academic and industry researchers.

Strengthening social and behavioral sciences will allow FDA to better equip American consumers and health professionals with the information they need to make sound decisions about using, prescribing, and dispensing products. This has the potential to decrease preventable adverse events related to FDA-regulated products.

Studying Pediatric Brain Function

FDA researchers John Chelonis, Ph.D. (left), and Merle Paule, Ph.D. (right), show the instrument they use to test complex brain function in children at their lab in Little Rock, Arkansas. The same apparatus is used to test monkeys at their National Center for Toxicological Research lab.

The National Center for Toxicological Research (NCTR) laboratory is developing important insights into pediatric brain function that will help scientists evaluate the effects of pediatric drugs on psychological processes—specifically, their memory, attention, motivation, and time perception.

Tests in animal models (e.g., monkeys) to assess potential effects of new drugs on pediatric brain function are often different from those given to humans, making it difficult to translate animal test findings to humans. Therefore, NCTR Operant Test Battery (OTB) uses the same tests to assess brain function in animals and children in an effort to better predict the effects of drugs in humans using animal data. The aim is to determine whether children's responses vary following use of the medication under study.

Population Assessment of Tobacco and Health

FDA is collaborating with the National Institutes of Health to prospectively study tobacco use and how it affects the health of Americans. This study, called the Population Assessment of Tobacco and Health (PATH), is following a nationally representative sample of about 46,000 people ages 12 years and over (both tobacco users and non-users) through annual in-person interviews that occur over at least three years. Each year the researchers invite participants to take part in additional interviews designed to determine a variety of behaviors, e.g., why some people use tobacco and others don't; how and why people start using tobacco and switch from one product to another; how

people quit using tobacco, and differences in tobacco use-related attitudes, behaviors, and health among men and women.

Disclosure Regarding Additional Risks in DTC Prescription Drug TV Ads

FDA is investigating the effectiveness of reducing the length and complexity of broadcast advertisements that include risks of agency-regulated products. This study evolved from concern that the major statements in such direct-to-consumer ads are too long, reduce consumer comprehension, and minimize important risk information. These drawbacks might therefore lead to therapeutic non-compliance due to fear of side effects.

The agency is investigating the effectiveness of a "limited risks plus disclosure strategy" that limits the risks in the major statement to those that are serious and actionable, and includes a disclosure to alert consumers that there are other product risks not included in the ad.

Experimental Study of DTC Advertising Directed at Adolescents

Current theoretical and empirical data support treating adolescences as a unique life stage with vulnerabilities that affect informed decision-making. The goal of this study is to determine how adolescents use risk and benefit information for health-related decisions in the context of product marketing messages aimed at this age group.

A randomized, controlled study of two different medical conditions will assess adolescents' perceptions following exposure to DTC prescription drug advertising that varies in benefit and risk onset and risk severity. We plan to compare adolescents' perceptions with the perceptions of their young adult counterparts.

Session 3: Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security

Medical countermeasures (MCMs) are FDA-regulated products (biologics, drugs, devices) that may be used in the event of a potential public health emergency caused by a terrorist attack with a biological, chemical, or radiological/nuclear material, a naturally occurring emerging disease, or a natural disaster.

FDA Medical Countermeasures Initiative (MCMi) Regulatory Science Program

The Medical Countermeasures Initiative (MCMi) coordinates agency-wide medical countermeasure development, preparedness, and response. Led by the Office of Counterterrorism and Emerging Threats in the Office of the Chief Scientist, MCMi helps ensure that medical countermeasures are safe, effective and secure. Our program includes strong collaborations with outside agencies and academic institutions.

Medical countermeasures often present unique and complex challenges with respect to developing data necessary to support regulatory decision-making. For example, many of the high-priority threats for which medical countermeasures are being developed do not occur naturally to an extent that would support the conduct of field efficacy studies; nor is it ethical to conduct human challenge studies with such threat agents. Instead, efficacy studies must be done in animals and the results extrapolated to humans.

The goal of the MCMi Regulatory Science Program is to develop the tools, standards, and approaches to assess medical countermeasure safety, efficacy, quality, performances and to help translate cutting-edge science and technology into innovative, safe, and effective medical countermeasures. Priority research areas being supported under the MCMi Regulatory Science Program include:

- Developing animal models and tools to evaluate safety and efficacy
- Identifying and qualifying biomarkers for safety and efficacy
- Using protein engineering to stabilize vaccine proteins
- Developing methods to assess medical countermeasure product quality and related product release assays
- Validating next-generation in vitro diagnostics platforms
- Assessing the performance of emergency medical equipment
- Enhancing emergency preparedness and response capabilities, including risk communication and tracking and evaluating the safety and clinical benefit of medical countermeasures used during public health emergencies

MCMi Regulatory Science Research Portfolio

FDA has established a broad and robust intra- and extramural research portfolio under the MCMi Regulatory Science Program to meet its goals in these priority research areas. Examples of ongoing intramural and extramural research include:

- Developing models of radiation damage in lung, gut, and bone marrow organs-on-chips and using these

models to test candidate medical countermeasures to treat such damage

- Mapping immune responses to certain biothreat agents and medical countermeasures in humans and animal models to create species-specific immune function maps
- Examining the scientific basis for the instability of the protective antigen that has hindered efforts to develop next-generation anthrax vaccines and using protein engineering to stabilize the antigen
- Developing new approaches for measuring the quality of next-generation smallpox vaccines
- Developing new methods for evaluating the purity and sterility of novel cell substrates that can be used to produce vaccines
- Developing new and improved tests to detect viruses and mycoplasma in biological samples including cell substrates and other starting materials to support assessment of product quality, safety, and consistency
- Constructing and characterizing highly qualified and validated nucleic acid panels for the molecular characterization of bacterial biothreat agents that will aid in the development of diagnostic devices for biothreat agents
- Developing methods for real-time detection of medical device surface contamination to decrease the potential for transmitting infection between patients and between patients and health care workers
- Developing a mobile device (e.g., smartphone) application for reporting adverse events associated with medical devices to FDA, including an in-depth module for products associated with medical countermeasures
- Cataloging the most likely and serious difficulties that may complicate emergency administration of MCMs and developing communication strategies to help ensure appropriate public use of life-saving MCMs in emergency situations
- Investigating decontamination and reuse of respirators in public health emergencies and optimizing respirator decontamination to ensure supplies for emergency preparedness
- Developing methods to obtain safety and limited efficacy data from patients who receive a medical countermeasure during a public health emergency through a collaboration with the United States Critical Illness and Injury Trials Group (USCIITG) and critical care physicians at 20 hospitals throughout the United States

For more information, visit www.fda.gov/medicalcountermeasures, email AskMCMi@fda.hhs.gov, or follow MCMi on Twitter @FDA_MCMi.

Session 4: Implement a New Prevention-Focused Food Safety System to Protect Public Health

FDA's Office of Foods and Veterinary Medicine (OFVM) leads a unified foods program designed to enhance the agency's ability to successfully face current and future challenges and opportunities in food and feed safety, nutrition, and other critical areas.

The goal of OFVM is to support public health efforts to reduce illnesses and deaths in humans and animals by creating new knowledge through research that improves health, nutrition, and safety of regulated products, including human and animal foods and cosmetics. To fill knowledge gaps in these areas of regulatory science, OFVM research includes collaborative projects with outside agencies, academic institutions, and industry.

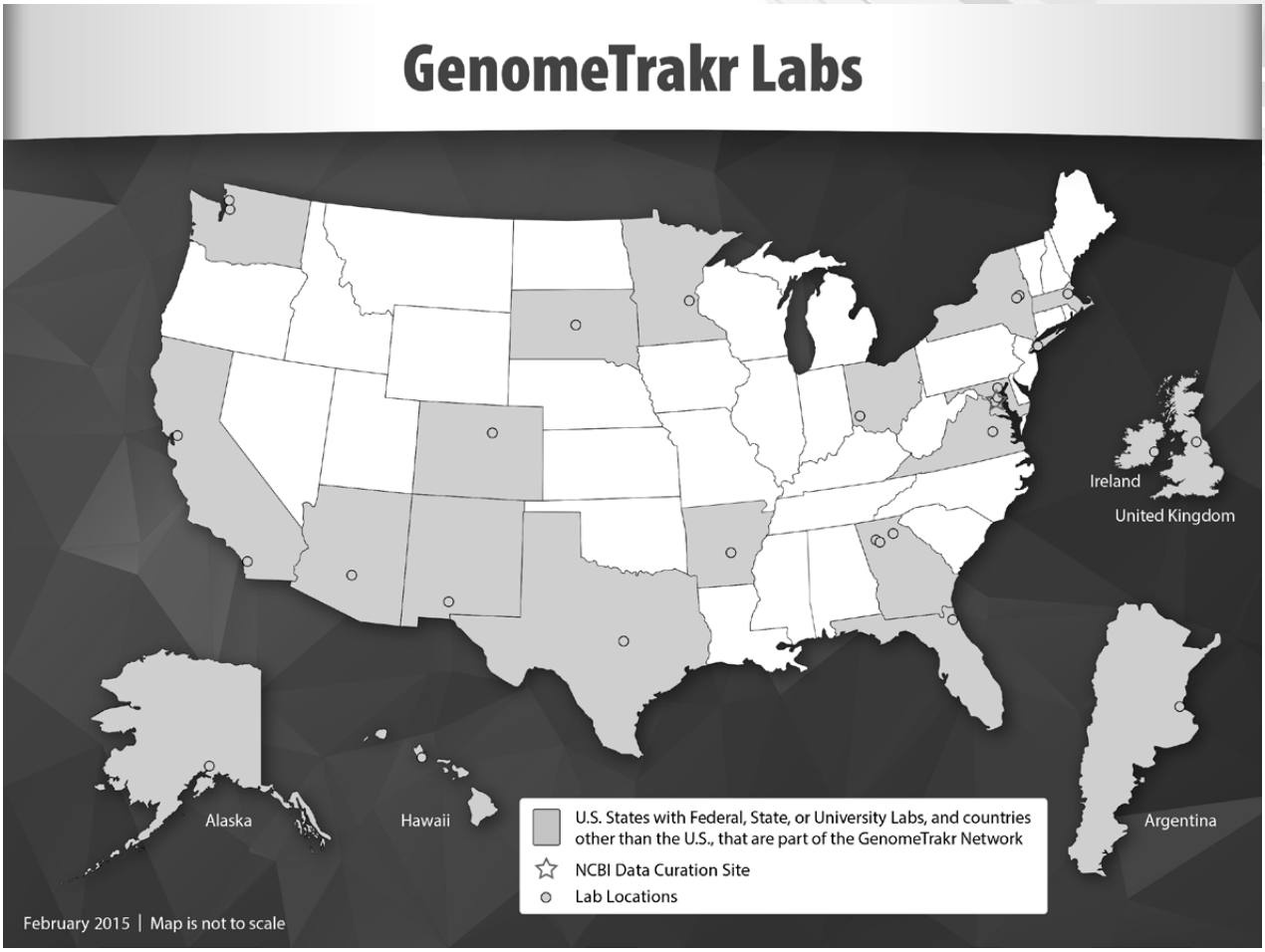
Whole Genome Sequencing

Whole genome sequencing (WGS) greatly speeds differentiating among and between species of infectious organisms with a precision unmatched by other technologies. FDA uses WGS as a molecular epidemiological tool to identify associations between food, environmental, and clinical microbial isolates during foodborne illness outbreaks, and to aid research efforts designed to reduce foodborne illnesses and deaths in the U.S. and globally. It is also used to address anti-microbial resistance concerns. Pulse field gel electrophoresis (PFGE) has been the predominant method for tracking outbreak pathogens and patterns. However WGS can now offer a greater level of specificity unmatched by PFGE. WGS can differentiate virtually all strains of foodborne pathogens regardless of species. The most promising application with far-reaching public health impact lies in its ability to pair a foodborne pathogen's genomic information with its geographic location and applying the principles of evolutionary biology to determine the relatedness of the pathogens. Knowing the geographic areas that pathogens are typically associated with can greatly facilitate tracking down the source of food contamination. This is especially important in the case of multi-ingredient food products whose ingredients come from different states or countries. Speed and specificity and coordination are essential to identifying sources of contamination and removing them from the food supply to reduce illnesses and avert deaths. FDA has responded to these needs by initiating GenomeTrakr, a network of laboratories created by scientists at the Center for Food Safety and Nutrition (CFSAN) that enlists the power of WGS to protect the public from foodborne contamination.

GenomeTrakr Network

GenomeTrakr is the first distributed network of state and federal public health laboratories to use WGS to collect and share the identity of foodborne pathogens. The network comprises six state and nine FDA field laboratories, with plans to expand the number of participating laboratories from states, federal agencies and the World Health Organization. Their data are accessible through the National Center for Biotechnology Information public databases for real time comparison and analysis of genomes.

GenomeTrakr enables public health officials to do rapid traceback to contamination sources, which could reduce the number of illnesses and deaths. The genomic information points investigators to specific food products potentially related to an outbreak, and provides insight into the origin of the contaminated food, e.g., pinpointing the source of a *Salmonella* outbreak in spicy tuna and spiced meat.



WGS and Antibiotic Resistance

WGS is fostering new research into non-culturable microorganisms, the response of the microbiome to different conditions, nucleotide polymorphisms signifying phylogenetic relationships, the complex dynamics of interrelated microbial ecosystems, facilitating timely diagnostics and leading to new insights into antibiotic drug development and resistance.

Using WGS, it is possible to identify the top 200 serotypes causing salmonellosis by sequence analysis of the genomic loci encoding the relevant surface antigens without the use of high quality typing sera. In addition, WGS provides a complete picture of acquired traits, such as the complement of known virulence and antibiotic resistance traits. Recent FDA studies showed a high correlation between the presence of specific antimicrobial resistance genes and antimicrobial resistance traits that can be measured using standardized clinical microbiological methods.

In addition, FDA is using a metagenomics approach to investigate the intestinal microrganisms (the microbiome), the types of antimicrobial resistance genes in the bacteria (the resistome), and the pieces of special DNA that transfer antimicrobial resistance genes between bacterial species (the mobilome), before and after exposure to antimicrobials. This will allow us to assess the potential of antimicrobial resistance reservoirs contributing to the development of clinical resistance.

Seafood Science

Fish-related illnesses

A team of OFVM scientists, medical officers, and consumer safety officers at CFSAN), the Coordinated Outbreak Response and Evaluation (CORE) Network, and the Office of Regulatory Affairs (ORA) established a program to track and study seafood-related illnesses linked to ciguatera fish poisoning, puffer fish poisoning, and rhabdomyolysis (Haff disease), primarily associated with buffalo fish. The team solicits meal remnants and associated clinical and epidemiological information from health care providers and local and state health and fisheries agencies to confirm origins of these illnesses, characterize the responsible toxins, better develop testing methodologies, and refine hazards controls (where appropriate).

For example, FDA scientists assisted public health officials in Minneapolis, MN investigate puffer fish poisoning of four individuals who had consumed illegally imported dried puffer fish they had purchased while visiting New York City. Based on their genetic analysis the scientists confirmed the product as a prohibited species of puffer fish and chemical analysis determined it was contaminated with high levels of tetrodotoxin.

DNA-based seafood identification

Fish SCALE (Seafood Compliance and Labeling Enforcement)

The OFVM Fish SCALE project develops and implements regulatory genetic methods to enable FDA, other regulatory agencies, and the seafood industry to confirm proper seafood labeling and identify where in the supply chain seafood misbranding is occurring. Among the ways the Fish SCALE team protect the consumer are identifying processed fish filets using state-of-the-art forensic DNA testing, validating protocols for identifying fish and crustaceans (shrimp, crab, and lobster), and producing reference standards, standard operating procedures, and educational materials to disseminate inside and outside the agency.

FDA seafood inspectors:

National Antimicrobial Resistance Monitoring System (NARMS)

NARMS is a national public health surveillance system that tracks antibiotic resistance in foodborne bacteria through a collaboration of FDA, the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture (USDA).

NARMS monitors antimicrobial susceptibility of enteric bacteria (*Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus*) from

humans, retail meats, and food animals. It also collaborates with antimicrobial resistance monitoring systems in other countries to harmonize international testing and reporting.

Initial data from the animal component of NARMS show that the program will advance our understanding of sources of foodborne illnesses, improve detection of emerging problems where mitigation steps are possible, and greatly improved the scientific basis for food-animal antibiotic use policies and regulations.

Section 5: Support New Approaches to Improve Product Manufacturing and Quality

Novel science and technologies are supporting innovation in both manufacturing and the development of products that are themselves innovative and often complex. In addition, the continual evolution of analytical technologies is rapidly improving their sensitivity, resolution, precision in determining product structure, and detecting contaminants.

To foster these innovations, FDA collaborates when appropriate with outside researchers to assess how these new technologies affect product safety, efficacy, and quality. The goals of this work are to 1) support development and evaluation of novel and improved manufacturing methods; 2) develop new analytical methods; 3) reduce risk of microbial contamination of products.

Single-Cell mRNA Profiling

The ability to identify reliable biomarkers of differentiation stages on cells is a potential solution to the challenge of determining which cells are appropriate for a specific therapeutic use. The mesenchymal stem cell consortium (MSCC) at FDA, which comprises seven laboratories at the Center for Biologics Evaluation and Research, is studying the relation between various molecular attributes and their use to reliably predict developmental pathways. One of MSCC's powerful tools is single-cell mRNA profiling (single cell RT-PCR, single cell RNA seq). This technology can be adapted to identify many RNA transcripts simultaneously in each of up to 96 individual cells in a single experiment. The goal is to correlate patterns of RNA transcripts with specific cells having the desired biological characteristics. Such information could be very effective in identifying those cells ready for therapeutic use. This, in turn, would support development of manufacturing protocols that produce cells with the desired pattern of attributes, as well as strategies to verify the safety and potential effectiveness of specific populations of therapeutic cell products.

Inhalation Pharmaceuticals

Nasal sprays, metered-dose inhalers (M.D.I), dry powder inhalers (DPI), and nebulizers, are currently used to treat asthma and chronic obstructive pulmonary disease (COPD). They are now being considered as a way to deliver vaccines, migraine medication, insulin, and other products. FDA is evaluating both sources of variability in current methodologies and new technologies to characterize inhalation products. This will improve regulatory decision-making and help the agency develop industry guidances that ensure quality and equivalency of inhalation products.

Transdermal Drug Delivery Systems (TDDS)

The critical factors affecting the ability of transdermal drug delivery systems (TDDS) to deliver effective amounts of drug across the skin are permeability and adhesion. Poor patch adherence causes low drug permeation and lack of efficacy. In contrast, heat, occlusion, or compromised skin can cause overdoses. FDA is aware of numerous cases of "adhesion lacking" for several TDDS. In response to such problems, FDA is developing novel in vitro testing methods to evaluate critical attributes of drug release and adhesion of TDDS. Such methods would be especially valuable because of the time, money, and safety issues involved with in vivo human testing.

Nanotechnology

The Nanotechnology Core Facility (Jefferson, Arkansas) supports nanotechnology toxicity studies, develops analytical tools to quantify nanomaterials in complex matrices, and develops procedures for characterizing namomaterials in FDA-regulated products Jefferson Arkansas Laboratories

3D Printing

FFDA is using 3D printing to expand both FDA research efforts and FDA's ability to review innovative medical products. The Office of Science and Engineering Laboratories (OSEL) is investigating how this technology could affect the commercial manufacture of medical devices in the future.

OSEL uses 3D printing technologies to modify designs of devices to study the effects of such changes on the safety and performance of devices when they are used in different patient populations. This work will help FDA to develop product standards and other aspects of product safety.

Advanced Analytical Techniques for Pharmaceutical Analysis

- Solid state and flow-NMR
- Ion mobility screening (IMS)
- Scanning electron microscopy coupled to Raman spectroscopy
- Advanced separation techniques to enhance LC-MS of complex drug products
- Online LC-MS to compare characterization of closely related therapeutic glycoproteins; LC-MC and statistical modeling analysis to compare innovator and generic lots of complex products
- Chemometric methods to compare differences in highly similar 2D NMR spectra
- Ion mobility-mass spectrometry to characterize higher order antibody structure
- Top-down mass spectrometry for primary structure confirmation and to enable the use of secondary structure as a quality attribute
- Deep UV Resonance Raman Spectroscopy assessment of monoclonal antibodies
- Lectin microarray profiling to characterize glycosylated products
- High resolution capillary electrophoresis to characterize protein heterogeneity

Session 6: Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes

Clinical development programs are lengthy, expensive and fraught with uncertain outcomes that can disrupt or halt efforts to get safe and effective new treatments to patients. Innovative statistical approaches can advance product development and appropriately identify subgroups of patients most likely to benefit from novel therapies. Bioinformatics, high-throughput screening, and powerful new genomic techniques have dramatically improved the efficiency of new drug candidate discovery and development. But targeted therapies using specific genomic technologies to select patients that respond are only in their infancy.

The ability to supplement traditional lab testing with clinically meaningful measures that are often based on physiologic, imaging, or genomic endpoints is critical to developing new therapies. It is also critical to ensuring the accuracy and consistency of analytical measurements, while reducing inter-platform and inter-site variability.


Personalized Medicine

Regulatory scientists at FDA have extensive experience evaluating agency-regulated product submissions. This gives them a unique advantage in facilitating development of knowledge and clinical evaluation tools (e.g., advanced informatics and innovative statistical approaches) needed to translate discoveries into safe and effective products. However, since the amount of data and effort needed to develop, validate or qualify clinical evaluation tools is substantial, intramural efforts are and should be supplemented by collaborative projects involving an array of external partners including academia, industry, and global regulatory agencies. These collaborations are aimed at developing the new tools and approaches needed to support personalized medicine and modernize and advance the science and conduct of clinical trials.

Advances in Statistical Methodology

- Describe performance of new products within subgroups defined by demographics, genomics, other biomarkers, or other patient characteristics in a clinical trial
- Enrich a clinical trial based on interim findings for one or more biomarker-defined subgroups to enhance chances of trial success
- Evaluate diagnostics used to select patients for trials, including 2nd–of-a-kind (me-too) companion diagnostics
- Inform trial designs that can be used for biomarker qualification.

Genetic Basis of Therapeutic Protein Safety and Efficacy

The safety and efficacy of recombinant therapeutic proteins often depend on their primary sequence, as well as their three-dimensional structure. For example, a substantial number of patients treated for inherited bleeding disorders with replacement protein therapy experience safety or efficacy problems. FDA is elucidating the genetic bases for these problems in individual patients. In one case, ethnic differences in safety profiles of some products that treat Hemophilia A have been observed in the African American population. This has been traced to greater genetic diversity in individuals of black African descent.

Genetic Risk Factors and Their Association with Clinical Outcomes

Genetic risk factors associated with specific adverse events in clinical trials are a long-standing challenge to drug product development and delivery to the public. The ability to identify such genetic risk factors in participants before they enter clinical trials might help investigators avoid this problem. FDA is working with outside collaborators to overcome that challenge. For example, the agency is collaborating with Kaiser Permanente Northern California and the University of California San Francisco on a case-control study to evaluate the human genome of individuals who experienced a febrile seizure 7-10 days after measles-containing vaccination (MMR or MMRV). The study's main objective is to identify genetic risk factors associated with febrile seizure following MMR or MMR and Varicella vaccination in children 6 month to 5 years old.

Source: http://www.fda.gov/scienceresearch/specialtopics/personalizedmedicine/default.htm

Genotyping and Pharmacogenetics

FDA does a broad range of research in combining genotyping information (e.g. inherited genes associated with drug metabolism) and PK/PD models to inform drug dosing. Examples of specific research areas include:

- Developing genome sequence analysis pipelines to examine antiviral drug resistance patterns
- Modeling pharmacogenetic interactions from drug interaction studies to facilitate individualized dosing
- Mapping variability in the druggable genome to validate targets and preempt response heterogeneity
- Evaluating population-based approaches to conduct safety pharmacogenomic studies

Evaluating Next Generation Sequencing Diagnostics

The agency is studying the diagnostic potential of next generation sequencing devices and is developing ways to evaluate them. The agency is addressing the bioinformatics challenges posed by such products.

For example, using genomic technology, they are working to develop algorithms to identify patients more likely to experience a particular adverse event with a specific product.

NCTR scientist analyzing readout of Microarray data

Session 7: Modernize Toxicology to Enhance Product Safety

The occurrence of serious and sometimes rare adverse events during clinical trials or following product approval demonstrates the need to close critical gaps in preclinical toxicology studies. The goal of this priority is to 1) develop better models of human adverse response; 2) identify and evaluate more reliable biomarkers and endpoints for non-clinical and clinical evaluations; 3) use and develop computational methods including in *silico* modeling. We have a variety of powerful tools available to address these goals.

Functional Models: to better translate preclinical toxicity findings into clinical correlates, a number of in vivo and ex vivo technologies are employed to measure drug effects on physiological function in animal models used in regulatory research. These include:

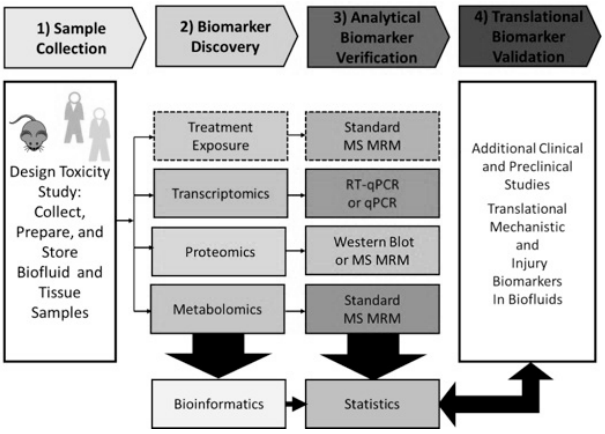
- Wireless in vivo battery charging telemetry implant for constant monitoring of pressures and bio-potentials (e.g., EKG, EEG, arterial pressure, heart rate, nerve impulse) in free-moving rats
- Isolated heart apparatus for cardiac studies on whole hearts of small rodents
- Laser capture dissection microscope for sampling specific cell types from harvested tissues
- High-resolution Doppler ultrasound imaging to assess rodent cardiac function, size and consistency of internal organs, and monitoring tumor growth
- Bioimaging (MRI, microPET, etc.) to follow, over time, adverse events in nonclinical species. Since this is non-invasive, it can be used in humans as well

Model systems using 3D cultures of human cells, humanized rodents, iPS-derived stem cells and zebrafish: These are used to evaluate biological drug products, drug-induced liver injury (DILI) and drugs that affect other organs.

Systems Biology

Systems biology can provide biomarkers that increase our understanding of health-to-disease status and help develop precision medicine by discovering translatable injury biomarkers in non-invasively obtained biofluids.

In addition, ex vivo imaging of tissues can be used to discover location of drugs, drug metabolites and biomarkers related to disease or toxicity. This imaging information can be used to enhance the sensitivity of other imaging techniques (i.e MRI/MRS) and be combined with the current golden standard – histopathology.



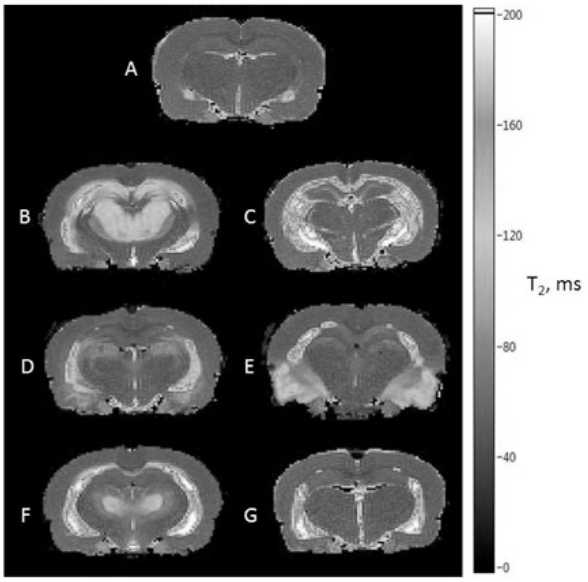
Biosystems view of liver toxicity studies.

Non-Invasive Brain Toxicity Studies

FDA uses non-invasive MRI to study neurotoxicity of various substances in animal models. The technique enables us to observe in vivo, the life cycle (onset, development, regression) of toxin-related lesions in the same animal. This technique has potential applications in pre-clinical studies identifying adverse effects of candidate drugs. Being translatable, it could also be used in the clinical setting. In addition, it might support development of an imaging

biomarker of neurotoxicity.

Figure 1.



Animals that showed signs of brain alterations in response to treatment with known neurotoxicants (responders). ***(A)*** Control animal treated with saline (2 ml/kg, once) 48 h before imaging; ***(B)*** animal treated with 3-nitropropionic acid (NP, 20 mg/kg, s.c., daily for 3 days); imaging on day 4; ***(C)*** animal treated with hexachlorophene (HC, 30 mg/kg, p.m. daily for 5 days); imaging on day 6; ***(D)*** animal treated with kainic acid (KA, 10 mg/kg, i.p., once); imaging on day 3; ***(E)*** animal treated with domoic acid (DA, 2 mg/kg, i.p., once) imaging on day 3; ***(F)*** animal treated with pyrithiamine (PT, 0.25 mg/kg i.p. daily for 2 weeks + thiamine free diet); imaging at 4 weeks; ***(G)*** animal treated with trimethyltin (TM, 12 mg/kg, i.p., once) imaging at 3 weeks.

Bioanalysis

Understanding drug effects requires knowledge of drug concentrations at the site of action. For topical and local acting drugs, this requires the development and validation of more sensitive bioanalytical methods that can measure low concentrations of drug in small sample volumes. FDA scientists are using a number of technologies to provide quantitative measurements of the exposure levels that are associated with drug safety and efficacy in in vivo and in vitro systems, including:

- Robotic high-throughput liquid chromatography analyses of biological matrices
- Clinical and animal PK/PD, bioequivalence, drug metabolism, transporters, in vivo/in vitro correlations and drug-drug interaction studies
- Improved methods for obtaining biological samples, such as the use of dried blood spots and metabolic cages that freeze urine as it is collected for assaying drugs and biochemical

Biomarker Analysis

FDA scientists are working to identify and evaluate candidate biomarkers and their measurement in animals and humans as part of the agency's effort to qualify new tools for drug development and to improve precision medicine. Research includes:

- Determining data standards and laboratory best practices needed to analyze biomarker performance and the endpoints they are intended to report
- Detecting metabolites in animals and humans that might serve as biomarkers of adverse drug events and disease
- Evaluating protein biomarkers of kidney injury, cardiac injury, and skeletal muscle injury quantitated in biofluids using highly sensitive electrochemiluminescence
- Identifying bioimaging biomarkers in nonclinical species
- Microarray technology and high throughput nucleic acid sequencing technology
- PCR profiling of microRNAs in biofluids and tissues quantitating

microRNA, mRNA, and DNA

- Laser capture microdissection for genomic analysis of tissue cells

Bioinformatics and Systems Pharmacology

To address regulatory questions of product safety, efficacy and quality, FDA scientists are taking an integrated and holistic approach to improve predictive models:

- Data mining to generate disproportionality scores for drugs and adverse events
- PKPB modeling to improve translation from animals to humans to special populations
- Predicting off- target binding of drugs, automated searches of literature, FDA labels, and other drug databases to generate drug safety hypotheses
- Mechanistic modeling of disease processes at the level of pathway, cell, and organ.

Session 8: Harness Diverse Data through Information Sciences to Improve Health Outcomes

FDA receives enormous amounts of data on product submissions, adverse event reports, de-identified patient data from health care providers, and results from surveys and basic scientific research.

The ability to integrate and analyze all the information from these disparate sources would enable FDA to extract more new knowledge and insight available from any single source. A few of the untapped opportunities FDA is exploring to enhance the agency's ability to do so include 1) monitoring adverse event trends and disease outbreaks; 2) combining data from multiple clinical trials, post-market studies, and preclinical data; 3) evaluating and comparing effectiveness and safety of medical and veterinary products in particular sub-populations, including sex/gender and race/ethnicity analysis, and ultimately host genomics and/or genomic response data; 4) large-scale active surveillance for rare events and data- and text- mining for a variety of research purposes.

FDA is in the early stages of constructing the Information Technology (IT) infrastructure to integrate this type of complex data. This will support FDA's ability to do sophisticated data mining that can support an enormous number of simultaneous queries of a large set of indexed data sources. The agency is looking for opportunities to work with collaborators, whether in other agencies, academic institutions, or industry.

FDA uses a powerful genomics data technology called HIVE to analyze very large sequence databases.

Characterizing Resistance Pathways for Drugs in NDA

FDA is using two next generation sequencing (NGS) analysis pipelines (using CLC Genomics Workbench and High-performance Integrated Virtual Environment--HIVE), to do independent analyses of all resistance data generated by NGS. The goal is to identify and carefully characterize resistance pathways for all drugs that come in as New Drug Applications. The results of the independent analysis are part of the regulatory review process and these results are compared to those provided by the sponsor, to inform labeling.

PACES Initiative

FDA is undertaking projects that have enormous potential to unlock the data from product applications reviewed by FDA. The goal is to provide new information that will help industry to reduce future product development costs by billions of dollars. One of the programs is the academic Partnership in Comparative Effectiveness Science (PACES) project funded by FDA. PACES facilitates pilot projects of advanced analyses aimed at helping to determine which interventions will be most effective for which patients under specific conditions. This would reduce many trial-and-error complications in studies designed to identify the most effective treatments for specific patients.

FDA Microbial Regulatory-Grade Reference Sequence Database (MicroDB): Supporting Development and Validation of Infectious Diseases Diagnostic Tests.

FDA, in collaboration with the National Center for Biotechnology Information (NCBI), the Department of Defense (DoD) and the Institute for Genome Sciences at the

University of Maryland, established a publicly available, well-curated reference database (FDA-ARGOS: dAtabase for Regulatory Grade micrObial Sequences; BioProject 231221) of 600 regulatory-grade-quality sequences from diverse infectious microorganisms. This database, which is still growing, comprises data collected from multiple collaborators.

Diagnostic test manufacturers develop sequence-based tests to identify infectious agents and/or to detect resistance or virulence markers. They will use this database to advance their development programs and to support the regulatory science review of such tests. This work will facilitate the development of these tests, which are aimed at improving patient outcomes.

Continued success of the database and adoption by the community is contingent on populating and curating the database, which in turn will support development of guidance and standards for clinical use of this technology. Discussions held at a related public workshop on advancing regulatory science for high throughput sequencing devices for microbial identification and detection of antimicrobial resistance markers were essential to establishing the safety and effectiveness of these devices.

MicroArray Quality Control (MAQC) Standards and Quality Measures

Microarray and next-generation sequencing technologies are core technologies in pharmacogenomics and toxicogenomics; however, these technologies cannot be reliably and successfully used in clinical practice and regulatory decision-making in the absence of standards and quality measures. FDA established the MicroArray Quality Control (MAQC) project to foster the development of standards and quality measures that can validate the use of these technologies in the discovery, development, and review of regulated products. The third phase of MAQC project, also known as the Sequencing Quality Control (SEQC) project, assessed next-generation sequencing technologies for RNA-sequencing in collaboration with 180 participants from 73 organizations and 12 countries. The consortium published eight manuscripts, all of which are now available in a *Nature Collections* special issue. Additional collaborators are welcome to participate in the MAQC project.

FDA Fellows Association

The FDA Fellows Association is a community representing the interests of all FDA fellows, including Oak Ridge Institute for Science and Education (ORISE) postdoctoral and post-baccalaureate fellows, Staff Fellows, Visiting Fellows, and Commissioner's Fellows.
As members of the Fellows Association, we provide Fellow representation on relevant FDA committees and are part of the FDA training infrastructure.

We foster cooperation and collaboration among FDA Fellows, help promote scientific communication between Fellows and with the FDA scientific community at large, and provide information on career and professional development for Fellows in coordination with FDA's Office of Scientific Professional Development.

FDAFellowsAssociation@fda.hhs.gov
http://research.cber.fda.gov/FellowsAssociation

FDA Student, Fellowship, and Senior Scientist Programs

Whether you're an undergraduate looking to pursue a career in science, a graduate science student seeking experience in regulatory science, a postgraduate looking for fellowship opportunities, or a senior scientist pursuing research experience in your field of expertise, FDA offers you many paths to learning about the exciting field of regulatory science.

Student Programs

- Center for Veterinary Medicine Student Profile
- Device Evaluation Intern Program
- FDA Veterinary Clerkship program
- Federal Information Privacy Internship Program
- Interdisciplinary Toxicology Program
- JIFSAN Student Internship Program
- Medical Device Fellowship Internship Program
- Oak Ridge Institute for Science and Education (ORISE) Research Participation Program at CBER, CVM, CDRH, CDER, CFSAN, OCS, ORA, and NCTR.
- Office of Policy Internship
- Pathways Program for Students
- Pharmacy Student Experiential Program
- Regulatory Science Student Internship Program
- Science Internship Program at NCTR
- Student Intern Program at CDRH-OSEL
- Summer Student Research Program at NCTR
- Veterinary Medicine Student Program – The Windows to Regulatory Research Internship Program
- Visiting Pediatric Pharmacology Fellows Rotation Program

Fellowship Programs

- Commissioner's Fellowship Program
- FDA – National Cancer Institute Inter-Agency Oncology Task Force Joint Fellowship Program
- Medical Device Fellowship Program
- Oak Ridge Institute for Science and Education (ORISE) Research Participation Program at CBER, CVM, CDRH, CDER, CFSAN, OCS, ORA, and NCTR.
- Pathways Program for Recent Graduates
- Post-graduate Research Program at NCTR
- Regulatory Pharmaceutical Fellowship
- Service Fellowship Plan for FDA
- Tobacco Regulatory Science Fellowship

Senior Scientist Programs

- Alzheimer's Disease Regulatory Science Fellowship
- Faculty Research Program at NCTR
- Service Fellowship Plan for FDA
- Tobacco Regulatory Science Fellowship

For information about FDA's programs:
- Visit FDA.gov
- Navigate to the "About FDA" section
- Select "Working at FDA"
- Select "Student, Fellowship and Senior Scientist Programs"

Office of the Chief Scientist
Intramural and Extramural Grants and Contracts Information

Office of the Chief Scientist Intramural Grant Programs

As part of FDA's Regulatory Science Initiative, five Office of the Chief Scientist (OCS) Intramural Grant programs support research projects throughout the agency.
http://sharepoint.fda.gov/orgs/OC-OCS/OCSGrants/FDAwide/info/default.aspx

Nanotechnology Collaborative Opportunities for Research Excellence in Science (CORES) Grants

- One year grants awarded in range from $100K to $200K, with 24 grants awarded since 2011.
- CORES Grants have led to the development of regulatory science tools such as assays, assessment methodologies, and test protocols that the agency is using to evaluate nanotechnology in FDA-regulated products.
- CORES Grants have increased overall collaboration across FDA, with academia, and with the US Government.

Medical Countermeasures Initiative (MCMi) Challenge Grants

- Medical countermeasures (MCMs) are products that can be used to diagnose, prevent, protect from, or treat conditions associated with chemical, biological, radiological, or nuclear (CBRN) threats, or emerging infectious diseases.
- The MCMi Regulatory Science Program–including intramural challenge grants–develops the tools, standards, and approaches to assess MCM safety, efficacy, quality, and performance.

www.fda.gov/medicalcountermeasures
Contact Rakesh.Raghuwanshi@fda.hhs.gov

Chief Scientist's Challenge Grants

The main purpose of the Chief Scientist Intramural Challenge Grants is to enable exceptional and innovative research. We are particularly supportive of research that:

- Involves cross-center collaboration.
- Aims for project outcomes that promise broad impact in more than one area of FDA
- Will further regulatory science efforts of other stakeholders.
- Have a practical application and the potential for regulatory impact.

Contact OCS at York.Tomita@fda.hhs.gov

Office of Minority Health Challenge Grants

- Supports research on the assessment of safety and efficacy of FDA regulated products and how these relate to racial and ethnic subgroups.
- Supports research advancing health literacy and cultural competency, including for audiences with limited English proficiency, for FDA communications /or their providers for comprehension and usability

Contact OMH at
Christine.Merenda@fda.hhs.gov

Office of Women's Health (OWH) Intramural Scientific Research Program

- Supports FDA women's health research initiatives to facilitate regulatory decision-making and advance scientific understanding of sex differences in the safety and efficacy of FDA regulated products.
- OWH funded research has contributed to the identification of sex differences in the safety and efficacy of FDA regulated products, development of guidance for industry, labeling changes and the development of tools to enhance the evaluation of medical products used by women.

Contact OWH at
Emmanuel.Fadiran@fda.hhs.gov

Extramural Programs In Collaboration with FDA PERSI (Program for Extramural Regulatory Science and Innovation)

Contract

FDA Broad Agency Announcement (BAA)

The Broad Agency Announcement (BAA) is an opportunity to promote regulatory science by leveraging expertise from industry and academia that creates innovative advancements in technology, materials, processes, methods, devices or techniques.

- Proposals reviewed by FDA subject matter experts and rated based on technical merit, program relevance and past performance/offerors capabilities.
- BAA contracts are awarded based on program interests within the nine FDA scientific priority areas and funds availability.
- Twenty-four ongoing projects were funded during FY14, totaling over $22 million.

Contact ORSI at
Shaila.Shaheed@fda.hhs.gov

Grant

Centers of Excellence in Regulatory Science and Innovation (CERSI)

FDA's Centers of Excellence in Regulatory Science and Innovation (CERSIs) are joint efforts between FDA and academic institutions to work collaboratively on projects that promote regulatory science, including innovative research, education, and scientific exchange under a cooperative agreement (U01) at:

UCSF-Stanford University CERSI:
Johns Hopkins University CERSI:
Georgetown University CERSI:
University of Maryland CERSI:

CERSI

CERSIs offer many opportunities for FDA scientists:

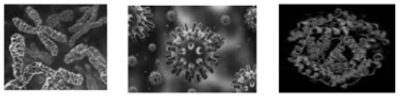
1. Collaborate on research projects:
   - Regulatory science research projects can be proposed by CERSIs or FDA Centers.
2. Academic Regulatory Science Programs and Educational Events:
   - Masters in Regulatory Science including engineering (UMD) & food safety (JHU)
   - Co-sponsored workshops and lectures (with CMEs)
3. Scientific Exchanges
   - Access to the scientific core facilities at CERSI institutions
   - Become a visiting scientist or host fellows and students at FDA

Please contact ORSI at
Donna.Blumkemelor@fda.hhs.gov

FDA Technology Transfer (T2)
Creating Collaborations-Protecting Inventions

Alice Welch, Ph.D.; William Ronnenberg, J.D.
Technology Transfer Program, Office of Regulatory Science and Innovation, OCS,OC, FDA

Your Regulatory Science Resource



FDA T2 is a valuable resource for FDA scientists pursuing regulatory science projects. It ensures that FDA inventions are protected and benefit the Agency and the US taxpayer.

FDA T2 experts in the Centers and ORA help implement agreements to share resources and for collaborations. The experts are guided by the Director and the Deputy Director, FDA Technology Transfer Program, ORSI, OCS, OC.

Regulatory Science Collaborations

FDA T2 implements agreements to:

(1) share unique research materials
(2) collaborate with outside scientists
(3) obtain funds and resources for FDA research and apply for grants.

FDA's scientific collaborations include scientists from academia, research foundations, local, state and foreign governments, industry and other Federal agencies.

Appropriate legal agreements are negotiated and executed within the framework of applicable laws and policies.

Invention Portfolio

FDA T2 manages FDA's invention portfolio of 600+ inventions, patents and licenses. Each FDA invention is evaluated for patentability and marketability.

Inventions are actively marketed to produce public health solutions through commercialization, generating license royalties in the process that pay patenting costs and support FDA research.

What We Do

We advance R&D for public health by assisting both FDA scientists & potential FDA collaborators

Help share research materials & reagents **(MTA)**

Protect confidential information when exploring possible collaborations **(CDA)**

Implement agreements for collaborative projects having defined goals, duties, timelines & outcomes **(RCA)**

Implement synergistic collaborations that provide the partners access to expertise, technologies, funds **(CRADA)**

Draft interagency agreements for collaborative projects with other USG agencies **(IAG / IAA)**

Help FDA scientists receive grant funds **(GARR)**

Protect the commercial and intellectual value of FDA inventions **(EIR & Patents)**

Execute income-producing licenses to FDA inventions

Protect Agency's integrity and independence when implementing collaborations.

Principal T2 Agreements	
Material Transfer Agreement	MTA
Confidential Disclosure Agreement	CDA
Research Collaboration Agreement	RCA
Cooperative Research & Development Agreement	CRADA
Interagency Agreement	IAG / IAA
Employee Invention Report	EIR

How to Get Started

FOR FDA STAFF

Collaborations

1. Define a mission-relevant project and identify a potential collaborator
2. Identify goals, materials, FTE load, proposed budget, timeline, outcomes
3. Identify potential conflicts of interest to address or manage
4. Use your FDA CONTACT for advice and guidance

Inventions

1. Obtain Employee Invention Report (EIR)
2. Work with your FDA T2 CONTACT to complete the EIR
3. Submit complete EIR packet for patentability and marketability evaluation
4. Respond to evaluator's requests

FOR COLLABORATORS OUTSIDE FDA

1. Define a potential collaboration and identify an FDA investigator
2. Include goals, materials, FTE load, proposed budget, timeline
3. Work with FDA investigator and FDA CONTACT to identify and negotiate appropriate collaborative agreement

FDA T2 Contacts

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1. Ensure FDA's Readiness to Evaluate Innovative Emerging Technologies

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2. Strengthen Social and Behavioral Science to Help Consumers and Professionals Make Informed Decisions about Regulated Products Topics

Lee Zwanziger, OC/OPP (Chair)
Sherry Ferguson, NCTR
Katherine Margolis, CTP
Joelle Robinson, CTP

3. Facilitate Development of Medical Countermeasures to Protect Against Threats to U.S. and Global Health and Security Topics

Rakesh Raghuwanshi, OC/OCET (Chair)
Patrick Regan, ORA

4. Implement a New Prevention-Focused Food Safety System to Protect Public Health Topics

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David White, OFVM(Chair)
Donald Zink, CFSAN (Chair)
Steven Foley, NCTR
Timothy McGrath, ORA

5. Support New Approaches to Improve Product Manufacturing and Quality Topics

Sau (Larry) Lee, CDER (Chair)
Carolyn Wilson, CBER (Chair)
George Salem, ORA

6. Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes Topics

Lisa LaVange, CDER (Chair)
William Mattes, NCTR
Martin Mendoza, OC/OMH
Estelle Russek-Cohen, CBER
Zuben Sauna, CBER

7. Modernize Toxicology to Enhance Product Safety Topics

Thomas Colatsky, CDER (Chair)
Donna Mendrick, NCTR (Chair)
James Weaver, CDER (Chair)
Xin Fu, CTP

8. Harness Diverse Data through Information Sciences to Improve Health Outcomes Topics

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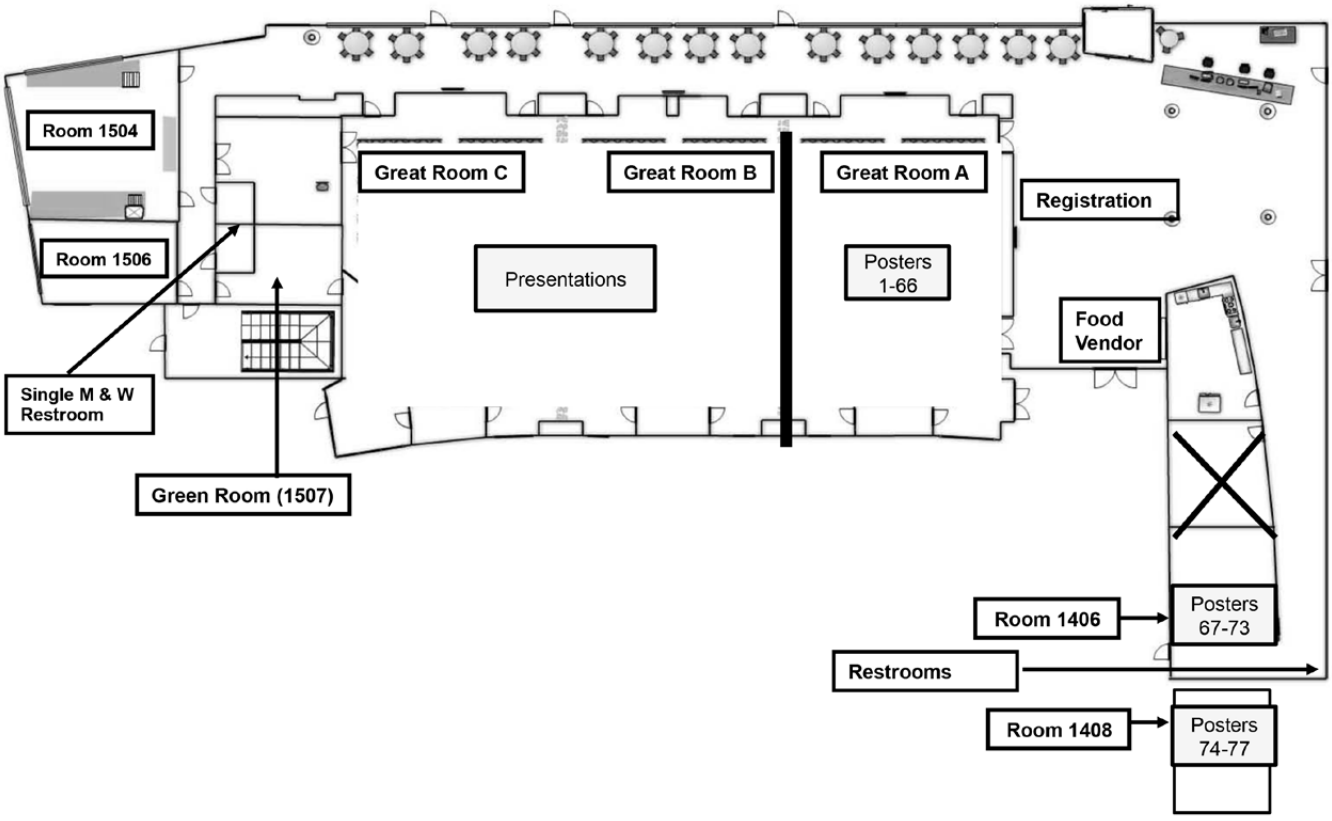
Map of Great Room and Surrounding Areas



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U.S. Food and Drug Administration
Office of Regulatory Science
www.fda.gov/regulatoryscience