



U.S. FOOD & DRUG
ADMINISTRATION

2019 FDA Science Forum

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Transforming Health : Innovation in FDA Science

September 11 & 12, 2019

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2019 FDA Science Forum

Transforming Health: Innovation in FDA Science

Wednesday, September 11, 2019
& Thursday, September 12, 2019

FDA White Oak Campus, Great Room

www.fda.gov/scienceforum

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A Message from FDA's Acting Commissioner



Norman E. "Ned" Sharpless, MD
Acting Commissioner, FDA

I'm honored to welcome you to FDA's 2019 Science Forum. The Science Forum gives our outstanding scientists an opportunity to share with the public the critical scientific activities and research that we perform daily to protect, promote, and advance public health across our entire regulatory portfolio, from medical products and foods to tobacco products.

As we all know, the pace of scientific innovation is accelerating. In just the past few years, we've seen transformative technologies like artificial intelligence, whole genomic sequencing, and cell- or gene-based therapies result in FDA-approved or cleared products that are advancing precision medicine--delivering the right intervention to the right patient, at the right time.

What may not be as well appreciated is that many of these technologies are transforming the way FDA conducts our mission, as they create new opportunities for us to do our work more effectively and efficiently. For instance, new digital ledger technologies offer the potential to help FDA determine the source or origin of foodborne illnesses by tracking and tracing food from farm to table in seconds instead of days or weeks. Predictive artificial intelligence algorithms can help our investigators to better target products for examination and potentially stop unsafe food and medical products, including illegal drugs, offered for import at the border, before they reach our communities; identify facilities that should be prioritized for inspection; or to predict and analyze risks in clinical trials to support more

efficient risk-based monitoring.

As these transformative technologies are developed, FDA is increasing our expertise and research in these areas to enable optimal review and regulation of products incorporating from cutting-edge technology, and to help us leverage these tools, where appropriate, to maximize our public health impact.

FDA's research is critical for developing the translational science strategies and regulatory standards needed to assess innovative technologies that might not fit into older regulatory approaches. Our research is advancing our understanding of these novel technologies and helping create the modern regulatory approaches needed to assess their safety and performance.

I'd like to mention just a few of FDA's groundbreaking scientific research efforts highlighted in our two-day Science Forum:

Researchers in FDA's Center for Food Safety and Applied Nutrition (CFSAN) are developing high-resolution forensic tools to help link foodborne illnesses that would have previously gone undetected. These tools, including whole genome sequencing (WGS), enable the comparison of complete pathogen genomes to better establish commonalties of illnesses and assist with determining the source of food contamination, including individual farms or facilities and specific geographic locales.

To support this effort, FDA created Genome-Trakr, an integrated network of state and federal laboratories that uses whole genome

sequencing to enhance traceback of foodborne pathogens. GenomeTrakr comprises several government food safety agencies and nearly three-dozen state, academic, and international partners. This network is creating a publicly available, global database containing the genetic sequences of hundreds of thousands of foodborne disease-causing bacteria, including Salmonella, Listeria and shiga toxin-producing E. coli.

FDA's Center for Devices and Radiological Health is developing computational modeling and simulation tools that can impact the entire product life cycle, from accelerating innovation in early research phases to undertaking in silico clinical trials to reduce the time and cost of novel medical devices. The Center is also advancing the uptake of appropriate verification and validation methods of these tools so that they can be used across the entire community in a reliable way.

FDA's Center for Drug Evaluation and Research is piloting the use of new technologies and tools to organize and analyze real-world data to produce evidence in support of regulatory decision-making. This approach currently enables us to identify or rule-out potential health risks in the post-market setting; it could also make product labeling more informative for patients and providers. In addition, the Center has developed and is currently piloting mobile technology tools to facilitate the flow of real-world data. CDER is also exploring the use of machine learning and natural language processing, for example, to help regulators and sponsors organize and make sense of data from a variety of sources, including electronic health records, claims data sets, wearable devices, and biosensors.

While FDA continues to work closely with our partners across government and the private sector to promote and protect public health, our Science Forum spotlights the scientific activities that FDA is uniquely positioned to perform, simply because they fall outside the scope of traditional applied or basic research.

FDA's regulatory science research may not

always grab headlines like these other efforts, but it is equally vital, and this Science Forum gives us an ideal opportunity to show you the unique contribution that FDA's stellar researchers make to protecting, promoting, and advancing our nation's public health.

Bio of Norman E. "Ned" Sharpless, MD

Norman E. "Ned" Sharpless, M.D., became Acting Commissioner of Food and Drugs on the afternoon of April 5, 2019. Previously, he was confirmed as the 15th director of the National Cancer Institute (NCI) on October 17, 2017. Prior to his NCI appointment, Dr. Sharpless served as the director of the University of North Carolina (UNC) Lineberger Comprehensive Cancer Center, a position he held since January 2014.

Dr. Sharpless was a Morehead Scholar at UNC-Chapel Hill and received his undergraduate degree in mathematics. He went on to pursue his medical degree from the UNC School of Medicine, graduating with honors and distinction in 1993. He then completed his internal medicine residency at the Massachusetts General Hospital and a hematology/oncology fellowship at Dana-Farber/Partners Cancer Care, both of Harvard Medical School in Boston.

After 2 years on the faculty at Harvard Medical School, he joined the faculty of the UNC School of Medicine in the Departments of Medicine and Genetics in 2002. He became the Wellcome Professor of Cancer Research at UNC in 2012.

A Message from FDA's Chief Scientist



RADM Denise Hinton

Acting Chief Scientist, Office of the Chief Scientist, OC, FDA

We are delighted to welcome you to FDA's 2019 Science Forum. This biannual event gives FDA the unique opportunity to share with the American public the extraordinary range of scientific activities and research FDA does every day to protect, promote, and advance public health.

At our White Oak headquarters and across the country, FDA's 11,000 scientists are investigating and applying new scientific tools to inform our regulatory decision making—and drive innovation. With industry focused on product development and academia focused on foundational research, FDA's regulatory science research concentrates on creating and assessing the tools, processes, and approaches that can ensure that our regulated products are safe and effective for consumers and patients—and that the harm from regulated tobacco products is reduced.

The science we do at FDA is critical for product quality and safety, particularly because it is seldom done by industry and academia. Our recent achievement in food safety is one example. Between 2013 and 2015, large and complex multistate cyclosporiasis outbreaks occurred, involving 1,206 cases in the U.S., with more than 70 hospitalizations. These outbreaks were linked to consuming different types of FDA-regulated fresh produce. Lacking a tool to detect and characterize the parasite *Cyclospora cayetanensis* in fresh produce, FDA researchers set to work. In 2016, they validated a method that can be used in the field by laboratories to help trace the sources

of this food contamination and significantly improve epidemiological investigations.

FDA scientists are also coming up with innovative methods to mine and analyze new data that will increase our understanding of the patient experience. An FDA research team is applying qualitative research methods with neurolinguistic processing in data mining to access unstructured data from FDA sources like advisory committee meetings, public docket comments, and a range of social media platforms. These data have the potential to inform clinical trial design and trial endpoint development, and they will also support regulatory reviews, including benefit-risk assessment.

In the area of precision medicine, FDA has also identified critical knowledge gaps. For example, we need to improve our understanding of the safety and efficacy of many medical products given to newborns, infants, and pregnant mothers. To this end, FDA has established the Perinatal Health Center of Excellence to improve product study design and analyses, perinatal genetic testing, and health communication, and to expand data sources.

And we're doing much more. As increasingly innovative products make their way into everyday use, FDA has adopted a proactive stance, working closely with all our stakeholders. We aim to be prepared for the new technologies and the advancing science that undoubtedly will affect the products we regulate in the coming decades. We are

building the expertise needed to understand and appropriately regulate those products and enable the U.S. public to access them in as safe a manner — and as quickly as possible.

During the next two days, I hope you'll gain a richer appreciation for the many types of regulatory science research underway at FDA, for its challenges, and for the many opportunities for collaboration. We also look forward to sharing with you some of the exciting scientific advances we're making with our partners in the scientific community.

Bio of RADM Hinton

RADM Denise Hinton is FDA's Chief Scientist. 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 and Medical Countermeasures Initiatives, health informatics, scientific professional development, scientific integrity, laboratory safety, and technology transfer.

RADM Hinton previously served as Deputy Director of the Office of Medical Policy (OMP) in FDA's Center for Drug Evaluation and Research (CDER), where she concurrently served as Acting OMP Director from 2014 to 2016. There, she led the development, coordination, and implementation of medical policy programs and strategic initiatives, including the efficient integration of rapidly evolving science and new technologies into the drug development and regulatory review processes. RADM Hinton's work involved close collaboration with other CDER program areas, FDA product centers, and a broad variety of stakeholders.

RADM Hinton joined FDA in 2002, serving in CDER's Division of Cardiovascular and Renal Products and, later, served in the center's former Division of Training and Development. Before coming to FDA, she was an officer in

the U.S. Air Force. RADM Hinton earned her Bachelor of Science in Nursing from Florida State University and her Master of Science degree from Boston University.

Opening Remarks



Amy P. Abernethy, MD, PhD

Principal Deputy Commissioner of Food and Drugs, Chief Information Officer

As the Principal Deputy Commissioner of Food and Drugs, Dr. Amy P. Abernethy, M.D., Ph.D., helps oversee the agency's day-to-day functioning and directs special and high-priority initiatives that cut across offices overseeing FDA's regulation of drugs, medical devices, tobacco and food.

Dr. Abernethy, a hematologist/oncologist and palliative medicine physician, is an internationally recognized clinical data expert and clinical researcher. Her areas of expertise include cancer data, real world evidence, clinical trials, health services research, patient reported outcomes (PROs), clinical informatics, and patient-centered care.

Before coming to FDA, Dr. Abernethy served as chief medical officer, chief scientific officer, and senior vice president for oncology at Flatiron Health (a member of the Roche Group), where she led the research oncology, clinical operations and data science teams, and contributed to the overall strategic vision of the company, including directing their research vision on real world evidence.

Prior to that, Dr. Abernethy was professor of medicine at Duke University School of Medicine, where she ran the Center for Learning Health Care in the Duke Clinical Research Institute and the Duke Cancer Care Research Program in the Duke Cancer Institute. At Duke, she pioneered the development of technology platforms to spur novel advancements in the care of people with cancer and other serious life-limiting illnesses.

Dr. Abernethy was formerly an appointed member of the National Academy of Medicine's National Cancer Policy Forum, an elected member of the American Society for Clinical Investigation, and Past President of the American Academy of Hospice & Palliative Medicine.

Dr. Abernethy received her M.D. at Duke University, where she also did her internal medicine residency, served as chief resident, and completed her hematology/oncology fellowship. She received her Ph.D. from Flinders University in Australia, with a focus on evidence-based medicine and clinical informatics, and her bachelor's degree from the University of Pennsylvania.

Keynote Speaker



Francis Collins MD, PhD
National Institutes of Health
FDA and NIH: Partners in Transformation

In this keynote lecture, National Institutes of Health (NIH) Director Francis S. Collins will highlight how the longstanding partnership between his agency and the Food and Drug Administration (FDA) is serving to advance biomedical research in ways that will improve human health. Areas with transformative collaboration potential include: gene therapy and gene editing; the *All of Us* Research Program; the HEAL (Helping to End Addiction Long-termSM) Initiative; the Accelerating Medicines Partnership (AMP), and the Partnership for Accelerating Cancer Therapies (PACT). Dr. Collins will also discuss the role of the FDA-NIH Joint Leadership Council in facilitating the translation of scientific discoveries.

Bio of Francis Collins MD, PhD

Francis S. Collins, M.D., Ph.D. was appointed the 16th Director of the National Institutes of Health (NIH) by President Barack Obama and confirmed by the Senate. He was sworn in on August 17, 2009. On June 6, 2017, President Donald Trump announced his selection of Dr. Collins to continue to serve as the NIH Director. In this role, Dr. Collins oversees the work of the largest supporter of biomedical research in the world, spanning the spectrum from basic to clinical research.

Dr. Collins is a physician-geneticist noted for his landmark discoveries of disease genes and his leadership of the international Human Genome Project, which culminated in April

2003 with the completion of a finished sequence of the human DNA instruction book. He served as director of the National Human Genome Research Institute at NIH from 1993-2008.

Before coming to NIH, Dr. Collins was a Howard Hughes Medical Institute investigator at the University of Michigan. He is an elected member of the National Academy of Medicine and the National Academy of Sciences, was awarded the Presidential Medal of Freedom in November 2007, and received the National Medal of Science in 2009.

2019 FDA Science Forum Agenda

• Day 1: September 11, 2019 •

8:30 a.m.–8:35 a.m.	Introduction Rokhsareh Shahidzadeh, MSN
8:35 a.m.–8:45 a.m.	Welcome FDA Chief Scientist, RADM Denise Hinton
8:45 a.m.–8:55 a.m.	Opening Remarks Amy Abernethy, M.D., PhD, FDA Principal Deputy Commissioner, Chief Information Officer
8:55 a.m.–9:10 a.m.	Remarks and Introduction of Keynote Speaker FDA Acting Commissioner Ned Sharpless, MD
9:10 a.m.–9:40 a.m.	Keynote Speaker: Francis Collins MD, PhD, Director, National Institutes of Health FDA and NIH: Partners in Transformation
9:40 a.m.–9:50 a.m.	Break

Poster Session 1

Great Room Section C and Room 1504

Time	Topics
9:50 a.m.–10:50 a.m.	Precision Health Advanced Technology

Concurrent Sessions 1 & 2 (10:50 a.m.– 12:50 p.m.)

Concurrent Session 1: Precision Health

Great Room Section A

Session Chairs/Moderator: Rhonda Moore, PhD (CDER), William Mattes, PhD, DABT (NCTR)

Time	Presentation	Speaker
10:50 a.m.–10:55 a.m.	Introduction	Rhonda Moore, PhD CDER-FDA
10:55 a.m.–11:10 a.m.	Regulatory Perspective on Digital Health for Precision Medicine	Bakul Patel, MSEE, MBA CDRH-FDA
11:10 a.m.–11:25 a.m.	Sex and Gender Differences in Health and Disease	Beverly Lyn-Cook, PhD NCTR-FDA
11:25 a.m.–11:40 a.m.	Clinical Trials In 200 Microliters – Extending Approval in Rare Diseases Using <i>In Vitro</i> Data.	Jim Weaver, PhD CDER-FDA
11:40 a.m.–11:55 a.m.	Genomic Biomarker Use in Cardiovascular Disease Clinical Trials	Oluseyi Adeniyi, PhD, PharmD CDER-FDA

chart continued on next page

11:55 a.m.–12:25 p.m.	Learning Healthcare Systems and Big Data: Advancing the Goal of Precision Pain Medicine	Sean Mackey, MD, PhD Stanford University
12:25 p.m.–12:50 p.m.	Panel Discussion /Q&A	Bakul Patel, MSEE, MBA Beverly Lyn-Cook, PhD Jim Weaver, PhD Oluseyi Adeniyi, PhD., PharmD Sean Mackey, MD, PhD

Concurrent Session 2: Advanced Technology

Great Room Section B

Session Chairs/Moderator: Darón Freedberg, PhD. CBER-FDA

Time	Presentation	Speaker
10:50 a.m.–11:15 a.m.	Accelerating Innovation in Manufacturing Technology for Biomanufactured Products: Manufacturing U.S. and NIST	Kelley Rogers, PhD National Institute of Standards and Technology
11:15 a.m.–11:30 a.m.	MetagenomeTrakr Pilot Program for Rapid Foodborne Pathogen Detection	Paul Morin, PhD ORA-FDA
11:30 a.m.–11:45 a.m.	The Promise of Microbial Genomics: How Microbiology is Standing Up to the Many Challenges of a 21st Century Food Supply	Marc Allard, PhD CFSAN-FDA
11:45 a.m.–12:00 p.m.	Editing the Genome without DNA Breaks	Jakob Reiser, PhD CBER-FDA
12:00 p.m.–12:15 p.m.	Computational Modeling for Medical Devices	Pras Pathmanathan, PhD, CDRH-FDA
12:15 a.m.–12:30 p.m.	Avian Influenza A Susceptibilities to Pulmonary Surfactant Protein D: Confirmation of N-glycan sub type as a Pathogenic Factor in Influenza	John Cipollo, PhD CBER-FDA
12:30 p.m.–12:50 p.m.	Panel Q & A, Advanced technology at FDA: Potential Utility and Regulatory Challenges	Moderator: Glenn Black, PhD, CBER-FDA Kelley Rogers, PhD Paul Morin, PhD Marc Allard, PhD Jakob Reiser, PhD Pras Pathmanathan, PhD John Cipollo, PhD Anil Patri, PhD, OC-FDA

12:50 – 1:40 p.m. Lunch

Concurrent Session 3 & 4 (1:40 p.m.– 3:40 p.m.)

Concurrent Session 3: Product Accessibility, Integrity, and Security

Great Room Section A

Session Chairs/Moderator: Stephen Perrine, M. S. ,and Leslie Rivera Rosado, PhD

Time	Presentation	Speaker
1:40 p.m.–1:45 p.m.	Introduction Product Accessibility, Integrity, and Security	Leslie Rivera Rosado, PhD, CDER-FDA Stephen Perrine, M.S. CFSAN-FDA
1:45 p.m.–2:00 p.m.	Violent Non-State Actor Use of Food as a Delivery System: Comparing ideological and Non-Ideological Perpetrators	Markus Binder, M.S. University of Maryland
2:00 p.m.–2:15 p.m.	Product Availability: A Drug Shortage Perspective	Hyun J. Son Pharm.D CDER-FDA
2:15 p.m.–2:30 p.m.	Bio-Terrorism Regulations and Food Security	Desmond Brown, M.S. ORA-FDA
2:30 p.m.–2:45 p.m.	FDA Food Defense Efforts – A Preventive Approach to Food Terrorism	Ryan Newkirk, PhD CFSAN-FDA
2:45 p.m.–3:00 p.m.	CBER-Regulated Products: Preventing and Mitigating Shortages	Anita Richardson, MAS CDER-FDA
3:00 p.m.–3:15 p.m.	On the 'Cyber-Securability' of Medical Devices	Eugene Vasserman, PhD Kansas State University
3:15 p.m.–3:40 p.m.	Panel Discussion	<i>Moderators:</i> Leslie Rivera Rosado, PhD Stephen Perrine, M.S. <i>Panel members:</i> Markus Binder, M.S. Hyun J. Son Pharm.D. Desmond Brown, M.S. Ryan Newkirk, PhD, CFSAN-FDA Anita Richardson, MAS Eugene Vasserman, PhD

Concurrent Session 4: Predictive Tools

Great Room Section B

Session Chairs/Moderator: Donna Mendrick, PhD

Time	Presentation	Speaker
1:40 p.m.–2:10 p.m.	Developing Digital Measures from Person-Generated Health Data	Luca Foschini, PhD Evidation Health
2:10 p.m.– 2:25 p.m.	MRI In Nonclinical Safety Assessment	Serguei Liachenko MD, PhD NCTR-FDA
2:25 p.m.–2:40 p.m.	The VICTRE Project: The First All-In-Silico Imaging Clinical Trial	Aldo Badano, PhD CDRH-FDA
2:40 p.m.– 2:55 p.m.	Use of The MHC Associated Peptide Proteomic Assay to Understand the Immunogenicity Risk of Therapeutic Proteins	Zuben Sauna, PhD CBER-FDA
2:55 p.m.– 3:10 p.m.	Cardiac and Hepatic Cellular Systems to Model Human Drug Effects	Alexandre Ribeiro, PhD CDER-FDA
3:10 p.m.– 3:25 p.m.	C. elegans for Rapid Developmental Neurotoxicity Assessment of Mixtures	Piper Hunt, PhD CFSAN-FDA
3:25 p.m.– 3:40 p.m.	Determination of Seafood Decomposition by Mass Spectrometry with Sensory-Driven Modeling	Randy L. Self, PhD ORA-FDA

Poster Session 2

Great Room Section C and Room 1504

Time	Topics
3:40 p.m.– 4:40 p.m.	Advanced Technology Product Accessibility, Integrity, and Security Predictive Tools

End of Day One

• Day 2: September 12, 2019 •

Poster Session 3

Great Room Section C and Room 1504

Time	Topics
9:00 a.m.–10:00 a.m.	Predictive Tools Advancing Digital Health and Artificial Intelligence

Concurrent Session 5 & 6 (10:00 a.m.– 12:00 p.m.)

Concurrent Session 5: Advancing Digital Health and Artificial Intelligence

Great Room Section A

Session Chair/Moderator: Qi Liu, PhD / Richard Forshee, PhD

Time	Presentation	Speaker
10:00 a.m. – 10:05 a.m.	Welcome to Advancing Digital Health and Artificial Intelligence	Qi Liu, PhD CDER-FDA
10:05 a.m. – 10:45 a.m.	Deep Learning for Polypharmacy and Drug Repurposing	Marinka Zitnik, PhD Stanford University
10:45 a.m. – 11:05 a.m.	FDA's Real-World Evidence Program – Technology and Innovation as a Cornerstone	Jacqueline Corrigan-Curay, J.D., MD CDER-FDA
11:05 a.m. – 11:25 a.m.	Assessment of Devices that Rely on Artificial Intelligence / Machine Learning	Berkman Sahiner, PhD CDRH-FDA
11:25 a.m. – 12:00 p.m.	AI at FDA: Potential Utility and Regulatory Challenges	Richard Forshee, PhD, CBER-FDA Yaning Wang, PhD, CDER-FDA Berkman Sahiner, PhD, CDRH-FDA Errol Strain, PhD, CVM-FDA Rhonda Moore, PhD, CDER-FDA Joshua Xu, PhD, NCTR-FDA Marinka Zitnik, PhD, CDER-FDA

Concurrent Session 6: Outbreak!

Great Room Section B

Session Chairs/Moderator: Surender Khurana, PhD

Time	Presentation	Speaker
10:00 a.m.–10:30 a.m.	Innovation in Science: Protecting People from Emerging Infectious Disease Threats	Christopher R. Braden, MD Centers for Disease Control and Prevention (CDC)
10:30 a.m. –10:45 a.m.	Foodborne Outbreak Investigations in the Whole Genome Sequencing Era	Jennifer Beal, MPH CFSAN-FDA
10:45 a.m. –11:00 a.m.	Immune Responses to Zika Infections	Steven Wood, PhD, CDRH-FDA
11:00 a.m.–11:15 a.m.	Tracking antibiotic resistance in Salmonella: The role of the National Antimicrobial Resistance Monitoring System.	Patrick McDermott, PhD, CVM-FDA
11:15 a.m.–11:30 a.m.	Emerging & Pandemic Threat Preparedness	Jerry Weir, PhD, CBER-FDA
11:30 a.m.–11:40 a.m.	Strengthening Regulatory Science to Support the Development of Medical Countermeasures for Emerging Infectious Diseases	Tracy MacGill, PhD, OC-FDA
11:40 a.m.–12:00 p.m.	Panel Discussion	Panel Discussion Moderator: Chad Nelson, PhD, OC-FDA Christopher R. Braden, MD Steven Wood, PhD Patrick McDermott, PhD Jennifer Beal, MPH

12:00 – 1:00 p.m. Lunch

Poster Session 4

Great Room Section C and Room 1504

Time	Topics
1:00 p.m.– 2:00 p.m.	Advancing Digital Health and Artificial Intelligence Outbreak! Addiction Impacting Public Health Through Electronic Media: Empowering Consumers, Patients, and Other Stakeholders

Concurrent Sessions 7 & 8 (2:00 p.m.– 4:00 p.m.)

Concurrent Session 7: Addiction

Great Room Section A

Session Chairs/Moderators: Katherine Bonson, PhD, Chad Reissig, PhD

Time	Presentation	Speaker
2:00 p.m.–2:05 p.m.	Introduction	Katherine Bonson, PhD, CDER-FDA
2:05 p.m.–2:20 p.m.	Drug abuse in the U.S.	Chad Reissig, PhD, CDER FDA
2:20 p.m.–2:35 p.m.	FDA response to the opioid crisis	Marta Sokolowska, PhD, CDER-FDA
2:35 p.m.–2:50 p.m.	Assessing the structural and pharmacological similarity of newly identified drugs of abuse to controlled substances using PHASE	Christopher Ellis, PhD, CDER-FDA
2:50 p.m.–3:10 p.m.	Preclinical pharmacology of novel synthetic opioids appearing in clandestine drug markets	Michael Baumann, PhD National Institute on Drug Abuse (NIDA)
3:10 p.m.–3:25 p.m.	FDA assessment of the abuse potential of drugs, including opioids	Katherine Bonson, PhD, CDER-FDA
3:25 – 4:00 p.m.	Panel Discussion	Chad Reissig, PhD Marta Sokolowska, PhD Christopher Ellis, PhD Michael Baumann, PhD Katherine Bonson, PhD

Concurrent Session 8: Impacting Public Health Through Electronic Media: Empowering Consumers, Patients, and Other Stakeholders

Great Room Section B

Session Chair/Moderator: Ryan Kennedy, PhD

Time	Presentation	Speaker
2:00 p.m.– 2:20 p.m.	Tobacco Regulatory Science – Understanding the Role of Flavor in E-Cigarette Marketing	Ryan Kennedy, PhD Johns Hopkins Bloomberg School of Public Health
2:20 p.m.– 2:35 p.m.	Using Content Analysis to Understand Tobacco Industry Use of Technology to Engage Consumers	Mario Navarro, PhD, CTP-FDA
2:35 p.m.– 2:50 p.m.	Consumers' Use of Personal Electronic Devices in the Kitchen	Amy Lando, MPP, CFSAN-FDA Michael Bazaco, PhD, CFSAN-FDA
2:50 p.m.– 3:05 p.m.	Assessment of Patient Perspective on Risks and Benefits Associated with High Intensity Focused Ultrasound (Hifu) for The Ablation of Prostate Tissue in Men With Localized Prostate Cancer	Charles Viviano, M.D., PhD CDRH-FDA
3:05 p.m.– 3:20 p.m.	Clinical Outcome Assessments in Medical Product Development	Elektra Papadopoulos, MD, CDER-FDA
3:20 p.m.– 3:35 p.m.	Collect Once, Use Many Times: Challenges and Opportunities for the Use of Real-World Evidence to Improve Healthcare	Gregory Pappas, MD, PhD, CBER-FDA
3:35 p.m.– 4:00 p.m.	Panel Discussion	Ryan Kennedy, PhD Mario Navarro, PhD Amy Lando, MPP Michael Bazaco, PhD Charles Viviano, MD, PhD Elektra Papadopoulos, MD Gregory Pappas, M.D., PhD

End of 2019 FDA Science Forum

Speakers Bios and Abstracts

Concurrent Session 1: Precision Health

Regulatory Perspective on Digital Health for Precision Medicine



Bakul Patel, MBA

Associate Director of Digital Health
FDA, The Center for Devices and Radiological Health

Bakul Patel is Associate Director for Digital Health in the Food and Drug Administration's (FDA) Center for Devices and Radiological Health (CDRH). Mr. Patel leads regulatory policy and scientific efforts at the Center in areas related to emerging and converging areas of medical devices, wireless, and information technology. This includes responsibilities for mobile health, health information technology, cyber security, medical device interoperability, and medical device software.

Mr. Patel is the FDA liaison between the Federal Communications Commission (FCC) and the Office of the National Coordinator (ONC). He has chaired the International Medical Device Regulators Forum (IMDRF) "software as a medical device" working group, a global harmonization effort, since its inception in 2013.

Before joining FDA, Mr. Patel held key leadership positions in the telecommunications, semiconductor capital equipment, wireless, and information technology industries. His experience includes Lean Six Sigma, creating long- and short-term strategy, influencing organizational change, modernizing government systems, and delivering high-technology products and services in fast-paced, technology-intensive organizations. Mr. Patel earned an MS in Electronic Systems Engineering from the University of Regina, Canada and an MBA in International Business from The Johns Hopkins University.

Abstract: Regulatory Perspective on Digital Health for Precision Medicine

Smart interconnected personalized digital health devices are on the cusp of revolutionizing the clinical market. To facilitate this transition, FDA proactively invested in increasing the number and expertise of digital health staff, launching the digital health software precertification pilot program ("Pre-Cert") and issuing guidance to modernize our policies to support innovation of digital health technologies. This talk will provide a high-level overview of a novel proposed regulatory framework for digital health, including Excellence Appraisal (EA) and Pre-Cert, Review Pathway Determination (RD), Streamlined Review (SLR) and Real-World Performance (RWP). Barriers and examples of real-world impact will be discussed with special emphasis on regulatory and policy efforts for personalized AI/ML-based digital health devices that continuously learn.

Sex and Gender Differences in Health and Disease

Beverly D. Lyn-Cook, PhD

FDA, National Center for Toxicological Research

Beverly Dawkins-Lyn Cook, PhD is a Senior Interdisciplinary Research Biologist with vast research experience in the areas of cell and molecular biology, pharmacogenomics, and epigenomics at the FDA/National Center for Toxicological Research (NCTR), located in Jefferson, Arkansas. She received her MS (1979) and PhD in 1981 from Atlanta University, Atlanta, Georgia. She conducted her post-doctoral studies in the Department of Biochemistry at the University of North Carolina School of Medicine, Chapel Hill, NC from 1981-1984 and later was a Research Associate Scientist at the Lineberger Cancer Center, Chapel Hill before joining FDA. For the last 30 years with FDA/NCTR her research interests have included: sex differences in adverse drug reactions, epigenetics, and health disparities in diseases (pancreatic cancer, cardiovascular disease, lupus and breast cancer). Currently, her laboratory is addressing sex/gender differences in the expression of drug transporters genes and proteins as it relates to adverse drug reactions to chemotherapeutics, triple-negative breast cancer, and the role of epigenetics in lupus, with the goal of identifying new targets for drug therapy. Dr. Lyn-Cook plays an active role in the Center's mentoring programs, including recruiting students as interns and participants in NCTR's summer research programs, serving as a mentor for FDA's Fellows program, outreach and mentoring of junior scientists (post-doc) and the developing research collaborations between NCTR and the colleges and universities in Arkansas. She is very active with the American Association for Cancer Research, where she has served as Chair of MICR, member of WICR, and currently serves on the AACR Science Education Committee and again on the MICR council.

Abstract: Sex Differences in Precision Medicine and Health

The inclusion of women in research studies has greatly improved over the last 20 years; however, preclinical research continues to use male animals and male-derived cells, which may cause the results to be sex-biased. Sex, as a biological variable, must be considered in health and diseases. Precision medicine involves giving the appropriate dose of a drug to an individual who will benefit the most with the least side effects. Sex differences in the expression of drug metabolizing enzymes and transporters are thought to be one the most important determinants accounting for individual differences in clinical pharmacology, pharmacokinetics, and toxicokinetics leading to differences in drug efficacy and safety. A number of studies have been conducted in liver, but the kidney is also a major pharmacokinetic organ with potential impact on adverse drug reactions indicating that further investigations are needed. Kidney disease is the 9th leading cause of death in the United States and it has been estimated that 31 million people in the United States have chronic kidney disease. Kidney toxicity is one of the leading causes of drug failure. It is believed that drug-induced nephrotoxicity is responsible for 30-50% of all drug failures in phase III clinical trials. Sex differences involved in pharmacokinetics in this organ are not fully understood. Sex differences

in the epigenetic regulation in drug metabolizing and transporting enzymes can be a critical determinant in how individuals respond to drugs. Our laboratory was the first to investigate epigenome-wide methylation in normal human kidney tissue and this resulted in the identification of sex-specific epigenetic variations. 429 differentially methylated sites with significant sex-based differences were involved in several biological pathways. Ingenuity Pathway Analysis of 316 genes associated with the 429 sites revealed a number of genes involved in kidney toxicity, disease, and biological function. We have provided a reference methylome for normal kidney that may be utilized to improve our understanding of renal disease and assessing the overall safety and effectiveness of drugs in the kidney.

Clinical Trials In 200 Microliters – Extending Approval in Rare Diseases Using In Vitro Data



James L. Weaver, PhD

FDA, Center for Drug Evaluation and Research

James L. Weaver obtained BA and MS degrees from the University of Vermont and a PhD 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 FDA's Center for Drug Evaluation & Research as a staff fellow in 1989 and became a Research Pharmacologist in 1994. He is currently doing biomarker, antibiotic resistance, 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.

Abstract: Clinical trials in 200 microliters – Extending approval in rare diseases using in vitro data

Cystic fibrosis (CF) and Fabry's Disease (FD) are single-gene disorders caused by hundreds of different and often rare variants. For the rarer variants, traditional clinical trials with appropriate numbers of participants with a given mutation are impossible. Ivacaftor was approved to treat CF patients with responsive class III variants in cystic fibrosis transmembrane conductance regulator (CFTR) and migalastat was approved to treat FD patients with amenable variants in galactosidase alpha (GLA). Both approvals were based on a small number of treated patients. The sponsors of each drug also developed in vitro cell systems to produce functional data on the responsiveness of variant gene products not studied clinically. Specific criteria were used by the FDA to determine if the in vitro data was sufficient to support expansion of approval. These criteria included a clear understanding of the disease, the overall clinical safety and efficacy of the drug; and the mechanism of action of the drug. Further, it should be demonstrated that clinical trials are not feasible for the investigated gene variant(s) and data should be generated from responsive and non-responsive variants so predictive power of the in vitro method can be determined. In addition, the in vitro cell assay must be a direct measure of target protein function; the performance metrics must be established through validation studies; bidirectional sequencing must be used for variant and gene context confirmation; and raw data must be available for reanalysis by the FDA. Using these evaluation criteria, treatment expansion based on submitted in vitro data was approved for ivacaftor and migalastat. These expansions have allowed CF and FD patient access to treatment that otherwise would not have been possible.

Genomic Biomarker Use in Cardiovascular Disease Clinical Trials

Oluseyi Adeniyi, PharmD, PhD

FDA, Center for Drug Evaluation and Research

Oluseyi Adeniyi received her PharmD and PhD training from the University of Michigan, where she studied ways to improve vaccine and gene delivery by cell-specific targeting and circumventing subcellular barriers. She joined FDA as a Commissioner's Fellow in 2015 to characterize the potential of biomarker-based drug development in the pharmaceutical pipeline for common chronic diseases. She is currently a reviewer in the Genomics & Targeted Therapy Group in the Office of Clinical Pharmacology where she contributes to advancing biomarker-based drug development strategies in the regulatory review of investigational new drugs and new drug/biologic applications.

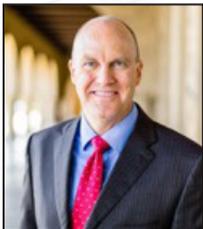
Abstract: An Overview of Genomic Biomarker Use in Cardiovascular Disease Trials

For many cancer drugs, knowledge of genetic variation has enhanced the development of targeted therapies for subsets of patients. However, drug development for common, chronic conditions like cardiovascular disease have not been similarly impacted by the genomics revolution. Drug development for CVD is in decline and the use of genomic biomarkers to select subsets of patients for clinical trials may enhance the efficiency of developing CVD drugs.

We aimed to characterize the extent to which genomic biomarkers are used in CVD clinical trials and identify potential for enhanced CVD drug development by evaluating CVD clinical trials for the application genomic biomarker strategies.

This talk will highlight how genomics has been applied in drug development, as well as recently-published findings evaluating the prospective use of biomarker in trials for CVD and related conditions that underscore potential opportunities for targeted drug development.

Learning Healthcare Systems and Big Data: Advancing the Goal of Precision Pain Medicine



Sean Mackey, MD, PhD

FDA, Center for Drug Evaluation and Research

Sean Mackey, MD, PhD, is Chief of the Division of Pain Medicine and Redlich Professor of Anesthesiology, Perioperative and Pain Medicine at Stanford University. Dr. Mackey received his BSE and MSE in Bioengineering from the University of Pennsylvania and his PhD in Electrical and Computer Engineering, as well as his MD, from the University of Arizona. He completed his Anesthesiology residency and Pain Medicine fellowship at Stanford and joined the faculty in 1999.

Under Dr. Mackey's leadership, the Stanford Pain Management Center has been twice designated a Center of Excellence by the American Pain Society for the Center's innovative approach in comprehensive, interdisciplinary, and outcomes-based care. He has served as principle investigator on multiple NIH awards where he has overseen efforts to map the specific regions of the brain and spinal cord that perceive and process pain.

Dr. Mackey is author of over 200 journal articles and book chapters in addition to numerous national and international lectures. Currently, he is developer of a free, open-source learning health system—CHOIR (<http://choir.stanford.edu>)—to transform the care of people with pain, and serve as a platform for research in real-world clinic patients.

Dr. Mackey is Past-President of the American Academy of Pain Medicine (AAPM). He co-authored the Institutes of Medicine's report on Relieving Pain in America. He was Co-Chair of the Oversight Committee for the HHS/NIH National Pain Strategy (NPS), an effort to establish a national health strategy for pain care, education and research. He has received multiple awards for leadership, teaching, research and clinical care. In the last two years he has received the American Pain Society Wilbert E. Fordyce Clinical Investigator Award; the AAPM Pain Medicine Fellowship Award and Distinguished Service Award, and the NIH Directors' Award for his efforts on the NPS.

Abstract: Learning Healthcare Systems for Optimized Care and Real-World Research Discovery and Innovation

The United States National Academy of Medicine (NAM) has called for the development of national patient registries and Learning Healthcare Systems (LHS) for acute and chronic pain. I will describe the rationale and the power of patient registries and LHSs. As envisioned by the NAM, LHS leverages an integrated digital infrastructure to provide data-based and coordinated care that is available just-in-time to the clinician and that is centered on the patient.

In this session, I will survey the field of LHS platforms, and illustrate its power using the Collaborative Health Outcome Information Registry (CHOIR). CHOIR is an open source and free platform created at Stanford, available to academic institutions. Attendees will learn about the digital infrastructure implemented in CHOIR and deployed at

Stanford and multiple other academic institutions. Attendees will learn about some of the opportunities and barriers in institutional buy-in and deployment across CHOIR institutions. We will share our perioperative and chronic pain experiences with big clinical data for: clinical decision support, research-grade clinical data, aggregation of data, and models to advance the goal of precision pain management.

We will share our early experiences with LHS at the bedside. Attendees will learn about the clinical decision support features of CHOIR, and the utility of obtaining research-grade clinical data as a part of routine clinical care. We will describe use of LHSs for pragmatic clinical trials and rapid piloting of clinical interventions. We will share our experiences in real-time aggregation and summarization of LHS data to provide on-going decision support in the perioperative and outpatient environments. Finally, attendees will learn about research efforts and 20+ resulting publications that are not only powered by but that are made possible by large-scale LHS platforms like CHOIR.

Learning Objectives:

- Identify defining features of patient registries and learning healthcare systems (LHS), and recognize the roles LHS platforms play in acute and chronic pain management.
- Evaluate the impact of LHS platforms on patient care and research activities.
- Delineate the aspects of registries and LHS and appreciate the data and process utility of them, both as a tangible IT infrastructure and as a profound cultural change in care delivery.
- Describe features of model open source LHS platforms and how to implement them in their home institution.
- Describe results from both a chronic and perioperative registry identifying factors of high risk and good pain care

Speakers Bios and Abstracts

Concurrent Session 2: Advanced Technology

Moderator:

Darón Freedberg, PhD

FDA, Center for Biologics Evaluation & Research

Darón Freedberg received his bachelor's degree in 1990 from UCSD in Chemistry, where he studied stereodynamics with Jay Siegel. In 1994, he earned his Ph.D. at UCLA where he studied conformational isotope effects and helium encapsulated in Buckeyballs, under Frank Anet. After a postdoctoral fellowship studying protein structure and dynamics at the NIH with Dennis Torchia, he took a position at the FDA. Since 1997, he has been combining his experience in stereochemistry, conformational analysis, structure and dynamics. He is now a Principal Scientist who reviews pharmaceutical polysaccharide-based vaccines and leads a research team in NMR studies of oligo- and polysaccharide structure-function relationships.

Accelerating Innovation in Manufacturing Technology for Biomanufactured Products: Manufacturing U.S. and NIST



Kelley Rogers, PhD

National Institute of Standards and Technology

Kelley C. Rogers, PhD, is the Technical Program Manager for the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), a Manufacturing USA institute sponsored by the National Institute for Standards and Technology (NIST). In this role within the Office of Advanced Manufacturing at NIST, Kelley provides oversight on the technical portfolio of the institute, whose mission is to accelerate biopharmaceutical manufacturing innovation in the United States.

Kelley also serves as the Technical Program Director for Biosciences and Health within NIST's Material Measurement Laboratory. In this position, she advises on strategic direction and partnering opportunities to maximize the NIST laboratory programs that support measurement science for biotechnologies.

Before her federal service, Kelley worked in the pharmaceutical industry as a Principal Investigator identifying novel targets for antimicrobial drugs. She completed her doctoral research in the Department of Molecular Biophysics and Biochemistry at Yale University, and holds a Bachelor of Arts with an emphasis in Chemistry from Hendrix College. She was a post-doctoral fellow and staff fellow in the National Institute of Digestive, Diabetes, and Kidney Diseases (NIDDK), at the National Institutes of Health. Kelley's research background is in bacterial protein synthesis and gene expression.

Abstract: Accelerating Innovation in Manufacturing Technology for Biomanufactured Products: Manufacturing USA and NIST

We live in an exciting era for medical breakthroughs and scientific discovery; CRISPR, precision medicine, cancer immunotherapies, stem cell therapies – all present new opportunities to advance public health and support the US economy. However, moving emerging types of biologically manufactured therapies through development into

widespread clinical use requires the ability to make them with consistent quality at an appropriate scale. Without that manufacturing capability, promising therapies will be available only for small patient populations for limited indications, and at great cost. As the US metrology institute, NIST contributes to both the measurement science that underpins the manufacturing and rigorous evaluation of these products, and partners with the Manufacturing USA network to build and innovate the manufacturing and skilled workforce capabilities needed to capture the full economic, public health, and national security benefits of emerging therapies. Two Manufacturing USA institutes, NIIMBL (the National Institute for Innovation in Biopharmaceuticals), and BioFabUSA together represent nearly a half-billion-dollar commitment to address enabling manufacturing challenges for complex biologic products such as cellular and gene-therapies. This talk will focus on progress made, as well as the 'art of the possible' for these partnerships to fuel the innovation ecosystem for better analytical and bioprocessing capabilities to support consistent manufacturing capabilities at scale.

MetagenomeTrakr Pilot Program for Rapid Foodborne Pathogen Detection

Paul M. Morin, PhD

FDA, Office of Regulatory Affairs

Paul M. Morin is a regulatory microbiologist at the FDA Northeast Food and Feed Laboratory (NFFL) located in Jamaica, NY. He earned his doctoral of science degree at the Harvard School of Public Health with a major in immunology and a minor in tropical public health. He continued his academic studies working as a post-doctoral fellow at the Albert Einstein College of Medicine studying microbial pathogenesis and tuberculosis vaccine development in a murine model. At NFFL, he serves as the lead for their GenomeTrakr team performing whole genome sequencing on all foodborne pathogens isolated from foods and environmental swabs. He is an active participant in their Select Agent program serving as an alternate responsible official and assisting with method development and method validation studies in their biosafety level 3 facility. He participates in many workgroups that involve regulatory science and collaborates on next generation sequencing projects. He also serves as the Northeast Regional Coordinator for the Food Emergency Response Network (FERN) providing continual support to federal, state and local food testing laboratories.

Abstract: MetagenomeTrakr Pilot Program for Rapid Foodborne Pathogen Detection

There is a critical need to develop novel technologies for recovering and identifying foodborne pathogens as well as understanding the microbial ecology of food and environmental matrices. Metagenomics has the potential to serve as transformative technology which can provide accurate unbiased data about an entire microbial community, including pathogens, commensals and organisms that cannot be cultured using traditional methods. The MetagenomeTrakr pilot project investigates the microbiomes of seafoods from various countries as well as environmental swab culture enrichments from food manufacturing facilities. Metagenomic DNA is extracted using mechanical lysis allowing for purification of high-quality microbial community DNA. We use both 16S rRNA amplicon and shotgun metagenomic sequencing to analyze these samples and to compare with traditional culture-based sampling methods. 16S rRNA amplicon libraries were prepared using eubacterial conserved primers that target the V1-V3 variable regions and shotgun metagenomic libraries were prepared using the Illumina Nextera XT kit. Sequencing is performed using the Illumina MiSeq instrument. 16S rRNA gene amplicon and shotgun metagenomic data are analyzed using QIIME2, COSMOSID, and in-house software programs. The data from this project will be publicly available in the MetagenomeTrakr Bioproject at the National Center for Biotechnology Information (NCBI).

The Promise of Microbial Genomics: How Microbiology is Standing Up to the Many Challenges of a 21st Century Food Supply



Marc Allard, PhD

FDA, Center for Food Safety and Applied Nutrition

Marc W. Allard is a Senior Biomedical Research Services Officer in the Division of Microbiology in FDA's Office of Regulatory Science. In 2008, Dr. Allard joined FDA, where he uses Whole Genome Sequencing (WGS) of foodborne pathogens to identify and characterize outbreaks of bacterial strains, particularly Salmonella, E. coli, and Listeria. Dr. Allard specializes in both phylogenetic analysis and the biochemical laboratory methods which generate the WGS information. Dr. Allard helped develop the first distributed network of laboratories that use whole genome sequencing for pathogen identification and traceback called the GenomeTrakr database, which is part of the NCBI Pathogen Detection web site. These tools are used daily for outbreak investigations and compliance. Dr. Allard acts as senior scientist to advise FDA on both WGS and phylogenetic methods as they apply to public health.

Abstract: The Promise of Microbial Genomics: How Microbiology is Standing Up to the Many Challenges of a 21st-Century Food Supply

High-resolution forensic tools are essential for aiding in tracing foodborne contamination events back to their source, and in this regard, whole genome sequencing (WGS) is rapidly transforming microbiological subtyping in the food safety laboratory. When coupled with powerful bioinformatic pipelines, accurate and stable genetic changes can be identified across pathogen genomes that can distinguish strains to the source level including individual farms or facilities and specific geographic locales. This is true even among highly homogeneous strain populations such as Salmonella Enteritidis and other salmonellae that have remained largely recalcitrant to differentiation by conventional typing approaches. Numerous published examples illustrate the ability of WGS to discern genetic relatedness of otherwise indistinguishable isolates and point to WGS as an important tool in the traceability of contamination events. To this end, FDA has created an integrated pilot network of state and federal laboratories to use whole genome sequencing to enhance traceback of foodborne pathogens. Known as GenomeTrakr and now comprising several government food safety agencies as well as nearly three-dozen state, academic, and international partners, the network is creating a publically available, global database containing the genetic makeup of tens of thousands of foodborne disease-causing bacteria including Salmonella. At present, WGS impacts regulatory science in FDA's Foods Program in several ways including: (i) support of traceability efforts during foodborne contamination events; (ii) enhanced regulatory casework for high-risk commodities and compliance standards; and (iii) quality assurance of food microbiological sampling programs. Taken together, regular applications of WGS underscore its extraordinary utility in food safety as well as the potential for complete characterization of bacterial pathogens as they emerge in the food supply.

Editing the Genome without DNA Breaks

Jakob Reiser, PhD

FDA, Center for Biologics Evaluation & Research

Dr. Reiser received a PhD in Biochemistry from the University of Basel in Switzerland, after which he obtained training in virology and protein chemistry as an American Cancer Society Fellow in the Department of Biochemistry, Stanford University School of Medicine. While at Stanford he studied proteins encoded by Simian virus 40—work that supported development of the original Western blot technique. He also worked out a novel chromatin immunoprecipitation assay that maps binding sites of specific DNA binding proteins on viral chromatin. His research at FDA/CBER has focused on two of the major limitations of lentiviral vectors for clinical gene therapy, including the potential of such vectors to, 1) activate oncogenes during random integration into the host genome and, 2) transduce cells in off-target tissues *in vivo*. To address these shortcomings, he has been developing safer HIV-1-based lentiviral vectors by, 1) limiting their integration to well-defined sites in the human genome, and 2) narrowing their tissue tropism through targeted transduction.

Abstract: Editing the Genome without DNA Breaks

Traditionally, genome editing strategies have relied on the introduction of a DNA double strand break (DSB) using engineered nucleases, resulting in insertions and deletions (indels) at the desired target site. To achieve gene correction, these strategies typically require the co-delivery of a repair template along with the nuclease. However, the off-target activity by engineered nucleases, resulting in mutations at sites other than the intended on-target site is a major concern, especially for clinical applications. CRISPR systems include RNA-guided endonucleases that allow the introduction of a DSB at a desired location in the genome. In CRISPR systems, Cas endonuclease proteins form complexes with “guide RNA” molecules, which direct the resulting complex to bind to and cleave a target nucleic acid sequence. We are pursuing strategies for genome editing that do not require DSBs. The ability of catalytically inactive Cas9 proteins to precisely target specific genomic sequences, while also delivering additional functional domains has resulted in novel molecular tools. For example, Cas9 has been repurposed to allow somatic hypermutation at desired target sequences by using catalytically inactive versions of Cas9 referred to as dCas9 to recruit variants of activation-induced cytidine deaminase (AID). Using such programmable hypermutators, we have introduced sequence diversity at defined genomic loci in a mammalian cell context. In a parallel approach, we are investigating site-specific recombinases for genome editing. In contrast to DNA nucleases, direct catalysis by recombinases typically does not provoke error-prone DNA repair processes that result in indel formation and is not dependent on the endogenous cellular DNA repair machinery. To test the recombinase approach, we are using the catalytic domains of small serine recombinases fused to dCas9. Recombinase-mediated genome modification has the potential for more precise and predictable genomic alterations compared to nuclease-based genome editing.

Computational Modeling for Medical Devices



Pras Pathmanathan, PhD

FDA, Center for Drug Evaluation & Research

Dr. Pathmanathan is a scientist at the Office of Science and Engineering Laboratories (OSEL) in FDA's Center for Devices and Radiological Health. His research is on methods for establishing the credibility of computational models, with a focus on models of cardiac electrophysiology. He leads or collaborates in numerous initiatives aiming to advance computational modeling; most notably he co-founded and co-Chairs FDA's Modeling and Simulation Working Group, which has brought together computational modeling scientists across the Agency. He received a BA in mathematics from Cambridge University and an MSc and PhD in computational biology from Oxford University.

Abstract: Computational Modeling for Medical Devices

Computational modeling and simulation (M&S) is at the forefront of the digital revolution in medical devices. Computational simulations of medical devices have been used for many years in regulatory submissions to FDA, as supporting evidence for safety or effectiveness of the device. Now, computational models are also used within software platforms, serving as clinical decision support tools, and are being embedded in medical devices. One major goal is clinical trial reduction using in silico clinical trials, where a device is tested on a cohort of virtual patients. This talk will overview research and initiatives at CDRH that is advancing M&S for medical devices, and discuss research into the reliability of M&S, including model validation and applicability for clinical implementation.

Avian Influenza A Susceptibilities to Pulmonary Surfactant Protein D: Confirmation of N-glycan sub-type as a Pathogenic Factor in Influenza

John Cipollo, PhD

FDA, Center for Biologics Evaluation & Research

Dr. John F. Cipollo received his PhD in 2000 from the State University of New York at Albany. He performed his post-doctoral work at Boston University (BU) School of Dental Medicine under Professors Catherine Costello in mass spectrometry and Carlos Hirschberg in biochemistry. He served as Assistant Professor at B.U. from 2005-2007 before moving to CBER FDA, where he is currently a Principal Investigator and Research Chemist. He has published over forty scientific articles in the areas of carbohydrate structural analysis and glycomics with strong emphasis in carbohydrate mass spectrometry. Dr. Cipollo has worked extensively in the glycomics of a series of organisms including human, *Caenorhabditis elegans*, *Entamoeba invadens*, *Entamoeba histolytica*, and several yeast species. His current interests include the function of glycoprotein antigens interactions in adaptive and innate immunity and the impact of those functions in vaccine development and performance as revealed through investigation of molecular structure using a suite of mass spectrometric and other chemical and physicochemical based methods. Other interests include novel chemistries for improvement of polysaccharide conjugate and other glycoconjugate based vaccines. As there are few broadly accepted informatics platforms for glycomics analysis the Cipollo group has partnered with members of HIVE FDA and HIVE George Washington University to develop and make publicly available a suite of glycomics software for processing of mass spectrometry and glycan array glycomics data. Dr. Cipollo serves as a Product Specialist for CBER FDA primarily as a product reviewer for bacterial polysaccharides and polysaccharide conjugate vaccines.

Abstract: Title: *Avian Influenza A Susceptibilities to Pulmonary Surfactant Protein D: Confirmation of N-glycan sub-type as a pathogenic factor in Influenza*

Seasonal Influenza Avian Virus (IAV) carrying key hemagglutinin (HA) head region high mannose glycans can be removed from the lung by pulmonary surfactant protein D (SP-D) whilst those without these glycans are not. Little is known about HA head glycosylation of low pathogenicity A type influenza virus (LPAIV) subtypes. These can pose a pandemic threat through reassortment and emergence in human populations. Since the presence of head region high mannose glycosites dictates SP-D activity predictability of these glycosite glycan subtypes may be of value. Here we investigate the SP-D activities of recombinant hn-SPD and rh-SP-D forms against representative LPAIV of different HA subtypes, including H2N1, H5N1, H6N1, H11N9, an avian H3N8 and a human seasonal H3N2 subtype. Using mass spectrometry, we determined the glycan subclasses and heterogeneities at each head glycosylation site. Sequence alignment and molecular structure analysis of the HAs were performed for LPAIV strains in comparison to seasonal H3N2 and avian H3N8. Intramolecular contacts were determined between protein backbone and glycosite glycan based on available three-

dimensional structure data. We found that glycosite “N165” (H3 numbering) is occupied by high mannose glycans in H3 HA but by complex glycans in all LVIIV HAs. SP-D was not active on LVAIV. Since SP-D affinity for influenza HA depends on the presence of high mannose glycan on the head region our data demonstrate that SP-D may not protect against virus containing these HA subtypes. Our results also demonstrate that glycan subtype can be predicted at some glycosites based on sequence comparisons and three-dimensional structural analysis. Such methods may be useful for prediction of collectin activities towards key pathogen proteins.

Panel Q & A, Advanced Tehcnology at FDA: Potential Utility and Regulatory Challenges

Glenn Black, PhD

FDA, Center for Biologics Evaluation & Research

Glenn Black is the Associate Director for Research at the U.S. Food and Drug Administration's Division of Food Processing Science and Technology jointly located with IFSH at the National Center for Food Safety and Technology in Bedford Park, IL. In this role, he provides guidance and support for research conducted at the center in the areas of food microbiology, processing, chemistry, packaging and serves as a subject matter expert for food processing related issues. Prior to joining the agency, Dr. Black served various roles in the food industry related to the evaluation and recommendation of processing equipment and parameters, as well as research and development in microbial food safety.

Anil Patri, PhD

FDA, Office of Commissioner

Dr. Anil Patri serves as the Chair, Nanotechnology Task Force and Director of Nanocore, at FDA's National Center for Toxicological Research. His laboratory is very active in regulatory science research to understand material characteristics, safety and efficacy of products containing nanomaterial, and provides training to scientists and reviewers at FDA. He serves on the U.S. National Nanotechnology Initiative (NNI) NSET Subcommittee and NEHI working group for inter-agency coordination. He is a member of ISO TC229 and serves on the executive committee of ASTM E56 to facilitate standards development in Nanotechnology. He co-chairs the EU-US Nanomedicine and Characterization Communities of Research. Before joining FDA in 2014, Dr. Patri served as the Deputy Director, Nanotechnology Characterization Laboratory at the Frederick National Laboratory for Cancer Research. In a decade-long tenure at NCL, he assisted collaborators from industry and academia towards clinical translation of drug products using nanotechnology, many currently in clinical trials. From 2006-2014, he served as a guest scientist at the National Institute of Standards and Technology (NIST). Dr. Patri developed nanotechnology-based targeted drug delivery and imaging agents for cancer until 2004 at the Center for Biologic Nanotechnology, University of Michigan Medical School. He obtained his PhD from University of South Florida, conducting basic research on dendrimer synthesis and characterization.

Panel Abstract: Potential Utility and Regulatory Challenges

Innovations in technology are transforming health care across the world. "Advanced Technologies," including genomics, computer modeling, additive manufacturing, and data analytics show great promise in improving the performance of existing medical products. The most recent advances in technologies need to be incorporated into the regulation process. This panel will discuss Advanced Technologies, as well as regulatory challenges in reviewing submissions related to technological innovations.

Speakers Bios and Abstracts

Concurrent Session 3: Product Accessibility, Integrity, and Security

Violent Non-State Actor Use of Food as a Delivery System: Comparing ideological and Non-Ideological Perpetrators



Markus Binder, MS
University of Maryland

Markus K. Binder is a Senior Researcher with the Unconventional Weapons and Technology Division (UWT) within the University of Maryland's National Consortium for the Study of Terrorism and Responses to Terrorism (START). His research focus is Violent Non-State Actor (VNSA) use of Chemical and Biological agents and he manages two START databases tracking this phenomenon. Before joining START, Mr. Binder was an independent consultant providing expertise in the areas of WMD nonproliferation, chemical and biological terrorism, and the spread of MANPADS. From 2004 to 2007 he was Deputy Director of the Chemical and Biological Weapons Nonproliferation Program (CBWNP) at the James Martin Center for Nonproliferation Studies (CNS) in Monterey, California. He has an MA in Political Studies from the University of Auckland, New Zealand.

Abstract: Violent Non-State Actor use of Food as a Delivery System: Comparing Ideological and Non-Ideological Perpetrators

Ideological and non-ideological perpetrators of violence utilizing chemical or biological agents demonstrate distinctly different patterns in terms of agent choice and mode of use when employing food products or systems as a delivery system. These differences have implications in terms of the threat likelihood, perpetrator characteristics and detectability, targeting choices, and potential for harm.

This presentation will touch on all of the above points to illustrate the need for defensive efforts to be set up in such a fashion that they can accommodate, and respond to, two very different patterns of attack against the U.S. food system.

Product Availability: A Drug Shortage Perspective



Hyun J. Son, PharmD
FDA, Center for Drug Evaluation and Research

Hyun Son has been with FDA since 2005. She started as a Regulatory Project Manager in the Division of Special Pathogen and Transplant Products in 2005 before becoming the Safety Regulatory Project Manager in 2009 when the division re-organized as the Division of Transplant and Ophthalmology Products. In 2013, she joined the CDER Drug Shortage Staff, working to coordinate and prevent nation-wide shortages of medically necessary products. She graduated from University of Maryland with a BS in Chemistry in 1998 and graduated from Howard University School of Pharmacy with a PharmD in 2003.

Abstract: Product Availability: A Drug Shortage Perspective

CDER Drug Shortage Staff supports FDA's mission of ensuring that safe and effective drugs are available to patients. DSS oversee and facilitate the resolution of all drug shortage situations. DSS facilitates temporary and long-term strategies to address shortages and coordinate for timely and comprehensive risk/benefit decisions. DSS also distributes information on our drug shortage web postings. Patient and practitioner access to life-saving medication is our #1 priority.

Bio-Terrorism Regulations and Food Security



Desmond Brown, MS
FDA, Office of Regulatory Affairs

Desmond Brown joined the FDA/Division of Food Defense Targeting ten years ago. He started his career with FDA as a Consumer Safety Officer/Reviewer and after three years he was promoted to a Supervisory Consumer Safety Officer. His responsibilities include reviewing data of high-risk imported food shipments for possible links to Bioterrorism to minimize the risk of serious illnesses or death from intentionally contaminated food shipments that enter the U.S. commerce. He also investigates firms and entities that violate the Federal Food, Drug, and Cosmetic Act under the prior notice regulations [section 801(m)] and food facility registrations [801(l)].

Before joining FDA, Desmond Brown worked with United States Department of Agriculture (USDA) for eight years. Some of his duties included, investigating and disqualifying grocery store owners who were exchanging food stamp benefits for cash from participating into the food stamp program. He also inspected imported agriculture and animal products to prevent the introduction and spread of harmful foreign pests into the United States.

Originally from Jamaica, Desmond Brown came to the United States in 1991. He graduated from the University of Illinois in 1999 with an MS in Horticulture and from Cornell University 1997 with a B.S. in Plant Science.

When he is not working to protect public health, he enjoys spending his time watching soccer, reading, or preparing for his upcoming Toastmasters meetings.

Abstract: Bio-Terrorism Regulations and Food Security

Identifying imported foods that may be intentionally contaminated with biological or chemical agents continues to be of significant concern to the Food and Drug Administration (FDA). To help mitigate the risk of harmful food products entering the United States, the Division of Food Defense Targeting (DFDT) uses an advanced risk-based targeting Matrix to screen imported food shipments for security and safety.

Each year, thousands of food shipments enter the United States. Any incident involving the use of hazardous agents to attack the U.S. food supply could inflict significant economic damage, instill fear in consumers, and lower confidence in U.S. food safety.

The Bioterrorism Act of 2002 requires the FDA to receive prior notifications of all imported food shipments before they arrive in the U.S. Among other things, the Act requires food facilities engaged in manufacturing, packing, or holding food for consumption in the U.S. to register with FDA.

Using risk-based screening criteria; the DFDT targets firms, countries, products, and other Prior Notice (PN) data elements to identify for manual review -- imported food shipments that may pose the highest risk for terrorism or other threats. Matrix criteria are dynamic and can be easily changed to fit the current environment allowing DFDT to consistently target the highest risk food shipments at any given time.

During FY 2018, the FDA screened approximately 15 million PNs against high-risk criteria. More than 81,000 high-risk PNs were manually reviewed by DFDT to investigate any association with terrorism or other threats. During this same period, the DFDT refused more than 600 imported food shipments for PN data requirement violations.

As part of FDA's wider food safety program, the DFDT screens and vets PN data of high-risk imported food shipments for signs of intentional adulteration by terrorists or other threats.

FDA Food Defense Efforts – A Preventive Approach to Food Terrorism



Ryan Newkirk, PhD, MPH

**Senior Advisor for Intentional Adulteration
FDA, Center for Food Safety and Applied Nutrition**

Dr. Newkirk is the Senior Advisor for Intentional Adulteration at FDA's Center for Food Safety and Applied Nutrition. He led the Food Safety Modernization Act Intentional Adulteration rule writing workgroup and is a co-lead for the IA rule guidance writing workgroup. Before joining FDA, Dr. Newkirk held a post-doctorate position with the U.S. Department of Agriculture, Food Safety and Inspection Service. He completed his doctorate in epidemiology and food defense research at the Food Protection and Defense Institute at the University of Minnesota School of Public Health and completed a master's degree in infectious disease epidemiology at Saint Louis University School of Public Health.

Abstract: FDA Food Defense Efforts – A Preventive Approach to Food Terrorism

Intentional adulteration of the food supply, including acts of terrorism, may result in wide scale public health harm, significant economic damage, disruption of trade, and loss of confidence in government. While such an event is unlikely, the results can be catastrophic. FDA, along with the food industry, academia, and other government partners have been working to protect the food supply from intentional adulteration. One of the major activities in this area has been the finalization of the final rule "Mitigation Strategies to Protect Food Against Intentional Adulteration" stemming from the Food Safety Modernization Act. This presentation will include a brief background of FDA's food defense efforts and requirements of the final rule.

CBER-Regulated Products: Preventing and Mitigating Shortages

Anita Richardson, MAS

FDA, Center for Drug Evaluation and Research

Anita Richardson serves as the Associate Director for Policy in CBER's Office of Compliance and Biologics Quality, where she leads a policy team that is responsible for policy development and review, the program for CBER-regulated product shortages, and informatics and import monitoring. Before leading the policy team, Ms. Richardson spent three years as the Director of the Compliance Branch in the FDA's Baltimore District Office, and ten years as a compliance officer in CBER. Prior to joining FDA, Ms. Richardson worked in the blood banking industry for eight years.

Ms. Richardson holds a Bachelor of Science Degree in Medical Technology from Indiana University of Pennsylvania and a Master of Administrative Science Degree from the Johns Hopkins University.

Abstract: CBER-Regulated Products: Preventing and Mitigating Shortages

This presentation provides an overview of the Center for Biologics Evaluation and Research's (CBER) program for managing product shortages. The statutory and regulatory requirements for notification of a permanent discontinuance or an interruption in manufacturing are reviewed, as are the processes and procedures for handling such shortage notifications. The presentation also covers the number of CBER shortages, the most common causes of shortages, and the tools used by CBER for preventing or mitigating shortages, where possible. CBER's engagement in the agency's Drug Shortage Task Force, and other agency-wide efforts to manage shortages is discussed.

On the ‘Cyber-Securability’ of Medical Devices



Eugene Vasserman, PhD
Kansas State University

Eugene Vasserman is a subject matter expert in cybersecurity and computer networking. He came to FDA’s OSEL in 2016 as an ORISE fellow. In 2018, he returned as a Senior Staff Fellow to CDRH. He serves as a cybersecurity specialist consultant for device and software reviews and is a member of the CyberSecurity Working Group (CSWG), helping with cybersecurity incident response. He served on the St. Jude Medical Cybersecurity Response Team, which received the Commissioner’s Special Citation for their work.

In his spare time, Eugene is an Associate Professor in the Department of Computer Science at Kansas State University and is the director of the university-wide Center for Information and Systems Assurance. His research has resulted in over 40 peer-reviewed publications in computer science, psychology, and education. His public service history includes over 30 conference program committees and national and international standards committees.

Eugene received a BS in Biochemistry and Neuroscience (with a Computer Science minor) from the University of Minnesota in 2003. His MS and PhD in Computer Science are also from the University of Minnesota, in 2008 and 2010, respectively. His current research is chiefly in various aspects of security for medical and other cyber-physical systems, security usability, and user education. His past work spans the gamut from security vulnerabilities emergent from the BGP infrastructure of the internet, to energy depletion attacks in low-power systems, to secure hyper-local social networking, to privacy and censorship resistance on a global scale, supporting billions of concurrent users.

Abstract: On The ‘Cyber-Securability’ of Medical Devices

The cybersecurity aspects of medical device safety have been attracting attackers, and therefore gaining increased attention from multiple stakeholders. However, security features implemented by the device manufacturer tell only part of the story. Device security in the context of deployment in the intended usage contexts is a more appropriate rubric. We name this property “securability”, and argue that it is strictly stronger than security in the context of protecting devices and systems from attack. In this paper, we present our opinion of the utility of the concept of securability, and how it can be used to accelerate and simplify the tasks of multiple stakeholders working to improve security throughout the healthcare sector.

Speakers Bios and Abstracts

Concurrent Session 4: Predictive Tools

Developing Digital Measures from Person-Generated Health Data



Luca Foschini, PhD

Co-founder & Chief Data Scientist, Evidation Health

Luca Foschini is the Co-founder and Chief Data Scientist at Evidation Health, responsible for data analytics and research and development. At Evidation he has driven research collaborations resulting in numerous publications in the fields of machine learning, behavioral economics, and medical informatics. Previously, Luca held research positions in industry and academic institutions, including Ask.com, Google, ETH Zurich, and UC Santa Barbara. He has co-authored several papers and patents on efficient algorithms for partitioning and detecting anomalies in massive networks. Luca holds MS and PhD degrees in Computer Science from UC Santa Barbara, and ME and BE degrees from the Sant'Anna School of Pisa, Italy.

Abstract: Digital Biomarkers Discovery from Patient-Generated Health Data

Applications of artificial intelligence (AI) to healthcare and medicine are becoming widespread, both within traditional clinics and hospitals, and outside of those venues integrated into patient's everyday lives. By the end of 2019, tens of millions of Americans will have Apple Watches with AI-powered arrhythmia detection on their wrists, one of several examples of applications cleared by the FDA in the last year that relies heavily on machine learning.

This revolution is being enabled by a new kind of data becoming more prominent in medical research: patient-generated health data from wearable sensors, smartphone apps, and other IoT devices. Unlike traditional data sources, these data are high-frequency, continuously collected, and controlled by the individuals who generate them.

MRI In Nonclinical Safety Assessment

Serguei Liachenko MD, PhD

FDA, National Center for Toxicological Research

Serguei Liachenko, MD/PhD has graduated from Russian State Medical University in 1988 and has worked in the area of non-clinical bio-imaging in pharmaceutical industry and government settings since completing his postdoctoral training in this field at the University of Pittsburgh in 2002, where he was partially supported by individual National Research Service Award from NIH. He has successfully merged the bio-medical, and physics sciences in the process of developing of efficacy and toxicity imaging biomarkers in the areas of neuroscience, inflammation, hepatology, cardiovascular medicine, and other areas to support drug discovery and development at Pfizer, Inc. (2002-2009). He is currently the Director of Bio-imaging at National Center for Toxicological Research focusing on the development of bio-imaging approaches to support toxicity and drug safety research.

Abstract: MRI in Nonclinical Safety Assessment

Preclinical neurotoxicity assessment in drug development is typically accomplished using microscopic analysis, which can be time consuming and not always comprehensive. Such an approach can result in false-negative findings, meaning that small localized lesions are missed. Non-invasive MRI can provide unique information about the structure and function of the whole brain in vivo with sufficiently high resolution and contrast, and serve as the basis of potential translatable neurotoxicity biomarkers. A battery of known neurotoxicants with different mechanisms of action and pathology extent and localization were employed to test the ability of quantitative T2 MRI mapping to noninvasively assess neurotoxicity in rats. The extent and the intensity of the T2 changes in response to neurotoxicity in these rat models was compared to the classical histopathology readout to estimate the performance (sensitivity and specificity) of T2 mapping as a potential preclinical biomarker of neurotoxicity. Such imaging could be used as a supplement to guide current standard histopathology evaluations and has the potential to significantly modernize current approaches in neurotoxicity assessments in different areas, including drug discovery and development.

The VICTRE Project: The First All-In-Silico Imaging Clinical Trial



Aldo Badano, PhD

FDA, Center for Devices & Radiological Health

Aldo Badano holds a Senior Biomedical Researcher Service appointment at FDA and currently serves as Deputy Director of the Division of Imaging, Diagnostics, and Software Reliability, OSEL/CDRH. He received an MEng in Radiological Health Engineering and a PhD in Nuclear Engineering from the University of Michigan in 1999 and 1995 after obtaining a Chemical Engineering from the Universidad de la República, Montevideo, Uruguay in 1992. His primary interests are in the characterization and modeling of medical imaging acquisition and visualization systems. Aldo has published over 300 publications (70 in the last 5 years) cited 2,800 times, citations including a tutorial book on medical displays. In addition, he leads international consensus development efforts through standards activities. Aldo has provided significant training to young regulatory scientists and has successfully directed several doctoral theses. He received CDRH's Excellence in Supervisory (2018) and Mentoring Award (2013), and FDA's Excellence in Laboratory Science Award (2003).

Abstract: *The VICTRE project: the first all-in-silico imaging clinical trial*

Can in silico imaging trials play a role in the evaluation of new medical imaging systems? The VICTRE trial used computer-simulated imaging of in silico patients to compare digital mammography (DM) and digital breast tomosynthesis (DBT) and found an improved lesion detection performance favoring tomosynthesis for all breast sizes and lesion types. The simulated trial was designed to replicate a clinical trial that used human patients and radiologists. Images obtained with in silico versions of DM and DBT systems via fast Monte Carlo x-ray transport were interpreted by a computational reader detecting the presence of lesions. A total of 2986 synthetic virtual patients with breast sizes and radiographic densities representative of a screening population and compressed thicknesses from 3.5 to 6 cm were generated using an analytic approach in which anatomical structures are randomly created within a predefined breast volume and compressed in the craniocaudal orientation. A positive cohort contained a digitally inserted microcalcification cluster or spiculated mass.

The results of the VICTRE trial are consistent with the performance seen in the comparative trial. The increased performance for tomosynthesis was consistent with results from a comparative trial using human patients and radiologists. The study's findings suggest that in silico imaging trials and imaging system computer simulation tools can in some cases be considered viable sources of evidence for the regulatory evaluation of imaging devices. We provide evidence that state-of-the-art computational methods coupled with laboratory testing can lead to less burdensome regulatory evaluation approaches. While further research is needed to assess the generalizability of these findings, in silico imaging trials represent a viable source of regulatory evidence for imaging devices.

Use of The MHC Associated Peptide Proteomic Assay to Understand the Immunogenicity Risk of Therapeutic Proteins



Zuben Sauna, PhD

FDA, Center for Biologics Evaluation & Research

Zuben E. Sauna is a Principal Investigator and a CMC Reviewer at FDA. His research interests lie in understanding the pharmacogenetic basis of the immune response to proteins used in therapeutic interventions as these affect efficacy and safety. His laboratory uses a combination of computational, in vitro and ex vivo approaches to understand why some individuals and/or sub-populations develop immune responses while others do not. Work from his laboratory has been published in high impact journals such as Nature Biotechnology, Nature Medicine, Science, Science Translational Medicine and Nature Reviews Genetics. He received his PhD from Poona University, India with subsequent training at the National Cancer Institute, Bethesda, Maryland.

Abstract: Use of the MHC Associated Peptide Proteomic Assay to Understand the Immunogenicity Risk of Therapeutic Proteins

The immune response to protein-therapeutics (immunogenicity) is an important safety and efficacy concern during drug development and regulation. Non-clinical assays that can be used in the early stages of clinical development and to identify at-risk individuals and sub-populations in the clinic are an important unmet need. The so-called MHC Associated Peptide Proteomic (MAPPs) assay directly identifies peptides derived from a protein of interest on a donor's MHC-II proteins. Here we have applied this technique to address several questions related to the use Factor VIII (FVIII) replacement therapy, in the treatment of hemophilia A. Over a dozen FVIII products are marketed but most fall into 3 categories. Our findings show that differences in the clinical immunogenicity of these products are consistent with the differences in the FVIII peptides found on the MHC-II proteins.

Cardiac and Hepatic Cellular Systems to Model Human Drug Effects



Alexandre Ribeiro, PhD

FDA, Center for Drug Evaluation & Research

Alexandre Ribeiro is a researcher in FDA's Division of Applied Regulatory Science since January 2017 and he supervises the FDA Integrated Cellular Systems Laboratory, where research is developed on evaluating and using physiological and human in vitro cellular systems for drug development. Alexandre Ribeiro received a PhD degree in Biomedical Engineering from Carnegie Mellon University in 2010, where he researched the mechanics of the nucleus of adult stem cells and cancer cells. He then became a postdoctoral fellow in the Stanford Microsystems Laboratory at Stanford University – Pruitt Lab – to develop microfabricated devices that engineer biological properties of mammalian cells and analyze cell mechanobiology. Dr. Ribeiro later joined the Srivastava Lab at the Gladstone Institute of Cardiovascular Disease in 2013 to study stem cell-derived heart muscle cells (cardiomyocytes) with engineered physiological microsystems and he leveraged novel tools to mature and functionally analyze stem cell derived cardiomyocytes. At FDA, Dr. Ribeiro has been continuing his research in stem cell derived cardiomyocytes and is also evaluating microphysiological systems as drug development tools.

Abstract: Cardiac and Hepatic Cellular Systems to Model Human Drug Effects

Mammalian cells have the potential to model human physiology in vitro and can provide mechanistic insight on the clinical effects of drugs. For this purpose, function of cellular systems must be physiologically relevant and functional measurements should translate to clinical data on drug effects. The cellular microenvironment is key in regulating the function of cellular systems and bioengineered devices have been developed in the last decade to improve the physiology of cells in culture. However, the regulatory use of cellular systems with enhanced physiology requires robustness in their use and reproducibility of their results to reliably investigate the predictability of clinical drug studies. Here, research on tuning the microenvironment of stem cell-differentiated heart muscle cells (cardiomyocytes) and on evaluating liver microphysiological systems will be presented to demonstrate how engineering the microenvironment can improve the use of cellular systems in the regulatory field. Heart and liver are the main targets of drug adverse effects and the field of drug development lacks cellular models that can better predict toxicity in these organs. By engineering the microenvironment of cardiomyocytes or hepatic cells to match tissue-specific conditions, we observed an improvement of cellular function. When using the same cells, different systems operate robustly and seem to have a reproducible response to drugs. However, lack of standardized quality control assays for cells to be used in engineered systems is the main roadblock in the field and should be the object of future research.

C. elegans for Rapid Developmental Neurotoxicity Assessment of Mixtures



Piper Hunt, PhD

FDA, Center for Food Safety and Applied Nutrition

Dr. Hunt develops alternative toxicity models and assays with the potential to reduce toxicity testing on vertebrates while still providing information of equivalent or better scientific quality and relevance that will support regulatory decision making. She holds a PhD from the Johns Hopkins doctoral program in Human Genetics and Molecular Biology. Her post-doctoral work utilized *C. elegans* to assess the effects of nutraceuticals on conserved adaptive cellular response pathways and health span. She joined FDA in 2011 to develop and evaluate new approaches that use alternative toxicity testing models, including *C. elegans* as well as suspended and 3D human cell cultures, and has expertise in using these models to assess metal toxicity, nanoparticle toxicity, dermal sensitizers, and carcinogens. Her semi-automated method for *C. elegans* LD50 assessment replicated rat LD50 ranking for studied compounds. Dr. Hunt recently developed a promising novel assay for developmental neurotoxicity (DNT) assessment and is heading a blinded-chemical study in collaboration with NTP/NICEATM to validate this assay for use in predicting mammalian DNT response.

Abstract: Caenorhabditis elegans for Rapid Developmental Neurotoxicity Assessment of Mixtures

New toxicological tools that better predict human response with reduced time and expense ratios will allow increased evaluation of individual compounds of concern as well as increased toxicity testing of mixtures. The 2016 update to the Toxic Substances Control Act mandates that U.S. Federal agencies develop leading edge predictive toxicology methods that can replace the use of vertebrate models. The U.S. Food and Drug Administration's Predictive Toxicology Roadmap supports the integration of emerging methods and new technologies into regulatory safety and risk assessments. Many pathways involved in organismal development, neuronal function, and cellular metabolism are conserved from worms to people. The digestive tract of the *Caenorhabditis elegans* nematode has several features that are analogous to the mammalian digestive system, making this simple model organism a potential candidate for predictive oral toxicity testing. *C. elegans* studies have demonstrated concordance for developmental toxicity or altered motor activity when exposed to mammalian developmental toxins or neurotoxins. We have designed a novel worm Development and Activity Test (wDAT) that maps the timing of *C. elegans* developmental milestone acquisition as well as stage-specific activity levels. The wDAT was able to detect both developmental delay and hyperactivity for arsenic, lead, and mercury, developmental neurotoxins that have been associated with hyperactivity in children. Binary mixtures of arsenic, lead, and mercury produced at most additive effects on developmental delay and/or hyperactivity; no synergistic effects were detected. The wDAT can be completed by a single technician in 4 days using a relatively inexpensive activity tracker, features that would make it a cost-effective addition to integrated approaches to toxicity testing. A planned 20-compound, blinded qualification study will help determine how the wDAT might provide "fit-for-use" data to support developmental neurotoxicity testing strategies.

Determination of Seafood Decomposition by Mass Spectrometry with Sensory-Driven Modeling



Randy L. Self, PhD
FDA, Office of Regulatory Affairs

Randy Self received a BS in chemistry from Western Washington University, with research work in hydrodesulfurization catalysis. In 2001, he joined FDA at the Office of Regulatory Affairs, Pacific Northwest Laboratory (ORA-PNL). On the regulatory side at PNL, he was involved in a wide range of programs, including mycotoxins, chemotherapeutics, food safety, pesticides, and drug chemistry, as well as pharmaceutical inspection activities. In 2010, Randy joined the research arm of the laboratory with the Applied Technology Center (ATC). At ATC, he conducts research on method development for a wide scope of food safety applications. His work focuses primarily on development of novel high-resolution mass spectrometry (HRMS) techniques. Some previous projects have included biogenic amines in seafood, pesticide analysis in fruits and vegetables, and work with the Direct Analysis in Real Time (DART) technique to screen for phthalates and glycols. Currently, Randy is developing techniques to determine decomposition in seafood using mass spectrometry with sensory-driven predictive modeling.

Abstract: Determination of Seafood Decomposition by Mass Spectrometry with Sensory-Driven Modeling

United States Food and Drug Administration (USFDA) testing laboratories currently perform sensory analysis to determine the decomposition status of seafood samples and ensure product safety. Sensory evaluation is an accurate, specific, and effective analytical tool; however, the availability of qualified personnel and extensive training requirements can be a critical limiting factor to the program. Furthermore, other methods currently employed to compliment this work, for example histamine or indole analysis, have limited utility and do not always offer results which are directly comparable to sensory results. Currently, studies are underway to explore two novel alternative techniques to address this issue.

First, a method was developed using liquid-chromatography with high-resolution mass spectrometry (LC-HRMS) with widely-focused sample extraction and instrumental conditions, and an untargeted data-processing approach. The second technique under study employs a compact mass spectrometer (CMS) equipped with vapor-phase atmospheric pressure chemical ionization (vAPCI). Using this apparatus, samples were tested directly via headspace analysis.

Samples of a wide variety of seafood products were subjected to varying degrees of controlled decomposition. These were then evaluated and scored by a USFDA National Seafood Sensory Expert (NSSE), immediately before processing in parallel via each of the study methods. For each method, mass spectrometry data were used in conjunction with sensory scores to create sensory-driven statistical models in the R programming

language. These models were then used to calculate sensory-like decomposition scores for each sample.

The accuracy of each technique was evaluated via comparison to sensory data, and false positive or negative rates were determined. Reproducibility was demonstrated via triplicate processing and analysis on separate days. Each of these evaluations shows promising results for both methods.

Speakers Bios and Abstracts

Concurrent Session 5: Advancing Digital Health and Artificial Intelligence

Assessment of Devices that Rely on Artificial Intelligence/ Machine Learning



Qi Liu, PhD

FDA, Center for drug Evaluation & Research

Dr. Qi Liu is a team leader in the Office of Clinical Pharmacology (OCP), CDER, FDA. During her 12-year career at the FDA, Qi contributed to over 200 NDA/sNDA reviews, 20 BLA/sBLA reviews, and numerous IND reviews to support oncology drug development. She co-authored about 30 manuscripts and presented on many topics at FDA Advisory Committee meetings and national conferences. She worked on several working groups for FDA guidance documents and Manual of Policies & Procedures (MAPP) development. She is the vice chair of the OCP Biologics Oversight Board. Qi is interested in the application of clinical pharmacology principles, innovative tools (e.g., modeling/simulation, machine learning), big data and real world evidence to facilitate drug development and advance precision medicine.

Before joining FDA, Qi was a senior pharmacokineticist at Merck & Co. Inc. She obtained her Ph.D. degree in Pharmaceutics (focus on Pharmacokinetics/Pharmacodynamics Modeling and Simulation) and a concurrent Master's degree in Statistics from the University of Florida. In addition, Qi has a Master's degree in Pharmaceutics (focus on bioanalysis) and a Bachelors' degree in Clinical Pharmacy from West China University of Medical Sciences.

Deep Learning for Polypharmacy and Drug Repurposing



Marinka Zitnik, PhD
Stanford University

Marinka Zitnik (<http://stanford.edu/~marinka>) is a postdoctoral research scholar in Computer Science at Stanford University. In the Winter of 2019, she will join Harvard University as a tenure-track assistant professor in Artificial Intelligence for Medicine. Her machine-learning methods have had a tangible impact in biology, genomics, and medicine, and are used by major biomedical institutions, including Baylor College of Medicine, Karolinska Institute, Stanford Medical School, and Massachusetts General Hospital. She received her PhD in Computer Science from University of Ljubljana while also researching at Imperial College London, University of Toronto, Baylor College of Medicine, and Stanford University. Her work received several best paper, poster, and research awards from the International Society for Computational Biology. She was named a Rising Star in EECS by MIT and also a Next Generation in Biomedicine by The Broad Institute of Harvard and MIT, being the only young scientist who received such recognition in both EECS and Biomedicine. She is also a member of the Chan Zuckerberg Biohub at Stanford.

Abstract: Deep Learning for Polypharmacy and Drug Repurposing

Large datasets are being generated that can transform biology and medicine. Artificial intelligence and machine learning methods are necessary to unlock these data and open doors for scientific discoveries.

In this talk, I will start by describing a new methodology for large-scale predictive modeling of polypharmacy. Polypharmacy, the use of drug combinations, is common to treat patients with complex or co-existing diseases. However, a major consequence of polypharmacy is a high risk of adverse side effects, which emerge because of drug-drug interactions, in which activity of one drug changes if taken with another drug. Furthermore, polypharmacy is recognized as an increasingly serious problem in the health care system affecting nearly 15% of the U.S. population and costing more than \$177 billion a year in the U.S. alone in treating side effects. To tackle this challenge, we have captured molecular, drug, and patient data for all drugs prescribed in the U.S. We then developed deep learning methods that have allowed us to, for the first time, predict safety and side effects of any drug combination. I will show how we can validate predictions about polypharmacy in the clinic using real patient data.

These new graph neural networks move beyond prevailing deep learning methods and set sights on new frontiers in sciences. In the second part of the talk, I will discuss how the new methods have enabled us to predict what diseases a new drug could treat and have given us insights into mechanisms of drugs' therapeutic effects. The methods operationalize insights that diseases are not independent of each other, and that the effects of drugs are not limited to proteins to which they directly bind in the body; instead, these effects spread throughout biological networks in which they act. I will show how the new methods make correct predictions for a large number of recently



repurposed drugs, and can operate even on the hardest, yet extremely important, cases when a drug has no indicated disease or when a disease does not yet have any drug treatment. In all studies, I collaborated closely with experimental biologists and clinical scientists to give insights and validate predictions made by our methods.

FDA's Real-World Evidence Program – Technology and Innovation as a Cornerstone

Jacqueline Corrigan-Curay, J.D., MD
FDA, Center for Drug Evaluation & Research

Jacqueline Corrigan-Curay, J.D., M.D., serves as Director of CDER's Office of Medical Policy (OMP). She leads the development, coordination, and implementation of medical policy programs and strategic initiatives. She works collaboratively with other CDER program areas, FDA centers, and stakeholders on enhancing policies to improve drug development and regulatory review processes.

OMP comprises the Office of Prescription Drug Promotion (OPDP) and the Office of Medical Policy Initiatives (OMPI). OPDP oversees the regulation of prescription drug promotion and advertising. OMPI provides oversight and direction for new and ongoing policy initiatives in broad-based medical and clinical policy areas.

Before joining FDA, she served as supervisory medical officer with the Immediate Office of the Director, National Heart, Lung and Blood Institute (NHLBI), at National Institute of Health's (NIH) where she focused on developing policies and procedures to enhance the clinical trial enterprise. She also served as the Director of the Office of Biotechnology Activities (OBA), Office of Science Policy at NIH, where she was executive secretary of the NIH Recombinant DNA Advisory Committee. She has held positions as an attending physician with the VA Medical Center, a policy analyst with the Congressional Office of Technology Assessment, and a practicing attorney in Washington, D.C.

Dr. Corrigan-Curay earned her law degree from Harvard Law School, her medical degree from University of Maryland School of Medicine, and a bachelor's degree in history of science from Harvard/Radcliffe College in Cambridge, MA. She completed her training in internal medicine at Georgetown University Medical Center, where she also served as a clinical assistant professor of medicine. She continues to practice internal medicine part-time at the Veterans Affairs Medical Center in Washington, D.C.

Abstract: FDA's Real-World Evidence Program – Technology and Innovation as a Cornerstone

The 21st Century Cures Act (Cures Act), is intended to facilitate the development of medical products and to leverage the latest technological innovations to maximize the utility of available real-world data (RWD). The ultimate goal is to bring therapeutics to patients who need them in faster and more efficient ways. The FDA created a framework for evaluating the potential use of real-world evidence (RWE) to help support the approval of a new indication for a drug already approved under section 505(c) of the FD&C Act or to help support or satisfy drug postapproval study requirements. CDER view this mandate as a multi-faceted effort that will require the convergence of technologies, tools and expertise. For example, electronic health records (EHRs) are considered a major source of RWD, yet they typically lack standardization and data input can vary. Technological innovations, such as machine learning and natural

language processing could be effective tools to help organize and make sense of these data. Another example that will be addressed in this talk is that the potential utility of RWD will be explored across different types of experimental study designs, such as traditional randomized clinical trials (RCTs), studies incorporated in health-care delivery settings, and observational studies. Each design has strengths and weaknesses that technology will be key to help us understand. Another major source of RWD can be digital tools, such as sensors and mobile trackers that are increasingly used, characterizing these digital sources of data and the data generated from them to determine what is fit-for use, is a challenge that technological innovations will be essential in addressing.

Assessment of Devices that Rely on Artificial Intelligence/ Machine Learning



Berkman Sahiner, PhD

FDA, Center for Devices & Radiological Health

Berkman Sahiner is an Electrical Engineer with the Office of Science and Engineering Laboratories (OSEL) at CDRH. He has a PhD in electrical engineering and computer science from the University of Michigan, Ann Arbor. Before joining FDA, he was an Associate Professor with the Department of Radiology at the University of Michigan. At the Division of Imaging, Diagnostics and Software Reliability at CDRH/OSEL, he performs research related to the evaluation of medical imaging and computer-assisted diagnosis devices, including devices that incorporate machine learning and artificial intelligence. He has authored/co-authored over 120 peer-reviewed journal publications. His interests include machine learning, computer-aided diagnosis, image perception, clinical study design, and performance assessment methodologies.

Abstract: Assessment of Devices that Rely on Artificial Intelligence / Machine Learning

Design and utilization of systems that rely on artificial intelligence (AI) and machine learning (ML) in medical applications go back multiple decades. Earlier approaches that used information-rich sources such as medical images or physiological measurement data relied heavily on feature engineering. With the introduction of techniques that can automatically learn useful feature representations directly from the data, the need for handcrafted features has been largely reduced or eliminated, resulting in the proliferation of AI/ML-based systems considered as software as a medical device (SaMD). After providing a background on AI/ML as SaMD, I will first present some example devices that were recently approved/cleared/granted by CDRH. I will discuss some of the current principles in the evaluation of these devices, many of which are in CDRH's guidance on computer-assisted detection devices applied to radiology images. I will then discuss some themes and challenges that are common to these application areas, including training/test datasets and curation, robustness and generalization, limited data set size, explainability/interpretability, and continuous learning. Among these challenges, I will particularly focus on continuous learning. I will discuss some of the ideas from a discussion paper recently released by CDRH on a proposed regulatory framework for modifications to AI/ML based SaMD. I will then present examples of work from our laboratory at the Office of Science and Engineering Laboratories at CDRH which explore some of the themes discussed above. I will conclude by summarizing the potential impact of AI in medical devices, as well as some of the areas that need careful consideration so that results of studies involving AI can be correctly interpreted.

AI at FDA: Potential Utility and Regulatory Challenges

Richard Forshee, PhD

FDA, Center for Biologics Evaluation & Research

Richard Forshee leads the Analytics and Benefit-Risk Assessment Team for the Office of Biostatistics and Epidemiology in the Center for Biologics Evaluation and Research at the U.S. Food and Drug Administration. He works on a wide range of issues related to the risks and benefits of blood and blood products, vaccines, and human cell and tissue products. Dr. Forshee has won numerous awards including the FDA Service Award and the CBER Hope Hopps Memorial Award, and he has published more than 60 scientific articles. Before joining FDA, he was the Director of the Center for Food, Nutrition, and Agriculture Policy at the University of Maryland, College Park.

Yaning Wang, Ph.D.

FDA, Center for Drug Evaluation & Research

Yaning Wang is the Director of the Division of Pharmacometrics in the Office of Clinical Pharmacology at FDA. Before joining FDA, Dr. Wang received his PhD in Pharmaceutics and master's degree in Statistics from the University of Florida from 1999 to 2003. He also obtained a master's degree in Biochemistry (1999) from National Doping Control Center and a bachelor's degree in Pharmacy (1996) from Peking University in China. Dr. Wang oversees reviews, research projects, and policy development within the Division of Pharmacometrics for all disease areas. During his 16 years of service at FDA, Dr. Wang received numerous awards, including Award of Merit and FDA Outstanding Service Award. Dr. Wang served as a committee member for multiple PhD candidates from various universities. He mentored more than 50 former research fellows (visiting scholars, post-doctoral scholars, and PhD candidates) at FDA. Dr. Wang has published over 70 papers and given over 190 presentations at various national and international meetings. He served as a board member of the International Society of Pharmacometrics. He is a member of the Advisory Committee for Chinese Pharmacometrics Society and a member of the Editorial Advisory Board for the Journal of Pharmacokinetics and Pharmacodynamics.

Errol Strain, Ph.D.

FDA, Center for Veterinary Medicine

In 2019, Dr. Errol Strain joined the Division of Animal and Food Microbiology in the Office of Research at FDA's Center for Veterinary Medicine, where he is responsible for bioinformatic analysis of drug-resistant pathogens for the National Antimicrobial Resistance Monitoring System (NARMS). From 2010 to 2018 he was the Supervisor for the Biostatistics and Bioinformatics Staff in the Office of Analytics and Outreach at the Center for Food Safety and Applied Nutrition where he helped to create bioinformatics pipelines and workflows for the GenomeTrakr program. He received his Bachelor of Science degree in Biochemistry from Purdue University in 1998 and his PhD in Bioinformatics from North Carolina State University in 2006. Dr. Strain's research at FDA has focused on the application of genomic methods for surveillance and prediction of antimicrobial resistance in foodborne bacterial pathogens.

Rhonda Moore, Ph.D.**FDA, Center for Drug Evaluation & Drug Research**

Rhonda Moore is a medical anthropologist, data science ethnographer and social scientist who combines anthropological methods, computational social science, narrative and clinical medicine to understand the experience and meaning of the patient experience with implications for regulatory decision making. Her current work focuses on the integration of ethnographic methods and data science to understand consumer use behaviors and enhance the development of regulatory intelligence and the ethical implications of the Quantified self for AI for social good for vulnerable patients in the context of pain and palliative care. She has spoken at Marketing and Public Policy, Serious Play, Games for Change, American Association for Clinical Oncology, International Association for the Study of Pain and several other large conferences. She is also editor of the following: Handbook of Pain and Palliative Care (Springer, 2012), Biobehavioral Approaches to Pain (Springer 2009) and Cancer Culture and Communication (Springer 2004).

Joshua Xu, Ph.D.**FDA, National Center for Toxicological Research**

Dr. Xu is currently the Branch Chief for Research-to-Review (R2R) at the Division of Bioinformatics and Biostatistics of NCTR. He has 20 years' experience developing bioinformatics software and systems and conducting research in genomics and image analysis. He specializes in data mining, image analysis, and machine learning. At NCTR, he has led several system development projects including SNPTrack, which is an integrated solution for managing, analyzing, and interpreting genetic association study data. His recent endeavor has been with the FDA-led Sequencing Quality Control Phase 2 (SEQC2) project to evaluate the technical reliabilities and scientific applications of the next generation sequencing (NGS) technologies. He is leading a SEQC2 Working Group to assess the reproducibility and detection sensitivity of onco-panel sequencing including liquid biopsy. The Working Group consists of over 200 participants from academia, government agencies, and industry including 8 companies providing onco-panels and 30 testing laboratories. The project aims to provide recommendation in support for FDA's mission in regulatory oversight of NGS diagnostic tests. He also oversees two cross-center collaborative projects developing artificial intelligence to enhance ORA product inspection.

Panel Abstract: AI at FDA: Potential Utility and Regulatory Challenges

Recent advances in artificial intelligence (AI) have resulted in systems that approach and sometimes exceed human levels of performance across a variety of application areas. These advances show great promise in improving the performance of existing medical devices such as computer-aided diagnosis systems, all the way to novel application areas such as drug discovery or development of an autonomous "AI doctor" that continuously learns while deployed. The day-to-day business practices at the agency can similarly benefit from AI, for instance by using natural language processing to comb through thousands of existing files from sponsors or social media to find patterns of interest. This panel will discuss these applications of AI, as well as regulatory challenges in reviewing AI-related submissions.

Speakers Bios and Abstracts

Concurrent Session 6: Outbreak!

Innovation in Science: Protecting People from Emerging Infectious Disease Threats



Christopher R. Braden, MD

Centers for Disease Control & Prevention (CDC)
Deputy Director of the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)

Christopher Braden, MD, is the Deputy Director of the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID). A medical epidemiologist at CDC, Dr. Braden assumed this position in March 2016, and is part of the leadership team overseeing the center charged with the prevention and control of a broad spectrum of infectious diseases, including Ebola, Zika and anthrax, and more common conditions like foodborne diseases and healthcare-associated and antibiotic-resistant infections. In his new role, Dr. Braden often coordinates NCEZID response to large or cross cutting outbreaks.

Dr. Braden has previously served as the director of the Division of Foodborne, Waterborne, and Environmental Diseases, associate director for science in the Division of Parasitic Diseases, and chief of outbreak response and surveillance within the Enteric Diseases Epidemiology Branch, Division of Foodborne, Bacterial and Mycotic Diseases at the National Center for Zoonotic, Vector-Borne, and Enteric Diseases. Dr. Braden also served as a medical epidemiologist in CDC's Division of Tuberculosis Elimination. Dr. Braden rose to the rank of captain in the U.S. Public Health Service but retired from the Commissioned Corps in July 2016.

Abstract: Innovation in Science: Protecting People from Emerging Infectious Disease Threats

FDA and the Centers for Disease Control and Prevention (CDC) routinely collaborate on investigations and scientific projects important to the mission for both agencies for advancing public health. Advances in detection and molecular characterization of pathogens has improved identification and investigation of foodborne outbreaks, hospital-associated infections, antimicrobial resistant pathogens, and many other pathogen types. The two outbreaks of E. coli O157:H7 infections due to romaine lettuce in 2018 exemplified collaborative investigations including advances in environmental assessment and testing. CDC and FDA conduct surveillance and studies related to medical products and procedures to advance the safety and efficacy of vaccines, blood transfusions, and organ and other tissue transplants. Our agencies collaborate on advancing diagnostic and pharmacologic science by providing critical materials and information to scientists globally, including a wide range of antimicrobial resistant pathogens and materials from the AR Isolate Bank. These are a few of the many areas of scientific collaboration between FDA and CDC; the opportunities will continue to expand.

Foodborne Outbreak Investigations in the Whole Genome Sequencing Era

Jennifer Beal, MPH

FDA, Center for Food Safety & Applied Nutrition

Jennifer Beal is the Senior Epidemiologist in FDA/CFSA's Office for the Coordinated Outbreak Response and Evaluation (CORE) Network. Jennifer received her MPH in Epidemiology from George Washington University School of Public Health in 2008 and completed a Presidential Management Fellowship with FDA's Office of Regulatory Affairs (ORA) as an epidemiologist from 2008-2011. She's been a member of the CORE Signals and Surveillance team since CORE began in 2011. Before CORE, she participated in various FDA Incident Management Groups for large outbreak responses, including roles as Deputy Incident Coordinator, Deputy Planning Section Chief, and Adverse Events Lead. As an epidemiologist on the CORE Signals team and subsequently as the CORE senior epidemiologist, Jennifer has focused on the early detection of foodborne outbreaks related to FDA-regulated foods. Her specific areas of interest include developing novel investigate methods to identify the causes of foodborne outbreaks, and supporting interagency collaborative efforts to improve foodborne outbreak detection and response.

Abstract: Foodborne Outbreak Investigations in the Whole Genome Sequencing Era

Microbial contamination in FDA-regulated foods is responsible for thousands of illnesses and deaths in the United States every year. When the same contaminated food makes more than one person sick, the event is referred to as a foodborne illness outbreak. CFSAN's Office for the Coordinated Outbreak Response and Evaluation (CORE) Network, established in 2011, leads FDA's foodborne outbreak investigation activities. Foodborne outbreaks constitute both public health threats but also opportunities, because it is much easier to identify a food that is making people sick in an outbreak investigation than for a single case. Outbreak investigations are therefore critical to identifying which contaminated food is responsible for the illnesses, take appropriate steps to remove the food from commerce, and address deficiencies in the food production supply chain. In addition, lessons learned from outbreak investigations can be further utilized to inform policy-making geared towards preventing similar events in the future. As the next decade rapidly approaches, foodborne outbreak investigations stand at a pivotal moment, with the primary molecular subtyping tool for identifying outbreaks changing for the first time in 20 years. Nationwide, laboratories are phasing out Pulsed Field Gel Electrophoresis (PFGE) and replacing it with Whole Genome Sequencing (WGS), a much more sensitive and specific subtyping method. This transition presents both opportunities and challenges for foodborne outbreak investigations. Recent experience has demonstrated that the use of WGS for foodborne outbreak investigations leads to an increased number of outbreaks detected (often with smaller numbers of cases per outbreak), but has also resulted in a greater number of outbreaks being solved. This presentation will explore the impact that WGS has had on



foodborne outbreak investigations from an FDA-perspective, including summarizing the current state of the WGS transition, and concluding with projections for the future of foodborne outbreak investigations specifically and food safety in general.

Immune Responses to Zika Infections

Steven Wood, PhD

FDA. Center for Devices & Radiological Health

Dr. Wood has established a bio-defense research program that incorporates the broader scope of FDA's public health mission and bio-terrorism mandates. Specifically, he is interested in the rapid detection of Zika and Ebola and threat mitigation. He has a PhD from the University of Connecticut in Nutritional Biochemistry and received post-doctoral training at the Medical College of Virginia and New York University in immuno-toxicology and immunology, respectively. He joined CBER first and then moved to CDRH. Dr. Wood has been funded externally by Defense Advanced Research Projects Agency (DARPA), the Biodefense Advanced Research Agency (BARDA), and the National Institutes of Allergy and Infectious disease (NIAID).

Abstract: Immune Responses to Zika

Zika virus (ZV) is a mosquito transmitted disease responsible for an outbreak of congenital microcephaly manifested recently in Brazil and the Caribbean. Detection of antibodies to ZV is crucial for identifying infected patients. There are significant cross reactivity issues in the current testing with other Flaviviruses such as Dengue, West Nile and Chikungunya. Additionally, the time required to perform the testing is also an issue that takes several hours or days for the assays. The focus of this presentation is to review technologies that will enhance the specificity to detect and reduce the time for analysis; the recent outbreak of ZV has emphasized the need for rapid diagnostics. Significantly, sensitivity issues related to antibody cross reactivity issues with other Flaviviruses, which complicates the testing, will also be discussed. In the absence of an effective vaccine, Zika continues to be a significant issue for neurological complications following in utero exposure and the development of Guillain-Barré syndrome. Personnel protective equipment, PPE, can also play a role in ZV Infection control. In sum, recent studies have focused on the evaluation rapid test methods of ZV which has implications for diagnostics, vaccine development and PPE performance.

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Plain Language Summary: Zika virus (ZV) is a mosquito-borne infection that is responsible for microcephaly and Guillain Barre syndrome. The focus of this presentation is to review technologies that may enhance diagnostic performance. Personnel protective equipment can also play a role in ZV Infection control.

Tracking antibiotic resistance in Salmonella: The role of the National Antimicrobial Resistance Monitoring System



Patrick F. McDermott, MS, PhD

FDA. Center for Devices & Radiological Health

Dr. Patrick McDermott is Director of the Division of Animal and Food Microbiology and Director of the National Antimicrobial Resistance Monitoring System (NARMS) at the Center for Veterinary Medicine Office of Research. NARMS was established in 1996 and is an interagency collaborative effort between the FDA, USDA and CDC that tracks antibiotic resistance in bacteria from retail meats, food producing animals and human clinical cases of infection. He led studies to develop the first standardized antimicrobial susceptibility testing methods for *Campylobacter*, conducted work to show the impact of antimicrobials in animals, and coordinated the implementation of whole genome sequencing into NARMS national surveillance. His collaborative work aims to understand the mechanisms of antimicrobial resistance in foodborne microorganisms, how they emerge and spread, and the impact of interventions designed to limit resistance in food animal production. He is a fellow of the American Academy of Microbiology, and recipient of FDA's Francis Kelsey award for excellence and courage in protecting the public health.

Abstract: Tracking antibiotic resistance in Salmonella: The role of the National Antimicrobial Resistance Monitoring System

The National Antimicrobial Resistance Monitoring System (NARMS) is an inter-agency program of the FDA, USDA and CDC that tracks antibiotic resistance in foodborne bacteria from foods, animals and human clinical cases using harmonized methods. NARMS is moving towards a One Health model of resistance monitoring, which incorporates data on animal pathogens and environmental testing. This resistance is tracked using in vitro antimicrobial susceptibility testing methods that have not changed for a hundred year. Today, whole-genome sequencing (WGS) technology is replacing many traditional microbiological methods. FDA conducted studies comparing WGS and classical susceptibility testing data for *Salmonella* and *Campylobacter* and demonstrated a very high concordance between clinical resistance and the presence of known antimicrobial resistance genes. This indicates that resistance can be predicted from any genomic sequence. In NARMS alone, hundreds of genomes are being uploaded into the public domain weekly. Furthermore, pilot studies to explore the strengths and limitations of metagenomic methods are promising, holding the possibility to monitor the resistome directly without the need to cultivate microorganisms for phenotyping. NARMS is using both genomic and metagenomic approaches to provide new insights into the ecology of antibiotic resistance and helping to establish best practices for One Health antibiotic resistance monitoring.

Emerging & Pandemic Threat Preparedness

Jerry Weir, PhD

FDA, Center for Biologics Evaluation & Research

Dr. Jerry P. Weir is the Director of the Division of Viral Products (DVP), Office of Vaccines Research and Review with FDA's Center for Biologics Evaluation and Research (CBER). He received his PhD in Biochemistry from Vanderbilt University and did postdoctoral research in virology at the National Institutes of Health. He joined FDA in 1994. As Director of DVP, Dr. Weir manages the regulatory activities and research programs of the Division. As a Senior Investigator at CBER, he directs a research program pertaining to diverse viruses, including influenza, herpesviruses, and poxviruses. Dr. Weir frequently serves as an advisor to the World Health Organization on issues relating to influenza virus vaccines activities and vaccine standards.

Abstract: Emerging and Pandemic Threat Preparedness

CBER's public health mission includes the review and regulation of viral vaccines to ensure their safety and efficacy, as well as research intended to facilitate the development and evaluation of high priority vaccines that impact the public health. In recent years, several viral diseases have appeared, sometimes unexpectedly, and posed a serious global public health threat. Many of these pandemic threats are caused by either zoonotic or vector-borne viruses, and in most cases, effective vaccines are not available. Recent examples include the threat of avian influenza virus, ebola virus and zika virus. In each case, research in the Division of Viral Products at CBER was important in preparing for and responding to these emerging threats.

Strengthening Regulatory Science to Support the Development of Medical Countermeasures for Emerging Infectious Diseases



Tracy MacGill, PhD
FDA, Office of the Commissioner

Dr. Tracy MacGill is Director, Medical Countermeasure Regulatory Science for FDA's Office of Counterterrorism and Emerging Threats (OCET) and the Medical Countermeasures Initiative (MCMi). She leads the MCMi Regulatory Science Program, oversees intra- and extramural research programs, and works with FDA Centers, PHEMCE stakeholders, and other U.S. and international partners on medical countermeasure-related regulatory science issues. OCET is part of FDA's Office of the Chief Scientist, in the Office of the Commissioner.

Prior to joining OCET, Dr. MacGill served as a Program Officer in the Office of Biodefense Research Affairs (OBRA), at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), where she managed a portfolio focused on the development of biodefense animal models to support product development. Previously, Dr. MacGill was a Microbiologist in the Office of Counter-Terrorism and Emergency Coordination (OCTEC), in FDA's Center for Drug Evaluation and Research (CDER).

Dr. MacGill also served on active duty with the United States Army as a research microbiologist in the Department of Immunology at the Walter Reed Army Institute of Research (WRAIR), working with non-human primate malaria models. Following the anthrax mail attacks in 2001, she was assigned to a counterterrorism augmentation team at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland.

Dr. MacGill is now an officer in the Commissioned Corps of the United States Public Health Service and holds the rank of Captain. CAPT MacGill earned a doctorate in Cellular and Molecular Biology from the University of Nevada, Reno.

Abstract: Strengthening Regulatory Science to Support the Development of Medical Countermeasures against Emerging Infectious Diseases

The U.S. Food and Drug Administration (FDA) plays a vital and active role in protecting the United States from chemical, biological, radiological, nuclear (CBRN), and emerging infectious disease threats such as pandemic influenza Ebola virus, and Zika virus. The FDA is responsible for ensuring that medical countermeasures (MCMs) – including diagnostic tests, drugs, and vaccines – to counter these threats are safe, effective, and secure. The FDA also works closely with interagency partners and product developers to advance the development and availability of MCMs. In 2010, the FDA established its Medical Countermeasures Initiative (MCMi) to further the development of MCMs by establishing clear regulatory pathways for MCMs, instituting effective regulatory policies and mechanisms to facilitate timely access to available MCMs, and advancing MCM regulatory science to create the tools, standards, and approaches that support



regulatory decision-making. This presentation will describe how the MCMi Regulatory Science Program supports FDA response to emerging public health threats through advancements in regulatory science and coordination with federal partners, academia, and industry



Speakers Bios and Abstracts

Concurrent Session 7: Addiction

Drug Abuse in the U.S.

Chad J. Reissig, PhD Pharmacologist

FDA, Center for Drug Evaluation & Research

Dr. Reissig is behavioral pharmacologist with almost two decades of experience in abuse and addiction-related science and research. His research interests include behavioral pharmacology with an emphasis on tobacco products and other drugs of abuse including hallucinogens and caffeine. Dr. Reissig has served as a pharmacologist with the Controlled Substance Staff at FDA's Center for Drug Evaluation and Research (CDER) and as the Addiction Branch Chief in the Center for Tobacco Products (CTP). While at CDER, Dr Reissig provided expert guidance and consultation services to FDA review divisions and government agencies to develop regulatory policies to mitigate drug abuse risks. He also reviewed New Drug Applications (NDA) and Investigational New Drug Applications (IND) for abuse liability assessment and scheduling recommendations. As the Addiction Branch Chief, Dr. Reissig oversaw a multidisciplinary team of behavioral and clinical pharmacologists in the review of tobacco product marketing applications including ITPs, SEs, MRTPs, and PMTAs. Dr. Reissig was also involved in tobacco product guidance and regulation development, and research involving tobacco products, including studies examining the behavioral effects of nicotine, flavor ingredients, and other product design features. Before entering into public service, Dr. Reissig received his PhD in Pharmacology and Toxicology from the University at Buffalo and completed his postdoctoral training at the Behavioral Pharmacology Research Unit at Johns Hopkins University.

Abstract: Overview of drug abuse and addiction in the U.S.

Although definitions vary, addiction may be defined as a "chronic, relapsing disorder characterized by compulsive drug seeking, continued use despite harmful consequences, and long-lasting changes in the brain" (NIDA, 2019). Drug abuse and addiction are common in the US; according to the National Survey on Drug Use and Health, approximately 19.7 million Americans had a substance use disorder in 2017 including 14.5 million individuals with Alcohol use disorder and 7.5 million who had an illicit drug use disorder. Substance abuse and addiction have substantial economic and societal costs. Addiction-related costs to healthcare, crime, and lost productivity accounted for over 740 billion dollars in 2010. Drug overdose deaths in the US continue to increase, including deaths related to opioids (both licit and illicit), psychostimulants, and alcohol. This presentation will provide an overview of addiction in the US, focusing on the primary drug classes of abuse (opioids, stimulants, depressants, alcohol and marijuana). Discussion will highlight the present state of addiction in the US, beginning with a brief overview of addiction, its definitions, and neurobiological mechanisms hypothesized to be involved in addiction. Trends and changes in drug use will be discussed including opioids, and the emergence of novel drugs of abuse. An overview of the increased use of electronic nicotine delivery systems (ENDS) will also be provided.



FDA Response to the Opioid Crisis

Marta Sokolowska, PhD

FDA, Center for Drug Evaluation & Research

Marta Sokolowska, PhD, serves as Associate Director for Controlled Substances in the Center for Drug Evaluation and Research. She joined FDA in December 2018 and oversees the Controlled Substance Staff (CSS). CSS provides consultation throughout FDA on evaluating abuse potential of drugs and advises on all matters related to domestic and international drug scheduling.

Dr. Sokolowska is a recognized expert in drug abuse potential assessment and scheduling strategies. She has facilitated initiatives to improve public health by advancing the science of assessing abuse liability including organizing and participating in numerous scientific meetings with FDA, academia, and industry experts. Her past leadership roles include serving as Vice President of Medical and External Affairs at Depomed, Inc. and Head of Medical Affairs and the Center for Abuse Prevention and Evaluation at Grunenthal, USA. Dr. Sokolowska earned her doctoral degree in psychology from McMaster University in Canada.

Abstract: How is FDA Responding to the Opioid Crisis?

US opioid abuse epidemic is a public health emergency. Despite decreased opioid prescribing, it still poses multiple challenges. In 2017, 47,600 Americans died from opioids overdose, 11.1 million people reported misuse of prescription opioid pain medications, nearly 900,000 people used heroin, and 2.1 million people had an opioid use disorder. At the same time, an estimated 25 million Americans experience pain every day. For many of these individuals, pain interferes with physical and mental health, work productivity, and ability to engage in social activities. Over the years, FDA has taken multiprong actions to impact the opioid crisis reflecting the unique challenges of how opioids are being used, misused and abused.

The goal of this presentation is to overview FDA's current priorities and strategies to address this crisis. The key initiatives focus on decreasing exposure to prescription opioids and prevention of new addictions, supporting the treatment of those with opioid use disorder, fostering the development of novel pain treatment therapies as well as improving enforcement and risk benefit evaluation.



Assessing the Structural and Pharmacological Similarity of Newly Identified Drugs of Abuse to Controlled Substances Using PHASE



Chris Ellis, PhD

FDA, Center for Drug Evaluation & Research

Dr. Christopher Ellis is a computational chemist at FDA's Center for Drug Evaluation and Research (CDER). He is the Scientific Lead for the Public Health Assessment via Structural Evaluation (PHASE) project, which uses a computational workflow to evaluate the risk of a newly identified drug of abuse. Before joining FDA, Chris worked at the University of Maryland, Baltimore developing molecular dynamics techniques for investigating selectivity issues when designing Alzheimer's drugs. He has published peer-reviewed articles on a range of topics including drug-protein interactions, enzyme conformational dynamics, the effects of glycosylation on protein structure, and coarse-grain modeling of condensed phase systems. Chris holds a BS and PhD degrees in chemistry from the University of Central Florida and the Pennsylvania State University, respectively.

Abstract: Assessing the Structural And Pharmacological Similarity of Newly Identified Drugs of Abuse To Controlled Substances Using Phase

New synthetic opioids have become a significant threat to public safety. In particular, the emergence of new fentanyl derivatives on the street-drug market has led to a rapid increase in overdose deaths. The large influx of new fentanyl derivatives is attributed to their high potency and inexpensive synthesis. Moreover, slight chemical modifications to the parent drug evade control by national and international legislation and there are often little to no pharmacological and toxicological data available. The resource requirements to experimentally evaluate all possible fentanyl analogs are prohibitively high and a computational risk-assessment model is desirable. Therefore, the US FDA's Center for Drug Evaluation and Research (CDER) has developed the Public Health Assessment via Structural Evaluation (PHASE) protocol, a multi-pronged computational approach that uses molecular structure to assess a drug's risk to public health. PHASE is comprised of four components that calculate a new drug's structural similarity to all previously scheduled drugs, identify plausible biological targets with target prediction software, predict binding affinity at the mu opioid receptor with molecular docking, and integrate experimental and predicted data to generate an overall conclusion. The multicomponent approach provides a rapid, systematic evaluation of a new drug in the absence of significant in vitro or in vivo data. PHASE has been used to prioritize experimental inquiry into the potential effects of newly identified drugs of abuse and has the potential to inform law enforcement agencies with vital information regarding newly emerging illicit opioids.



Preclinical Pharmacology of Novel Synthetic Opioids Appearing in Clandestine Drug Markets



Michael Baumann, PhD
National Institute on Drug Abuse (NIDA)

Michael H. Baumann, PhD, is a Staff Scientist and Facility Head at the National Institute on Drug Abuse (NIDA), Intramural Research Program (IRP), in Baltimore, MD. Dr. Baumann has authored or co-authored more than 200 scientific publications and serves on the editorial board for the *European Journal of Pharmacology*, *Neuropharmacology*, and *Neuropsychopharmacology*. For more than 20 years, his research at the NIDA IRP was focused on the role of brain dopamine and serotonin systems in mediating the effects of therapeutic and abused stimulant drugs. In 2012, he established the Designer Drug Research Unit (DDRU), the goal of which is to collect, analyze and disseminate the most up-to-date information about the pharmacology and toxicology of newly-emerging synthetic drugs of abuse, more formally known as new psychoactive substances (NPS). Working with partner organizations such as the Drug Enforcement Administration and the National Drug Early Warning System, Dr. Baumann is kept informed about trends in the abuse of NPS. His research team has characterized the molecular mechanism of action and pharmacological effects for many of the “bath salts” cathinones and their replacement analogs. Ongoing research is aimed at determining the biological effects of emerging synthetic cannabinoids and opioids which pose serious public health risks.

Abstract: Preclinical Pharmacology of Novel Synthetic Opioids Appearing In Clandestine Drug Markets

Novel synthetic opioids (NSOs) are being encountered in clandestine drug markets worldwide. NSOs include various analogs of fentanyl (e.g., butyrylfentanyl) and non-fentanyl compounds (e.g., U-47700) which act as agonists at the mu-opioid receptor subtype in the brain and periphery. NSOs are found as standalone products, adulterants in illicit heroin, or in counterfeit pain pills. As new analogs appear on the street, there is a critical need for rapid and accurate pharmacological evaluation of the substances to inform the public and assess risks. This presentation will describe *in vitro* and *in vivo* laboratory strategies that can be used to determine potency and efficacy of NSOs in rodents. *In vitro* methods include radioligand binding at mu-, delta-, and kappa-opioid receptor subtypes, whereas *in vivo* paradigms include analgesia testing. An important conclusion is that *in vitro* binding affinity of a drug at mu-opioid receptors does not always predict *in vivo* potency. Furthermore, analgesic potency may not predict the propensity to induce adverse effects, suggesting new compounds should be tested in a battery of *in vivo* paradigms.



FDA Assessment of the Abuse Potential of Drugs, Including Opioids

Katherine Bonson, PhD

FDA, Center for Drug Evaluation & Research

Dr. Katherine Bonson is one of the founding members of the Controlled Substance Staff (CSS) at FDA. She has a PhD in pharmacology from SUNY-Buffalo and a bachelor's degree in psychology and English from the University of Iowa. After receiving her doctorate, she conducted research on drugs of abuse, first in animals at the National Institute of Mental Health (NIMH), and then in humans in the Brain Imaging Unit of the National Institute on Drug Abuse (NIDA), and the Department of Psychiatry at Johns Hopkins University. Since CSS was created in 2000, Dr. Bonson has been instrumental in creating regulatory science policy involving drugs of abuse and was the primary author of the 2017 Guidance for Industry: Assessment of the Abuse Potential of Drugs. In addition to providing abuse evaluations for hundreds of INDs and NDAs, she has written dozens of recommendations for scheduling placements of drugs with abuse potential under the Controlled Substances Act.

Abstract: FDA Assessment of the Abuse Potential of Drugs, Including Opioids

The assessment of whether a drug with central nervous system activity (CNS) has abuse potential is part of the safety evaluation of that drug. At FDA, the Controlled Substance Staff (CSS) is consulted by review divisions when a CNS-active drug has been submitted under an investigational new drug (IND) application or a new drug application (NDA). CSS works with drug sponsors to identify the appropriate preclinical and clinical studies that are required to fully evaluate their drug for abuse potential. Typically, these studies include an assessment of receptor binding, second messenger systems, animal behavior (general behavior, drug discrimination, and self-administration), pharmacokinetics, physical dependence, and abuse-related adverse events in clinical studies. If these studies produce abuse-related signals, it may also be necessary to conduct a human abuse potential study with the drug using recreational drug abusers as subjects. Although FDA has previously reviewed many INDs and NDAs for opioids, each one is evaluated individually for abuse potential, especially when the opioid is a new molecular entity or has a novel formulation. If CSS concludes from all available data that a drug has abuse potential, this will determine drug labeling in Section 9 (Drug Abuse and Dependence), as well as a recommendation from FDA regarding the scheduling of the drug under the Controlled Substances Act (CSA).

Speakers Bios and Abstracts

Concurrent Session 8: Impacting Public Health Through Electronic Media: Empowering Consumers, Patients, and Other Stakeholders

Tobacco Regulatory Science – Understanding the Role of Flavor in E-Cigarette Marketing



Ryan Kennedy, PhD

Johns Hopkins Bloomberg School of Public Health
Center of Excellence in Regulatory Science and Innovation

Ryan David Kennedy is a tobacco control researcher interested in the role policy plays in addressing the global tobacco epidemic. Kennedy received his PhD from the University of Waterloo (2010) and completed post-doctoral training at the Center for Global Tobacco Control in the Harvard School of Public Health. Since 2013, Kennedy has been faculty in the Department of Health, Behavior & Society at the Johns Hopkins Bloomberg School of Public Health. Kennedy works in low- and middle-income countries through his role with the Institute for Global Tobacco Control. Domestically, Kennedy has a program of research with the FDA's Center for Tobacco Programs, funded through the Johns Hopkins CERSI (Center for Excellence in Regulatory Science and Innovation) working to understand e-cigarette advertising of product features.

Abstract: Tobacco Regulatory Science – Understanding the Role of Flavor in E-Cigarette Marketing

The use of electronic nicotine delivery systems (ENDS) has increased rapidly over time. Most adolescents and adults report that their first ENDS was flavored like something other than tobacco, such as menthol, mint, candy, fruit, chocolate or other sweets. ENDS products are advertised direct to consumers and in traditional print media and online. This study sought to understand how flavors were used in ENDS advertising.

Researchers at Johns Hopkins School of Public Health purchased copies of ENDS advertisements from two advertising tracking services (Numerator and Mintel); ads ran during 2015, 2016 or 2017. Ad mediums included direct-to-consumer mail, direct-to-consumer emails, magazines, and social media (such as banner posts). Ads were coded for visual and lexical content related to flavor.

The study identified 1860 unique ENDS ads. Most of the ads (66%; n=1233) included a reference to flavor. The most common flavor advertised, "tobacco", was present in 45% of ads (n=549). The flavor menthol was present in approximately one third of ads (32%, n=393), followed by fruit/berry flavors (22%, n=274), spice/clove (11%, n=134), and mint (14%, n=168). Many ENDS products advertised (20%, n=244) featured flavor names that were unconventional such as "Sweet Lava" and "Meteor Milk".

ENDS products commonly feature flavors in their advertising. Additional research about the effect of marketing on consumer behavior, and the monitoring of ENDS ads can help inform tobacco regulatory activities.

Using Content Analysis to Understand Tobacco Industry Use of Technology to Engage Consumers

Mario Navarro, PhD

FDA, Center for Tobacco Products

Mario Navarro has a PhD in Psychology with a concentration in Applied Social Psychology and an MA in Psychology with a co-concentration in Applied Social Psychology and Evaluation from Claremont Graduate University. Since 2016, he has worked as a part of the Research and Evaluation team in the Office of Health Communication and Education in the US Food and Drug Administration's Center for Tobacco Products. Dr. Navarro contributes to the development research for campaigns tailored for LGBT young adults ages 18-24 (This Free Life), for multicultural youth ages 12-17 (Fresh Empire), and other campaigns. Mario has publications in journals in the realms of tobacco, psychology, communication, and public health.

Abstract: Content analyses of tobacco product brand marketing on mobile websites, smartphone apps, and social media

This research describes how tobacco companies reach people on smartphones. Generally, ENDS, hookah, and cigar brands marketed on mobile websites and social media, and rarely used age gating or displayed health warnings. Cigarette and smokeless brands marketed on mobile websites and apps, and often used age gating and displayed warnings.

BACKGROUND: Most youth and adults have access to smartphones and use them hours per day. Little is known about how tobacco marketing has evolved to reach people using these devices. The current research describes how cigarette, smokeless tobacco, hookah, and Electronic Nicotine Delivery Systems [ENDS] companies reach people using smartphone-optimized (mobile) websites, apps, and social media. **METHODS.** We conducted three content analyses to describe how tobacco companies use branded (1) smartphone-mobile websites, (2) smartphone apps, and (3) social media. We identified leading brands based on criteria such as, sales and advertising spending. We searched (1) for websites using a smartphone browser, (2) for apps on the Google Play and Apple app stores, and (3) for social media pages on the platforms of Instagram, Facebook, Twitter, YouTube, Pinterest, and Tumblr. **RESULTS:** Overall, about half of brands had mobile websites, and only cigarette and smokeless brands with the highest market share had smartphone apps. Websites offered internal social media, games, sweepstakes, and videos. All cigarette and most smokeless websites required age-verified accounts for entry, while few other websites did. Most ENDS websites required accounts for making online purchases. All cigarette and smokeless websites displayed health warnings, but no hookah and few ENDS and cigar websites did. All mobile apps provided time-sensitive, location-based coupons. Most ENDS, hookah, and cigar brands had at least one social media page, while very few cigarette and smokeless brands did. Many pages contained links to branded websites and online stores, and pages' posts



featured images of specific products. Few pages used any age gating, and less than one-quarter had a visible health warning. ENDS pages had the most engaged audience. CONCLUSIONS: Tobacco companies use websites, apps, and social media to market their products. Results can inform tobacco regulatory activities and prevention and cessation interventions.

Consumers' Use of Personal Electronic Devices in the Kitchen

**Amy Lando, MPP,
Michael Bazaco, Ph.D.**

FDA, Center for Food Safety & Applied Nutrition

Amy M. Lando is a Social Scientist on the Consumer Studies branch at FDA's Center for Food Safety and Applied Nutrition. She attended Duke University where she received a Bachelor's of Arts in public policy with a minor in chemistry. Upon graduation from Duke, Amy attended Georgetown University and completed her Master's in Public Policy with an emphasis on food and nutrition policy. Amy is the project director of the Food Safety and Nutrition Survey, a national survey of consumers' food safety and nutrition attitudes and behaviors. She has also directed a number of studies evaluating consumer understanding of food labels.

Michael Bazaco is an epidemiologist at FDA's Center for Food Safety and Applied Nutrition, where he is the emerging infectious disease lead for the Office of Analytics and Outreach. He received a Master's degree in food science and technology from Virginia Tech and a doctorate degree in epidemiology from the University of Pittsburgh. He also did his post-doc research as an FDA Commissioner's Fellow. In addition to his work at FDA, he is an adjunct professor at the University of Maryland School of Public Health, where he teaches emerging infectious diseases, epidemiology, and food policy.

Abstract: Consumers' Use of Personal Electronic Devices in the Kitchen

Smartphones, tablets, and other personal electronic devices have become ubiquitous in Americans' daily lives. These devices are used by people throughout the day including while preparing food. For example, they may be used to look at recipes and therefore be touched multiple times during preparation. Previous research has shown that cell phones can harbor bacteria including opportunistic human pathogens (such as *Staphylococcus* and *Klebsiella* spp.). Using data from the 2016 Food Safety Survey (FSS) and subsequent focus groups the frequency of consumers using their personal electronic devices in the kitchen while preparing food, what type of devices they use, and their handwashing behaviors after handling their devices were investigated. The 2016 FSS is the 7th wave of a repeated cross-sectional survey conducted by the Food and Drug Administration (FDA) in collaboration with the U.S. Department of Agriculture (USDA). The goal of the survey is to evaluate U.S. adult consumers' attitudes, behaviors, and knowledge about food safety. The FSS surveyed 4,169 adults using a dual frame (landline and cell phone interviews) random-digit-dial (RDD) sampling process. The personal electronics module was the first of three food safety topics discussed in each of eight consumer focus groups which were conducted in four U.S. cities in the fall of 2016. Results from the 2016 FSS found that of those who use personal electronic devices while cooking, only about a third reported washing their hands after touching their device before they continue cooking. This is significantly lower than the self-reported handwashing behavior after touching risky products such as raw eggs, meat, chicken, or fish. Results from the focus groups highlight the varied usage of these devices while cooking and the related strategies consumers are using to incorporate personal electric devices into their cooking routines.

Assessment of Patient Perspective on Risks and Benefits Associated with High Intensity Focused Ultrasound (Hifu) for The Ablation of Prostate Tissue in Men With Localized Prostate Cancer



Charles Viviano, MD, PhD

FDA, Center for Devices & Radiological Health

Dr. Viviano is Clinical Deputy Division Director in the Division of Reproductive, Gastro-Renal and Urologic Devices (future OHT3) in the Center for Devices and Radiological Health at the Food and Drug Administration. He is a board-certified Urologist. He was involved in the final FDA clinical review of the currently available HIFU devices and currently remains involved in the regulatory review of prostate ablation devices, in addition to serving as the senior medical officer in the division. Before joining FDA, he was an Assistant Professor in the Division of Urology at Duke University, where his practice focused on General Urology and Men's Health. He received his medical education at the University of Connecticut and his PhD in Toxicology from the University of North Carolina at Chapel Hill.

Abstract: Assessment of Patient Perspective on Risks and Benefits Associated with High Intensity Focused Ultrasound (HIFU) for the Ablation of Prostate Tissue in Men with Localized Prostate Cancer Background: Prostate cancer is the most common malignancy and the third leading cause of cancer-related deaths in men in the United States and Europe. FDA allowed to market two HIFU tools for prostate tissue ablation after rejecting prior premarket applications indicated to treat prostate cancer, as they did not demonstrate cancer-specific effectiveness. Benefit-risk balance is unclear in patients with prostate cancer. The adverse event profile is known but there are few studies with data on oncologic outcomes.

Problem: Patients and regulators must make decisions regarding HIFU treatment with a paucity of relevant clinical effectiveness data but in light of known potential adverse events and 12-month post-treatment prostate biopsy data.

Rationale: We plan to undertake a study that generates the patient perspective on available benefit data or tolerance of risks associated with HIFU. Such information may inform future premarket device evaluation of ablation tools, and delivery of better ablation devices sooner to patients.

Study Overview:

- Target Population includes men who have been diagnosed with organ confined (localized) Gleason 6 or 7 prostate cancer who have not undergone any prostate cancer treatment
- We will use the Discrete Choice Experiment method to generate patient input on this device via questions on a survey. Under this approach patients (survey respondents) are presented with treatment profiles that cover the full range of plausible prostate

ablation treatment profiles (benefits and risks), and their maximum risk tolerable, and minimum benefit acceptable is calculated

Sample Discrete Choice Experiment Question

Instruction: You have been diagnosed with localized prostate cancer and have been asked to consider some minimally invasive treatment options with different risk benefits profiles. What treatment device would you choose?

Attributes	Device A	Device B
Chance of negative biopsy after treatment	100%	50%
Chance of permanent erectile dysfunction after treatment (%)	35%	60%
Chance of permanent urinary incontinence after treatment (%)	0%	30%
Which alternative do you prefer?		

Regulatory and public health Impact: our study aims to increase the use and transparency of patient input in our decision making. This will help in benefit – risk decisions for market applications. In addition, patient preference information feedback to sponsors will facilitate and hasten improved device design/clinical trial protocols, all with the goal of delivery of better ablation devices sooner to patients.

Clinical Outcome Assessments in Medical Product Development

Elektra Papadopoulos, MD

FDA, Center for Drug Evaluation & Research

Elektra J. Papadopoulos, MD, MPH is the Associate Director for the Clinical Outcome Assessments (COA) Staff in the Office of New Drugs, Center for Drug Evaluation and Research, FDA. The COA Staff contributes to a culture that ensures the patient voice is integrated into drug development through COAs, including patient-reported outcomes, that are meaningful to patients, valid, reliable and sensitive to change. The Staff works collaboratively to provide consultation for COAs used across all stages of drug development and therapeutic areas, manages the COA Drug Development Tool Qualification Program to develop and qualify COAs for unmet public health needs, and provides education and outreach to advance the science of COA development and implementation in drug development.

Abstract: Clinical Outcome Assessments in Medical Product Development

An important component of patient-focused medical product development is the successful implementation of fit-for-purpose clinical outcome assessments (COAs), including patient-reported outcome instruments, that can be used to obtain valid, reliable, and meaningful endpoints in populations of interest. COAs measure outcomes that describe or reflect how an individual feel, functions or survives and can be used to determine whether or not a drug has been demonstrated to provide clinical benefit. The following are some considerations for what it means for a COA to be fit-for-purpose for use in drug development: (1) Is the COA appropriate for its intended use (e.g., study design and patient population)?; (2) Does it validly and reliably measure a concept that is clinically relevant, important to patients, and modifiable with treatment?; and (3) Is the assessment able to be communicated in labeling in a way that is accurate, interpretable, and not misleading? The presentation will introduce the components of a fit-for-purpose COA and highlight some novel approaches for clinical outcome assessment medical product development.

Collect Once, Use Many Times: Challenges and Opportunities for the Use of Real-World Evidence to Improve Healthcare



Gregory Pappas, MD, PhD

FDA, Center for Drug Evaluation & Research

Gregory Pappas, MD, PhD is the Associate Director for National Surveillance working on BEST (Biological Effectiveness and Safety System) at the Office of Biostatistics & Epidemiology, Center for Biologics Evaluation and Research (CBER). Dr. Pappas had a leadership role in establishment of the National Evaluation System for health Technology (NEST), for CDRH (Center for Devices and Radiological Health) working with broad set of stakeholders including other government agencies (ONC, CMS, NIH, AHRQ), industry, health care providers, patient groups, and data partners.

He previously served as the Senior Deputy Director of HAHSTA (HIV/AIDS, Hepatitis, STD, and TB Administration) for the District of Columbia, Department of Health. He has worked professionally in over 30 countries. His consultancies include work with WHO, USAID, and World Bank.

Gregory Pappas MD, PhD served as the Noordin M. Thobani Professor at the Aga Khan University (AKU), where he was the Chairman of the Department of Community Health Sciences in Karachi, Pakistan. Dr. Pappas was an author of President's Emergency Plan for AIDS Relief (PEPFAR) including contributing to the PEPFAR Five Year Strategy, a report to Congress. Dr Pappas served in a variety of positions over an 18-year period in the Department of Health and Human Services, including his role as Senior Policy Advisor to the Assistant Secretary for Health/Surgeon General, David Satcher. Dr Pappas directed the Office of International and Refugee Health, Department of Health and Human Services, serving on the Executive Board of UNICEF and PAHO, and as a delegate to the World Health Assembly. For ORC Macro, as Deputy Director of the Demographic and Health Survey (DHS) he implemented innovative surveys in Uganda, Mali, Uzbekistan, and Dominican Republic.

Dr. Pappas received his MD and PhD (Anthropology) from Case Western Reserve in Cleveland, Ohio. After doing his clinical training, he came to Washington DC, first as a fellowship in Epidemiology, then continuing as a scientist at the National Center for Health Statistics/CDC. Dr. Pappas is author of numerous articles, including his work in the New England Journal of Medicine "The increasing disparity in mortality between socioeconomic groups in the United States" and his book with Cornell University Press, *The Magic City: unemployment in a working class community*. Dr. Pappas is on the faculty of the Bloomberg School of Public Health in the Department of Health Policy and Management, and is on the faculty of Howard Medical School. Dr. Pappas served as Chair of the Science Board and member of the Executive Board of the American Public Health Association. His *Megacities and Global Health* (APHA Press) with Omar Khan was published in 2012.

Poster Session 1

Topic: Precision Health (Day 1, A.M.)

1. Near-infrared Cerebral Oximetry: Testing the impact of skin pigmentation and superficial tissue layers on performance using a 3D-printed phantom

Authors: Afshari, Ali, FDA/CDRH; Ghassemi, Pejman FDA/CDRH; Wang, Jianting FDA/CDRH; Mendoza, Gonzalo FDA/CDRH; Weininger, Sandy FDA/CDRH; Pfefer, Joshua FDA/CDRH

Plain Language Synopsis: We developed a phantom-based test method to evaluate the impact of skin pigmentation on cerebral oximeter performance. The phantom was composed of a 3D-printed cerebrovascular module and epidermis-simulating layers with biologically relevant optical properties. High levels of pigmentation consistently decreased oximeter saturation estimates up to 5-10%, depending on tissue geometry.

Abstract:

Cerebral oximetry based on near-infrared spectroscopy has emerged as an increasingly popular technology for non-invasive monitoring during neonatal, pediatric, and adult cardiac surgery. However, the utility of this approach remains controversial among clinicians for a variety of reasons, including a lack of consistency in values measured by different devices. This is likely due, at least in part, to differences in how devices account for patient-specific variations in epidermal pigmentation and thickness of superficial layers. Parametric testing of robustness to biological variables is not readily achieved in the small clinical studies performed for clearance purposes. Tissue-simulating phantoms, however, may provide insights into these confounding factors.

Phantoms based on a liquid matrix have been proposed for standardized testing of oximeters; whereas, we have focused on solid phantoms composed of a cerebrovascular module (CVM) and silicone-based superficial layers. Our phantom was developed to mimic the optical properties and layered structure of the neonatal/pediatric head. The CVM was fabricated by 3D printer and includes 148 cylindrical channels of

0.8 mm diameter each. While the optical properties of the CVM were in a biologically relevant range, the change in scattering with wavelength was slightly greater than optimal. Superficial layers were designed to represent scalp/skull and cerebrospinal fluid regions. An epidermis-simulating material was developed after spectrophotometric characterization of several absorber/silicone combinations. This material was used to mold 0.1-mm-thick layers simulating low, medium and high-melanin-content levels. The final measurement procedure involved injecting bovine blood at well-validated saturation levels into the CVM and performing measurements with neonatal and pediatric probes from two commercial cerebral oximeters.

Results from Oximeter #1 showed a high degree of precision with both neonatal and pediatric sensors, whereas accuracy degraded with thicker extracerebral tissue layers. Saturation measurements with this system decreased monotonically 5 to 10% with increasing pigmentation levels. Results from Oximeter #2 showed poor precision, yet greater robustness to variations in extracerebral layers and skin pigmentation. While some modifications in CVM optical properties may be needed, the consistency of our results with prior clinical and phantom studies indicates the promise of our modular phantom approach for evaluating cerebral oximeters.

2. Dysbiosis Of Commensal Microbes And Correlation With Increased Systemic Dissemination And Gastro-intestinal Pathology During Listeriosis

Authors: Alam, Mohammad, FDA/CFSAN; Carmen, Tartera, FDA/CFSAN; Jayanthi, Gangiredla, FDA/CFSAN; Tammy, Barnaba, FDA/CFSAN

Plain Language Synopsis: Listeriosis is a public health problem. Variation in human susceptibility to listeriosis is difficult to evaluate since human populations cannot ethically be used for clinical trials. This surrogate study of aging murine listeriosis

suggest that, in older mice the composition of gut microbiome alters and increases the susceptibility to invasive listeriosis.

Abstract:

Background: Foodborne *Listeria monocytogenes* (Lm) causes gastroenteritis, septicemia, meningitis, and chorioamnionitis, and is associated with high case-fatality rates in the elderly. We developed a geriatric murine model as a human surrogate for listeriosis and previously reported increased systemic infection in old mice due to imbalances in protective immune responses. **Purpose:** Listeriosis-induced perturbation of gut microbiota and pathological changes in gastrointestinal tissues were compared between young and old mice. **Methods:** Young and old C57BL/6 mice were dosed intragastrically with ~10⁶ CFU/mouse Lm. Spleen, liver and gastric tissues were collected. Spleen/liver tissues were cultured for viable Lm. Gastric tissues were H&E stained. Intestinal tissues were analyzed for cytokine mRNA by Real-time RT-qPCR. Fecal pellets were collected pre- and post-Lm infection for microbiome analysis via shotgun metagenomics sequencing using Illumina's MiSeq platform. **Results:** Metagenomic analysis of uninfected old mice showed a significant ($p \leq 0.05$; t-test: significance) reduction in Clostridiaceae and Lactobacillaceae families compared to young mice. Older mice had significantly higher systemic Lm counts in liver ($p=0.03$) and spleen ($p=0.05$). Porphyromonadaceae and Prevotellaceae were increased in infected young mice while members of the Ruminococcaceae and Lachnospiraceae families were significantly increased in old mice after infection. Genera *Blautia* and *Alistipes* were abundant in uninfected young and old mice respectively, but significantly ($p=0.027$, $p=0.032$) reduced post Lm infection. Immune-modulating bacteria, *Pseudoflavonifractor* and *Faecalibacterium* were significantly ($p=0.014$; $p=0.043$) increased only in the old infected mice correlating with increased inflammatory response and gastritis. IFN-gamma and

IL-10 mRNA was upregulated in intestinal tissues from old mice. Histologic analysis of gastric tissues showed extensive lesions in the Lm-infected old mice, moreso in the non-glandular region and fundus than in the pylorus. **Conclusion:** Listeriosis is a public health problem mostly affecting the elderly. Animal models of oral listeriosis have been developed to use as human surrogates for evaluating the role of gut microbiome alterations in the susceptibility to Lm infection. Listeriosis in old mice enhances the abundance of butyrate-producing inflammatory members of the Ruminococcaceae/Lachnospiraceae family bacteria while reducing beneficial commensals in the aging gut. Aging may affect the composition of gut microbiota and increase the risk of invasive *L. monocytogenes* infection.

3. Sex-based susceptibility of doxorubicin-induced cardiotoxicity correlates with transcriptome changes in a mouse model

Authors: Azevedo-Pouly, Ana, FDA/NCTR; Vijay, Vikrant, FDA/NCTR; Moland, Carrie, FDA/NCTR; Revollo, Javier, FDA/NCTR; Rao, Ashutosh, FDA/CDER; Aryal, Baikuntha, FDA/CDER; Han, Tao, FDA/NCTR; Fuscoe, James, FDA/NCTR; Desai, Varsha, FDA/NCTR

Plain Language Synopsis: Adult males are more susceptible to doxorubicin-induced cardiotoxicity than adult females. RNA-sequencing showed genes that control calcium levels and energy pathways in the heart were differentially expressed between the sexes. Furthermore, the apelin pathway (which prevents fibrosis) was induced by the drug in females and may explain cardio-protection.

Abstract:

Cardiotoxicity induced by the anti-cancer drug doxorubicin (DOX) shows a well-documented disparity between adult males and females. However, the mechanisms underlying sex differences are not fully understood. To address this, next generation RNA-sequencing was performed in hearts from male and female mice treated with

3mg/kg body weight DOX or an equivalent volume of saline (i.v.) once a week for 8 consecutive weeks (24 mg/kg cumulative dose). At necropsy, one week after the last dose, greater cardiotoxicity was observed in DOX-treated males compared to DOX-treated females. Only males exhibited cytoplasmic vacuolization in the myocardium and had 3.6-fold higher plasma level of cardiac troponin T, a marker of heart injury, compared to females. The hearts of DOX-treated males had a greater number of differentially expressed (DE) genes compared with females (836 in males vs. 26 in females at FDR \leq 0.1). IPA canonical pathway classification of expression profiles recapitulated previous findings of altered cardiac energetics (oxidative phosphorylation, fatty acid oxidation, and TCA cycle) with a statistically significant DOX effect only in males. IPA analysis also indicated activation of cell death/apoptosis of cardiomyocytes in males, which may explain significantly lower (9.7%) absolute heart weight in DOX-treated males (vs. saline), but not in DOX-treated females. DOX influenced the transcript levels of sarcoplasmic reticulum (SR) genes involved in calcium homeostasis, such as *Atp2a1*, *Atp2a2*, *Ryr2*, and *Pln*, that were significantly down-regulated in male hearts with a significant sex difference in *ATP2a2* transcript. Changes in these genes may lead to SR stress as exemplified by a significant 1.2-fold up-regulation of *Eif2ak2* (SR stress marker) in the male heart. A noteworthy finding was an up-regulation ($p = 0.02$) of the apelin signaling pathway in DOX-treated female heart. Apelin is thought to inhibit cardiac fibroblast differentiation, thereby preventing formation of fibrosis. A significant up-regulation of apelin (1.24-fold) and its receptor (1.47-fold) only in heart of DOX-treated females may have a role in cardio-protection in female mice.

These data provide information of novel genetic contributors that may influence differential vulnerability of mouse heart to DOX toxicity between the sexes and may be important in understanding clinical

limitations during chemotherapy.

4. Validating human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) as a preclinical screening model of drug cardiotoxicity

Authors: Bozza, William, FDA/CDER; McSweeney, Keisha, FDA/CDRH; Zhang, Baolin, FDA/CDER

Plain Language Synopsis: Cardiotoxicity is a major safety concern in drug development. Although the traditional safety screening methods can identify some cardiotoxic drug candidates, they cannot accurately represent the human heart in many aspects. There remains a high demand for preclinical screening models that possess both high human physiological relevance and potential for high throughput applications.

Abstract:

A major barrier to studying off-target cardiotoxicity has been the lack of appropriate model systems. Animal models are expensive and low throughput and exhibit species-specific differences in both drug metabolism and cardiac structure and function. In particular, significant differences between mouse and human cardiac system in electrophysiology and contractile features limit the extrapolation of findings from studies in murine systems to humans. Alternative approaches include using human cardiac myocytes, but access to these cells is extremely limited, and their long-term maintenance in culture is a major technical challenge. An ideal model system would possess both high human physiological relevance and potential for high throughput applications. Recently, human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have emerged as a powerful tool to model cardiac toxicity in highly physiologically relevant human cells. These cells express major human cardiac ion channels and sarcomeric proteins, suggesting that they may have high human physiological relevance. The objective of this study was to validate the iPSC-CMs system as a preclinical screening model to assess drug

cardiotoxicity.

We tested a panel of four chemotherapeutic agents of the anthracycline family, including doxorubicin, idarubicin, daunorubicin, and epirubicin. These agents are highly effective chemotherapy drugs; however, their clinical use is complicated by well-established cardiotoxic side effects. The FDA recommended life-time cumulative dose limits are: idarubicin = 150 mg/m², doxorubicin = 400 mg/m², epirubicin = 900 mg/m², and daunorubicin = 550 mg/m². Using an impedance-based technology, we assessed the effects of these agents on iPSC-CMs cellular index, beating amplitude, and beating rate. The derived EC₅₀ values for the anthracycline agents are in agreement with their known clinical dose limits. The iPSC-CMs model recapitulates many of the cardinal features of the adverse drug reaction including changes in cardiomyocyte morphology and contractility. These results support the utility of the iPSC-CMs model for screening protective agents against the adverse drug reactions, as well as identification of novel biomarkers that can predict cardiac risks in individual patients prior to the therapy.

5. **Epidemiological Cutoff Value Analysis of 229 MICs From Standard Testing of *Flavobacterium columnare* Isolates by Four Laboratories**

Authors: Crosby, Tina, FDA/CVM; Declercq, Annelies, Ghent University, Belgium; Gaunt, Patricia, Mississippi State U; Hawke, John, Louisiana State U; Hasbrouck, Nicholas, FDA/CVM; Giesecker, Charles, FDA/CVM

Plain Language Synopsis: Antimicrobial resistance is a major public health concern; therefore, it is critical to have standard testing methods and criteria to monitor for resistance. We tested an aquatic bacterium against 10 antimicrobials to determine the lowest concentration required to prevent growth. These values were approved for use in standardized testing methods.

Abstract:

Antimicrobial resistance is a major public

health concern. Therefore, it is critical to have standard methods and criteria, called epidemiological cutoff values (ECVs, a.k.a. ECOFFs) to monitor for resistance. To develop ECVs for the fish pathogen *Flavobacterium columnare*, we tested the susceptibility of 229 isolates using the standard broth dilution susceptibility testing method in the Clinical Laboratory and Standards Institute (CLSI) VET04-A2 guideline. Using that method, we determined the minimum inhibitory concentration (MIC) against 10 antimicrobials: ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, gentamicin, ormetoprim/sulfadimethoxine, oxolinic acid, oxytetracycline, and trimethoprim/sulfamethoxazole. We analyzed frequency distributions for each antibiotic to estimate an ECV that separates the wild-type non-resistant isolates from the non-wild-type resistant isolates using two statistical methods: ECOFFinder (Turnidge et al., 2006) and Normalized Resistance Interpretation (Kronvall, 2010). ECVs were estimated for ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, oxolinic acid, and oxytetracycline. The estimated ECVs showed that the isolates categorized similarly among the quinolones (enrofloxacin, flumequine, and oxolinic acid) as wild-type and non-wild-type. Therefore, laboratories could potentially use only one of these antimicrobials to monitor quinolone resistance. An ECV for the potentiated sulfonamides were not estimated because the potentiators mask resistance to sulfonamide. Similarly, an ECV for gentamicin was not evaluated because there are currently no existing quality control ranges to verify the results. The CLSI Veterinary Antimicrobial Susceptibility Testing subcommittee approved the ECVs proposed from our analysis; these will be included in the next revision of the VET04-supplement document.

6. **Analyzing the Role of Gut Microbiota on the onset and treatments of Autoimmune Diseases Using TNFΔARE Murine Model.**

Authors: Smith, Dylan, FDA/CDER; Edward, Vivienne, FDA/CDER; Tiffany, Linda, FDA/

CDER; Phue, Jenie, FDA/CBER; Wu, Wells, FDA/CBER, Santana-Quintero, Luis, FDA/CDER; Engel Odile, FDA/CDER, Clouse, Kathleen, FDA/CDER

Plain Language Synopsis: Biologic therapeutics for treatment of RA and IBD have shown variable efficacy and response in patients. We hypothesize that the microbiome could play a role in the variations observed and we conduct an in vivo model study to assess our hypothesis.

Abstract:

Background: While infectious diseases are decreasing, the prevalence of autoimmune diseases have been steadily rising. Biologic therapeutics have been successfully used to treat the latter diseases but have shown variable efficacy and responses in patients. Dysbiosis in the gut microbial community is associated with inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). In a previous study, we found that there were taxonomic differences between healthy mice treated with different TNF-antagonists.

Purpose/Objectives: Using the same study experimental design, we analyzed the role of gut microbiome in the development of autoimmune disease pathology. We hypothesized that transplanting fecal bacteria from mice with IBD and RA into germ-free (GF) and conventional mice will lead to dysbiosis and possible disease manifestation, which could then be treated with TNF antagonists.

Materials/methods: C57BL/6 adult male and female mice were used. Fecal samples from TNFΔARE mice, a model of engineered mice exhibiting spontaneous IBD and RA, were collected under anaerobic conditions. Fecal Microbiota Transplantation (FMT) was performed on WT and GF mice by oral gavage, using TNFΔARE and control mice fecal matter. Composition of the microbiome of these transplanted mice is analyzed before and after FMT, as well as during the course of injection treatment, using next generation sequencing (NGS) and bioinformatics analysis. Histology, flow cytometry, and ELISA

assays were also used to further investigate the microbiome changes.

Results: Our analysis of the microbial composition before FMT shows differences in the gut microbiome between WT and TNFΔARE mice. These sick mice exhibit a particular microbiome profile. After FMT, we observed anatomical changes reflecting a partial transmission of RA and IBD, linking the microbiome to the diseases.

Conclusions: This study highlights the importance of the gut microbiome in the onset of autoimmune diseases (RA and IBD) and the variation occurring during treatment with biologic therapeutics. Further studies are needed to better understand the interactions mechanistically among the microbiome, the various diseases, and treatment with biologic therapeutics, to potentially lead to the identification of biomarkers of the response in treated patients.

7. Development and validation of a novel, sensitive and specific LC-MS/MS assay for quantitative measurement of fialuridine in mouse serum

Authors: Gandhi, Adarsh FDA/OCP; Cai, Yan, FDA/OCP; Knapton Alan D, FDA/OCP; Patel Vikram, FDA/OCP; Howard Kristina E, FDA/OCP

Plain Language Synopsis: Differences in cytochrome P450 enzymes expression can lead to erroneous conclusions on drug safety or efficacy. The lack of sensitive bioanalytical assays to quantify hepatotoxic agents poses significant challenges to support pharmacokinetic/toxicokinetic studies. We developed a LC-MS/MS assay to quantify fialuridine (a mitochondrial hepatotoxicant) in mouse serum.

Abstract:

Background: Interspecies differences have limited the predictive utility of hepatotoxicity studies performed using standard rodent models. Differences in cytochrome P450 enzymes and function can lead to erroneous conclusions on the safety, or lack thereof, for human use. Lack of sensitive bioanalytical

assays to quantify hepatotoxic agents poses significant challenges to support pharmacokinetic (PK)/toxicokinetic (TK) studies with serial sampling. Here, we describe development and validation of a novel and robust liquid-chromatography tandem mass spectrometry (LC-MS/MS) assay for fialuridine (FIAU, a mitochondrial hepatotoxicant) in mouse serum.

Methods: Stock solutions of FIAU and FIAU-13C3 (internal standard) were prepared in methanol at 1.00 mg/mL. Calibrators and quality controls (QC) were prepared in blank mouse serum. FIAU was extracted from 5 μ L serum using protein precipitation with 25 μ L of methanol containing 20 ng/mL FIAU-13C3. The analytes were resolved on SynergyFusion-RP C18 column with 0.05% formic acid in water (mobile phase A) and in acetonitrile (mobile phase B) in 2.0 min. Positive mode ESI with MRM transitions (m/z 373.0/239.0 for FIAU) and (m/z 376.0/242.0 for FIAU-13C3) were selected. The assay was validated according to 2018 FDA Guidance for Bioanalytical Method Validation.

Results: The validated assay was found to be robust and sensitive. Assay was linear from 1.00-1000.00 ng/mL, selective with no interference from blank matrix, intra- and inter-batch variabilities were <15%, and accuracies were 85-115% for 6 replicates at 4 different QC levels. Mean recovery was ~95% with no matrix effect. Stability studies met the acceptance criteria for bench-top, auto-sampler, long-term storage and freeze-thaw conditions. Dilution integrity test passed when diluted 20x. There was no LC-injection carryover.

Conclusion: We developed and validated a novel, robust and sensitive LC-MS/MS assay for detecting FIAU in mouse serum. The high precision and accuracy with 5 μ L sample, no chemical derivatization, and assay run time of 2 min presents a highly amenable assay for quantitation of FIAU in a high-throughput capability. This assay was applied to determine serum concentrations of FIAU in a TK study with C57Bl/6, hCYP3A4, TK-NOG, and hepatic-humanized mice treated with

FIAU (100 mg/kg, oral) for up to 28 days.

8. Setting Epidemiological Cutoff Values for Monitoring Antibiotic Resistance of *Aeromonas hydrophila* Isolates Collected From Fish

Authors: Giesecker, Charles, FDA/CVM; Gaunt, Patricia, Mississippi State University; Hawke, John, Louisiana State University; Crosby, Tina, FDA/CVM; Hasbrouck, Nicholas, FDA/CVM; Stine, Cynthia, FDA/CVM; Grim, Christopher, FDA/CFSA

Plain Language Synopsis: Criteria called epidemiological cutoff values (ECVs) are needed for standard antimicrobial susceptibility testing to determine if bacterial isolates have developed antibiotic resistance. To create ECVs for the pathogen *Aeromonas hydrophila*, we tested the susceptibility of 104 *A. hydrophila* isolates with standard minimal inhibitory concentration and zone of inhibition testing against eight antibiotics. We then analyzed frequency distributions for each antibiotic to estimate a cutoff value. An ECV was estimated for six of the eight antibiotics tested. ECVs combined with the standard test methods allow for effective surveillance of antibiotic resistance, which promotes judicious use of antibiotics that farmers need for managing the health of their fish.

Abstract:

Antimicrobial resistance is a major public health issue that has created concerns about the use of antibiotics in aquaculture. Therefore, laboratories need standard methods and criteria called epidemiological cutoff values (ECVs, a.k.a. ECOFFs) to monitor for the development of resistance. ECVs are a critical part of standard susceptibility tests as they are used to interpret if an isolate has lost susceptibility to an antibiotic. To create ECVs for the pathogen *Aeromonas hydrophila*, we gathered 286 isolates from various fish health laboratories and confirmed the isolates identity with *rpoD* and/or *gyrB* gene sequencing. One hundred four isolates were confirmed as *A. hydrophila*. Using Clinical Laboratory Standard Institute (CLSI) guidelines, we tested the

susceptibility of these isolates with standard minimal inhibitory concentration (MIC) and zone of inhibition (ZOI) testing against eight antibiotics: erythromycin, florfenicol, gentamicin, oxytetracycline, enrofloxacin, oxolinic acid, ormetoprim / sulfadimethoxine, and trimethoprim / sulfamethoxazole. We then analyzed frequency distributions for each antibiotic to estimate a cutoff value that separates the wild-type isolates without resistance from the non-wild-type isolates that have developed resistance. We determined a cutoff value for six of the eight antibiotics tested. No ECV was estimated for the erythromycin ZOI due to excessive intra-laboratory variation. ECVs were not estimated for the two potentiated sulfonamides since the potentiator could mask sulfonamide resistance. The ECVs proposed from this study are being reviewed by CLSI to be included in a guideline for standard testing of aquatic bacteria. If approved, the ECVs will be included in the next revision of the guideline. Standard test methods and interpretive criteria allow for effective surveillance of antibiotic resistance, promoting judicious use of antibiotics that farmers need for managing the health of their fish.

9. **The Role of Core 3 β 3-N-Acetylglucosaminyltransferase in Colorectal Cancer**

Authors: Su-Ryun Kim, FDA/CDER; Guozhang Zou, FDA/CDER; Tongzhong Ju, FDA/CDER

Plain Language Synopsis: The O-linked glycans on glycoproteins play important roles in many biological processes. Current ongoing research will address the importance of the link between suppression of beta3GnT6 and Tn antigen in the process of o-glycan synthesis in the progression and metastasis of human colorectal cancer.

Abstract:

O-GalNAc glycosylation, also known as mucin-type O-glycosylation (O-glycosylation), which is characterized by alpha-GalNAc linked to serine, threonine or tyrosine residues in proteins, is a major type of protein glycosylation. The O-glycans on

glycoproteins play important roles in many biological processes. The common O-glycans are either Core-1, Galbeta1-3GalNAc-alpha-R or Core-3, GlcNAcbeta1-3GalNAc-alpha-R based structures. Core-1 O-glycans are the most predominant ones found in all animal cells, while Core-3 O-glycans appear to be restricted in the epithelial cells from the gastrointestinal tract. Notably, the Core-3 O-glycans were reported to play significant suppressive roles in colorectal tumor biology. But the mechanisms underlying Core-3 O-glycans' tumor suppression are not well understood. Core-3 N-acetylglucosaminyltransferase gene (C3GnT, beta3GnT6) encodes the enzyme responsible for the synthesis of Core-3 O-glycans. It is not known how 3GnT6 in intestinal epithelial cells is transcriptionally regulated, and what biochemical properties the enzyme possesses. Furthermore, existing cell lines do not express beta3GnT6. Therefore, we firstly established cell lines with the expression of beta3GnT6 and performed characterizations. Notably, ectopic overexpression of 3GnT6 eliminated the expression of Tn antigens in the Cosmc-deficient cells and led to synthesise of Core-3 O-glycans as evidenced by mass spectrometry (MS) analysis data. Our ongoing studies will address the link between the suppression of beta3GnT6 and the progression and metastasis of human colorectal carcinoma. Overall, this study will lead to our better understanding of important role of 3GnT6 in colon cancer, and the development of potential therapeutics.

10. **BSL-2 animal pregnancy model of Ebola infection to test therapeutics in high-risk groups**

Authors: Lewkowicz, Aaron, FDA/CDER/OBP/IDI COE; McWilliams, Ian, FDA/CDER/OBP; Manangeswaran, Mohanraj, FDA/CDER/OBP; Verthelyi, Daniela, FDA/CDER/OBP

Plain Language Synopsis: Evidence suggest that the uterus, placenta and fetus in the pregnant patient are particularly susceptible to Ebola infection and the virus may not be cleared as effectively by some candidate

therapeutics. We developed an animal model to test the efficacy of therapeutics that target the Ebola glycoprotein in pregnancy.

Abstract:

Ebola virus (EBOV), of the filoviridae family, is a highly contagious pathogen (BSL4) that causes Hemorrhagic Fever and has a fatality rate ranging from 50-100%. The virus causes great devastation in stricken communities and international concern with its ease of communicability, short incubation period, and high fatality rates. Filovirus infections during pregnancy have been associated with preterm labor and perinatal death. During the 2013 Ebola outbreak, cases were reported of pregnant women who cleared EBOV Disease but delivered stillbirths featuring high levels of Ebola RNA. This suggests the need to establish whether therapeutics targeting EBOV are safe and effective during pregnancy. Most therapeutics currently being tested for EBOV target the glycoprotein that determines its tissue tropism. To develop a model to assess treatments of EBOV during pregnancy, we used rVSV-ZEBOV-GP, a BSL2 safe recombinant pseudotype Vesicular Stomatitis Virus that expresses the Zaire Ebola glycoprotein. Initial in vitro experiments demonstrate that the pseudovirus rVSV-EBOV-GP can infect and replicate in human trophoblast cell line, HTR-8, modeling the placental tropism of EBOV. To establish an EBOV glycoprotein driven infection model during pregnancy, we inoculated 6-8 week old C57BL/6J pregnant mice with rVSV-EBOV-GP. Adult mice proved resistant to infection even when pregnant; however, administration of a single dose of anti-IFNAR1 antibody that induced a transient low-grade immunosuppression 24h prior to infection rendered them susceptible. In pregnant mice challenged with rVSV-ZEBOV-GP late in gestation (E15-17), replicating virus was found only in the uterus; however, those challenged at a mid-gestation (~E10) had live virus in the uterus, placenta and fetuses. This model will provide product developers and regulators with a platform to evaluate candidate Ebola therapeutics for pregnant women at different

stages of gestation.

11. Characterization of syndecan I (CD138) positive T cells in systemic lupus erythematosus

Authors: Lunhua, Liu, FDA/CBER/OVRR/DBPAP; Kazuyo Takeda, FDA/CBER/OVRR/DVP; Mustafa, Akkoyunlu, FDA/CBER/OVRR/DBPAP

Plain Language Synopsis: We identified a new T cell subset expressing the molecule CD138 in lupus prone-MRL/Lpr mouse. Compared to CD138- cells, CD138+ T cells were impaired in their ability to stimulate B cells. Adoptively transferred CD138+, but not CD138- cells slowed down the progression of disease in young recipient MRL/Lpr mice.

Abstract:

Abnormally high levels of syndecan I (CD138), a member of the heparan-sulfate proteoglycan family, have been reported in systemic lupus erythematosus (SLE) patient sera. Moreover, their levels positively correlate with disease severity. But the origin and biological function of circulating CD138 in SLE disease remain unclear. We identified a CD138-expressing TCR+ cell population in various organs of lupus-prone MRL/lpr mice. We showed that the frequency of TCR+CD138+ cells progressively expanded with age and disease severity. Although some B cell surface molecules such as B220, BAFFR, and BCMA were also expressed on TCR+CD138+ cells, we concluded that the origin of TCR+CD138+ cells was T cells because they expressed high levels of T, but not B cell-related transcription factors. Compared to TCR+CD138- T cells, TCR+CD138+ T cells exhibited reduced proliferation and early apoptosis, slower activation kinetics, and diminished IFN γ and TNF α secretion after activation. The in vitro differentiation of TCR+CD138+ cells into Th1, Th17, and Treg cells was also impaired compared to TCR+CD138- cells. When co-cultured with lupus B cells, the ability of activated TCR+CD138+ cells to promote plasma cell formation was less than those of TCR+CD138- cells. Finally, adoptively

transferred TCR+CD138+ T cells slowed down the disease progression in recipient young MRL/lpr mice. We hypothesize that the TCR+CD138+ T cells may be modulating lupus progression, possibly by shedding CD138 into circulation. Future experiments will focus on CD138 cleavage mechanisms and the biological activity of soluble CD138.

12. Computational Drug Repositioning for Rare and Neglected diseases at NCTR

Authors: Zhichao Liu, FDA/NCTR; Liyuan Zhu, FDA/NCTR; Hong Fang, FDA/NCTR; Weida Tong, FDA/NCTR

Plain Language Synopsis: Computational solutions for rare and neglected disease therapy development

Abstract:

Rare and neglected diseases affect a small proportion of the population but are severe and life-threatening. There is a tremendous unmet need for treatment development for rare and neglected diseases. Unlike a de novo drug discovery paradigm, drug repositioning provides a quicker, safer, and more affordable approach for therapy development. The conventional drug repositioning strategies are mainly based on close clinical observation and are serendipitous in nature. Here, we provide computational solutions for systematically exploring drug repositioning opportunities for rare and neglected disease treatment development. We propose the key bioinformatics steps essential for discovering valuable repositioning methods. The proposed steps (repurposing with a purpose, repurposing with a strategy and repurposing with confidence) are aimed at providing a standardized repurposing pipelines. We will elaborate on and exemplify our proposed in silico approaches including (1) reuse of ontology drugs for rare disease treatment development; (2) AI-based text-mining for rare and neglected disease information retrieval; (3) translation of novel genetic findings for promoting rare and neglected disease therapy. The proposed computational solutions could be easily migrated for other regulatory application.

13. Advancing the regulatory science of 'omics tests via crowdsourcing on the precisionFDA platform

Authors: Johanson, Elaine, FDA/OC; Bandler, Ruth, FDA/OC; Tezak, Zivana, FDA/CDRH; Sichtig, Heike, FDA/CDRH; Yan, Yi, FDA/CDRH; Strain, Errol, FDA/CFSAN; Didion, John, DNAnexus; Maier, Ezekiel, Booz Allen Hamilton

Plain Language Synopsis: TheprecisionFDA platform, a secure cloud-based collaborative environment, is advancing the regulatory science of 'omics tests through crowdsourcing. Six community challenges completed on precisionFDA have generated a total of 425 responses from 176 participants. These challenges have enabled the evaluation of novel algorithms, and the development of best practices.

Abstract:

The rapid advancement of next-generation sequencing (NGS) technology has fueled innovation of 'omics-based diagnostic tests for guiding more individualized care, known as precision medicine. However, the safety and efficacy of precision medicine treatments must be ensured, requiring FDA to address the challenge of understanding the strengths and limitations of 'omics-based diagnostic tests.

To enable advancement of precision medicine regulatory science, FDA developed the precisionFDA platform, a secure cloud-based collaborative environment. This platform provides scalable computation and data storage, access to over 40 terabytes of reference data, and shared spaces for collaboration. Since its public launch on December 15, 2015, precisionFDA has gained over 3,000 community members, representing NGS instrument manufacturers and test providers, standards-making bodies, pharmaceutical & biotechnology companies, healthcare providers, academic medical centers, research consortia, and government agencies. precisionFDA engages the public to advance regulatory science through crowdsourcing challenges, soliciting

voluntary contributions from a group of individuals.

Six community challenges have been completed on precisionFDA, which have generated a total of 425 responses from 176 participants. The first two challenges, the Consistency and Truth challenges, assessed the reproducibility and accuracy of bioinformatics software pipelines for identifying genetic variants. The Hidden Treasures – Warm Up challenge evaluated variant calling pipelines on a targeted set of in silico injected variants. These challenges included the evaluation of a pipeline that uses deep neural networks to identify variants and the development of best practices for benchmarking variant calls. The most recent challenges have expanded the focus and impact of precisionFDA challenges. The CFSAN Pathogen Detection Challenge evaluated bioinformatics pipelines for accurate and rapid detection of foodborne pathogens in metagenomics samples. The CDRH ID-NGS Diagnostics Biothreat Challenge addressed the issue of early detection during pathogen outbreaks by evaluating algorithms for identifying and quantifying emerging pathogens, such as the Ebola virus, from their genomic fingerprints. Finally, in collaboration with NIH/NCI, the NCI-CPTAC Multi-omics Enabled Sample Mislabeling Correction Challenge addressed the issue of sample mislabeling, which contributes to irreproducible research results and invalid conclusions, by evaluating algorithms for accurate detection and correction of mislabeled samples using rich multi-omics datasets.

14. Geospatial Analysis of Poison Exposure Calls among Children <5 Years Old Due to Tobacco Products and State-specific Prevalence of Tobacco Product Use

Authors: Niazi, Mehran, FDA/CTP; Crosby Lynn, FDA/CTP; Persoskie, Alex, FDA/CTP

Plain Language Synopsis: In this research, the geographical distribution of poisoning exposure calls among children <5 years of age for tobacco products due to cigarettes,

e-cigarettes, snuff, cigars and chewing tobacco was described. Oklahoma, Oregon, and Wyoming had the highest rates of child-poisoning for most of the tobacco products.

Abstract:

Given regional differences in tobacco product use rates across the United States, rates of children's accidental tobacco exposure are likely to vary; yet analysis of the geographic variation in children's poisoning from tobacco products is limited. We analyzed data from the National Poison Data System (NPDS) from 2010 to 2016 to describe the geographical distribution of poisoning exposure calls among children <5 years of age due to cigarettes, e-cigarettes, snuff, cigars, and chewing tobacco as reported to poison control centers across the United States, normalized using U.S. Census population counts for children <5 years old. For e-cigarettes, given that use rates have changed dramatically since 2012, we also compared the normalized rates of exposure calls to state-specific use rates to examine whether e-cigarette use prevalence would correlate strongly with increases in state-specific exposure calls among the 50 states and DC. We found that Oklahoma, Oregon, and Wyoming had the highest rates of child-poisoning for most of the tobacco products. In 2014-15, there was a strong correlation between state-specific use rates and rates of exposure calls for e-cigarettes (n=51; Pearson r = 0.77; p<0.001); however, this correlation decreased by 27% (n=51; Pearson r = 0.50; p<0.001) in 2016. This 27% reduction in correlation may be explained by the introduction of legislation requiring child-resistant packaging for e-liquids containing nicotine or by growing consumer awareness of the poisoning risks related to nicotine. Given that NPDS is a passive surveillance system relying on voluntary reporting, caution should be exercised when interpreting the data.

15. NFAT nuclear translocation is impaired in murine neonatal B lymphocytes

Authors: Sakai, Jiro, FDA/CBER; Coleman, Adam, FDA/CBER; Akkoyunlu, Mustafa, FDA/

CBER

Plain Language Synopsis: An explanation of the neonatal vulnerability to bacterial infection

Abstract:

Introduction: Immune response to vaccines is dampened in neonates and infants compared to adults, resulting in high susceptibility to infection. B lymphocytes have a central role in humoral immunity to immunization and infection. B cell receptor (BCR) signaling mediates B cell survival, proliferation, and differentiation. T cell-independent type 2 (TI-II) antigens activate B lymphocytes through BCR. Neonatal B cells, however, are unable to respond to TI-II antigens compared to adult counterpart, partly due to suboptimal BCR activation. The transcription factor Nuclear Factor of Activated T-cells (NFAT), which is essential for antigen-specific B cell responses, is activated by BCR-mediated calcium signaling. In this study, we sought to investigate NFAT nuclear translocation in BCR-stimulated neonatal B cells to identify the mechanism negatively regulating the neonatal humoral immunity.

Methods: Splenic B cells purified from neonatal and adult mice were stimulated with BCR ligand anti-mouse IgM f(ab')₂ or the calcium influx inducer Ionomycin in in vitro assays. NFAT nuclear translocation was assessed by western blotting and confocal microscopy.

Results: BCR activation resulted in ablated nuclear translocation of NFAT in neonatal B cells independent of the differences in splenic B cell subsets between neonate and adult mice. Neither anti-IgM stimulation nor Ionomycin stimulation elicited a significantly different calcium influx profile between neonatal and adult B cells, suggesting that the factors downstream of calcium influx are likely responsible for the suppression of NFAT activation. We found that calcium-dependent phosphatase calcineurin, which regulates NFAT activation, was inhibited by highly auto-phosphorylated Ca²⁺/Calmodulin-dependent protein kinase II (CaMKII) in

neonatal B cells. Supporting a role for CaMKII mediated suppression, the CaMKII inhibitor KN-93 restored the ablated NFAT nuclear translocation.

Conclusion: Our results suggest that the auto-activated CaMKII suppresses neonatal B cell responses by inhibiting NFAT activation.

16. Evaluation of tau neuropathology and other key proteins in the olfactory bulb and correlations with hippocampus in human Alzheimer's disease

Authors: Sumit Sarkar, FDA/NCTR; James Raymick, FDA/NCTR; Bonnie Robinson, FDA/NCTR; Elvis Cuevas, FDA/NCTR Hector Rosas-Hernandez, FDA/NCTR

Plain Language Synopsis: Alzheimer's disease (AD) is one of the most debilitating neurological diseases, leading to impairments in cognitive, sensory and motor functions. Olfactory dysfunction (OD) in humans has been recognized as a potential biomarker for the early detection of AD. Thus, an attempt has been made to investigate expression of key proteins in the olfactory bulbs at different stages of AD.

Abstract:

Background: Alzheimer's disease (AD) is one of the most debilitating neurological diseases, leading to impairments in cognitive, sensory and motor functions. Olfactory dysfunction (OD) in humans has been recognized as a potential biomarker for the early detection of AD. Olfaction is a process that originates from a sensory neuron input to the olfactory bulb (OB) that is then decoded in the piriform cortex, followed by downstream stimulation of neurons in the hippocampus.

Methods: OB and hippocampus tissue samples of individuals diagnosed with different stages of AD (Braak I-II; n=4, Braak III-IV; n=10; Braak V-VI, n=9) along with their age matched controls (n=3) were investigated using Fluoro-styrylbenzene (FSB) labeling and Western Blot/ Dot Blot using phosphorylated-Tau (pTau) antibodies PHF1, CP13, AT8, TNT2, pTau.

Results: We observed an extensive number

of neuro-fibrillary tangles in the OB mostly in the glomerular layer. Most of those tangles were either ghost or flame tangles that resemble tangles in the hippocampus. The highest number of FSB positive structures were present in the Braak V-VI stages. Immunohistochemical analysis using antibodies against different epitopes of phosphorylated Tau revealed different morphological features of the neurites or tangles in the olfactory bulb. While PHF-1 displayed most of the tangles of various sizes, AT8 revealed most of the neurites and big swelling of neuronal threads of pTau-containing axons, pTau antibodies labeled most of the globose axons and pre-tangles and few ghost tangles; however, TNT-2 showed most of the thick axons present in the glomerular layer of the olfactory bulb. Western Blot and Dot Blot analysis suggested a significant increase in the level of expression (pTau, AT8, CP13, PHF-1, TNT2, and oligomeric complex, such as TOC1) in the hippocampus and OB. Specifically, there was minimal expression of phosphorylated Tau in age matched control samples; however, there were significant changes in the hippocampus as early as Braak I-II and the expression gradually increased along the higher Braak stages (V-VI). In the olfactory bulb, there were increased in the level of phosphorylated Tau in the AD individuals (Braak I-III) and V-VI; however, the amount of the increase was much lower in the olfactory bulb than seen in the hippocampus. The ubiquitin proteosomal system was activated in the hippocampus as revealed in the Dot blot/Western Blot, and a similar phenomenon has been observed in the OB. Ubiquitin immunolabeling also showed ubiquitin containing granules in different layers of OB along with few stained neurites.

Conclusion: The present results corroborate the hypothesis that olfaction may be an early marker for AD and future studies will decipher the role of olfactory dysfunction as an early detection of AD.

17. Transient Alanine Aminotransferase Increases Following Acetaminophen Treatment in Rats

Authors: Mattes, William, FDA/NCTR; Regev, Arie, Eli Lilly and Company; Church, Rachel, Institute for Drug Safety Sciences/UNC; Watkins, Paul, Institute for Drug Safety Sciences/UNC; Avigan, Mark, FDA/CDER; Mendrick, Donna, FDA/NCTR; Greenhaw, James, FDA/NCTR; Shi, Qiang, FDA/NCTR

Plain Language Synopsis: Many drugs can damage the liver. In some individuals, no liver damage occurs, and in others, liver damage recovers much faster after injury. An animal model that was developed to mimic these clinical responses is being used to explore new blood-based tests to predict such highly individualized liver responses.

Abstract:

A persistent concern in pharmaceutical development is the appropriate response to serum alanine aminotransferase (ALT) increases observed in patients given a new drug in clinical trials. While ALT increases can portend serious liver injury, it is well known that for certain drugs, such increases in clinical settings reverse with continued treatment, with no further evidence of liver injury. This well-documented phenomenon is often referred to as adaptation. A means to distinguish those ALT increases that will resolve from those that portend serious liver injury is needed. We report here a pilot animal model of such transient ALT increases. Sprague-Dawley rats were given daily oral doses of 1.0, 1.5 and 2.0 g/kg acetaminophen (APAP). Interim blood samples were collected at days 1, 3, 6 and 7 of treatment and rats were sacrificed after 8 days of treatment. Liver tissue and blood were collected for histopathology or clinical chemistry and microRNA (miRNA) profiling, respectively. Minimal to mild centrilobular necrosis was observed in terminal liver samples collected from most APAP-treated animals. Interestingly, dose-independent ALT increases with miR-122-5p and miR-192-5p elevations were seen only on day 3 in APAP-treated rats. Despite continued treatment with APAP, these increases returned to baseline by day 6. No increases in total bilirubin were seen at any point in the

study. Three miRNAs (miR-27a-5p, -326-3p, and -31a-3p) showed gradual elevations in APAP-treated rats, compared to controls. The serum levels of all three miRNAs were highest at necropsy. This study represents the first report of transient ALT increases in a rodent model and offers the possibility of discovering miRNA biomarkers of adaptation.

18. Effect of Data Analysis Methods on Functional MRI Reproducibility

Authors: Soltysik, David

Plain Language Synopsis: This study evaluated the effect of different analysis methods on the reproducibility of functional MRI (fMRI) activation metrics. The goal is to improve the precision of fMRI for clinical applications. The results showed a significant increase in fMRI reproducibility when no spatial smoothing was applied.

Abstract:

Background: CDRH continually faces new technologies to evaluate scientific evidence in support of potential diagnostic claims. One such technology is functional magnetic resonance imaging (fMRI), a type of functional brain imaging. Currently, fMRI is used to assist in presurgical planning for tumor resection or epilepsy surgery. In addition, clinical fMRI biomarkers have the potential to diagnose neurological disorders and guide and evaluate treatment.

Purpose: The purpose of this study was to evaluate the effect of different analysis methods on the reproducibility of fMRI activation metrics. Determining the analysis method that maximizes reproducibility will help clinicians improve the precision of fMRI results that they use in presurgical planning as well as improve the precision of potential fMRI-based biomarkers.

Methodology: This study used a publicly available test-retest fMRI data set in which ten subjects were scanned using five different task-based fMRI runs. The data were analyzed using different methods for motion correction, spatial smoothing, statistical regression, and thresholding. For each case,

the fMRI activation metrics of activation volume and the center of mass of activation were determined. The difference in these metrics were computed across the different data analysis strategies.

Results: To minimize the difference in the activation volume or center of mass, it was found that no spatial smoothing was optimum for two of five runs. The mean differences in activation volume and center of mass for data sets using spatial smoothing were significantly greater ($p < 0.05$) and had up to large effect sizes. To minimize the difference in activation volume, it was found that regression with restricted maximum likelihood (REML) was optimum in four of five runs. The mean difference in activation volume using other regression methods was significantly greater ($p < 0.05$) and had up to medium effect sizes.

Conclusion: To maximize the reproducibility of activation volume, fMRI data analysis methods should avoid spatial smoothing and should use regression with REML. To maximize the reproducibility of the center of mass of activation, fMRI data analysis methods should avoid spatial smoothing. These findings can help sponsors improve the precision of fMRI used in submissions.

19. Evaluation of Worldwide Clinical Trials by Gender: An FDA Perspective

Authors: Ayuso, Emily, FDA/OC/OWH; Geller, Ruth, FDA/OC/OWH; Wang, Junyang, FDA/CDER/PASE; Whyte, John, FDA/CDER/PASE; Jenkins, Marjorie, FDA/OC/OWH

Plain Language Synopsis: Together, FDA OWH and CDER studied worldwide clinical trial demographic data. The results showed an increasing number of women participating in clinical trials. FDA is using this data to support multiple projects to encourage more women and more diverse individuals to join clinical trials.

Abstract:

The US Food and Drug Administration (FDA) has undertaken efforts to promote representation of women in clinical trials.

The objectives of this research are to assess women's participation in clinical trials from a global perspective and to analyze the demographic characteristics of clinical trial participants.

FDA's Center for Drug Evaluation and Research-Professional Affairs and Stakeholder Engagement (CDER/PASE) and Office of Women's Health (OWH) collaborated to evaluate demographic data (race, ethnicity, gender, and age) of pivotal trials of New Molecular Entities (NMEs) approved in 2015-2016 by geographic location. One hundred fifty-four pivotal clinical trials supporting 66 NMEs were identified, and the research team analyzed demographic characteristics of 131,749 participants from 70 countries.

U.S. sites contributed 31% of the 131,749 study participants. On the country level, the U.S. contributed the largest number of participants and other individual countries contributed 5% or less of the total trial population. Overall, 43% (n=56,272) of the 131,747 clinical trial participants were women. Of the 40,833 U.S. participants, 49% were women as compared to 40% of the 90,914 non-U.S. participants. Similar levels of participation were seen after the exclusion of sex-specific drug indications, and by therapeutic area for U.S. and non-U.S. sites.

Clinical trials are becoming increasingly multi-national, and the increasing representation of women across countries is promising. FDA approval processes ensure that global data used in the drug approval process meets regulatory standards and that data can be generalized to the U.S. population.

20. Defining the molecular features of circulating tumor cells (CTCs)

Authors: Twomey, Julianne, FDA/CDER; Zhang, Baolin, FDA/CDER;

Plain Language Synopsis: Circulating tumor cells (CTCs) are the cells detached from the primary tumor, surviving in the blood stream, to seed the metastatic spread. In-depth molecular characterization of CTCs can identify novel targets to advance CTC

detection technology towards developing reliable biomarkers that monitor patient responses to FDA-regulated oncology drug products.

Abstract:

The accelerating number of targeted cancer therapies being approved by the Food and Drug Administration (FDA), accounting for about 35% of new molecular entities approved in 2017 alone, drives the need of predictive biomarkers that stratify individuals into sub-populations that are likely or unlikely to respond to a specific drug treatment. Circulating tumor cells (CTCs) have great potential as a "liquid biopsy" for monitoring a patient's disease progression or treatment response; being evaluated as a surrogate endpoint in more than 140 clinical trials. Recent evidence shows that CTCs are vastly heterogeneous, displaying distinct molecular and phenotypic features, posing a challenge in CTC isolation and data interpretation. An in-depth molecular characterization of CTCs can inform stakeholders regarding the development of novel CTC isolation technology toward moving CTC utility into routine clinical practice for advancing precision cancer medicine.

Here we present our recent work that identified a subpopulation of cancer stem cells (CSC) within CTCs. We developed an in vitro model of CTCs, in which a panel of breast cancer cell (BCC) lines are cultured in suspension to simulate the circulating environment in the blood stream, and cell lines in monolayer culture were used to simulate the primary tumor cells. BCCs were analyzed for viability, cancer stem cell surface markers, and gene expression and signaling pathway changes. Among the cell lines tested, the MCF7 and MDA-MB-231 cells exhibited high levels of CSC markers following suspension condition. Gene expression was analyzed to identify a molecular signature of cell surface proteins that were significantly upregulated under suspension condition. We are collaborating with Johns Hopkins to validate the protein signatures in primary CTCs from blood samples of cancer patients.

Upon confirmation, these proteins may be used as a more universal signature to identify and isolate circulating CSCs to further their use as a surrogate biomarker for monitoring treatment responses. These projects directly address CDER's research priorities to develop predictive tools that aid in evaluating drug safety and efficacy, as well as to strengthen partnerships and engage stakeholders.

21. Elucidation of the mechanism of nitinol toxicity in human fallopian tube cells

Authors: Saritas, Banu, FDA/CDRH/OIR; Wood, Steven, FDA/CDRH/OSEL

Plain Language Synopsis: Nitinol, the nickel-titanium alloy, is used in cardiovascular and reproductive stents. The nitinol-based devices inserted in fallopian tubes sometimes induce hypersensitivity responses for unknown reasons. Therefore, establishing a molecular toxicology profile of nickel in fallopian tube cells is essential to understanding the hypersensitivity reactions and developing biomarker based diagnostic tests.

Abstract:

Nitinol, the nickel-titanium alloy, is the major constituent of multiple medical devices including cardiovascular and reproductive stents. The cardiovascular devices have been used routinely with minimal adverse clinical effects; however, the nitinol-based devices inserted in female reproductive tracts (i.e., fallopian tubes) cause severe inflammatory and hypersensitivity responses. The reason that reproductive tissue reacts much differently to nitinol is not known. Therefore, establishing a molecular toxicology profile of nickel in fallopian tube cells is essential to understanding the source of hypersensitivity and developing biomarker based diagnostic tests. For this purpose, we exposed fallopian-derived ovarian cancer cells to increasing concentrations of nickel (0.25-20 mM) for 5 hours to 5 days. This mimics in vivo exposure conditions where the cells in the vicinity are exposed to higher concentrations and the internal ones are exposed to lower. We evaluated the phenotypic variations with microscopy, which showed that the nickel

toxicity has two components; it is toxic to the cells at high concentrations at short exposure times (5-20 mM, for 5 hours) but also is toxic at low concentrations at extended exposures (0.25 mM, for 5 days). With flow cytometry, we established that nickel promotes its toxicity through cellular internalization followed by cell death. The internalization of nickel is a function of exposure time and concentration with significant uptake observed at even the lowest concentration (0.25 mM) and the shortest exposure time (5 hours). This is linearly correlated to programmed cell death because the membrane flipping, studied with annexin V antibody and flow cytometry, increased with nickel internalization. Expression of fallopian lineage-specific genes was unchanged during 5h exposures; however, majority of genes including PAX8, WFDC2, CP, CLDN3, KRT7, and MSLN were significantly down regulated (75-90%) with increasing concentrations in 24-hour exposure.

Thus, the data indicate that nickel toxicity in fallopian tube cells is dependent on nickel release and that CP and PAX8 expression might serve as diagnostic biomarkers for hypersensitivity reactions.

22. Developing of a Database of Pharmacogenomics Biomarkers with Minority Groups Specific Information

Authors: Xu, Joshua, FDA/NCTR; Liu, Zhichao, FDA/NCTR; Ramamoorthy, Anuradha, FDA/CDER; Tong, Weida, FDA/NCTR; Fang, Hong, FDA/NCTR; Ning, Baitang, FDA/NCTR; Amur, Shashi, FDA/CDER

Plain Language Synopsis: Pharmacogenomics studies how the differences in peoples' genes affect the way in which their bodies respond to a drug or a biologic. This project aims to create a database of 1) pharmacogenomics information from people across multiple ethnicities and 2) distribution of such pharmacogenomics-related genetic variations among all ethnicities.

Abstract:

Numerous drug labeling contains pharmacogenomics (PGx) information to

aid prescribers in the safe and effective use of the drug. However, the pivotal and supportive trials for such PGx biomarkers are usually conducted with a predominant White study population. It is a great challenge to understand the implications of the PGx knowledge in minority groups and apply them to improve the health of such groups. The first step toward addressing this challenge is to gather information on the distribution of genetic variants in these minority groups, which will pave the way toward understanding the population-wide utility of the PGx biomarkers in minority groups. In this project, a bioinformatics approach has been developed to enhance the data collection of PGx information related to minority groups. As the first step, PGx information in minority groups was collected from drug labeling and extensive literature research. The distribution of genetic variants in minority groups was then extracted from public resources. The collected PGx data was then integrated to provide the scientific foundation for reviewers and researchers better prioritizing the PGx knowledge that is applicable to different racial/ethnic groups. A database is under development to host the information of known PGx biomarkers, such as demographics, in their pivotal and supportive trials, their related genetic information in the minority groups, and applicability assessments based on ethnic difference comparison. A web-based application will also be developed to facilitate easy query and access to the database, with links to external databases such as NCBI Entrez Gene functional annotation and FDALabel for drug labeling. This database will provide the scientific foundation for reviewers and researchers to better prioritize PGx information for minority groups and to assess the utility for clinical application of the PGx biomarkers to the minority groups.

23. Transcriptomic profiling reveals p53 as a key regulator of doxorubicin-induced cardiotoxicity

Authors: Xu, Qing, FDA/CDER; McSweeney, Keisha; FDA/CDRH, Bozza, William, FDA/

CDER; Zhang, Baolin, FDA/CDER

Plain Language Synopsis: Cardiotoxicity is a safety concern for chemotherapy agents. This project aims at identifying biomarkers that can predict cardiac risks in individual patients prior to therapy.

Abstract:

Doxorubicin is a highly effective chemotherapy that is widely prescribed for treating a variety of malignancies. Unfortunately, it causes cumulative and dose-dependent cardiotoxic side effects. As the population of cancer survivors who have been exposed to treatment continues to grow, there is increased interest in assessing the long-term cardiac effects of doxorubicin and understanding the underlying mechanisms at play. In this study, doxorubicin-induced transcriptomic changes were investigated using RNA-sequencing (RNAseq) and a cellular model composed of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Analyses of predicted upstream regulators identified the p53 protein as a key regulator of transcriptomic changes induced by doxorubicin. Clustering and pathway analyses showed that increased expression of the TNF family death receptors (DRs), including TNFR1, Fas and DR4/DR5, and enrichment of the extrinsic apoptotic pathway are significantly associated with doxorubicin-induced cardiotoxicity. Increased expression of p53 and DRs were confirmed via immunoblotting. The data pinpoints increased DR expression as an early transcriptomic indicator of cardiotoxicity. Under physiological conditions, DRs are known to transmit death signals from their cognate ligands, such as TNF-alpha, Fas ligand, and TNF-related death inducing ligand (TRAIL). We hypothesize that individuals with an elevated serum levels of TNF cytokines, which could occur under certain disease and treatment conditions, may be predictive of cardiac risks in individual patients prior to doxorubicin therapy. We continue to evaluate this novel cardiac safety biomarker through collaboration with clinical institutions.

24. Sex-Specificity in Safety of Vagus Nerve Stimulation: An Investigation on Cardiovascular and Immune Systems

Authors: Yaghouby, Farid, FDA/CDRH; Jang, Kee, FDA/CDRH; Hoang, Uyen, FDA/CDRH; Vasudevan, Srikanth, FDA/CDRH

Plain Language Synopsis: Biological sex differences in safety assessment of vagus nerve stimulation (VNS) have been primarily neglected, despite sex-specific anatomical and physiological differences. In this animal study, we investigated the off-target effects of VNS on cardiovascular and immune systems and the role of sex differences in safety assessments.

Abstract:

Introduction: Vagus nerve stimulation (VNS) has been approved by FDA for the treatment of epilepsy, depression and headache. While VNS has been reported as an effective treatment for other conditions such as rheumatoid arteritis, tinnitus and migraines, the importance of biological sex differences for off-target effects has been overlooked, despite the existence of anatomical and physiological differences between men and women. Our objective is to study the biological sex differences associated with VNS on the cardiovascular and immune systems. This is being studied using chronic rodent models implanted with wireless electrocardiogram (ECG) devices and VNS cuff implants.

Materials and Methods: Under approval by the Institutional Animal Care and Use Committee (IACUC) at FDA, male and female Lewis rats (n = 16) were implanted with a wireless physiological monitoring device for continuous ECG recording. After three weeks, each animal was implanted with a custom cuff electrode around the left cervical vagus nerve. After complete recovery, the daily stimulation protocol was triggered and continued for 8 weeks using a wireless programmable pulse generator. To characterize the immune system effects, blood samples were collected on a weekly basis and the expression of inflammatory cytokines was examined. Cardiovascular

variables and cytokine concentration levels were compared between male and female animals at different time points of the experiment to evaluate VNS effects.

Results and Discussion: Two groups of rats including treatment (n=8) and sham (n=8) were used in this study, with an equal number of male and female animals (n=4) in each group. Comprehensive analysis of ECG signals was performed to quantify cardiovascular effects. ECG features and cytokine levels were averaged for each week and compared between groups at different time points of the experiment. Gender-specific cardiovascular responses were observed for selected ECG variables. Additionally, immune system effects reflected by cytokine concentrations showed a notable increase after surgeries with slight differences between male and female rats.

Conclusions: In a cohort of male and female rats, significant differences were observed for heart rate variability. However, inflammatory biomarker assessment did not reveal significant effects differentiating between genders.

25. Creatinine Based Renal Function Formula: Can They be Used to Predict Drug Clearance?

Authors: Zhang, Yifei, FDA/CDER; Zhang, Alena, FDA/CDER; Mehta, Neha, FDA/CDER; Sherwin, Catherine, Dayton Children's Hospital, Department of Pediatrics; Liu, Xiangyu, FDA/CDER; Khurana, Mona, FDA/CDER; Wang, Jian, FDA/CDER; Wang, Yaning, FDA/CDER

Plain Language Synopsis: How well does eGFR predict drug clearance in children, if the drug is predominantly renally eliminated? With four antibiotics that are > 90% renally eliminated, our results demonstrated that the drug clearance was significantly correlated but consistently lower than the eGFR value calculated by the Schwartz equation.

Abstract:

Objectives: Clinical assessment of renal function mainly relies on calculation of

estimated glomerular filtration rate (eGFR) from serum creatinine (SCR) concentration. Our goal is to compare the accuracy of currently available eGFR equations and to evaluate their ability to predict the clearance of predominantly renally-eliminated drugs in the pediatric population.

Methods: Individual drug clearance was obtained from population PK models using NONMEM. Eight different SCR-based equations, such as Schwartz equation, were used to calculate eGFR for each individual. The obtained eGFR values were compared with the normal range of GFR for each age group. The eGFR were also compared with observed clearance of drugs that are >90% renal eliminated, including gadobutrol, gadoterate, amikacin and vancomycin. The performance of eGFR equations in predicting drug clearance was evaluated by linear regression and concordance analysis.

Results: The observed clearance of all four renally-eliminated drugs in children was significantly correlated but consistently lower than the eGFR value calculated by the Schwartz equations. The Leger and Cockcroft-Gault equations over-predicted drug clearance by more than two-fold for most individuals. The Schwartz equations also over-predicted drug clearance, but to a less extent. Further, individual eGFR calculated by the Modified Schwartz equation ($k=0.413$) had multiple outliers that dramatically exceeded the upper limit of the normal range of GFR (mean + 2SD). However, drug clearance did not show a significant difference from other individuals. Those subjects typically have SCR < 0.3 mg/dL and relatively low body weight and body surface area.

Conclusions: The Schwartz equation results in a significant proportion of subjects that are above the normal GFR range in pediatrics. Using the bedside creatinine-based formula over-predicts clearance for the tested renally-eliminated drugs. Caution should be taken if applying the calculated eGFR to derive the dose of renally eliminated drugs in children with low serum creatinine levels.

26. Identification, genetic characterization, and validation of diverse HIV viruses collected from Cameroon for HIV panel development

Authors: Zhao, Jiangqin, FDA/CBER; Lee, Sherwin, FDA/CBER; Huang, Hanxia, FDA/CBER; Ragupathy, Viswanath, FDA/CBER; Biswas, Santanu, FDA/CBER; Mbondji, Christelle, FDA/CBER; Wang, Xue, FDA/CBER; Hewlett, Indira, FDA/CBER.

Plain Language Synopsis: The continued global spread of HIV diversity poses significant challenges to HIV prevention. Dynamic evolution of emerging variants in Cameroon could potentially impact the epidemic globally in the future. Many HIV positive plasma samples from this region were collected and characterized in CBER Lab for HIV reference panel studies.

Abstract:

Background: The continued global spread and evolution of HIV pose significant challenges to diagnostic and vaccine strategies. The dynamic evolution of emerging variants in Cameroon is demonstrated by the prevalence of all known subtypes and circulating recombinant forms (CRFs) in this region. Emergence of new variants could potentially impact the epidemic in this region and globally in the future. Recent studies indicate that HIV-1 subtype should be considered for new HIV therapeutics, prevention modalities, and vaccines. We obtained HIV positive plasma and viruses from this region for characterization and identification of high divergent HIV strains. The strains provide valuable reference reagents for CBER to develop validation panels for detection and quantitation of HIV variants.

Goal: To identify and characterize genetically diverse HIV viruses and positive plasma for development of globally diverse and dynamic CBER validation and reference panels.

Methods and Results: A total of 163 viral strains were cultured in our laboratory using HIV positive plasma or PBMC (viruses were isolated from patient PBMC) to high-titer/high-volume using donor PBMCs. A total of 922 viruses were cultured and supernatants

were harvested at different time points. Initially, 101 out of 922 viruses (59 strains) covering the period from 2005 to 2011, were characterized and categorized by genotype, recombinant forms, and time point of harvest. Results showed that those viruses represented very high viral load (VL), p24 values, and bead-based AlphaLISA reads. The VL range was 0.36-398.9x10⁷ copies/mL, p24 value was 0.2-173.2 ng/mL and AlphaLISA was 0.2-1134 ng/mL. Twelve HIV-1 near full-length genomic sequences were identified and analyzed using bioinformatics tools. The phylogenetic analysis demonstrated that the most common recombinants were CRF02_AG or CRF02 containing unique recombinant forms (URFs) including CRF02+CRF06, CRF02+F2, and CRF02+G, six of these viruses contain CRF02_AG clusters. There were four pure sub/subtypes (F2, G, and D), five CRFs (CRF02, CRF06, and CRF22), and two URFs.

Summary: Current studies provide reference reagents of highly diverse HIV strains that have been extensively characterized using viral load, p24 antigen values and sequencing data for a variety of subtypes/CRFs/URFs. These reagents and sequence data reflect the current dynamic and complex HIV epidemic in Cameroon and future global epidemics and will be used for development of CBER validation and HIV reference panels.

Poster Session 1

Topic: Advanced Technology (Day 1, A.M.)

27. Multi-Domain Simulation Framework for Evaluating Performance of Photoacoustic Breast Imaging Systems

Authors: Akhlaghi, Nima, FDA/CDRH/OSEL/DIDSR, Vogt, William C., FDA/CDRH/OSEL/DBP, Wear, Keith A., FDA/CDRH/OSEL/DAM, Pfefer, Joshua, FDA/CDRH/OSEL/DBP, Garra, Brian S., FDA/CDRH/OSEL/DIDSR,

Plain Language Synopsis: Photoacoustic imaging is a promising emerging modality for breast cancer detection, but the underlying light and sound transport mechanisms are not sufficiently understood. To address this knowledge gap, we developed and validated an integrated optical-acoustic simulation modeling tool to quantitatively evaluate how system design parameters and tissue properties impact performance.

Abstract:

Breast cancer is the second leading cause of cancer-related death in American women. Photoacoustic Imaging (PAI) is a noninvasive hybrid imaging modality that combines the high contrast of optics with the deep penetration and high resolution of ultrasound. PAI is being studied for use in breast lesion detection and classification through multispectral detection of vasculature and oximetry mapping. Despite recent advances in system development, the understanding of underlying optical and acoustical transport phenomena is still limited. Current performance assessment approaches (e.g. tissue-mimicking phantoms, animal studies, and clinical trials) are invaluable, but each approach requires trade-offs between ability to predict real-world performance and study complexity/cost. Computational modeling is a powerful, inexpensive alternative to benchtop experiments and may provide faster and more flexible ways to evaluate key performance issues of PAI devices. We developed a dual-domain computational modeling framework by combining a previously developed 3D Monte Carlo model of tissue light transport with an acoustic wave propagation model implemented in k-Wave, an open-source MATLAB toolbox. The modeling framework was validated against experimental image

data acquired with the custom PAI system, then used to investigate the effects of key system design parameters on performance (i.e., image quality, resolution, and depth of penetration). Specific design parameters included optical parameters (laser beam geometry, wavelength) and ultrasound parameters (detector geometry and frequency response). Results demonstrated that the modeling framework can accurately predict spatial resolution and penetration depth measured with the custom PAI system. Parametric studies indicated that optical beam geometry is a critical design consideration, with large elliptical beams offering better image uniformity than circular or small elliptical beams. Ultrasound detector center frequency and bandwidth were found to significantly affect spatial resolution and signal-to-noise ratio as functions of depth. These results demonstrate the utility of dual-domain computational tools for improving understanding of underlying PAI mechanisms and provide insight into device design consequences. Thus, these techniques will make a major impact as the field progresses. The availability of computational modeling tools will enable device optimization, streamline regulatory evaluation, and accelerate patient access to innovative PAI medical devices.

28. The next Wi-Fi revolution: non-contact respiratory rate estimation using Wi-Fi signals

Authors: Al-Kalaa, Mohamad Omar, FDA/CDRH/OSEL; Fujimoto, Kyoko, FDA/CDRH/OSEL

Plain Language Synopsis: Wi-Fi is a wireless technology used worldwide in environments like homes, healthcare facilities, shopping malls, etc. Recent advances in hardware and signal processing allow novel uses of Wi-Fi like tracking, fall detection, and vital signs monitoring. This work describes an experimental platform for respiratory rate estimation using commercial Wi-Fi routers.

Abstract:

The use of wireless technology like Wi-Fi and

Bluetooth is prevalent in many healthcare applications. However, wireless signals are primarily used for data communication that enables transfer of medical data and alarms that inform the patient and care-givers. Other uses include delivering electric power to batteryless implants. Recent innovations in non-contact vital signs monitoring using radiofrequency signals promise to extend the usability of ubiquitous wireless infrastructure like Wi-Fi and LTE to deliver non-invasive estimation of a patient's respiratory rate and heart rate. The literature includes reports on techniques for non-contact monitoring like Doppler radar, Wi-Fi channel state information, wireless channel impulse response, and time reversal of wireless signals. In addition to vital signs estimation, these techniques can be used for localization, tracking, fall detection, and sleep monitoring.

This research aims to address regulatory science questions relevant to the design and evaluation of medical devices that use wireless signals for non-contact vital signs estimation. To do so, the Electromagnetics and Wireless Technology laboratory at CRDH/OSEL initiated the development of an experimental platform for respiratory rate estimation using commercial Wi-Fi routers. The electromagnetic waves emitted by a Wi-Fi device undergo varying reflections due to the minute changes in the environment caused by the human chest movement when breathing. Therefore, a Wi-Fi receiver can detect these changes with custom software and use signal processing to estimate the patient's respiratory rate. This technology requires only a software upgrade to commercially available Wi-Fi routers. Accordingly, the scale of its deployment opportunity is massive given the widespread use of Wi-Fi.

This technology can enable beneficial applications like the detection of obstructive sleep apnea, non-contact sleep monitoring, and monitoring the wellness of infants and elderly patients. Preliminary results indicate that there are several challenges that impact its performance, including the presence of multiple subjects, whether in the same room

or in nearby rooms, large movements in the environment, and wireless coexistence with other wireless medical and non-medical systems.

29. Taking the laboratory to the field: Investigating the use of field-deployable analytical instruments at the International Mail Facilities to screen packages.

Authors: Falconer, Travis, FDA/ORR; Kern, Sara, FDA/ORR; Lanzarotta, Adam, FDA/ORR; Thatcher, Michael, FDA/ORR

Plain Language Synopsis: This poster summarizes the work being accomplished with analytical instruments deployed in the field at U.S. ports of entry to increase the number of packages screened entering the country and reduce the volume of illicit drugs and pharmaceuticals entering the U.S. supply chain.

Abstract:

In 2017, the FDA Commissioner challenged the agency to increase the number of packages screened at the International Mail Facilities (IMFs) and Express Courier Hubs (ECHs) from 15,000 to 100,000 per year. As part of this effort, the Forensic Chemistry Center began evaluating several field-deployable analytical instruments to support the Import CSOs and OCI Import Agents at these locations. These devices would provide field personnel with the ability to visually and chemically analyze products containing illicit medications, foreign unapproved products, and counterfeit pharmaceutical products, including opioid dosage forms. A recommendation was made to establish IMF beta test sites to incorporate selected devices into the field. These sites would be used continuously to evaluate and test methods and instrumentation prior to being transitioned into routine use at all IMFs and ECHs.

To date, hand-held Raman and portable Fourier-transform infrared spectrometers and hand-held and portable mass spectrometers have been evaluated for this work. Methods were developed and validated, and the instruments were field-tested during a mail blitz operation that occurred

at multiple ports of entry. This poster will summarize the equipment selected for evaluation, the methods developed, the results of the blitz operation, and how the lessons learned will be used to implement more extensive pilot studies at the IMF beta test sites. This will include the current status of creation of instrumentation toolkits, development of training courses for CSOs and ORS chemists, and proposed changes in sample process flow at the IMFs.

30. Characterization of Display Performance in Virtual and Augmented Reality Devices Medical Applications

Authors: Beams, Ryan, FDA/DIDSR; Kim, Andrea, FDA/DIDSR; Cheng, Wei-Chung, FDA/DIDSR; Badano, Aldo, FDA/DIDSR

Plain Language Synopsis: Augmented and virtual reality devices are being explored for medical applications including therapy, training, diagnostics, and surgery. Our goal is to develop characterization methods to determine the performance of these devices to ensure their safety and effectiveness. This effort emphasizes characterizing the color and the rendering process.

Abstract:

While virtual reality (VR) and augmented reality (AR) devices are primarily being developed for consumer and entertainment applications, AR/VR devices have demonstrated potential for medical training, therapy, diagnostics, and surgery. The underlying technology of head-mounted displays (HMDs) raises new questions regarding the efficacy and quality of the devices. An HMD consists of two stages: the visualization pipeline in the rendering software and the resulting optical performance of the hardware. Both are critical for accurate examination of 3D image datasets in diagnostic or surgical procedures. One image quality aspect that spans rendering and optical hardware properties is color. In a VR environment, the color of a virtual object depends on several rendering properties including lighting, materials, physics models, and

camera geometry present in the scene. This adds complexity to the rendering of precise medical data. After pixels are rendered into the display, the optical components in the VR HMD add color-dependent magnification known as transverse chromatic aberration (TCA). Proper characterization of the entire imaging chain requires consideration of the rendering and optical performance and their effects on image quality. We addressed these challenges in two steps. First, we developed a virtual environment for characterizing the color transfer from inside the virtual scene to the VR HMDs. Second, a measurement technique is proposed as a bench test methodology for characterizing the TCA observed through various HMDs. The purpose of these tools is to help establish controlled virtual environments suitable for conducting optical measurements and for assessing performance variability in HMD devices. This work is an initial step toward color reproducibility and image consistency in medical VR and AR devices.

31. 2- and 3-Monochloropropanediol (MCPD) Esters and Glycidyl Esters: Analysis and Occurrence in Infant Formulas and Other Processed Foods

Authors: Beekman, Jessica, FDA/CFSAN; Granvogl, Michael, Technical University of Munich; MacMahon, Shaun, FDA/CFSAN

Plain Language Synopsis: Many vegetable oils undergo processing to remove components that could negatively impact product quality. However, processing can result in the formation of potentially carcinogenic contaminants. This research aims to determine the occurrence of these contaminants in infant formulas and processed foods in order to evaluate any risk to consumers.

Abstract:

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization step of

the refining process, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in refined oils and foods a potential health risk. Dietary exposures to bound 3-MCPD and glycidol from consumption of infant formulas are of particular interest because formulas are the sole or primary food source for some infants. Research efforts over the last several years have focused on the analysis of these contaminants in refined vegetable oils and complex food matrices containing these oils (including infant formulas) in an effort to estimate levels of exposure. Current research in the Center for Food Safety and Applied Nutrition (CFSAN) has focused on developing a method for the extraction and analysis of 3-MCPD and glycidyl esters in infant formula to produce occurrence data for products found on the U.S. market and worldwide. Details of the extraction methodology and liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection method will be presented, along with the results of several occurrence studies performed between 2015 and 2019, which show a wide range of 3-MCPD and glycidyl ester concentrations across a variety of infant formulas, as well as occurrence data in other processed foods.

32. Determination of aniline, p-cresidine, and 2-methoxy-5-methyl-4-nitroaniline in FD&C Red No. 40 using LC-MS/MS

Authors: Belai, Nebebech, FDA/CFSAN/OCAC; McClure, Corina, FDA/CFSAN/OCAC; and Richardson, Nicole, FDA/CFSAN/OCAC

Plain Language Synopsis: A new LC-MS/MS method was developed to determine aniline, p-cresidine, and 2-methoxy-5-methyl-4-nitroaniline in FD&C Red No. 40.

Abstract:

FD&C Red No. 40 (R40, disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid, Allura Red AC) is a color additive permitted for use in foods, drugs, and cosmetics. R40 is batch certified by FDA to ensure compliance with regulatory requirements listed in the

Code of Federal Regulations. We developed and validated a new ultra-high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) method to directly determine aniline, p-cresidine, and 2-methoxy-5-methyl-4-nitroaniline in R40 following extraction, using a modified QuEChERS (“Quick, Easy, Cheap, Effective, Rugged, and Safe”) technique. These analytes may be determined by a published labor-intensive method that includes chloroform extraction, derivatization, and analysis of the reaction products by reversed-phase high-performance liquid chromatography. The new method is more sensitive than the published method and does not use chloroform, and the ready-to-use extraction kits make our method faster and less labor intensive. The new method may be used to screen for these analytes as potential good manufacturing practice violations. We used the new method to analyze samples from certified R40 batches from domestic and foreign manufacturers that requested certification. Results for the analytes obtained by the new method will be presented.

33. Acoustic Wave Separation: An Emerging Technology in Biomanufacturing for Continuous Clarification of CHO Cell Bioreactor Material

Authors: Berilla, Erica, FDA/CDER; Hong, Jinsung, FDA/CDER; Faison, Talia, FDA/CDER; Powers, David, FDA/CDER; Angart, Phillip, FDA/CDER; Brown, Lindsey, FDA/CDER; Arden, Nilou Sarah, FDA/CDER; Agarabi, Cyrus, FDA/CDER

Plain Language Synopsis: A new technology for cell separation using acoustic (sound) waves to separate cells from culture fluid is being developed for application in biomanufacturing. We explored benefits and challenges of acoustic wave separation technology for use in biomanufacturing using a CHO cell line that produces a model monoclonal antibody.

Abstract:

Therapeutic protein and monoclonal antibody drug products are typically manufactured

in mammalian cells, the most common of which are Chinese hamster ovary (CHO) cells, cultured in bioreactors. An essential step in the manufacturing process is the initial bulk separation of the therapeutic drug from the cells and cell debris. Cell separation technologies in biomanufacturing fall primarily into two categories, centrifugation and filtration. While these technologies are widely used and have been improved and adapted to biomanufacturing processes, they can still be problematic and are not always well-suited for continuous operations. A new technology for cell separation using acoustic (sound) waves to separate cells from culture fluid is being developed for application in biomanufacturing. While acoustic wave separation (AWS) may offer benefits in biomanufacturing applications, such as reduced footprint, decreased product loss, reduced buffer use, and decreased cleaning, its use involves changes to traditional bioprocess schemes of currently approved biotechnology products on the market. Thus, to best understand benefits and challenges of AWS technology for use in biomanufacturing and potential introduction into GMP environments, we tested AWS using a CHO cell line that produces a model monoclonal antibody. We explored settings on the acoustic separator, such as power and feed flow rate, as well as bioreactor culture parameters, such as duration of run and CHO cell density. We aimed to determine when and where potential failures could occur with the system, especially with prolonged running of the instrument, very low and high flow rates, and high cell densities of $> 50 \times 10^6$ cells/mL.

34. Characterizing Human PBMC Based Reference Control Materials for Comparable and Quantitative Cytometry Measurements

Authors: Bhardwaj, Rukmini, FDA/CBER/OTAT; Wang, Lili, NIST; Mostowski, Howard, FDA/CBER/OTAT; Bauer, Steven, FDA/CBER/OTAT; Degheidy, Heba, FDA/CBER/OTAT

Plain Language Synopsis: Characterizing multiple production lots of PBMC's from different manufacturers to establish a biological reference standard for product

characterization based on CD4 and CD19 expression using Flow cytometry. Expression of CD4 and CD19 on normal blood donors was used as a comparison.

Abstract:

Background: Rapid advances of cell-based therapeutics have increased the need for high quality, robust, validated measurements for cell characterization, and flow cytometry has emerged as an important platform. However, it is challenging to address measurement assurance due to lack of adequate biological and non-biological reference materials and the complexity of the cytometer instrumentation. Therefore, this study was designed to quantify CD4 and CD19 expression in three potential biological reference preparations (PBMCs). These preparations were used to test their performance as reference biomarkers, and the level of expression of these two markers was compared to their expression on normal blood donors.

Methods: Flow cytometry requires proper controls and standards for measurement assurance. Under the flow cytometry quantitation consortium, NIST and FDA are jointly characterizing human PBMC-based cell reference materials including Veri-Cells PBMC, Cyto-Trol Control Cells, and FACSCyto PBMC for product characterization. The expression of CD4 and CD19 was evaluated using three different lots of custom made unimolar CD4 PE 1:1 and CD19 PE 1:1 monoclonal antibodies. Three lots of PBMC from three different manufacturers were obtained along with normal donor blood specimen from the NIH blood bank.

Each lot of PBMC was tested in duplicate on three different days by three operators using three different lots of antibodies. Stained cells were acquired on a FACSCanto 10. Quantibrite PE beads were used to adjust the PMT voltage for the PE channel and to transfer PMT target values between experimental days. The expression level of CD4 and CD19 in terms of mean fluorescence intensities (MFIs) was obtained. Percent CVs were calculated to

estimate the variability between operators, days, lots of reagents, and lots of PBMC cell preparations.

Results& Conclusions: The consistency/variation between different lots of PBMCs from different manufacturers was tested for their CD4 and CD19 expression. Our analysis disclosed that acceptable CVs were obtained incorporating all variabilities stated above. With fully characterized PBMC-based cell reference materials, users can choose for their application needs the most suitable reference PBMC material as their biological assay control for standardization, reagent quality control, panel characterization, and longitudinal studies across different instruments and centers.

35. Control of O-glycosylation of therapeutic proteins to improve drug safety, and quality.

Authors: Biel, Thomas, FDA/OBP; Zou, Guozhang, FDA/OBP; Ju, Tongzhong, FDA/OBP

Plain Language Synopsis: Protein therapeutics are linked to sugar molecules. Some of these sugars are known as O-glycans. The impact of O-glycans on therapeutic safety and potency remains unknown. Here, a manufacturing relevant method was developed to evaluate O-glycan's impact on therapeutic protein safety that can be used by drug manufactures.

Abstract:

Most monoclonal and bispecific antibodies, fusion proteins, and enzymes are therapeutic protein drugs produced using Chinese hamster ovary (CHO) cell lines. This cell line generates "human like" carbohydrate moieties, known as glycans, on therapeutic proteins. N-linked and O-linked glycans are carbohydrate structures attached to an asparagine residue and serine/threonine residues, respectively. N-linked glycans are known to play a crucial role in therapeutic safety and efficacy, while the impact of O-glycans on therapeutic proteins remains unknown. To investigate the effects of O-glycans on therapeutic protein potency and safety, we generated five clonally-

derived, genetically-modified CHO cell lines that can produce proteins with different and defined O-glycan structures. Immunoblot analysis confirmed the generation of 5 novel O-glyco-engineered CHO-K1 cell lines: 1) ST6GalNAc1 overexpressing cells, 2) C3GnT overexpressing cells, 3) Cosmc knockout (KO) cells with and without (4) ST6GalNAc1 or (5) C3GnT overexpression. Flow cytometry and mass spectroscopy data indicates that each O-glyco-engineered CHO cell line successfully modulated the O-glycan biosynthesis pathways in a predictive manner. Collectively, this data establishes the generation of a novel CHO cell line platform for drug manufacturers to develop therapeutic proteins with defined O-glycosylations to improve the potency, stability, safety and immunogenicity mitigation of drug products.

36. Influence of nanoscale surface roughness on the properties of surface-adsorbed fibrinogen and albumin

Authors: Boehm, Ryan, FDA/CDRH; Skoog, Shelby, FDA/CDRH; Goering, Peter, FDA/CDRH; Dair, Benita, FDA/CDRH

Plain Language Synopsis: The number of medical devices containing nanomaterials submitted to the FDA has grown in recent years. To gain scientific insight into nanomaterial-biological interactions, this study investigated how nanoscale surface roughness impacts the interactions between materials used in implants and some of the initial proteins they are exposed to following implantation.

Abstract:

Over the past decade, FDA has received a growing number of nanomaterial-related medical device submissions. These devices incorporate nanomaterials with various functionalities, ranging from antimicrobial properties to enhanced osseointegration, and present both discrete particle and nanostructured surface forms. The goal of this research project has been to assess how varied size domains of nanoengineered surfaces impact early biological responses,

such as adsorption of proteins. Deposition of surfaces with distinct nanoscale roughness was investigated. The titanium-based materials that are the subject of this study have applicability to orthopedic, dental, and cardiovascular devices.

Nanostructured coatings of these materials were deposited on a quartz crystal microbalance with dissipation (QCM-D) sensors. Physico-chemical characterization of the nanomaterials was conducted to assess the vertical and lateral scales of nanoscale surface roughness, nano-topographical morphology, and atomic composition of the materials. As expected, there was a measurable increase in projected surface area as a function of increasing nanoscale surface roughness. Additionally, contact angle measurements indicated an increase in surface hydrophilicity with increasing surface roughness.

Given that material-protein interaction is a key first step in the body's biological response to foreign materials, the adsorption properties of serum albumin and fibrinogen, two major proteins that readily come into contact with any implant surface, were measured on the nanorough surfaces. Measurements were made with QCM-D monitoring to determine the amounts of surface-adsorbed protein and the viscoelastic properties of the adsorbed protein layers. We found significant differences in the amounts of adsorbed protein and viscoelastic properties between surfaces having differing degrees of nanoscale surface roughness and also between protein types when examining binding behavior of single- and multi-protein solutions of albumin and/or fibrinogen. These data suggest that very small differences in nanoscale surface roughness may influence the properties of these adsorbed protein layers. The binding behavior of these proteins has downstream impacts on other biological processes (e.g., cell adhesion, thrombogenicity) that affect the success of implant integration into the body.

37. Usability of Augmented Reality for Surgery: A Systematic Review

Authors: Brown, Ellenor, FDA/CDRH; Fujimoto, Kyoko, FDA/CDRH; Benz, Heather, FDA/CDRH

Plain Language Synopsis: Surgeons can use augmented reality (AR) tools to plan and perform surgeries by displaying virtual medical images in the real environment. The goal of this literature review is to understand how researchers have assessed the visual, mental, and physical strains that AR device use may place on the surgeon.

Abstract:

In augmented reality (AR), virtual objects are superimposed onto a real environment. The use of AR technology in surgical applications may enhance diagnosis and planning of surgical interventions through interactions with virtual, patient-specific models, facilitate intra-operative tool navigation based on medical images registered to the patient's anatomy, and improve monitoring of patient vital signs compared to conventional surgical applications with visual displays. While the potential technological benefits are promising, safety and effectiveness questions remain regarding the visual, cognitive, and physical loads placed on the user. The purpose of this review is to describe the user assessments that have been applied to AR and related technologies for surgery, and to identify knowledge and methodology gaps for future research. For this review, 81 usability articles for surgical planning and procedures, published from 1995 to 2018, were analyzed. Technology usage is split between AR (41 articles) and virtual reality (virtual objects in a virtual environment, 40 articles), but AR has become more prevalent in the last three years (20 AR vs 11 VR). The most commonly used hardware for 3D visualization is head-mounted displays (20 articles overall, 12 in the last three years), monitors (38 and 10), and glasses (11 and 3). Sixty articles include expert surgeons. Fifty of these articles specify the number of users, while only nine include the number of women or the ages of the users. Across all analyzed articles, user

assessments addressed task performance (46 articles), user experience (42), time management (30), system performance (17), visual effects (13), task efficiency (11), test validity/reliability (9), cognition (6), and objective measures of physical load (1). These results suggest that AR technologies and HMD devices are gaining popularity in the surgical literature, which may indicate future increases in related FDA device submissions. Regarding usability, reporting of complete user demographic data is lacking, which limits understanding of the results and their generalizability. Also, the use of objective measures of cognitive and physical loads has been limited. These findings show a general lack of objective user-focused data that should be addressed in future studies.

38. Quality Evaluation of 3D Printed Inhalation Delivery Systems

Authors: Cao, Leo N.Y. , FDA/CDER; O'Connor, Thomas , FDA/CDER; Siddiqui, Akhtar , FDA/CDER; Tian, Geng , FDA/CDER; Coowanitwong,Intira , FDA/CDER; El-Shafy, Mohammed Abd , FDA/CDER; Delvadia, Renishkumar , FDA/CDER; Witzmann, Kimberly , FDA/CDER; Conti, Denise , FDA/CDRH; Coburn, James, FDA/CDRH; Prima, Matthew Di , FDA/CDER; Lee Sau (Larry), FDA/CDER; Liu Xiaofei , FDA/CDER;

Plain Language Synopsis: A solution-type MDI was used to compare the aerosol performance of 3D-printed actuators to commercial actuators from FDA-approved MDI products. Data generated using 3D-printed inhalation device components has the potential to support the scientific review of new drug applications and abbreviated new drug applications for OIDs submitted to the Agency.

Abstract:

Background: 3D printing technology has advanced to potentially enable FDA-regulated medical products to be printed by both medical product manufacturers and individual patients. To ensure consistent quality of 3D printed inhalation drug delivery systems, the impact of construction

materials, device design, and manufacturing process parameters on product quality and performance must be evaluated.

Goal: In this study, 3D printing technology was used to manufacture metered dose inhaler (MDI) devices with various design elements. These devices were then evaluated for the impact on product quality and performance. Specifically, commercial MDI devices were used as benchmarks for 3D printing with the widely used types of 3D printing technology, and with printing materials having various mechanical, chemical and biocompatible properties.

Method: Both commercial and 3D-printed MDI devices were tested by 3D imaging and micro CT scan to evaluate the spray nozzle orifice, surface roughness, and 3D internal structure. Meanwhile, the spray pattern, particle size distribution, and emitted dose were measured for in vitro drug performance evaluation. Commercial and 3D printed products were compared.

Results: The 3D-printed and commercial MDI actuators produced spray patterns with a mean ellipticity ranging from 1.038 to 1.078. Nozzle diameters for the actuator in the commercial MDI and the 3D-printed actuator (grey material, vertical orientation) are 257 μm and 267 μm , respectively. The 3D-printed actuator performed similarly to the benchmark commercial actuator in terms of cumulative particle size distribution profiles.

Conclusion: This study suggested the printed models may be useful in identifying critical quality attributes in orally inhaled drug products (OIDPs). The outcome of this study will potentially impact the current MDI device sameness recommendation for generic MDI products from a product performance perspective, and inform FDA reviewers about the potential risk areas in the 3D printing processes. This will facilitate science-based decision making on the applications for 3D-printed inhalation drug delivery systems submitted to the FDA.

39. The use of DART-HRMS for the identification of BFRs in several food contact polymers and food matrices

Authors: Paseiro-Cerrato, Rafael, FDA/CFSAN, DeJager, Lowri, FDA/CFSAN, Begley, Timothy, FDA/CFSAN.

Plain Language Synopsis: DART-HRMS was used to rapidly identify BFRs in food contact materials, food, and food simulants

Abstract:

Direct analysis in real time (DART) coupled to a high-resolution mass spectrometer (HRMS) is an ambient mass spectrometric sample introduction technique that can identify a wide variety of chemicals in few minutes. No sample treatment is required to conduct the analysis. DART-HRMS has been successfully used in the field of food contact materials (FCM), as well as food as screening technique. BFRs are a group of compounds commonly used to prevent flammability of certain industrial products, and consequently, to decrease the risk of fire. These types of compounds have been detected in FCM as well as food. The use of BFRs in FCM, and therefore as food additives, is not allowed in the United States. DART-HRMS was used to rapidly identify BFRs in FCM, food, and food simulants. The DART source temperature was 500 °C. The HRMS was set in the negative mode and the mass range was from 87 m/z to 1300 m/z. Several BFRs standards diluted in toluene and methanol (0.2 – 5 mg/L) were used to validate the method. BFRs were identified in FCA polymers, as well as in the food and food simulants. The results suggest that DART-HRMS can be used as a rapid screening technique to identify BFRs in the proposed matrices.

40. Understanding the Molecular Basis of Atypical Activity Properties of FVIII in Hemophilia A Gene Therapy

Authors: Chattopadhyay, Maitreyi, FDA/OTAT; Shestopal, Svetlana, FDA/OTAT; Sarafanov, Andrey G., FDA/OTAT; Ovanesov, Mikhail V. FDA/OTAT

Plain Language Synopsis: A discrepancy between two assays prevents reliable

measurement of FVIII activity in plasma of hemophilia A patients treated with gene therapy products. The culture conditions of cells expressing a variant of codon-optimized FVIII and /or the FVIII sequences might be the reasons for this assay discrepancy.

Abstract:

A significant discrepancy between two Factor (F) VIII activity assays--one-stage clotting (OC) and the chromogenic substrate-based (CS) assay--may result in a misdiagnosis of hemophilia A (HA) severity in patients with mild or moderate FVIII deficiency. A discrepancy between OC and CS assays was also observed in the plasma of HA patients on recombinant B-domain deleted (BDD) FVIII concentrate therapy. A typical ratio of CS/OC >1 is observed, with a degree of this discrepancy determined by the sequence of truncated B-domain "linker". Interestingly, in a recent clinical study, a gene therapy (GT) that encoded a BDD-FVIII variant with a well-characterized "SQ" linker, the assay discrepancy in patient plasma showed a reverse (CS/OC <1) of what was previously known for the purified SQ-FVIII protein (CS/OC = 1.4). In our previous study, a similar reversed assay discrepancy was observed for another BDD FVIII variant, which was found to contain new post-translational modifications (PTMs). To understand the molecular basis for CS and OC assay discrepancies in GT therapy patients, we used a lentiviral-based expression system under the conditions that were previously associated with the unusual CS/OC discrepancy. But this time we encoded a codon-optimized (CO) SQ variant of BDD FVIII. Our preliminary data show that the CS/OC FVIII activity ratio can be measured in the cell culture media, enabling us to assess culture conditions and/or FVIII sequences that may be responsible for FVIII activity assay discrepancy.

41. Mutagenicity of silver nanoparticles evaluated using whole genome sequencing in mouse lymphoma cells

Authors: Chen, Tao, FDA/NCTR; Pan, Bohu, FDA/NCTR; Kaldhone, Pravin, FDA/NCTR;

Guo, Xiaoqing, FDA/NCTR; Chen, Ying, FDA/NCTR; Yan, Jian, FDA/NCTR; Zhou, Tong, FDA/CVM; Robison, Timothy, FDA/CDER

Plain Language Synopsis: The mutagenicity of silver nanoparticles is difficult to evaluate because the Ames test, the first-line assay for gene mutation, is not suitable for testing nanoparticles. In this study, we used whole genome sequencing to evaluate the mutagenicity of silver nanoparticles and found that silver nanoparticles significantly induce mutations over the control.

Abstract:

The increasing medical and food applications of silver nanoparticles (AgNPs) raise concerns about their safety, including potential health consequences of human exposure. Our previous study found that AgNPs are negative in the Ames test due to both the inability of nanoparticles to penetrate bacterial cell walls and their excessive toxicity to bacteria. Thus, the mutagenicity of AgNPs is still not clear, although they apparently induce chromosome damage, as suggested by many previous genotoxicity studies. In this study, the researchers used whole genome sequencing (WGS) to analyze the mutagenicity of AgNPs in mouse lymphoma cells expanded from single-cell clones. The cells were treated with AgNPs, vehicle control, and 4-nitroquinolone-1-oxide (4-NQO) as the positive control. AgNPs and 4-NQO significantly increased mutation frequencies over their concurrent vehicle controls by 11% and 450% (440% and 18000% per cell replication), respectively. 4-NQO induced a high percentage of G:C > A:T and G:C > T:C mutations, while AgNPs increased G:C > T:A, G:C > C:G, G:C > A:T, and deletion mutations significantly over their concurrent control. The mutations induced by AgNPs at G:C mainly occurred at CpG sites, suggesting an oxidative mechanism, while the sequencing context of the mutations at G:C sites for 4-NQO treatment was mostly at GpG sites. The results suggest that the WGS mutation assay can sensitively detect the weak mutagenicity of AgNPs. Also, the data for mutagenicity of AgNPs can be in the

risk assessment and regulation of consumer products that contain AgNPs.

42. **Detection of peanut in legume containing food products**

Authors: Cho, Chung, FDA/CFSAN; Panda, Rakhi, FDA/CFSAN; Ivens, Katherine, FDA/CFSAN; Eischeid, Anne C., FDA/CFSAN; Garber, Eric A.E., FDA/CFSAN; MacMahon, Shaun, FDA/CFSAN; Noonan, Gregory O., FDA/CFSAN

Plain Language Synopsis: The reliable detection of peanut, a potentially deadly food allergen, in the presence of related legumes using ELISAs is very challenging due to cross-reactivities. To address this problem, orthogonal methods including xMAP technology and DNA-based technology were evaluated for their ability to detect peanut in legume-containing regulatory samples.

Abstract:

Food allergies affect 4% of adults and 8% of children, and about 0.6% of adults and 0.8% of children suffer from peanut allergies in the U.S. Each year, about 29,000 cases of anaphylaxis occur in allergic individuals, resulting in roughly 150 deaths. Peanut allergy is one of the most severe allergies and can be potentially life-threatening. Patients do not generally outgrow peanut allergy, and since there is no cure, avoidance of peanut is the only option for the allergic population. The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) mandates that manufacturers label major allergens on food labels. However, inadvertent cross-contact of allergens is still possible, threatening the health of allergic individuals. Peanuts belongs to the legume family and contain homologous proteins to those present in other legume species. In recent years, foods were recalled due the presence of peanut in legume-containing products. Current commercial ELISA methods face challenges in detecting peanut in legume-containing products due to cross-reactivity issues. In this study, the limitations of the ELISA method have been addressed by using orthogonal methods, including the multi-

analyte profiling food allergen detection assay (xMAP FADA) and a DNA-based PCR method specific for peanut chloroplast genome, to detect the presence of peanut in legume-containing food products.

43. Genomic Typing of *Cyclospora cayetanensis* via Targeted Next Generation Sequencing of Mitochondria Genomes

Authors: Cinar, Hediye (Nese), FDA/CFSAN; Gopinath, Gopal, FDA/CFSAN; Almeria, Sonia, FDA/CFSAN; Ewing, Laura, FDA/CFSAN; Durigan, Mauricio, FDA/CFSAN; Murphy, Helen, FDA/CFSAN; DaSilva, Alexandre, FDA/CFSAN.

Plain Language Synopsis: *Cyclospora cayetanensis* is a parasite responsible for large outbreaks in the US. Despite public health importance of *C. cayetanensis*, little genetic information was available due to technical difficulties. This new genomics tool will facilitate epidemiologic investigations by helping to link *C. cayetanensis* identified in clinical and food samples during outbreaks.

Abstract:

Cyclospora cayetanensis is a coccidian apicomplexan responsible for large food borne outbreaks globally, including the most recent US event affecting 2299 individuals in 2018. Only one-third of the cases could be linked to specific food exposures through epidemiologic investigations, due to the lack of molecular epidemiology tools. Hence, the development of genomic typing tools to track the source of *C. cayetanensis* contamination in foods is essential for the prevention and management of outbreaks. Our previous work, based on geographical metadata, showed that SNP profiles of multi-copy mitochondria genomes of *C. cayetanensis* exhibit significant discriminatory power. In this study we developed a very sensitive genomics workflow to obtain complete mitochondrial genome sequences for typing of *C. cayetanensis* isolates from foods and water contaminated with oocysts and from clinical stool samples. The 6274 bp *C. cayetanensis* mitochondrial genome was amplified using Illumina Ampliseq Targeted

Next Generation Sequencing technology. Genomic DNA was extracted directly from various food matrices such as fresh produce and prepared dishes spiked with known numbers of *C. cayetanensis* oocysts. Targeted sequencing libraries of the samples were prepared using the Illumina Custom Targeted Panel and sequenced using MiSeq. Sequence reads were mapped to a reference *C. cayetanensis* mitochondria genome, and analyzed using the Geneious program. This targeted sequencing approach allowed us to obtain near-complete mitochondrial genomes directly from food samples seeded with as few as five *C. cayetanensis* oocysts, the level that has been detected in contaminated food samples during recent outbreak events. Achieving this level of sensitivity tied to the collection of high resolution genome data with discriminatory power is a critical milestone in genomic typing of *Cyclospora*. This new tool will facilitate epidemiologic investigations by helping to link *C. cayetanensis* identified in clinical and food samples during outbreaks.

44. Positive control(s) for synthetic condom-lubricant compatibility testing

Authors: Lin, Alexander, UNC/Chapel Hill; Mandadi, Hyma, UMD/College Park; Herberbermann, Erich, UMD/College Park; Peiling Chen, UMD/College Park; Sarkar Das, Srilekha, FDA/CDRH/OSEL;

Plain Language Synopsis: Synthetic condoms - the first-line of defense for users with latex allergy - has no established positive control for testing compatibility with personal lubricants. This study evaluated properties of synthetic condoms in the presence of major ingredients of common commercial personal lubricants to identify the best-suited positive control.

Abstract:

Background: Mineral oil, an established positive control in condom-lubricant compatibility testing of personal lubricants with latex condoms, is inert to polyurethane and therefore, it is not an appropriate positive control for testing compatibility of polyurethane condoms with personal

lubricants. The goal of the current project is to identify positive control(s) to produce a comparable effect to that of commercial personal lubricants (PLs) on polyurethane and other synthetic condom materials.

Methods: Swelling, tensile modulus at various strain rates, and stress reduction at a constant stretch of condom materials were determined before and after absorption of major ingredients of common commercial PLs.

Results: Dimensional changes due to swelling are similar in the natural rubber and synthetic poly-isoprene condoms; however, they were different for polyurethane condoms. For tensile testing, changes in the elastic property were detectable at low strain rate in the presence of ingredients that produced vigorous swelling in the condom materials. For polyurethane samples however, percent stress reduction was prominent in the presence of ingredients which caused minimal swelling at room temperature.

Conclusions: Viscoelastic properties of natural and synthetic poly-isoprene condoms are similar in the presence of most lubricant ingredients. Extent of property changes, including the effect of mineral oil on latex, can be modified by percent composition of ingredients, and sensitivity of detection can be prominent at lower strain rates. Certain ingredients can be a precursor of prominent stress reduction in polyurethane condoms under constant stretch, and therefore can be considered as positive controls for polyurethane condom testing.

45. Evaluation of Ventricular Assist Device Performance using a Mock Circulatory Loop

Authors: D'Souza, Gavin, FDA/CDRH; Rinaldi, Jean, FDA/CDRH; Retta, Stephen, FDA/CDRH; Herbertson, Luke, FDA/CDRH

Plain Language Synopsis: Advancements in ventricular assist device (VAD) technologies and features require more comprehensive benchtop test methods to characterize and assess VAD flow performance. To address this need, we have developed a mock circulatory loop to simulate physiologic

conditions and assess VADs as well as other cardiovascular devices.

Abstract:

Background: Ventricular assist devices (VADs) are used to provide short- and long-term therapy for heart failure (HF) patients. Newer versions of these devices can incorporate advanced functionalities, including feedback control, retrograde flow monitoring, suction detection, and variable pulsatile flow modes, to provide enhanced patient care. The existing VAD performance test methods do not necessarily account for recent technological advances or the pathophysiologic conditions of different patient populations. A lack of comprehensive testing increases the risk of post-market device failures and serious adverse events. To mitigate this risk and improve pre-clinical bench testing, a versatile mock circulatory loop (MCL) has been developed in the Division of Applied Mechanics (CDRH/OSEL) to simulate a range of cardiovascular conditions. The objective of this study is to conduct in vitro tests for VADs using the MCL to comprehensively characterize device performance under clinically-relevant conditions.

Methods: Left heart function within the MCL is generated using a piston pump in conjunction with atrioventricular chambers and heart valves. Compliance chambers and flow-control valves are incorporated within the loop to simulate vascular compliance and resistance, respectively, allowing us to obtain physiologic pressure and flow waveforms. The inflow of the test VAD is connected to the MCL at the apex of the left ventricle and the outflow reattaches on the ascending aorta. Cardiovascular and VAD pressures and flows are simultaneously recorded using pressure and flow sensors coupled to a data acquisition system. A feedback controller interacts with the sensors and actuators to automate the MCL and VAD for robust and reproducible testing.

Results: Different heart failure conditions were simulated by varying the cardiac output, and the left ventricular, left atrial, and aortic

pressures, in the MCL. For each disease condition, multiple support modes of the VAD were tested to simulate the intended device operation. The results from these performance tests will strengthen FDA-recognized standards, such as ISO 14708-5, enabling device manufacturers to develop safer and more effective VADs. Additionally, the test methods developed in this study will enhance current regulatory practices within CDRH for evaluating the hydrodynamic performance of emerging VAD technologies.

46. Evaluation of Consumer, Near-Infrared Portable Devices for Food Applications

Authors: Ellsworth, Zachary, Univ. MD/ JIFSAN; Yakes, Betsy Jean, FDA/CFSAN

Plain Language Synopsis: In the future, the public may have questions about the effectiveness of consumer devices. Understanding these technologies will allow FDA to be prepared for these questions and to determine if these devices are efficacious and could play a role in food safety evaluation for FDA investigators/scientists.

Abstract:

Recent advances in the miniaturization of existing technologies have allowed the development of pocket-sized, consumer-marketed spectrometers. These spectrometers connect wirelessly to smartphones and are operated through applications downloaded onto the smartphone. The producers of these devices capitalize on their small size and wireless connectivity to market the spectrometers to consumers as a quick and easy way to verify food integrity. This includes determining the freshness of meat and fish, sweetness of fruit, and potentially even food adulteration. FDA must understand this technology and be prepared if the public has questions regarding efficacy. Additionally, these hand-held devices may hold promise for use in food safety and field screening applications. To fully vet the efficacy of this technology, research is needed to understand the advantages and limitations of the miniaturized analytical instruments. Our initial investigations are focused on

marine oil dietary supplements, seafood freshness, milk powder, and olive oil. This poster will provide an overview the project and elucidate the current capabilities of the devices.

47. Test methods for predicting gold nanorod photodamage at 'tissue-safe' laser exposures during photoacoustic imaging

Authors: Fales, Andrew, FDA/CDRH; Vogt, William, FDA/CDRH; Wear, Keith, FDA/CDRH; Ilev, Ilko, FDA/CDRH; Pfefer, Joshua, FDA/CDRH

Plain Language Synopsis: Gold nanorods are one of the most commonly used contrast agents in nanobiophotonics. However, nanorod photostability under nanosecond-duration pulsed laser excitation used by many optical diagnostic and therapeutic modalities raises major concerns regarding safety and effectiveness. Phantom-based test methods were developed to characterize nanorod damage during multispectral photoacoustic imaging.

Abstract:

Photoacoustic imaging (PAI) is a rapidly emerging technology with broad clinical applications, including vascular imaging, tissue oximetry, and cancer detection. Gold nanorods (GNRs) are a commonly investigated PAI contrast agent due to their strong, narrowband plasmon resonance peak. PAI has also been proposed as a preclinical imaging tool for evaluating safety and effectiveness of gold nanoparticles intended as diagnostic or therapeutic drugs. However, GNRs can undergo reshaping when exposed to nanosecond-duration laser pulses used by PAI devices, which may degrade GNR detectability and modify in vivo biotransport. Our goal was to determine the onset of GNR damage in turbid media and how such damage may impact PAI device performance. We developed an intralipid-based turbid phantom with channels containing solutions of 45/10-nm length/width GNRs at depths of 4 to 15 mm. The phantom was exposed to 5-ns pulses at radiant exposures up to 30 mJ/cm² (near standardized laser

safety limits) and imaged with a custom multispectral PAI system. Damage-induced changes in PAI signal – a blue shift in the absorption spectrum and a decrease in signal intensity – were observed for channels up to 12.5 mm deep. Particle damage was produced for radiant exposures as low as 4 mJ/cm² (~8× below laser safety limits) at a depth of 4 mm, due to scattering-induced fluence enhancement in superficial regions. Simulations of optical fluence within the phantom indicated that fluences above the GNR damage threshold produced strong PAI signal decreases within 1-5 pulses, while fluences near the threshold produced more gradual reductions over 1000 pulses. Overall, changes in PAI signals due to GNR damage may occur at very low exposure levels (including levels far below standard safety limits) and cause rapid and significant impacts in superficial tissue regions. However, damage effects in deeper regions will likely be limited. The developed test methods and study results have contributed to improved regulatory science knowledge that will facilitate the review of emerging nanoparticle products and help inform the design of PAI devices when combined with GNRs for clinical applications or preclinical development.

48. FDALabel: Database on the Amazon Cloud for Drug Labeling information

Authors: Fang, Hong, FDA/NCTR; Turner, Steven FDA/NCTR; Meehan, Joe FDA/NCTR; Harris, Stephen, FDA/NCTR; Yang, Junshuang, FDA/NCTR; Zhou, Guangxu, FDA/NCTR; Ingl, Taylor, FDA/NCTR; Liu, Zhichao, FDA/NCTR; Joshua, Xu, FDA/NCTR; Wu, Leihong, FDA/NCTR; Mehta, Darsha

Plain Language Synopsis: FDA/NCTR developed a bioinformatics tool and database - FDALabel. The tool is made available on Amazon Cloud (open to the public) and allows reviewers, regulatory agencies, and scientists to access the most up-to-date drug labeling information (including human prescription drug and biological products) to promote translational medicine and public health.

Abstract:

FDA's Structured Product Labeling (SPL) archive, which stores drug labeling documents submitted by manufacturers, contains labeling information including product indications, dosing recommendations, contraindications, drug interactions, warnings and precautions, adverse reactions, and information for patients to help ensure the safe and effective use of the product. The continual increase in the number of labeling documents and large amount of data contained in these documents necessitates an advanced bioinformatics tool with powerful drug data management and search capabilities. We developed the FDALabel database as a web-based application, containing over 100,000 drug labeling documents, from FDA's SPL archive. FDALabel allows the public to perform customizable (any combination of sections, document types, and other information), full-text searches of product labeling on a relational Oracle database. A new version of FDALabel (v2.3), available at Amazon Cloud: <https://nctr-crs.fda.gov/fdalabel/>, was developed to search human prescription drug and biological product labeling and human over-the-counter (OTC) drug labeling. To demonstrate the FDALabel database's utility, we selected study cases including a pharmacogenomics study for Precision Medicine and an ADR (Adverse Drug Reaction) study that applied Medical Dictionary for Regulatory Activities (MedDRA) standard terminologies. We identified 261 drugs with 362 drug-biomarker pairs across different therapeutic areas, such as oncology (140), psychiatry (35), and infectious diseases (35). We also found severe ADRs were prevalent in MedDRA System Organ Classes, such as nervous system disorders, psychiatric disorders, and cardiac disorders. The FDALabel database search tool offers the public, researchers, and regulatory reviewers an efficient and user-friendly means to access and search the large amount of information on drug labeling. An Amazon Cloud version of FDALabel (v 2.3) supports and promotes

translational medicine and public health by employing advanced computer technologies to deliver end-users a reliable, effective, and efficient search tool.

49. **A Rapid, Univariate Near-Infrared Spectroscopy Method to Determine Moisture Content in Olive Oils**

Authors: Fardin Kia, Ali Reza, FDA/CFSAN; Karunathilaka, Sanjeewa, FDA/CFSAN; Yakes, Betsy Jean, FDA/CFSAN; Mossoba, Magdi, FDA/CFSAN

Plain Language Synopsis: A novel near-infrared spectroscopic procedure was developed to rapidly (<1 minute) quantify the moisture content in authenticated grades of olive oils and U.S. retail olive oil products. This proposed procedure provides a unique strategy to investigate the potential correlation between moisture content and the quality of the olive oil.

Abstract:

Worldwide, extra virgin olive oil (EVOO) is one of the most valuable edible oils. Historically, the cold pressed oil, extracted from the healthy olive fruit, has been used mainly for culinary purposes due to its unique aroma and taste. International organizations and governmental agencies, such as the International Olive Council (IOC), Codex Alimentarius (CODEX), the European Union (EU) and the U.S. Department of Agriculture (USDA), have set purity and quality criteria for olive oil. One of the quality characteristics of olive oil is the level of moisture. Traditional official methods for measuring the moisture content in foods and oils, such as the Karl Fischer titration and other dry heating techniques, are time consuming and labor intensive. In the present study, we developed a novel, rapid, and univariate Fourier transform near-infrared (FT-NIR) spectroscopic procedure to measure moisture content in olive oil. The method is based on quantifying a unique water O-H combination band observed near 5280 cm^{-1} . To quantify moisture content, a quadratic regression function over the range 0.00-0.22% H₂O (w/w) was developed. Using a

limited set of authenticated olive oil samples, we found the moisture content to be 0.099-0.12% H₂O (w/w) for EVOO, 0.025-0.033% H₂O (w/w) for refined olive oils, and 0.030-0.056% H₂O (w/w) for pomace olive oils. For 88 retail products analyzed, the moisture content ranged from 0.040% to 0.14% (w/w). This method may be used by FDA to investigate the correlation between moisture content and the quality of olive oil sold in the U.S.

50. **Assessing the feasibility and challenges of in vivo wireless communication of implantable devices using computational modeling**

Authors: Fujimoto, Kyoko, FDA/CDRH; Guag, Josh, FDA/CDRH; Bassen, Howard, FDA/CDRH; Al-Kalaa, Omar FDA/CDRH

Plain Language Synopsis: A recent study introduced implant-to-implant wireless communication within the human body. This technology enables innovative implant designs; however, unanswered regulatory questions remain when such devices are submitted to FDA. The goal of this study is to assess the feasibility and challenges of this wireless technology using computational modeling.

Abstract:

Wireless communication has become pervasive and many medical devices incorporate it. For example, FDA reviews premarket submissions for active implantable medical devices that use such technology to communicate with an external device for patient data transmission and device configuration. A recent study introduced an idea of implant-to-implant wireless communication technology within the human body by performing computational modeling and animal study. It is foreseeable that this technology will permit the development of novel implantable systems that exchange data within the human body for diagnosis or treatment of health conditions. This study aims to address regulatory science questions related to the feasibility and challenges of implant-to-implant wireless communication inside of the human body using computational modeling.

Two dipole antennas in vacuum capsules were modeled as implantable communication devices (receiver antenna and transmitter antenna) in a two-compartment phantom with the electrical properties of blood and muscle. Simulations were performed in the frequency range of 0.3 GHz to 3 GHz. Two different computational modeling methods, the Finite-Difference Time-Domain method (FDTD) and the Finite Element Method (FEM) were used to confirm the antennas' behavior over the wide range of frequencies. The results from both modeling methods indicated similar interactions between the two antennas over the investigated frequencies which confirmed our modeling setup. Further assessment will include computational modeling validation with bench testing and additional simulations with a detailed anatomical human model with implantable devices at different anatomical locations within the body. These results will be presented at the poster session.

51. An Automated Process to Standardize Investigational Drug Substance Names Reported to the US Food and Drug Administration Adverse Event Reporting System

Authors: Fung, Maggie, FDA/CDER; Chang, Sherry, FDA/CDER; Digital Safety Reporting Workgroup, FDA/CDER

Plain Language Synopsis: The FDA Adverse Event Reporting System (FAERS) Product Dictionary (FPD) indexes pre- and post-market drug products to facilitate safety reporting. We describe the proposed process for automated standardization of investigational drug substance names in FPD as part of a larger initiative to modernize safety reporting of these products.

Abstract:

Background: The FDA Adverse Event Reporting System (FAERS) Product Dictionary (FPD) indexes pre- and post-market suspect drug products to facilitate retrieval of Individual Case Safety Reports (ICSRs) for internal review. Sponsors of investigational new drug (IND) applications may initially submit ICSRs using proprietary

codes to identify the IND drug substances. During IND development, sponsors submit a proposed nonproprietary name to the US Adopted Names (USAN) Council and WHO-International Nonproprietary Names (WHO-INN) Expert Group for review. The FDA Global Substance Registration System (G-SRS) incorporates approved USAN/INN into its database as preferred names, which FPD uses to update substance code names. Codes that have not established a USAN/INN are grouped under an "Investigational Product" category within FPD to be manually reappointed to their respective USAN/INN once established. The current process of manually updating IND drug substance codes in FPD is insufficient, leading to inconsistencies with coding safety reports. An automated process is necessary to standardize and track IND substance names during development.

Objective: To describe the proposed process for the automated standardization of IND substance names in support of the modernization initiative for electronic IND safety reporting in FAERS.

Methods: Substance codes under the "Investigational Product" category of FPD are periodically referenced against G-SRS to determine the establishment of their preferred names. Substances with preferred names that do not exist in FPD are manually added. Respective code names are reclassified as synonyms for preferred names so that cases reporting the codes are redirected to the preferred names. IND drug substances identified only by a code remain in the "Investigational Product" category. The proposed automation establishes a G-SRS to FPD linkage to auto-update substance code names to preferred names when available.

Results: The automated process will improve updating of IND drug substance names by eliminating manual G-SRS cross-referencing and FPD changes. The new G-SRS-to-FPD linkage will enhance accuracy and timeliness of FPD updates, making searching and review of safety reports easy and consistent.

Conclusion: The proposed process for the automated standardization of IND drug substance names is expected to positively impact pharmacovigilance by improving product coding accuracy and efficiency.

52. Hepatocyte-like Cells Derived from Human Induced Pluripotent Stem Cells Using Small Molecules: Implications of a Transcriptomic Study

Authors: Gao, Xiugong, FDA/CFSAN; Yourick, Jeffrey, FDA/CFSAN; Sprando, Robert, FDA/CFSAN

Plain Language Synopsis: Hepatocytes were differentiated from induced pluripotent stem cells using only small molecules. The resultant cells were characterized using transcriptomics and compared to primary human hepatocytes and hepatocytes differentiated using growth factors.

The results suggest that hepatocytes differentiated using small molecules are potentially useful for toxicological applications but need further maturation.

Abstract:

Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (iPSCs) hold great promise in toxicological applications, as well as in regenerative medicine. Previous hepatocyte differentiation efforts have mostly relied on the use of growth factors to recapitulate developmental signals under in vitro conditions. Recently, the use of small molecules (SMs) has emerged as an attractive tool to induce cell fate transition, due to its superiority in terms of both quality and cost. In the current study to evaluate and identify ways to improve the efficiency of using SMs for hepatocyte differentiation, HLCs were differentiated from iPSCs using a protocol that involves only SMs. Gene expression changes during the course of the SM-driven differentiation were characterized using whole genome microarrays. Transcriptomic analysis of the SM-driven differentiation defined a hepatocyte-differentiation track that identified several key genes in major stages of hepatocyte differentiation. In addition, the

HLCs derived using the SM protocol (SM-HLCs) were scored with CellNet, an online tool for quantifying how closely engineered cell populations resemble their target cell type, and compared to primary human hepatocytes (PHHs), adult liver tissue, fetal liver tissue, HLCs differentiated using growth factors (GF-HLCs), and commercially available HLCs. Similar to GF-HLCs, SM-HLCs displayed a mixed phenotype of fetal and adult hepatocytes and had relatively low expression of metabolic enzymes, transporters, and nuclear receptors compared to PHHs. Overall, the present study demonstrated the usefulness of the SM-based hepatocyte differentiation method, offered new insights into the molecular basis of hepatogenesis and associated gene regulation, and suggested further improvements in hepatocyte differentiation to obtain more mature HLCs that could be used in toxicity testing of foods, dietary supplements and cosmetics.

53. Single Laboratory Validation of the Multiplex xMAP Food Allergen Detection Assay (xMAP FADA) with Incurred Food Samples

Authors: Garber*, Eric A.E., FDA/CFSAN; Cho, Chung Y., FDA/CFSAN; Rallabhandi, Prasad, FDA/CFSAN; Nowatzke, William L., Radix BioSolutions, Georgetown, TX; Oliver, Kerry G., Radix BioSolutions, Georgetown, TX.

Plain Language Synopsis: Analytical methods help assure the accuracy of labels and safeguard food allergic consumers. However, analyte-specific methods (e.g., ELISAs) are too time-consuming when testing for multiple allergens and potentially misleading with some foods due to cross-reactivities. The xMAP Food Allergen Detection Assay provides a solution to these problems.

Abstract:

The xMAP Food Allergen Detection Assay (xMAP FADA) was developed to meet analytical needs when responding to complaints by individuals with multiple food allergies and to address potential ambiguities associated with cross-reactive proteins. A Single Laboratory Validation (SLV) was conducted to examine the reliability

of the xMAP FADA in detecting 15 analytes individually, or as part of a mixture, at ≥ 6 concentrations, in four foods. The xMAP FADA reliably detected all the analytes despite the incurred dark chocolate and incurred baked muffins displaying recoveries of 10-20% and $< 60\%$, respectively. The high reliability for recoveries less than 60% in part reflects the statistical strength of the design of the xMAP FADA. Only crustacean, egg, and milk incurred in dark chocolate were not reliably detected using the PBST buffered-detergent protocol. Following the reduced-denatured protocol, no problems were encountered detecting milk, though egg did not display a dynamic response in dark chocolate.

The ruggedness of the xMAP FADA was ascertained by the ability of novice analysts to detect food allergens in baked rice cookies. Despite one analyst losing $> 80\%$ of the beads and the count for one bead set dropping to 7, the assay displayed only a decrease in precision (increased standard deviations) and a change in the ratios between complementary antibody pairs.

54. Product quality assessment in a continuous bioprocessing approach using a perfusion WAVE bioreactor and an acoustic wave separator

Authors: Hong, Jin Sung, FDA/CDER; Berilla, Erica, FDA/CDER; Faison, Talia, FDA/CDER; Powers, David, FDA/CDER; Angart, Phillip, FDA/CDER; Brown, Lindsey, FDA/CDER; Arden, Nilou Sarah, FDA/CDER; Agarabi, Cyrus, FDA/CDER;

Plain Language Synopsis: We assessed an acoustic wave separator for bulk cell separation, which enabled effective cell clarification/filtration and product recovery. We also test unwanted side effects of this technology, i.e., effects on product quality, cell damage, and particle generation. This study provided useful information about potential product quality concerns in continuous drug substance harvest clarification.

Abstract:

Continuous bioprocessing is an important

biopharmaceutical manufacturing technique for enhancing manufacturing speed and operation flexibility, and for reducing production costs. Continuous drug substance harvest clarification is critical for successful integration of upstream (cell culture) and downstream (purification) bioprocesses. This integration must occur without decreasing process performance and product quality: it is an important potential impediment to flow at high cell densities, which can restrict working flow rates and eventually build up pressure when using filtration. In this study, we implemented a novel, continuous upstream bioprocessing approach and explored potential regulatory concerns regarding potential risks to product quality. We integrated a perfusion GE WAVE 25 bioreactor that allows prolonged cell culture exposure to a PALL Cadence acoustic wave separator (AWS), thus providing continuous cell clarification of harvested material. Using the perfusion WAVE bioreactor, we achieved representative operating conditions and batch growth kinetics. The AWS allowed effective cell clarification/filtration and product recovery. Product quality was evaluated to determine potential impacts on the monoclonal antibody from the process. Lastly, the impact of the acoustic wave on cellular damage and its effect on generation of any cell debris were tested. Overall, we believe our assessment provides useful information for the implementation of a continuous cell clarification tool and further understanding of its impact on product quality.

55. Whole genomic sequencing of Salmonella ser. Enteritidis, Salmonella ser. Typhimurium, and Salmonella ser. Heidelberg from egg and chicken sources and predicted protein structures of target genes for Salmonella detection

Authors: Hu, Lijun, FDA/CFSAN; Cao, Guojie, FDA/CFSAN; Brown, Eric, FDA/CFSAN; Allard, Marc, FDA/CFSAN; Ma, Li, OSU/DEPP; Zhang, Guodong*, FDA/CFSAN

Plain Language Synopsis: The study explored the relationships of the top 3 Salmonella serotypes on DNA sequence level and

predicted protein structures of 6 target genes (*invA*, *ttrRSBCA*, *fimA*, *phoP*, *spvC*, and *agfA*) for *Salmonella* detection. These new discoveries will greatly enhance the development of new culture and molecular detection methods for *Salmonella*.

Abstract:

Background: Whole genome sequencing (WGS) has become an indispensable tool in foodborne pathogen surveillance, outbreak investigation, and the development of new detection and identification techniques.

Purpose: The purpose of this study was to 1) provide a detailed comparative genomic analysis of *Salmonella* ser. Enteritidis (SE), *Salmonella* ser. Typhimurium (ST), and *Salmonella* ser. Heidelberg (SH) sourced from egg and chicken; 2) determine the genomic variation of 6 *Salmonella* target genes (*invA*, *ttrRSBCA*, *fimA*, *phoP*, *spvC*, and *agfA*) among these isolates and their phylogenetic relationships based on each gene; 3) predict the protein structure of the selected genes to expound the positions of the mutations discovered.

Methodology: Genomic DNA from 143 isolates of 3 top *Salmonella* serotypes sourced from eggs and chickens was extracted using the DNeasy Blood and Tissue kit, then sequenced on the Illumina MiSeq/NextSeq platform. The phylogenetic trees were generated following the FDA CFSAN SNP pipeline. The predicted protein structures for 6 target genes were created by using Phyre2 and SWISS-MODEL software.

Results: The results showed that ST was more diversified phylogenetically than SE and SH. The *spvC* gene was primarily associated with SE isolates and absent in SH isolates. The WGS data also indicated that horizontal gene transfer (HGT) occurred among different *Salmonella* serotypes and the *ttrR* gene were more conserved than other *ttr* genes. A total of 45 non-synonymous mutations were identified from target genes, *invA* (13), *ttrRSBCA* (18), *fimA* (13), *phoP* (1), *spvC* (0), and *agfA* (0), among the 143 isolates. The top two frequent non-synonymous mutations

occurred between T↔C (15 times) and A↔G (13 times). Notably, there was one non-synonymous mutations (*fimA*-Mut.6) from all SE and SH, which happened at AA 166 position Glutamine (Q)-->Stop codon (TAG), caused by the change of C-->T (496 nt position). The predicted protein structures of the target genes illustrated the positions of these non-synonymous mutations.

Conclusion: These results are contribute to the understanding of the function of these genes and the development of new culture and molecular detection methods for *Salmonella*.

56. Inducible *Staphylococcus aureus* Cas9 mediated hypermutation

Authors: Iaffaldano, Brian, FDA/CBER; Marino, Michael, FDA/CBER; Reiser, Jakob, FDA/CBER

Plain Language Synopsis: We developed a system, using a CRISPR/Cas9 variant to introduce genetic diversity at defined regions of DNA in living cells, which adds to the CRISPR-based diversification toolbox. This genetic diversity can be selected upon to improve proteins and vectors used in gene therapy.

Abstract:

The ability of catalytically inactive *Streptococcus pyogenes* Cas9 (SpCas9) proteins to precisely target specific genomic loci, while also delivering additional functional domains has been adopted to yield numerous molecular tools. For example, the commonly used SpCas9 has been repurposed to enable somatic hypermutation at endogenous target sequences by using catalytically inactive versions that do not cut DNA, but instead recruit variants of activation-induced cytidine deaminase (AID). Using such programmable hypermutators, sequence diversity can be introduced at defined genomic loci in a mammalian cells, allowing evolution of proteins and the study of gene function, which may facilitate novel gene therapy approaches involving evolved viral envelope proteins, nucleases, and base editors.

In order to expand the number of loci and breadth of experiments that may be conducted with this strategy, we developed a doxycycline-inducible hypermutator using a catalytically dead *Staphylococcus aureus* Cas9 (dSaCas9). The use of an inducible approach may allow multiple rounds of evolution to be conducted, while minimizing toxicity. To do this, lentiviral vectors were used to stably deliver sequences encoding dSaCas9, a hyperactive AID variant fused with a MS2 coat protein (MCP) domain, as well as gRNAs containing MS2 aptamer sequences that recruit MCP-AID.

As a proof of principle, we targeted four regions of the EGFP coding sequence in HEK293 cells. Loss of EGFP expression was observed in doxycycline treated cells, indicating mutagenesis activity. The EGFP sequences of unsorted cell populations were then deep sequenced. Using four guide sequences we observed the expected increase in the mutation rates of guanine and cytosine bases within EGFP. Increases in substitution frequency of approximately 20-fold were observed within a window of 600 base pairs. The average substitution frequency across the 600 bp window was approximately 1 in 1,000.

The use of SaCas9 allows for additional experiments to be designed, as SaCas9 operates orthogonally to SpCas9. For example, such Cas9 orthologues could be used in parallel to evolve functionality in a mammalian cell context. Additionally, as SaCas9 has a different PAM sequence requirement than SpCas9, it expands the range of loci that can potentially be targeted by programmable hypermutators.

57. Methodology for objective, task-based evaluation of clinical FFDM and DBT systems using an anthropomorphic breast phantom

Authors: Ikejimba, Lynda, FDA/CDRH; Salad, Jesse, Deloitte; Ghamraoui, Bahaa, FDA/CDRH; Makeev, Andrey, FDA/CDRH; Glick, Stephen, FDA/CDRH

Plain Language Synopsis: A realistic breast phantom is needed to evaluate full-

field digital mammography and digital breast tomosynthesis imaging systems. Using inkjet printing, our group created an anthropomorphic breast phantom with masses and microcalcifications. This phantom allows for objective assessment of imaging systems for both pre- and post-market use.

Abstract:

Background: Many breast phantoms currently used to evaluate imaging systems have unrealistic texture or are expensive to fabricate. Furthermore, very few allow the insertion and removal of clinical pathologies such as micro-calcifications (MCs) and masses at desired locations. There is a need for a realistic breast phantom for assessing imaging devices in both pre-market and post-market applications.

Purpose: The purpose of this work is to introduce a novel, task-based methodology to evaluate field digital mammography (FFDM) and digital breast tomosynthesis (DBT) systems using an anthropomorphic inkjet-printed 3D phantom with removable clinically relevant signals.

Methodology: The phantom was first modeled analytically as a digital, 4-cm-thick compressed breast, then physically realized in a slice-by-slice fashion using inkjet printing with iohexol-doped ink. Signals consisted of micro-calcifications (MCs) and extended masses. MCs were made by arranging individual specks of calcium hydroxyapatite into regularly spaced clusters. Masses were created by printing with a KI solution. To test the physical realism of the phantom, the effective linear attenuation coefficient was measured for the phantom and MC materials.

The phantom was imaged on commercially available FFDM and DBT systems, with typical mammographic beam conditions for each device. A similar average glandular dose was maintained across the systems. Using the images, a four alternative forced choice (4AFC) reader study was performed with human observers to interrogate signal detectability with the different modalities.

Results: The effective linear attenuation coefficients of the phantom and MCs were found to be similar to reference values. Results of the 4AFC study showed the visibility of the micro-calcifications and ranged from easy to difficult.

Conclusion: An anthropomorphic breast phantom was created using inexpensive, easily available materials. Task-based assessment was performed on clinical FFDM and DBT systems. With the above components, this work provides a methodology for assessing image quality based on realistic diagnostic tasks. The methodology has a number of possible applications, such as 1) improving assessment of safety and effectiveness in regulatory applications, 2) helping optimize system and design parameters for maximizing performance, and 3) quality control testing to assure maximum clinical performance over time.

58. xMAP FADA: A multiplex method for simultaneous detection of 15 food allergens plus gluten

Authors: Ivens, Katherine O., FDA/CFSAN; Cho, Chung Y., FDA/CFSAN; Garber, Eric A.E., FDA/CFSAN

Plain Language Synopsis: The xMAP Food Allergen Detection Assay is a unique analytical platform developed to simultaneously detect 15 allergens plus gluten. Simultaneous detection of 16 target analytes offers a substantial reduction in cost of materials, labor, and time required to generate results compared to traditional antibody-based detection methods.

Abstract:

The xMAP Food Allergen Detection Assay (xMAP FADA) is a unique and powerful analytical platform for the simultaneous detection of almond, brazil nut, cashew, coconut, crustacean, egg, gluten, hazelnut, macadamia nut, milk, peanut, pine nut, pistachio, sesame, soy, and walnut in complex matrices. The assay is antibody-based, with capture antibodies conjugated to magnetic microspheres. A complex is formed between

the magnetic bead-bound capture antibody and the analyte, which is detected by a biotinylated antibody to which streptavidin-phycoerythrin binds to generate a fluorescent signal when irradiated. A strength of the xMAP is its redundancy: the use of at least two antibody capture-based assays per target analyte, except for crustacean, provides built-in confirmation of positive responses. Using multiple antibodies for each allergen permits the calculation of complimentary antibody bead set ratios, thereby allowing results to be interpreted, distinguished from cross-reactivity, and simultaneously confirmed. Recently, the xMAP FADA has been expanded and validated for the detection of sesame in both simple and complex matrices. The plug-and-play capability of xMAP FADA allows the user to add or remove analytes from the assay repertoire. The xMAP FADA is currently being used by FDA for the analysis of regulatory samples, providing a powerful analytical solution when the type and number of possible food allergens present is unknown. The ability of the assay to simultaneously detect 15 food allergens plus gluten and confirm data offers a substantial reduction in cost of materials, labor, and time required to generate results.

59. Determination of brominated fluorescein components in the color additive D&C Orange No. 5 using high-performance liquid chromatography

Authors: Lazo-Portugal, Rodrigo, FDA/OCAC; Richardson, Nicole, FDA/OCAC; McClure, Corina, FDA/OCAC; Weisz, Adrian, FDA/OCAC

Plain Language Synopsis: A new HPLC method was developed for determining brominated fluoresceins in the color additive D&C Orange No. 5.

Abstract:

D&C Orange No. 5 (05, Colour Index No. 45370:1) is a color additive allowed in mouthwashes, dentifrices, lipsticks, and externally applied drugs and cosmetics. 05 consists mainly of a mixture of 4',5'-dibromofluorescein, 2',4',5'-tribromofluorescein, and

2',4',5',7'-tetrabromofluorescein. Its manufacture involves condensation of phthalic anhydride (or acid) with two equivalents of resorcinol, followed by partial purification and bromination of the resulting fluorescein. During manufacture, mono- and other dibrominated fluorescein components may be produced and carried over into the final product, along with other impurities and some unreacted fluorescein. To ensure compliance with limiting specifications described in the Code of Federal Regulations, including those for the brominated fluorescein components, O5 is batch-certified by the U.S. Food and Drug Administration (FDA). Currently, a labor-intensive, multi-step procedure is used to determine the brominated fluoresceins in samples of the dye submitted to FDA for batch certification. The method requires streaking the dye solution on a 20 x 20 cm semi-preparative silica gel thin-layer chromatography (TLC) plate, developing and drying the plate, scraping off the color bands, extracting the colors from the silica gel, and quantifying them spectrophotometrically. The present study was aimed at developing a simpler, more modern method of determining the brominated fluoresceins in O5 through the use of high-performance liquid chromatography (HPLC). Several brominated fluoresceins were synthesized for use as reference materials and the components in O5 were quantified based on five-point calibration curves. The HPLC method was found to be significantly faster than the TLC procedure and generated less solvent waste. Most significantly, due to the better separation of the components, the method yielded more accurate results. The new HPLC method is expected to replace the TLC method for routine batch certification of O5.

60. Influences of simulated gastrointestinal environment on physicochemical properties of gold nanoparticles and their implications on intestinal epithelial permeability

Authors: Jiang, Xiumei, FDA/CFSAN *; Zhang, Xiaowei, FDA/CFSAN; Gray, Patrick, FDA/CFSAN; Zheng, Jiwen, FDA/CDRH; Croley,

Timothy, FDA/CFSAN; Fu, Peter, FDA/NCTR; Yin, Jun-Jie, FDA/CFSAN *

Plain Language Synopsis: Nanotechnology holds great promise for food, industrial, and biomedical applications. The gastrointestinal tract represents a likely route for the administration of nanoparticles, which are intentionally ingested or indirectly ingested from food-packing materials, into the human body. Thus, we investigated the influences of simulated a gastrointestinal environment on physicochemical properties of gold nanoparticles.

Abstract:

Gold nanoparticles (Au NPs) hold great promise in food, industrial, and biomedical applications due to their unique physicochemical properties. However, the influences of the gastrointestinal tract (GIT), a likely route for Au NPs administration, on the physicochemical properties of Au NPs has rarely been evaluated. Here, we investigated the influence of GIT fluids on the physicochemical properties of Au NPs (5, 50, and 100 nm) and their implications for intestinal epithelial permeability in vitro. The simulated human GIT fluids included fasted-state simulated gastric fluid (FaSSGF, pH=1.2), fasted-state simulated intestinal fluid (FaSSIF, pH=6.5) and fed-state simulated intestinal fluid (FeSSIF, pH=5.0). Au NPs incubated in FaSSGF aggregated in a time-dependent manner, while Au NPs in FaSSIF and FeSSIF had negligible changes during the 2 h incubation. Chemical catalytic activities of Au NPs towards H₂O₂ and superoxide anion were measured by ESR spectroscopy. Increased production of hydroxyl radical from H₂O₂ was observed in the presence of Au NPs incubated in FaSSGF, probably due to the low pH of the gastric fluids. Au NPs scavenged superoxide anion in a size-dependent manner and incubating 5 nm Au NPs in GIT fluids did not affect their superoxide anion scavenging activity. GIT fluid incubation of Au NPs affected the cellular uptake of Au NPs without inducing cytotoxicity in intestinal epithelial cells or disturbing their intestinal epithelial permeability.

61. Examples of Tissue Imaging of Neurotransmitters, Lipids, n-linked glycans, Drugs and Drug Metabolites in Model Systems.

Authors: Jones, E. Ellen FDA/NCTR; Schnackenberg, Laura FDA/NCTR; Sarkar, Sumit FDA/NCTR; Thorn, David FDA/NCTR; Begler, Richard FDA/NCTR

Plain Language Synopsis: Matrix assisted laser desorption ionization imaging mass spectrometry is an innovative, label-free technology that enables analysis of a variety of analytes across a tissue section. Proteins, peptides, lipids and other small molecule drugs and metabolites can be detected using this approach, and then overlaid with the tissue's histopathology. The value in imaging is the ability to have a localization component within and organ or tissue of interest to determine whether the molecule of interest may distribute differentially across a tissue section. MALDI IMS has been used to study a variety of diseases; however, its incorporation into toxicology studies is fairly new. Data from many FDA-relevant studies will be shown, including lipid and neurotransmitters in brains from a rodent Alzheimers model, n-linked glycans from an obesity model, and a drug toxicity study in a zebrafish model.

Abstract:

Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) is a label-free, evolving technology that produces 2D ion density maps representing the distribution of analyte(s) across a tissue section in relation to tissue histopathology. Although MALDI IMS was initially developed to spatially profile proteins and peptides, the variety of detectable analytes has greatly increased and includes lipids, n-linked glycans and small molecule drugs. Furthermore, the recent incorporation of high resolution instruments, such as the Fourier- transform ion cyclotron resonance (FTICR) mass spectrometer, within imaging workflows has also enabled the detection of unique and low abundance classes of analytes, such as neurotransmitters and metabolomic pathways Although this

technology has been used for some time to study different disease models, it has only recently been incorporated into toxicology studies. Determination of drug concentrations in tissue has historically been achieved using tandem-mass-spectrometry-based platforms, without laborious dissections. However, traditional MS/MS does not offer any information concerning drug localization. The value of MALDI IMS in these studies is its ability to provide images of the distribution of drugs or metabolites, which, when overlaid with histopathology, offer direct links to specific regions of interest in a tissue. Applications of MALDI IMS in a variety of different toxicological studies will be highlighted, including neurotransmitters and lipids in brains from an Alzheimer mouse model, drug and drug metabolites in a zebrafish study, n-linked glycans from a mouse obesity project, and preliminary imaging data from an ongoing mouse opioid project.

62. Analysis of pharmaceutical tablets for labeled and unlabeled active ingredients using surface enhanced Raman scattering (SERS) with handheld devices

Authors: Kimani, Martin, FDA/ORR; Lanzarotta, Adam, FDA/ORR; Batson, JaCinta, FDA/ORR

Plain Language Synopsis: Rugged, simple, and rapid identification of APIs in pharmaceutical products using surface-enhanced Raman spectroscopy (SERS)-based method.

Abstract:

The Forensic Chemistry Center (FCC) has encountered several low-dosage finished dosage tablets that were found to contain labeled and unlabeled active pharmaceutical ingredients (APIs), using established laboratory-based methods. To support Consumer Safety Officers and OCI Special Agents working at the international mail facilities (IMF) and express courier hubs (ECH), a field-friendly method to detect these APIs is needed. Unfortunately, sensitivity is the most significant limitation of Raman

handheld devices, which often precludes their use for detecting low-concentration APIs. The purpose of this study is to overcome this limitation by using surface enhanced Raman scattering spectroscopy (SERS) to screen and detect low-dose APIs. Using a handheld Raman spectrometer with an on-board matching algorithm, this method was used successfully for products containing benzodiazepines (alprazolam, diazepam, clonazepam, lorazepam and etizolam), opioids (tramadol, oxycodone, hydrocodone and fentanyl), lifestyle drugs (sildenafil, avanafil, vardenafil and tadalafil), and weight loss medications (sibutramine, phentermine HCl and other structurally similar compounds). SERS validation studies of standards revealed the lowest concentration of sildenafil in a water/colloid solution that yielded a "Pass" was 7.6µg/mL; fentanyl HCl 250ng/mL; alprazolam 1µg/mL; tramadol HCl 5µg/mL; and phentermine HCl 10µg/mL.

The use of SERS for rapid chemical identification at remote sampling sites, such as IMFs and ECHs, provides a rugged, simple, and practical method applicable to point-of-entry sampling. Preliminary results generated in this study indicate that this field-portable analytical method should be effective for package detentions or other regulatory actions.

63. Osseointegration for limb amputees: Load Characterization during Activities of Daily Living

Authors: Kontson, Kimberly, FDA/CDRH/OSEL; Niswander, Wesley, FDA/CDRH/OSEL; Baumann, Andrew, FDA/CDRH/OSEL

Plain Language Synopsis: Osseointegrated implants have potential to improve outcomes for limb amputees. However, limited data are available on implant loading or body kinematics as users perform common activities. This project will inform the development of standards for mechanical performance assessment of osseointegrated devices through collection of human kinematic and kinetic data.

Abstract:

Osseointegration (OI) is a new method of attachment for upper and lower limb prosthetic devices to a residual limb in which bone grows into an implanted device so it becomes rigidly anchored within the skeleton. Osseointegrated orthopedic implants are placed within the medullary canal of residual bone and connect to a limb prosthesis with a permanent transcutaneous abutment. Compared to sockets, OI implants may offer greater osseoperception, improved range of motion, easier donning/doffing, less soft tissue irritation, and are unaffected by size changes of the residual limb. Despite these potential benefits, data on expected loads and body kinematics during activities of daily living (ADLs) are limited. These data are critical to establishing standards or best practices for assessing the preclinical mechanical performance of OI orthopaedic systems. The overall goal of this research is to collect average load curves (forces and moments) and peak loads at the abutment site, as well as body kinematics and muscle activity for individuals implanted with various OI technology. The Human-Device Interaction lab in CDRH will leverage its experience with upper limb device evaluation to gather data on lower limb amputees. A pilot study will be initiated with n = 6 able-bodied individuals performing several ADLs associated with the lower limb (walking, stair ascent/descent, obstacle avoidance, ramp ascent/descent, and curb navigation) under normal conditions and simulated disability conditions that limit the degrees of freedom in the ankle and/or knee. Kinematic data will be collected using a 10-camera Vicon motion capture system. Bilateral muscle activity from the rectus femoris, semitendinosus, tibialis anterior, and lateral gastrocnemius will be recorded using a Delsys Trigno wireless electromyography system. Temporal, spatial, and pressure data of each footfall during ambulation will also be collected and analyzed using a 16-foot Protokinetics Zeno walkway. Various metrics will be computed and compared across the two conditions to understand those most informative in kinematic and kinetic analyses for the lower limb prosthesis user population.

The mention of commercial products, their sources, or use in connection with material reported herein is not to be construed as an actual or implied endorsement of such products by DHHS.

64. Analyte Extension Method Validation of Elemental Analysis Manual Method 4.7 for Six Additional Elements, Cobalt (Co), Strontium (Sr), Thallium (Tl), Tin (Sn), Uranium (U) & Vanadium (V)

Authors: Kovalenko, Anthony, FDA/NFFL; Stutts, Dominique, FDA/NFFL; Gray, Patrick, FDA/CFSAN

Plain Language Synopsis: A collaboration between laboratories to expand an existing analytical method for toxic elements in food.

Abstract:

FDA currently uses Elemental Analysis Manual (EAM) method 4.7 (Gray et al., 2015) to analyze elements in regulatory food samples, in accordance with FDA compliance programs. EAM 4.7 underwent a Level Four multi-laboratory validation (Gray et al, 2018) and is used for the regulatory analysis of eleven elements in food matrices. These elements include: arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium and zinc. The analyte extension project added strontium (Sr), cobalt (Co), vanadium (V), uranium (U), tin (Sn) and thallium (Tl) to the list of validated elements that can be analyzed using EAM 4.7.

Analyte extensions to existing validated methods require a Level Two validation and was performed as per FDA's Guidelines for the Validation of Chemical Methods for the FDA FVM Program (GVCM, 2015). In summary, method accuracy, precision, sensitivity, limits of detection and quantification, linearity, range, and ruggedness in variety of food matrices were established for strontium (Sr), cobalt (Co), vanadium (V), uranium (U), tin (Sn) and thallium (Tl). Foods from each category of the AOAC food matrix triangle were spiked at three different levels in triplicate. Certified reference materials from food matrices with

certified values for the target elements were also tested.

This Analyte Extension Method Validation of EAM 4.7 for Six Additional Elements, Cobalt (Co), Strontium (Sr), Thallium (Tl), Tin (Sn), Uranium (U) & Vanadium (V) followed all guidelines for a Level 2 validation and met all acceptance criteria for analyte extensions. Following the procedures and using specified equipment operated under recommended conditions as per EAM 4.7, the extension parameters can report new analytes, Co, Sn, Sr, Tl, U and V with acceptable repeatability and accuracy. The results presented demonstrated accuracy, limit of detection, limit of quantitation, linearity, and precision by successful analyses of method blanks, matrix spikes, unfortified samples, and reference materials.

65. CTP Integrated Research and Data System (CIRDS)

Authors: Vibha Kumar, FDA/CTP: Daniel St. Laurent, FDA/CTP: Deborah Sholtes, FDA/CTP : Priyadarshan Kadam, Cont./Mitre: Michael Schoenfel, Mitre: Wei Wu, Mitre

Plain Language Synopsis: This poster presents a proof of concept (POC) using MarkLogic's Enterprise NoSQL-based operational and transactional data integration platform. The POC addresses the obstacles that make it challenging for the Center for Tobacco Products to harness knowledge from disparate sources.

Abstract:

CTP introduced an Integrated Research Data System (CIRDS) platform that allows users to search, explore, discover, and disseminate information from multiple data sources, which currently include: CTP-funded research, CTP's internally developed Tobacco Constituent Knowledgebase, and a subset of the publicly available Truth Tobacco Industry Documents. The platform provides an advanced and customized search engine that facilitates the retrieval of structured data (e.g. tables or spreadsheets of information) and unstructured data, such as free text in publications, emails, and other document

sources. CIRDS searches across these disparate sources of information through an intuitive interface used for viewing, saving, and sharing search results. CIRDS is designed for ingestion of both structured and unstructured data. It ingests data from wholly separate data sources, harmonizes the data where appropriate, and stores the data in a CIRDS repository that can readily accept updates and changes that may occur to the source data. By compiling and harmonizing data from disparate systems, CIRDS enables the end user to receive a more holistic view of data related to their search query. The system handles many advanced functions of search, including stemming, Boolean operators, proximity concept (two different words close to each other, even if not next to each other), and a variety of filters. The robust search across harmonized data provides a powerful application that allows CTP to find and synthesize tobacco knowledge from a variety of sources.

To date, CIRDS has ingested:

- The CTP Research Tracking System (RTS) Oracle database (structured metadata regarding CTP funded research)
- The CTP Tobacco Constituents Knowledgebase (TCKB) Oracle database
- A subset of documents from the publicly available UCSF Truth Tobacco Industry Documents library

The proof of concept became available for CTP staff review April 2018. Based on feedback from the proof of concept, CTP is now planning to incorporate additional search enhancements and more data repositories, including confidential industry documents and CTP-funded research documents (unstructured data/documents not currently in the RTS system). The CTP Integrated Research and Data System shows promise for advancing knowledge by providing CTP personnel a consolidated search engine that compiles data and documents from many disparate systems into one user-friendly interface. This provides CTP personnel an efficient, more holistic search capability,

unearthing knowledge and data relationships that would otherwise be difficult or impossible to discover.

66. Terminology and Relationships for a Smokeless Tobacco Product Ontology

Authors: Vibha Kumar, FDA/CTP:Thomas Eads,FDA/CTP: Christine Keys,FDA/CTP: Deborah Sholtes, FDA/CTP: Christopher Chaney,Cont./Wise Design: Eric Fenzl,Wise Design: Esther Wisdom, Wise Design:John F. "Jack" Jones,Wise Design:

Plain Language Synopsis: This poster focuses on a prototype ontology for smokeless tobacco products and resolves thorny issues in standardizing tobacco product ingredient information.

Abstract:

CTP gains knowledge about tobacco products from industry submissions, review of marketing applications, and from research. Ontology provides the tools to organize and store that information in both machine and human readable forms, and to make it readily accessible for regulatory decision-making. This poster discusses a model for smokeless tobacco products that aids regulatory decision making.

The prototype ontology thoroughly describes all products in this product category. The description includes product structure, product ingredients, and harmful and potentially harmful constituents. Our curation of the highly variable ingredient data included deduplication, corrections to spacing, punctuation, spelling, and application of ingredient naming rules. Where appropriate, we added registry numbers such as the FDA Unique Ingredient Identifier (UNII), to provide interoperability with other substance information systems. The result was reduction of 12,000 ingredient names to less than a thousand. Above all, the thousand names now correspond to uniquely identified substances.

Doing this for other tobacco product categories will be much less work because of the rules.

We also added synonyms likely to be used by CTP staff and industry and structure for known and expected ingredient functions. Combined with ingredient name standardization, this enables precise description of concepts in the ontology, while preserving originally reported names, all in a product category-specific way.

The standardization methods will be submitted to the CTP Data Standards Work Group, so that others may benefit (through CTPedia) from the standardization work described here.

67. Comparative Genomic Analysis of Forty-Nine Salmonella Serotypes Isolated from Sick Animals

Authors: Cong Li¹, Chih-Hao Hsu¹, Thu-Thuy Tran¹, Heather Tate¹, Jason Abbott¹, Patrick McDermott¹, Shaohua Zhao¹, ¹Office of Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland, USA

Plain Language Synopsis: This purpose of the study was to help us understand genetic differences in Salmonella virulence and antibiotic resistance by animal source. The complicated genomic structures shed light on the strain characteristic contributing to the severity of disease in sick animals and the distribution of antimicrobial resistance determinants in different serotypes and animal types.

Abstract:

Salmonella is a leading cause of bacterial human and animal illness. To understand the genetic makeup of their virulence and resistance features, we sequenced 504 Salmonella strains recovered from sick animals, including cattle (n=175), pig (n=229), horse (n=25), goat (n=4), cat (n=1), chicken (n=30) and turkey (n=40) using Illumina MiSeq.

Forty-nine serotypes were determined by SeqSero2 (Deng Lab). The top five serotypes were Typhimurium (n=132), Dublin (n=51), Newport (n=42), Heidelberg (n=38), and Choleraesuis (n=35). Among 26 serotypes

with three or more isolates, all carried the same eight Salmonella Pathogenicity Islands (SPI-1,2,3,4,5,6,9,10) and none carried SPI-15, 20, 21. *S. Dublin* and *S. Enteritidis* carried the most SPIs (fifteen) while *S. Muenchen* and *S. Derby* carried the least (ten). Only *S. Typhimurium* contained SGI-1, which also harbored resistance genes. Four serotypes (*Typhimurium*, *Dublin*, *Choleraesuis*, *Enteritidis*) carried the *spv*-operon (*spvRABCD*), which is tightly linked to the presence of the *IncFII* plasmid. *Salmonella* serotype IIIa56:z4,z23 carried a *spv* operon with an *spvD* deletion located on the chromosome.

Ten plasmid types were identified by PlasmidFinder (Camacho et al.) (*IncFIA*, *FIB*, *HI1*, *HI2*, *I1*, *L/M*, *N*, *A/C*, *X* and *Q*). Plasmid type and distribution varied by serotype and source. Some plasmids were likely to be serotype specific – 100% of *S. Dublin* strains carried *incX*, whereas less than 21% of other serotypes had this plasmid, and 92% of *S. Choleraesuis* isolates carried *incQ*, and only 21% of other serotypes carried that plasmid. Some plasmids were likely to be host specific: among 105 *IncA/C2* detected from the top ten serotypes, 85 (81%) were detected in strains isolated from cattle and of 77 *incHI* plasmids identified, 71% were detected in strains isolated from pigs.

Resistance genotypes were identified using BLAST to search assembled genomes against the ResFinder database (Zankari et al). The isolates carry 41 genes encoding resistance to eight classes of antimicrobials.

In conclusion, *Salmonella* isolated from sick animals carried various SPIs, SGI-1, *spv* operon and MDR plasmids with resistance genes. Such complicated genomic structures shed light on the strain characteristics contributing to the severity of disease in sick animals and the distribution of antimicrobial resistance determinants in different serotypes and animal hosts.

68. Sensitive Penicillin Residue Detection in Limited Amount of Bovine Biopsy Tissues using Liquid Chromatography and Tandem Mass Spectrometry

Authors: Li, Linge, FDA/CVM; Howard, Karyn, FDA/CVM; Kilonzo, Christine, FDA/CVM; Gonzales, Raoul, FDA/CVM; Myers, Michael, FDA/CVM

Plain Language Synopsis: This tissue method is the Office of Research's first and fully validated assay that uses tissue weight as low as ~ 100 mg with penicillin's LLOQ of 10.0 ng/g. Also, it's our first kidney assay that compares the penicillin quantitative results obtained from kidney cortex, medulla, and whole kidney.

Abstract:

Penicillin has been widely used in veterinary medicine to treat bacterial infections in dairy cattle and continues to be the most persistent violative antibiotic residue in human food at slaughter. To better understand the correlation between label or extra-label dosing vs. violative penicillin residues, a residue depletion study in bovine tissues is proposed to compare penicillin residues. The analyses of these tissue samples are challenging, since they are collected via laparoscopic biopsy sampling approach, which only yields tissue weights from 50-150 mg. This poster presents a fully validated LC/MS/MS bioanalytical method for the quantitation of penicillin in limited amounts of bovine biopsy tissues.

Tissue homogenization was accomplished either by a blender or an OMNI-prep tip. Individually weighted tissues (range: ~ 100 mg [+/- 10 mg]) were fortified with a stable, isotopically-labeled internal standard. Analytes were extracted from biological matrix by protein precipitation coupled with an HLB PRiME one-step pass-through extraction plate for further clean up. The extracted analytes were separated on a Waters XSelect HSS T3 column (2.1 x 50 mm, 2.5 µm) and detected by an AB Sciex 4000 QTRAP Mass Spectrometer using Multiple Reaction Monitoring (MRM) with positively

charged ion mode. The total run time was approximately 4.0 minutes.

Assay precision and accuracy were evaluated using two types of quality controls samples (QCs). Type I QCs (LLOQ, Low, Mid, and High) were prepared by fortifying penicillin to pre-homogenized (bulk blended) blank tissue. Type II QCs (Low and High) were prepared by fortifying penicillin to non-homogenized tissue, later homogenized during protein precipitation extraction step using OMNI-prep tips. Inter-assay precision and accuracy (diff% from theoretical) were in the range of 2.26% to 10.0%, and 8.4% to 13.0%, respectively.

For kidney assay, the precision and accuracy results demonstrated no significant differences between cortex vs. medulla vs. whole kidney.

The assay was further investigated for other method specifications defined in FDA's "Bioanalytical Method Validation Guidance for Industry." This fully validated LC/MS/MS kidney tissue method will be used for sample analysis in support of an IACUC approved label vs. extra-label dosing penicillin depletion study in daily cattle.

69. Study of Honey and Lemon Juice Adulteration Using Cavity Ring Down Spectroscopy and Isotope Ratio Mass Spectrometry

Authors: Mantha, Madhavi, FDA/FCC; Kubachka, Kevin, FDA/FCC; Urban, John, FDA/FCC;

Plain Language Synopsis: Foods like honey and lemon juices can be adulterated with low cost sweeteners and/or other additives for monetary gain. The Forensic Chemistry Center (FCC) was able to detect the adulteration using cutting edge technology (CRDS) and compare to the traditional instrumentation (IRMS).

Abstract:

In the last several years, economically motivated adulteration (EMA) of foods has received increased attention. Honey and lemon juice have been targets of EMA, as they can be adulterated with low-cost ingredients

like sweeteners or exogenous citric acid.

Stable isotope ratio analysis (SIRA) provides a tool to detect adulteration in both honey and lemon juice, based on the premise that adulterants distort the natural $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) of the product. The most common instrumentation for this type of analysis is an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS).

Syrups from C4 plant sugars (i.e., corn or cane syrup) have been used to adulterate honey. The Forensic Chemistry Center (FCC) uses AOAC method 998.12 to analyze bulk honey and the corresponding protein isolate to determine their respective $\delta^{13}\text{C}$ values. The relative difference between these values is indicative of adulteration.

Potential adulterants for lemon juice are exogenous citric acid and/or C4 syrups. To detect adulteration with citric acid, methodology adapted from Doner, et al. has been used at the FCC.1 A $\delta^{13}\text{C}$ value for isolated calcium citrate greater than -23‰ is indicative of adulteration.

As honey and lemon juice samples have become a routine regulatory application, instrumentation offering lower cost and decreased maintenance compared to EA-IRMS was explored. One such potential alternative SIRA instrumentation is the combination of a combustion module (CM, analogous to an EA) coupled to a Cavity Ring-Down Spectrometer (CRDS). CM-CRDS has gained attention in the last five years as a viable alternative to EA-IRMS. This new technology offers a less expensive, more robust, and simpler analysis approach that could be transferred to other regulatory laboratories for routine use. Therefore, the FCC has evaluated the use of CM-CRDS to establish its suitability compared to EA-IRMS. The FDA validation protocol was followed to evaluate both honey and lemon juice methods, using CM-CRDS analysis. The $\delta^{13}\text{C}$ values obtained from CM-CRDS were equivalent to the EA-IRMS values within suitable accuracy and precision.

1) Doner, L., "Carbon Isotope Ratios in Natural

and Synthetic Citric Acid as Indicators of Lemon Juice Adulteration." *J. Agric. Food Chem.*, 22 (1985) 770-772.

70. Generation of Recombinant Ligand-Binding Fragments of Low-Density Lipoprotein Receptor-Related Protein 1 Using Co-Expression with its Chaperone Receptor-Associated Protein

Authors: Marakasova, Ekaterina, FDA/DPPT; Uceda-Cortez, Gabriela, FDA/DPPT; Shestopal, Svetlana, FDA/DPPT; Lee, Timothy, FDA/DPPT; Sarafanov, Andrey, FDA/DPPT;

Plain Language Synopsis: Deficiency in factor VIII (FVIII) results in excessive bleeding. Hemophilia A is treated by frequent infusions of FVIII. Our studies are aimed at elucidating the mechanisms of FVIII clearance in the circulation to facilitate generation and review of longer-acting therapeutic FVIII products.

Abstract:

Introduction: Concentrates of blood coagulation factor VIII (FVIII) are used to treat FVIII deficiency, a condition that causes excessive bleeding (Hemophilia A). Due to the short half-life of FVIII in the circulation, prophylaxis requires up to 4 intravenous injections of the concentrate per week. Understanding FVIII clearance mechanisms would facilitate generation of longer-acting therapeutic FVIII products. It was shown that the clearance of FVIII is mediated by a liver endocytic receptor low-density lipoprotein receptor-related protein 1 (LRP). The ligand-binding moiety of LRP is presented by complement-type repeats (CRs), grouped in four clusters, among which clusters II-IV are important for FVIII binding. To study this interaction and develop a method to test the quality of FVIII products, we aimed to generate recombinant clusters II-IV of LRP. Previously, expression of these fragments resulted in low yields because most of protein was expressed in non-functional, misfolded, multimeric form. To increase the quality and yield of these proteins, we tested their co-expression with a molecular chaperone of LRP, receptor-associated protein (RAP).

Methods: The LRP fragments were co-

expressed with RAP in a baculovirus system. The proteins were purified using metal-affinity chromatography and size-exclusion chromatography. The proteins' functional properties were studied by surface plasmon resonance (SPR).

Results: Co-expression with RAP resulted in significantly higher yield of cluster II monomeric form, compared to expression without RAP. Using RAP with an insect endoplasmic reticulum retention signal HTEL resulted in further increase of the yield. Clusters III and IV were then expressed using the same approach that also resulted in high yields. Functional properties of the proteins were confirmed with SPR by binding with RAP and FVIII.

Conclusion: We developed a method to generate recombinant fragments of LRP at high yields. The proteins were produced in correctly folded form, preserving the binding properties of LRP. These proteins are suitable for further study of mechanism of FVIII-LRP interaction and development of a method to test the quality of FVIII products by testing its binding to fragments of LRP.

Disclaimer: This is an informal communication and it represents the authors' own best judgment. These comments do not bind or obligate FDA.

71. Characterization of Silver Nanoparticles in a Variety of Feminine Hygiene Products

Authors: Ghorai, Suman, FDA/NCTR; Popescu, Ioana-Mihaela, FDA/NCTR; Mathew, Ammu, FDA/NCTR; Koonce, Nathan, FDA/NCTR; Patri, Anil, FDA/NCTR*

Plain Language Synopsis: Silver nanoparticles are shown to have antimicrobial activity and are used in many consumer products. While there are many potential beneficial properties associated with ionic and nano silver, the toxicity due to their exposure is not completely known. To evaluate the presence of silver in feminine hygiene products, a variety of such products were analyzed to quantitate the total amount of silver and ion release kinetics in different dissolution media, mimicking practical usage scenario.

This information is useful in evaluating in vitro and in vivo dosing concentrations to ascertain their safety.

Abstract:

Feminine hygiene products are found to contain silver. While silver and silver nanoparticles have antimicrobial properties, potential release of silver ion, particularly in vaginal pH conditions, may have beneficial or adverse effects, depending on the concentration and biodistribution potential. In this project, a variety of feminine hygiene products were screened for the presence of silver. The total amount of silver in these products was quantified, and ionic silver release was evaluated in different dissolution media by mimicking practical use conditions of the corresponding products through elemental analysis with inductively coupled plasma mass spectrometry (ICPMS). In addition, scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) was used to visualize and identify the presence of nanosilver in these products. Twenty-six different feminine hygiene products were analyzed and fifteen were found to contain silver. The rate of ionic silver dissolution from nanoparticle-based products increased significantly with acidity of the dissolution media. This work indicates that a significant amount of silver ions from products containing small (5-40 nm) nanoparticles can be released under test conditions mimicking a practical use scenario in vitro.

72. Techniques for Scaling Applications on High Performance Computing Clusters

Authors: Mikailov, Mike, FDA/CDRH; Petrick, Nicholas, FDA/CDRH; Luo, Fu-Jyh, FDA/CDRH

Plain Language Synopsis: Techniques for scaling Bioinformatics, Modeling & Simulation, Big Data analysis applications on High Performance Computing Clusters will be presented.

Abstract:

In their mission to protect and promote public

health, scientists at FDA increasingly rely on innovative techniques on HPC platforms. Thousands of CPUs can be marshaled to perform research computations on a scale and at speeds unthinkable in the recent past. Important benefits include: broadening the range of investigations that can be performed in silico; dramatically increasing the speed of innovation while reducing costs; potentially improving confidence in medical devices and drugs with highly-relevant evidence for regulatory decisions. The problems of increased computation time and large data associated with traditional M&S techniques are being overcome.

One of the greatest challenges in accomplishing the above-mentioned mission-critical tasks is not how powerful the clusters can be, but how HPC applications can take advantage of all resources in a scalable manner. Novel techniques for scaling bioinformatics, modeling & simulation, big data analysis applications of FDA on High Performance Computing Clusters will be presented.

73. Rapid prediction of low (<1%) trans fat content in edible oils and fast food lipid extracts by infrared spectroscopy and partial least squares regression

Authors: Mossoba, Magdi, FDA/ORS; Farris, Samantha, FDA/ORS; Karunathilaka, Sanjeewa, FDA/ORS

Plain Language Synopsis: FDA ruled that partially hydrogenated oils are no longer "GRAS" for any use in human food. A rapid screening procedure using infrared spectroscopy and chemometrics for the quantitative prediction of low concentrations of trans fat in edible oils and food lipid extracts was developed for regulatory purposes.

Abstract:

FDA ruled that partially hydrogenated oils (PHO), the major dietary source of industrially produced trans fat (TF), were no longer "generally recognized as safe (GRAS)" for any use in human food. Consequently, the objective of the present study was to develop

a rapid screening procedure using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy in conjunction with partial least squares regression (PLSR) for the quantitative, accurate prediction of low concentrations of trans fatty acid (TFAs) (<1% of total fatty acids [FAs]). Broad-based calibration models were developed for a combined set of samples consisting of edible oils and fast food lipid extracts. Predicted concentrations of TFAs in the two matrices showed good correlation with the primary reference data generated by gas chromatography (GC) ($R^2 > 0.99$) and high accuracy, as evidenced by low root-mean-square error of cross-validation (RMSECV) values. The lowest TFA concentration, determined by GC to be 0.13% of total FAs, was accurately predicted by ATR-FTIR/PLSR as 0.18% of total FAs. This simple, rapid ATR-FTIR/PLSR methodology could potentially be used as a screening alternative to conventional gas chromatographic methods for predicting the TFA content of edible oils and food lipid extracts for regulatory purposes and quality control of raw material and processed food.

74. Quantification of Phospholipid Degradation Products in Parenteral Liposomal Formulations by Liquid Chromatography – Mass Spectrometry (LC-MS)

Authors: Siriwardane, Dumindika, FDA/ORA; Wang, Changguang, FDA/ORA; Jiang, Wenlei, FDA/CDER; Mudalige, Thilak, FDA/ORA

Plain Language Synopsis: Phospholipids are the principal excipient in liposomal pharmaceutical formulations. Phospholipids are susceptible to hydrolysis/degradation forming free fatty acid and lyso-lipids during manufacturing and storage of formulations. The development of LC-MS-based analytical methods for quantitation of lipid degradation products in pharmaceutical formulations will be presented.

Abstract:

Identification and quantification of impurities in liposomal formulations are important, as they may impact the safety and efficacy

of the drug. Cholesterol and phospholipids are major excipients in liposomes and they may be vulnerable to oxidation and hydrolysis reactions, respectively. For phospholipids, the major degradation pathway is hydrolysis because saturated phospholipids in commercial liposomal drugs have less potential for lipid oxidation. Lysophospholipids and free fatty acids are the major degradation products formed through phospholipid hydrolysis during manufacturing or long-term storage of liposomal formulations. Here we report the development and application of a simple, fast, and sensitive method that can be used to quantitate lysophosphocholines (LPC 18:0 and LPC 16:0), lysophosphoglycerol (LPG 18:0), and free fatty acids (FFA 18:0 and FFA 16:0) in liposomal formulations. The liposome drugs were solubilized by direct dilution using chloroform:methanol (1:1) solvent, then further diluted with LC mobile phase to appropriate concentrations for liquid chromatography – mass spectroscopy (LCMS) analysis. The lysophospholipid was separated from other components in the liposomal formulations by C18 stationary phase, whereas free fatty acids were separated by using C8 stationary phase and quantified with accurate mass Q-TOF mass spectrometry. This method was validated according to USP compendial procedures and has been used to analyze seven commercial parenteral liposomal drug products. The limit of quantification (LOQs) for FFA 16:0, FFA 18:0, LPC 16:0, LPC 18:0, and LPD 18:0 are 5 ng/mL, 5 ng/mL, 6.5 ng/mL, 7.0 ng/mL, and 7.1 ng/mL respectively. The linearity range was 5-400 ng/mL. This method has the advantage of high specificity, short run time, and smaller sample size required for analysis, compared to some existing lysophospholipid analysis methods. In conclusion, this LC-MS method can be used to quantify lysophospholipids and free fatty acids in liposomal formulations with reasonable linearity, specificity, accuracy, and precision.

Poster Session 2

Topic: Advanced Technology (Day 1, P.M.)

1. Quantitative analysis of parenteral liposomal drug products for cholesterol oxidation products and desmosterol

Authors: Wang, Changguang, FDA/ORa; Siriwardane, Dumindika, FDA/ORa; Jiang, Wenlei, FDA/CDER; Mudalige, Thilak, FDA/ORa

Plain Language Synopsis: Cholesterol is an essential component of liposomal pharmaceutical products and is susceptible to oxidation to cholesterol oxidation products during manufacture and long-term storage. The development of LC-MS-based analytical methods for detecting and quantitating cholesterol oxidation products and desmosterol in liposomal pharmaceutical formulations will be presented.

Abstract:

Cholesterol is one of the major structural components of liposome bilayers. Cholesterol is vulnerable to oxidation during liposome preparation and/or storage, resulting in a variety of cholesterol oxidation products (COPs). The oxidation of cholesterol to COPs could cause the physical properties of liposome bilayers to change, resulting in "leaking" of the drug from the liposome. This altered liposome stability could further affect the safety and efficacy of the liposomal drug, and the presence of bioactive COPs could cause unwanted physiological responses. Herein, we report a liquid chromatography – mass spectroscopy (LC-MS) based analytical method for separating and quantifying COPs present in liposomal parenteral drug formulations from five different vendors. The results show that six COPs and desmosterol (cholesterol precursor) are present in liposomal drug products (LDPs). 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, 7-keto-cholesterol, and desmosterol were the major impurities detected in LDPs. It is worth noting that none of USP/NF grade cholesterol excipients contained COPs, which suggests that COPs are generated during liposome preparation and/or storage. This method has been validated using USP compendium validation procedures and consequently provides

referenceable information for quantitation of cholesterol-related impurities present in liposomal drug formulations.

2. Genomic Analysis of Emerging Florfenicol-Resistant *Campylobacter coli* Isolated from Cattle in the United States.

Authors: Mukherjee, Sampa, FDA/CVM ; Zhao, Shaohua, FDA/CVM ; Hsu, Chih-Hao, FDA/CVM ; Yang, Shenia, FDA/CVM ; Li, Cong, FDA/CVM ; Tate, Heather, FDA/CVM ; Morales, Cesars, USDA/FSIS; Haro, Jovita, USDA/FSIS ; Thitaram, Sutawee, USDA/FSIS; Tillman, Glenn, USDA/FSIS ;

Plain Language Synopsis: Florfenicol resistance in *Campylobacter* first appeared in beef cattle in 2013. Multidrug resistance gene, *cfr(C)* was recently reported to be responsible for florfenicol resistance in *Campylobacter*s. In this study we tested sixteen florfenicol resistant *Campylobacter coli* strains. We identified *cfr(C)* gene by whole genome sequencing (WGS) and studied the involvement of *cfr(C)* gene in florfenicol resistance by conjugation experiment. The purpose of this study was to perform genomic analysis of emerging florfenicol resistant *Campylobacter coli* isolated from beef cattle and characterize the *cfr(C)* gene associated with the multi-drug resistance plasmid.

Abstract:

Background: The U.S. National Antimicrobial Resistance Monitoring System (NARMS) tracks changes in antimicrobial resistance in foodborne pathogens, including *Campylobacter* isolated from food animals, retail meats, and humans. Florfenicol is one of the 9 antimicrobials included in the NARMS *Campy* testing panel. While florfenicol resistance (FFNR) is rare in *Campylobacter*, it has been increasing in isolates from beef since it first appeared in 2013. This study investigated the genomic basis of FFNR *Campylobacter coli* isolated from cattle and characterized the *cfr(C)* gene associated with a multi-drug resistance (MDR) plasmid.

Methods: Sixteen FFNR *C. coli* isolates recovered between 2013-2018 from beef cattle were identified and WGS was done

using MiSeq chemistry. Plasmids were closed for three genomes using the PacBio sequencing platform. Whole genome single nucleotide polymorphisms (SNP) and the structure of MDR plasmids were analyzed. Conjugation was carried out to determine the transferability of *cfr(C)* associated MDR plasmids and the spectrum of resistance encoded by the *cfr(C)* gene was further investigated by agar dilution.

Results: All 16 FFNR isolates exhibited co-resistance to ciprofloxacin, nalidixic acid, clindamycin, and tetracycline. All isolates had a mutation of *GyrA*, T86I and they shared the same resistance genotype, carrying *aph(3')-III*, *hph*, Δ *aadE*, *blaOXA-61*, *cfr(C)*, *tet(O)* genes. The *aadE* gene was truncated (Δ *aadE*) in each isolate. The *cfr(C)*, *aph(3')-III*, *hph*, Δ *aadE*, and *tet(O)* genes were on transferable MDR plasmids 48-50 kb in size. These plasmids showed high sequence homology with the common pTet *Campylobacter* plasmid, and carried several *Campylobacter* virulence genes, including *virB2*, *virB4*, *virB5*, *virB6*, *virB7*, *virB8*, *virB9*, *virB10*, *virB11*, and *virD4*. The *cfr(C)* gene was found to confer resistance to florfenicol (8-32 μ g/ml), clindamycin (512-1,024 μ g/ml), linezolid (128-512 μ g/ml), and tiamulin (1.024 μ g/ml). Phylogenetic analysis showed SNP differences ranging from 11-2,248 among the 16 isolates.

Conclusion: The *cfr(C)* gene located in the conjugative pTet MDR virulence plasmid is present in diverse strains, where it confers high levels of resistance to several antimicrobials, including linezolid, a critical drug for treating gram-positive bacterial infections in humans. This study highlights the power of genomic antimicrobial resistance surveillance to uncover the intricacies of transmissible co-resistance and provide information that is needed for accurate risk assessment and mitigation.

3. Chemical Characterization of Acrylonitrile Butadiene Styrene 3D Printed Medical Devices

Authors: Nahan, Keaton, FDA/CDRH;

Sussman, Eric, FDA/CDRH; Oktem, Berk, FDA/CDRH; Wickramasekara, Samanthi, FDA/CDRH

Plain Language Synopsis: To determine the safety of novel medical devices, 3D printed medical devices undergo extraction to elucidate the worst case amount of chemicals that can leach from them. This analysis determined that the 3D printing of polymer feedstock either introduces new chemicals or extracts chemicals that were previously unavailable.

Abstract:

Research on additive manufacturing, otherwise known as 3D printing, has become exceedingly popular in recent years. According to a recent Reuters news report, 3D printing has provided new applications for medical-imaging-based anatomical surgical models and medical devices. While 3D printing is becoming relevant to medical devices, a variety of factors can affect the biocompatibility of these products. Chemical characterization of medical device extractables by mass spectrometric techniques has emerged as a more selective alternative to traditional biocompatibility testing of medical devices, which uses biological test systems.

We investigated the extractables profile of two specific acrylonitrile butadiene styrene (ABS) feedstocks. These feedstocks were then 3D-printed into casts. The aim was to determine whether the extractables profile would be altered by 3D printing and thereby potentially affect biocompatibility.

Forearm casts were manufactured from biocompatible and consumer grade filaments by Fused Filament Fabrication (FFF). Materials tested included the filaments prior to printing, a 3D printed cast for each filament, and a post-processed 3D printed cast made from the consumer grade filament. Sample extraction was performed with 3 different solvents (water, isopropyl alcohol, and hexane) at 50C for 24h with gentle agitation. An Agilent 7890B GC/MS was used to determine volatile/semi-

volatile extractables with NIST 2017 spectral database for compound identification.

GC/MS analyses tentatively identified over 60 extractables in isopropanol and hexane extracts of all materials, including extractables such as acetophenone and styrene-acrylonitrile (SAN) trimer. No water soluble extractables were found by GCMS. Prints made of biocompatible filament showed fewer unique identified extractables than the prints made of consumer grade filament, based on the volatile extractables from hexane and isopropanol extractions. Further, the finished consumer grade 3D print contained fewer identified compounds than the unfinished consumer grade 3D print. This analysis determined that the printing process used here either introduces new compounds or makes otherwise unextractable analytes available. Ongoing work includes rapid analysis of extractables and leachables by DART-MS, as well as toxicological risk assessments of the identified extractables.

4. **Chemical Analysis of Medical Device Materials to Probe Material Equivalency**

Authors: Oktem, Berk, FDA/CDRH; Nahan, Keaton, FDA/CDRH; Sussman, Eric, FDA/CDRH; Wickramasekara, Samantha, FDA/CDRH

Plain Language Synopsis: Chemical analysis is becoming a more important alternative to animal testing of medical device materials. A data science-based approach described here was developed to make material equivalency judgements using state-of-the-art chemical analysis methods.

Abstract:

Biological evaluation of medical devices often includes chemical characterization, as described in ISO 10993-18. Device materials are subject to extraction, whereby materials, processing additives, residues, and material breakdown products may be released. These extractables have potential consequences on the biological response to the device during its use. Chemical characterization studies are followed by toxicological risk assessments that evaluate potential harm from exposure

to the extractables. In some cases, evaluation of material equivalence of similar device materials is required. Following extraction, chemical analysis is performed by various methods, such as GC/MS, FTIR, and LC/UV/MS. Current technology in high resolution mass spectrometry may improve these analyses. This presentation discusses development of improved data analysis methods to probe chemical analysis data from the equivalency of two materials.

Two batches of two medical device materials, polyether block amide (commonly known as Pebax) and polypropylene, were analyzed. Multiple rounds of extractions (24 hour at 50°C 200 rpm) were used to obtain non-volatile residue. Test extracts were introduced to different instruments: a GC/MS (7890B/5977B Agilent) and an UHPLC-QTOF-MS system (6540 UHD- Agilent). Amounts of extractables were estimated semi-quantitatively by comparing them to a set of internal standards.

Standards of various polymer additives were prepared and analyzed by the LC-UV-MS system, by which the UV absorbance and MS data were acquired together. Processed and raw Pebax materials were extracted by water and hexane, the total non-volatile residue was on the order of 60 mg /1.5 g sample. GC/MS analysis disclosed minor amounts of extractables (less than 20 µg/1.5 g sample). The GC/MS data were also processed for identification of analytes using the 2017 NIST Mass Spectral Library. Preliminary results showed more than 20 compounds, tentatively identified. Ongoing work includes the use of Q-TOF MS with greater resolution (~40,000) for unknown identification using a commercial E&L Database. A scoring scheme for binary comparison of the data was done using Pearson correlation.

5. **Method validation and new peak detection (NPD) for the multi-attribute method (MAM)**

Authors: Oyugi, Mercy, FDA/CDER; Wang, Xiaoshi, FDA/CDER; Yang, Xiangkun, FDA/CDER; Rogstad, Sarah, FDA/CDER

Plain Language Synopsis: Multi-attribute

method (MAM) is a liquid chromatography-mass spectrometry (LC-MS) peptide mapping technique that has been proposed as a replacement for traditional quality control methods used for protein therapeutics. Here, method validation of an in-house MAM protocol and evaluation of MAM's new peak detection (NPD) feature are discussed.

Abstract:

Background

Multi-attribute method (MAM) is a liquid chromatography-mass spectrometry (LC-MS) peptide mapping technique used for identifying and quantifying product quality attributes (PQAs) in protein therapeutics. MAM has been proposed as a replacement for conventional quality control (QC) and release methods for protein therapeutics. The Office of Testing and Research (OTR) has developed an in-house MAM using rituximab as the model protein.

Purpose

To evaluate MAM method validation and its new peak detection (NPD) feature as an impurity monitoring tool.

Methods

Three analysts each performed three tryptic digestions of rituximab followed by triplicate LC-MS analysis of each digest using a Thermo Accela LC system coupled with a Thermo QE hybrid quadrupole-orbitrap mass spectrometer. Chromeleon software was then used for relative quantitation of rituximab PQAs. Method validation criteria included selectivity, autosampler, and freeze-thaw stability, repeatability, and intermediate precision based on inter-day ($n = 9$), analyst-to-analyst ($n = 9$), and analyst-to-analyst inter-day ($n = 27$). For NPD parameters, m/z range and width, retention time, and maximum frames were held constant, while frame time width and peak intensity threshold were varied to test the effects of these variations on the number of new peaks detected.

Results

Selectivity was demonstrated by the absence

of overlapping peaks between blank and rituximab chromatograms. Repeatability and precision tests yielded $CV < 15\%$ for pyroglutamination, Lys451 clipping, Asn388 deamidation, Met256 oxidation, and all Asn301 glycosylations. Met20, Met34, Met81, Met432, and Met21 (LC) oxidation had $CV > 20\%$. Autosampler stability was demonstrated for all 21 PQAs, while freeze-thaw stability was established for 15/21 PQAs, excluding the six oxidation sites. For NPD, the number of new peaks decreased with increasing peak intensity threshold. There was variability in the number of new peaks detected between analysts and within some individual digests.

Conclusion

Overall, the method validation criteria were satisfied for all 21 PQAs, excluding the six Met oxidation PQAs. Additionally, NPD results show that MAM's non-targeted processing feature can potentially be used as an impurity monitoring tool, but that optimized parameters are critical for accurate NPD data.

6. Impact of various surface coatings on in vitro cell uptake and cytotoxicity of ultrasmall superparamagnetic iron oxide nanoparticles (USPION)

Authors: Palacios-Hernandez, Teresa, FDA/CDRH; Nguyen, Alexander, FDA/CDRH; Skoog, Shelby, FDA/CDRH; Wu, Yong, FDA/CDER; Tang, Xing, FDA/CDRH; Goering, Peter, FDA/CDRH

Plain Language Synopsis: Medical devices enabled with nanotechnology represent an emerging area for novel biomedical products. Understanding the potential adverse health responses to nanoparticles in devices is critical to establish their safety. We are investigating the effect of iron oxide nanoparticles with different coatings on cells to support device safety evaluation.

Abstract:

USPION are excellent candidates for medical applications due to their unique physicochemical properties (e.g., nanoscale size, highly reactive surfaces,

and superparamagnetism). However, the potential adverse health effects of USPION with different coatings, commonly applied to control their biological activity and stability, are not fully understood. Therefore, the goal of this study was to evaluate cellular uptake and cytotoxicity of carboxyl- and amino-coated USPION on human coronary artery endothelial cells (HCAEC) as a vascular cell model.

Both types of USPION were spherical with average diameters of ~30 nm as assessed by transmission electron microscopy (TEM) and hydrodynamic diameters of ~100 nm diameter as assessed by dynamic light scattering, and negatively charged (-36.3 and -6.5 mV for carboxyl- and amino-coated USPION, respectively) according to zeta potential analyses. After heat sterilization, the size and surface charge were unchanged for carboxyl-coated USPION; however, surface charge for amino-coated USPION increased (-0.66 mV) and aggregation was observed.

Concentration-dependent cytotoxicity was observed using the Alamar Blue assay. Cells exposed for 24 h to 25, 50, or 100 µg/mL of carboxyl-coated USPION exhibited viabilities of 100, 57, and 42 percent of control, respectively. Nanoparticle uptake assessed by TEM, confocal, and phase contrast microscopy showed perinuclear accumulation inside cytoplasmic vesicles of carboxyl-coated USPION. In contrast, cells exposed to amino-coated USPION exhibited minimal cytotoxicity at all concentrations tested and negligible particle uptake. These findings indicate that HCAEC injury is directly proportional to USPION exposure concentration and cellular uptake; however, this response depends on the type and chemical properties of the particle surface coating. Finally, Cas-3/7 was expressed, which is an early precursor to apoptotic cell death. This data will help build upon the current toxicological profile of USPION employed in medical products.

7. Quantifying Poly(ethylene glycol) Coating on Gold Nanocrystals Using High Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC-ELSD)

Authors: Palui, Goutam, FDA/NCTR; Ganguly, Aahana, FDA/NCTR; Raghavendra, Achyut, FDA/NCTR; Parti, Anil, FDA/NCTR

Plain Language Synopsis: The critical quality attributes for nanomaterials used in biomedical applications include size, shape, composition, purity, stability, and the surface properties. Minor changes to surface coatings will influence safety and efficacy of the products. Robust reproducible techniques are needed to quantify these surface coatings. Standardized methods and methodologies are a high priority that will facilitate regulatory review. Efforts toward developing quantifying surface-coated polyethylene glycol, a common coating polymer, will be presented.

Abstract:

Inorganic nanomaterials, such as gold and silver nanoparticles (NPs), have been explored extensively in various biomedical applications, including drug delivery, imaging, sensing, and radiation therapy, with many products in clinical trials. Many nanomaterials use poly(ethylene glycol) and their derivatives as coating materials for passivating the surface; but very few measure sets of parameters that are required to ensure the consistency and reproducibility during product development. It is important to measure parameters such as quantity, density, molecular weight, stability, and homogeneity. Slight variations in surface coatings on the nanomaterial may lead to differential recognition by the immune system in vivo and will lead to undesirable/ altered biodistribution, efficacy, and safety. Standardized methods and methodologies must be used to ensure adequate characterization of these organic coatings on nanomaterials. Consensus standards through stakeholder involvement can assist product development, quality control, and accelerate review of submission to FDA. Here, a robust and reliable test method for quantitative

analysis of a common surface coating ligand, polyethylene glycol (PEG), using HPLC-ELSD, is presented. A series of bio-compatible spherical gold nanoparticles (AuNPs) and gold nanorods (AuNRs) coated with different molecular weight PEGs and its derivatives have been prepared, followed by isolation, separation and quantitation of coatings using the HPLC-ELSD system. Various techniques are compared for appropriate sample preparation to quantify the surface coatings. These methods are being proposed to ASTM International as work items for standards development with stakeholder collaboration.

8. Development of a Method to Control Product-Related Impurities in FVIII Products

Authors: Pettersson, John R., ORISE/FDA; Shestopal, Svetlana A., FDA; Lee, Timothy K., FDA; Sarafanov, Andrey G, FDA

Plain Language Synopsis: Functional deficiency in factor VIII (FVIII) results in excessive bleeding (Hemophilia A), which is treated by infusions of FVIII concentrates. Previous data indicated these products contain significant amounts of inactive protein suggested to be immunogenic. We developed a method to quantify these impurities and improve product safety, and thus management, of Hemophilia A.

Abstract:

Introduction:

Products with blood coagulation factor FVIII (FVIII), used to treat Hemophilia A, cause an immune response in ~30% of patients. This correlates with the presence in the product of a significant fraction of protein (FVIII*) unable to bind von Willebrand Factor (vWF), a carrier of FVIII in plasma. FVIII* was proposed to be a structurally compromised protein, which may be immunogenic. FVIII* is not controlled by specifications of all current FVIII products, therefore, we aimed to develop a method to quantitate this impurity.

Methods:

vWF was covalently immobilized to agarose beads using specific chemistry. Several drug products based on recombinant FVIII were

analyzed using a chromatographic method with the vWF-agarose. The migration pattern of flow-through (FVIII*) and eluted (FVIII) fractions were analyzed by SDS-PAGE / Western Blot and tested for FVIII functional activity by a chromogenic substrate method.

Results:

For all tested FVIII products, the column-bound and eluted protein had high functional activity while the flow-through protein (FVIII*) had very low activity (<5% of that of eluted protein). At the same time, the band patterns between FVIII and FVIII* fractions displayed only small differences. The relative amounts of FVIII* were ~20% compared to FVIII protein.

Conclusion:

All tested FVIII products contain a significant fraction (~20%) of protein unable to bind vWF. This fraction (FVIII*) has significantly decreased functional activity and likely consists of denatured and aggregated protein. The method described here can be used by manufacturers of FVIII products to improve their safety and efficacy. Our data will facilitate FDA regulation of FVIII products for better treatment of Hemophilia A.

Disclaimer:

This is an informal communication and it represents the authors' own best judgment. These comments do not bind or obligate FDA.

9. Real-time quantification and supplementation of bioreactor amino acids to prolong culture time and maintain antibody product quality

Authors: Powers, David, FDA/CDER; Wang, Yifan, FDA/CDER; Fratz-Berilla, Erica, FDA/CDER; Velugula-Yellela, Sai Rashmika, FDA/CDER; Chavez, Brittany, FDA/CDER; Angart, Phillip, FDA/CDER; Trunfio, Nicholas, Sartorius Stedim North America Inc.; Cruz, Celia, FDA/CDER;

Plain Language Synopsis: We developed a method for measuring amino acid levels in bioreactors, which allowed us to study their consumption patterns. Using this information, we supplemented amino acids into bioreactors and found it could prolong culture

life with minimal effects on product quality.

Abstract:

Real-time monitoring of bioreactors allows for expedited responses required to correct for batch failure perturbations that might otherwise not be discovered until it is too late. Currently, analytical platforms are dedicated to real-time monitoring of media parameters such as pH, dissolved oxygen, temperature, nutrients such as glucose and glutamine, or metabolites such as lactate. Despite the importance of amino acids as the building blocks of the therapeutic protein product, their concentrations are not commonly measured (except for glutamine bioanalysis) due to costs and technical challenges. Here we present a study into amino acid monitoring, supplementation strategies, and how these techniques can impact the cell growth profiles and product quality. We used preliminary bioreactor runs to determine amino acid consumption patterns, the results of which were used to settle on a selected pool of species which are quickly depleted in the bioreactor. These amino acids were combined into blends which were supplemented into bioreactors during the run, the concentrations of which were monitored using an at-line method we developed to quickly assess amino acid concentrations from crude bioreactor media. We found that these blends could “rescue” bioreactors where the viable cell density was decreasing, resulting in a revitalization in cell viability that prolonged culture life and increased culture yield. We also explored how these strategies might impact protein product quality, such as the glycan profile. The amino acid consumption data were used with the final glycan profiles in principal component analysis to identify which amino acids are most closely associated with glycan outcomes.

10. Characterization of high-molecular weight polyethylene oxide in abuse-deterrent opioid formulations using asymmetric flow filed flow fractionation

Authors: Qu, Haiou, FDA/CDER/OPQ/OTR/

DPQR; Feng, Xin, FDA/CDER/OPQ/OTR/DPQR; Xu, Xiaoming, FDA/CDER/OPQ/OTR/DPQR; Cruz, Celia N, FDA/CDER/OPQ/OTR/DPQR; Faustino, Patrick J, FDA/CDER/OPQ/OTR/DPQR

Plain Language Synopsis: We developed an asymmetric flow field flow fractionation method to characterize the molecular weight distribution of high molecular weight polyethylene oxide that has been used in abuse-deterrent formulations. The acquired information can assist the evaluation of the stability of PEO under different manufacturing conditions.

Abstract:

High molecular weight (HMW) polyethylene oxide (PEO) has been widely used in solid oral opioid formulations to impart abuse-deterrent (AD) properties. Specifically, the molecular weight (MW) of PEO is one of the critical quality attributes that could affect the properties of the PEO polymer, as well as the performance of the product. For example, it is known that HMW-PEO may degrade under oxidative stress, such as during the manufacturing or manipulation (e.g. heat). As such, the PEO MW distribution may change, which could lead to undesired changes in solution viscosity (an important measure for injection abuse potential). Accurate determination of the MW distribution of PEO is therefore vital to understanding its role in abuse-deterrent formulations. Asymmetric flow field flow fractionation (AF4) is a high-resolution separation technique that has been demonstrated to be more effective than size-exclusion chromatography in characterizing high molecular weight polymers. However, there are currently no reports in the scientific literature that document the use of advanced physical separation techniques for HMW PEO analysis. The aim of this study is to develop a method based on AF4 to determine the MW distribution of PEO up to 7 million Da. PEO standards (up to 1.3 million Da) and polystyrene nanoparticles were used for method development. Experimental factors, including mobile phase composition and concentration, focus flow, focus/

injection duration, and cross-flow profile, were optimized. Online detectors including MALS, DLS, and RI were coupled to the AF4 to acquire information on the MW, radius of gyration, and hydrodynamic size of the polymer. Several commercial PEO products with nominal molecular weight from 300 kDa to 8000 kDa were stored at elevated temperature to induce thermal degradation. Decrease in the average molecular weight and increase of small PEO segments were observed. In summary, this regulatory science study evaluated AF4 as an emerging technology that shows promise in supporting ADF research. This study supported CDER Research Goal 3 (Improve product manufacturing and testing to help ensure the availability of high-quality products) and OPQ Research Initiative 4 (Advanced characterization of complex mixtures and biologics).

11. Standard Test Method Development for Lipid Quantitation in Liposomal Formulations

Authors: Raghavendra, Achyut, FDA/NCTR; Ramasahayam, Sunil, FDA/NCTR; Nasini, Udaya, FDA/NCTR; Patri, Anil, FDA/NCTR;

Plain Language Synopsis: Liposomes constitute 1/3rd of drug products containing nanomaterial submitted to FDA. The quality of these complex drug products depends on many factors, including composition of lipids. A fast, reliable and sensitive test method to quantify the lipids would support drug development and the review process. This poster describes a standard analytical procedure development through ASTM International, in collaboration with stake holders from industry and other government agencies.

Abstract:

Liposomal drug products have been approved for clinical use for over two decades and constitutes more than 1/3rd of the drug products containing nanomaterial submitted to FDA. Advantages of liposomal drug delivery include prolonged half-life, reduced toxicity, and potentially improved efficacy. It is known that beyond size, drug

encapsulation, and stability, the critical quality attributes include structure and composition of liposomal products to assure quality control. Standards are critical to assure consistency, quality, help with product development, and regulatory review. Herein, a fast, reliable, and sensitive test method for lipid quantitation in liposomal formulations, using high performance liquid chromatography (HPLC) with charged aerosol detector (CAD) is presented. This method can be used for other non-chromophoric material, such as other lipid compositions, to ensure quality control and to ascertain variations in lipid component profile for regulatory submissions.

12. A Portable Mid-Infrared Device for Food Safety and Quality Applications

Authors: Karunathilaka, Sanjeewa R, FDA/CFSAN; Yakes, Betsy Jean, FDA/CFSAN; Choi, Sung Hwan, FDA/CFSAN; Brückner, Lea, FDA/CFSAN; Ellsworth, Zachary, JIFSAN ; Mossoba, Magdi M, FDA/CFSAN.

Plain Language Synopsis: Two applications of a rapid portable ATR device in combination with chemometric in food safety and quality are discussed: 1) prediction of fatty acid (FA) concentrations in marine oil omega-3 dietary supplements, and 2) prediction of low (0.5%) total trans-fat contents in fast foods.

Abstract:

There has been increased interest in the development of novel and rapid analytical methods for food safety and quality. Among the methods evaluated, vibrational spectroscopy in combination with chemometric data analysis has gained much attention in recent years. Benefits of these methods compared to other conventional technologies (e.g., GC/MS) include their ready availability, low cost, high throughput, and robust, rapid analytical measurements. Two quantitative applications of a portable attenuated total reflection infrared (ATR-FTIR) spectroscopic device in combination with chemometric data analysis for food safety and quality are presented. In the first application, which uses the portable

device in combination with linear and non-linear regression approaches, calibration models were developed and used to predict the fatty acid (FA) concentrations in marine oil omega-3 dietary supplements. Such FAs included eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the sum of saturated, branched chain, and monounsaturated FAs and n-6 and n-3 polyunsaturated FAs. In the second application, ATR-FTIR spectral data in conjunction with multivariate partial least squares regression (PLS-R) was used to accurately predict low (0.5%) total trans-fat contents in the lipids extracted from 24 representative fast foods. PLS-R-predicted concentrations of total trans fatty acid methyl esters (FAME) were in good agreement with those determined by a primary reference gas chromatography (GC) method ($R^2 > 0.99$). This simple, fast, and nondestructive analytical method has the potential to be used for rapid screening of food products, quality assurance, and regulation of label claims.

13. Quantitative Evaluation of Gold Nanoparticle-Pulsed Laser Interaction in Biologically-Relevant Media: Cytotoxicity, Photomodification, and Implications for Emerging Medical Products

Authors: Rosa, Nicholas, FDA/CDRH; Fales, Andrew M., FDA/CDRH; Vogt, William C., FDA/CDRH; Wear, Keith A., FDA/CDRH; Pfefer, T. Joshua, FDA/CDRH; Ilev, Ilko K., FDA/CDRH

Plain Language Synopsis: Gold nanoparticles (GNPs) have seen increasing use in nanobiophotonics due to their unique optical properties. However, the pulsed laser irradiation used in many optical diagnostic and therapeutic techniques can induce photomodification of GNPs below current safety limits. Potential safety and performance impacts of GNP photomodification were studied in vitro with biologically-relevant media.

Abstract:

One of the most promising areas in the field of nanobiophotonics is the use of gold nanoparticles (GNPs) in laser diagnostics and

therapeutics. This combination has been used in several preclinical and clinical studies to enhance the performance of various laser-based diagnostic and therapeutic techniques. For example, in photoacoustic imaging, targeted GNPs have been used to enable molecular imaging. During photothermal therapy, GNPs can enhance light-to-heat transduction to improve the efficacy of treatment. Recently, GNP-mediated bubble generation was shown to be effective for optoporation and cancer cell destruction. While GNPs show potential for use in nanobiophotonic medical devices, challenges with photostability present a significant hurdle to their development and regulatory evaluation. The high peak irradiance levels generated by pulsed lasers during imaging or treatment can cause irreversible modification of GNPs size and shape above a laser exposure threshold. We showed that for some sizes of gold nanoparticles, these photomodification thresholds can be below the laser safety limit set by ANSI Z136.1:2014. However, questions remain about the safety of GNPs in nanobiophotonics: 1) where does the cytotoxicity threshold lie in relation to the thresholds for effects like bubble generation or photomodification and 2) at the threshold, what is the mechanism of cytotoxicity? To address these questions, we present evaluations of plasmonic GNPs in biologically-relevant media under irradiation from a 532 nm nanosecond-duration pulsed laser routinely used in nanobiophotonics applications. Using the standardized methodology developed in our lab, we investigated the effects of biological media on the damage and bubble generation thresholds and sought to establish a radiant exposure threshold for the onset of cytotoxicity. Further, we began preliminary investigations into the mechanism of cytotoxicity to define the future directions for safety evaluation. Theoretical models indicate that the bulk temperature change induced by pulsed-laser irradiation is insufficient to cause significant cell death. We instead focused on detecting photomechanical toxicity mechanisms. The results of this study will facilitate the

development of pulsed-laser irradiated plasmonic nanoparticle medical devices by providing methods to enable more rapid and informed assessment of the safety and efficacy of GNP-based nanobiophotonic techniques.

14. 3D Bioprinting of Human Multipotent Stromal Cells within Gelatin-Alginate-Collagen Hydrogel Constructs

Authors: Sawyer, Stephen, FDA/CBER; Degheidy, Heba, FDA/CBER; Takeda, Kazuyo, FDA/CBER; Alayoubi, Alaadin, FDA/CDER; Zidan, Ahmed, FDA/CDER; Bauer, Steven, FDA/CBER

Plain Language Synopsis: In this study, we optimized a gelatin-alginate-collagen material containing human multipotent stromal cells (MSCs) for 3D printing and evaluated how 3D printing and subsequent encapsulation of human MSCs affected their viability, proliferation, morphology, and differentiation.

Abstract:

Background/Purpose: 3D bioprinting is increasingly used to fabricate regenerative medicine products and in the development of micro-physiological systems. Most cell-based constructs proposed for clinical trials or drug screening are composed of cells plus extracellular matrix (ECM) with inherent tunable and modular properties. 3D bioprinting of stem cells may affect their differentiation potential and their commitment towards specific cell types, while being a versatile approach for making cellular tissues with controllable sizes to fit the purpose of screening or regenerative medicine applications. In this study, we optimized a gelatin-alginate-collagen material containing human multipotent stromal cells (MSCs) for 3D printing and evaluated how 3D printing and subsequent encapsulation of human MSCs affected their viability, proliferation, morphology, and differentiation.

Methodology: A 10% (w/v) gelatin, 1% (w/v) alginate, and 1.0 mg/ml collagen solution was optimized to be printed at temperatures between 15oC and 20oC at various pressures

using an EnvisionTEC Bioplotter. The optimized gelatin-alginate-collagen material was capable of being sterilized at 70oC with no disruption to the material properties, and human MSCs were added to the mixture at a final concentration of 1x10⁶ cells/ml. Material containing cells was printed at 20oC, cross-linked in a 3% CaCl₂ solution, and kept in culture media at 37oC with 5% CO₂ for up to two weeks.

Results/Conclusion: Our results revealed that we were able to consistently print identical constructs containing internal 2 mm crisscrossed lines at 20oC using 1.8 bar at 8 mm/sec. In addition, these printed constructs were stable in culture media for up to two weeks post-cross-linking. Encapsulated cells were shown to be viable directly after printing and after two weeks of culture. Proliferation studies showed that after one week of encapsulation, the human MSCs began to proliferate rapidly, suggesting that the gelatin-alginate-collagen environment was suitable for 3D culture. Morphology and differentiation studies of encapsulated MSCs showed that after two weeks of 3D culture, cells within the printed constructs formed large, interconnected colonies and were capable of robust adipogenic differentiation. Together, these results show promise for the use of 3D printing in the creation of regenerative medicine products.

15. Modernization of Color Additive Analysis

Authors: Schaufler, Lawrence, FDA/ORA/DENL; Clark, Susan, FDA/Retired; Storey, Joseph, FDA/Retired; Johnson, Aaron, FDA/ORA/DENL; Thomas, Terri, FDA/ORA/DENL; Carr, Justin, FDA/OSPOP

Plain Language Synopsis: Colors are added to foods, cosmetics, and other products to improve consumer appeal. Worldwide, the manufacturing industry uses some colors that are harmful to health, yet most traditional detection methods are decades old. We have developed an efficient technique using modern technology to analyze products for over 150 color additives.

Abstract:

Color additives have been used for centuries to increase the appeal of food, cosmetics, and other regulated products to the consumer. In the last century, however, our knowledge of the harmful nature of many of these additives has vastly increased. Worldwide, the manufacturing industry continues to use colors that have been found to be harmful to health. Yet many of the methods FDA uses to detect them are 25-50 years old and are limited in applicability and detection level. We developed a protocol to rapidly analyze foods, drugs, and cosmetics for over 150 color additives using modern UPLC-PDA detection with minimal solvent waste production. We successfully increased throughput, sensitivity, and efficiency, even with the most difficult sample matrices. We also developed a process using LC-MS/MS to identify new or unusual color additives that continue to appear, particularly in imported products. For example, we successfully identified Basic Violet 11:1, a color additive that could not be identified using traditional methodology, in an imported children's cosmetic, as well as other products. Industry advances require FDA to continue to maintain current methods to effectively regulate the approved use of color additives in foods and other products.

16. Wireless Coexistence Testing in the 5 GHz Band with LTE-LAA Signals

Authors: Seidman, Seth J, FDA/CDRH/OSEL; Al-Kalaa, Mohamad Omar, FDA/CDRH/OSEL

Plain Language Synopsis: LTE-LAA is a version of LTE that uses unlicensed spectrum that will compete with other unlicensed operators, such as Wi-Fi. We have presented methods to determine the wireless coexistence between LTE-LAA and Wi-Fi. These methods will be used to update standards that device manufacturers currently use to demonstrate wireless coexistence.

Abstract:

Exploiting unlicensed spectrum bands for cellular communication is a trend that has been rapidly embraced by industry stakeholders. Accordingly, the specifications

of Long Term Evolution (LTE) were extended in Release 13 to allow unlicensed spectrum operation, also known as LTE-Licensed Assisted Access (LAA).

LTE is widely adopted, and there is a potential for significant coexistence impact of LTE-LAA on users of unlicensed spectrum, including wireless medical devices, whether adopters of the new technology or incumbents. Therefore, work was initiated to revise and update the American National Standards Institute (ANSI) C63.27 standard for evaluation of wireless coexistence.

This poster details the experimental work conducted at the Electromagnetic and Wireless Laboratory, U.S. Food and Drug Administration, to investigate the use of LAA signals for wireless coexistence testing. A software-defined radio platform was deployed to generate realistic LAA signals and measure the wireless coexistence impact on the LAA communication link. The equipment under test (EUT) used IEEE 802.11ac as an example incumbent technology in the 5 GHz band. The standardized radiated anechoic chamber method was used for testing. Results highlight the mutual coexistence impact of LAA in the 5 GHz band and suggest that selecting an LAA signal with the maximum possible channel time occupancy and the highest possible modulation and coding scheme (MCS) has the most coexistence impact on both the EUT and the LAA system.

17. Assessing the Effect of Embedded Metals in Tissues Using Novel Spectroscopic Techniques

Authors: Smith, Diane, FDA/CDRH/OSEL/DBCMS, HJF; Hoffman, Jessica, USUHS/AFRRI; Kalinich, John, USUHS/AFRRI; Centeno, Jose, FDA/CDRH/OSEL/DBCMS

Plain Language Synopsis: Metals embedded in tissues are evaluated using highly sensitive chemical analysis. These materials are relevant to medical devices and military wounds, the long-term health ramifications of which are not fully understood.

Abstract:

Many medical devices contain metals that may interact with the body. The long-term health consequences of many of these materials are not thoroughly understood. Additionally, embedded metal fragments from military wounds are typically not removed, to avoid the risk of morbidity associated with invasive surgery. The aim of our study is to evaluate the distribution of metals in animal tissues as a model to establish health risk from metal exposure. Metal pellets were implanted in the gastrocnemius muscle of rats for up to 12 months. The muscle with the embedded metal fragment and distant tissues were harvested, flash frozen, and sectioned for analysis. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) and Fourier transform infrared spectroscopy (FTIR) microscopy were used to map the distribution of metals in rat tissues without the use of fixatives or stains, thereby preserving ultrastructural integrity of the tissues. This information will help bridge the gap in our understanding of the potential effects of select metals in the body. In addition, this study will contribute to our assessment of embedded metal fragments in military personnel.

18. MALDI-TOF Mass Spectrometry: An Emerging Technology for Rapid Species Identification of Human-Pathogenic Bacteria of Public Health Importance

Authors: Sulaiman, Irshad^{1,*}, FDA/ORA/SFFL; Hsieh, Ying-Hsin¹, FDA/ORA/SFFL; Banerjee, Pratik², School of Public Health/University of Memphis; Miranda, Nancy¹, FDA/ORA/SFFL; and Simpson, Steven¹, FDA/ORA/SFFL

Plain Language Synopsis: Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is an emerging technology that can provide microbial identification using either intact cells or cell extracts without performing any extraction or purification.

Abstract:

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-

TOF MS) is an emerging technology that can provide microbial identification using either intact cells or cell extracts, without performing extraction or purification. This new instrument has been employed as a rapid, high throughput, and efficient diagnostic method. Currently, MALDI-TOF MS systems are routinely used to identify different microbes isolated from clinical, environmental, and outbreak samples. It has also been found useful in strain typing, discriminating phylogenetic groups, and in identifying difficult-to-cultivate human-pathogenic bacteria that requires sequencing of the regions of ribosomal rRNA genes. In recent years, this platform was shown to be effective in discriminating species level identification of various Gram-positive and Gram-negative bacteria causing foodborne illnesses. Nevertheless, the technology has limitations, especially for identifying new isolates that may be absent in the reference database used for strain typing. In a previous study, the performance of two commercially available MALDI-TOF MS platforms (bioMérieux VITEK MS, and Bruker Biotyper) was evaluated. Furthermore, the 16S rRNA gene was used to sequence sixteen distinct *Campylobacter* species and the data generated was compared to the Biotyper and the VITEK MS systems. In a subsequent study, the VITEK MS system was used to identify *Staphylococcus aureus* and its related species recovered from food, environment, cosmetics, medical devices, and clinical samples. Additional analysis on a cosmetic product was performed for the presence of a human-pathogenic bacteria. The VITEK MS and 16S rRNA sequencing correctly identified the recovered Gram-positive bacterial isolates as *Lysinibacillus fusiformis*. More recently, fifty *Cronobacter*-like Gram-negative bacterial isolates from nine major categories of foods and forty-nine *Clostridium*-like Gram-positive bacterial isolates were recovered from food and environmental samples. Analysis of this data revealed that MALDI-TOF MS is a suitable platform for typing bacteria isolated from food and environmental samples and is of public health importance for the

implementation of effective food safety programs.

19. Characterization of Movement Trajectories of the Reverse Total Shoulder Arthroplasty

Authors: Kim Kontson, PhD, FDA/CDRH/OSEL; Alec Boyle BS, FDA/CDRH/OSEL; Fraser Bocell, PhD, FDA/CDRH/OPEQ; Edward McFarland, MD, JHU; Stephen Weber, MD, FDA/CDRH/OPEQ; Stacey JL Sullivan, PhD, FDA/CDRH/OSEL

Plain Language Synopsis: Accurately measuring arm movement in patients with upper limb joint replacements is critical to understanding how well these devices work for patients, and thus how to understand the benefits and risks of new device applications to FDA. This study uses motion capture technology to measure arm movement.

Abstract:

The use and design variations of reverse total shoulder arthroplasty (rTSA) have increased in the last two decades. Two-dimensional goniometric measurement has been the gold standard for upper extremity measurement in evaluating function of individuals with rTSA. This data is translated into functional outcome scores used to evaluate the safety and effectiveness of upper extremity devices that come to FDA. Patient-reported outcomes (PROs) that assess an individual's perception of functional abilities are also used to support medical claims. Two-dimensional goniometry, however, lacks accuracy and reproducibility, and perceived patient performance captured from PROs has never been compared to actual patient performance.

Therefore, the goals of this study are to create a data set that characterizes the motion path of rTSA patients using more accurate, reliable 3D optical motion capture technology, and relate motion path of rTSA patients to their perceived capabilities captured through PROs. In collaboration with The Johns Hopkins School of Medicine, subjects that are one-year postoperative from rTSA surgery, along with age-matched controls, will be recruited to participate in the study at FDA. PRO data will be collected

from each individual before they complete activities of daily living that mimic activities described in the PROs. Motion path data during task performance will be acquired from a 10-camera optical motion capture system (Vicon Motion Systems, Oxford, UK). The functional reach space of the arm and movement trajectories as participants complete these activities of daily living will be compared for different designs of rTSA and normal subjects. Different characteristics of motion (path length, peak angle, range-of-motion, etc.) will also be correlated with PRO responses. Results from a pilot study to assess the tasks and motion capture model will be presented here, along with preliminary data collected from normal controls and rTSA patients.

By collecting more robust, reliable movement data from individuals with these devices, we can critically evaluate in vivo performance of different rTSA designs and determine adequacy of PROs to accurately assess the potential for function after implantation of these devices.

20. Correlation of tissue factor- and phospholipid-activated thrombin generation assays with tail bleeding in hemophilia A mice treated with coagulation factor VIIa

Authors: Surov, Stepan, FDA/CBER; Rezaie, Alireza R., St Louis University School of Medicine, Saint Louis, MO, USA; Liang, Yideng, FDA/CBER; Ovanesov, Mikhail V., FDA/CBER

Plain Language Synopsis: Thrombin Generation Test (TGT) under different activation conditions was used to study the correlation between ex vivo and in vivo (tail bleeding model) effect of recombinant factor VIIa in hemophilic mice. Among all TGT conditions tested, only the Tissue Factor-free Phospholipid-based assay was able to predict the effect of FVIIa on tail bleeding

Abstract:

Background: Recombinant factor VIIa (FVIIa) is used to treat hemophilia patients with inhibitors. Traditional laboratory assays have not been useful in guiding FVIIa therapy

because dosing levels, frequency, and treatment duration are highly variable. The thrombin generation test (TGT) has been proposed as a candidate assay. Recent studies showed that TGT assay sensitivity to FVIIa concentration in human and mouse plasma improves significantly with the use of soluble tissue factor (sTF), versus either phospholipid (PL)- or tissue factor (TF)-based reagents.

Objective: Study the relationship between tail bleeding and TGT assays ex vivo in FVIII knock out (KO) mice treated with FVIIa.

Methods: FVIII KO mice (B6;129S-F8tm1Kaz/J, Jackson Labs) were treated with 0.125 to 10 mg/kg of recombinant FVIIa product (Novo Nordisk). FVIIa activity was measured by an sTF-based clotting StaClot (Stago) assay. TGT was studied using five activation conditions: PL, human full-length TF (FLTF), FLTF with PL, human sTF (hsTF) with PL, and mouse sTF (msTF) with PL. Clotting time and blood loss volume were evaluated in a parallel tail clip study.

Results: PL-, PL/FLTF- and PL/hsTF-TGT assays demonstrated a strong correlation with high levels of FVIIa activity, while PL/hsTF TGT assay was also sensitive to low FVIIa doses. Tail bleeding studies demonstrated the weak effect of low FVIIa doses (below 1 mg/kg). Good dose-dependent hemostatic effect on tail bleeding time and volume of blood loss were observed above 2.5 mg/kg. Of all the TGT assays tested, only the TF-free PL-based TGT assay was able to predict the effect of FVIIa on tail bleeding time.

Conclusions: Correlation of PL-based TGT with the hemostatic effect of FVIIa action appears to confirm previous observations of PL-dependent mechanism of human FVIIa in mice. This, and the lack of response to human doses (0.1-0.3 mg/kg), may be related to poor interaction of human FVIIa with mouse TF.

Disclaimer: This is an informal communication and represents the authors' best judgment. These comments do not bind

or obligate FDA

21. UVA-Riboflavin Corneal Cross-Linking as a Novel Optical Therapeutic Platform: Safety Issues Investigated by Optical Coherence Tomography with Optical Path Length Measurements

Authors: Tan, Xin, FDA/CDRH/OSEL; Agrawal, Anant, FDA/CDRH/OSEL; Hammer, X. Daniel, FDA/CDRH/OSEL; Ilev, Ilko, FDA/CDRH/OSEL

Plain Language Synopsis: Corneal Cross-Linking (CXL) has emerged as a new light-based treatment platform for corneal ectatic disorders characterized by progressive thinning of the cornea leading to visual loss. Approved by FDA in 2016 as a combination product, CXL has been undergoing continuous innovative developments. This study investigates important safety concerns of CXL.

Abstract:

Corneal cross-linking (CXL) using UVA irradiation with riboflavin photosensitizer has emerged as a new optical therapeutic paradigm for corneal ectatic disorders characterized by progressive thinning and weakening of the cornea that leads to visual loss. The thickness threshold for protection of intraocular structures has often been challenged with ongoing developments. Corneal thinning becomes an important safety concern, especially for patients with thin corneas. In this study with an ex vivo bovine eye model, we monitored corneal thinning and corneal refractive index change using optical coherence tomography (OCT) integrated with an adaptation of the optical path length method. CXL experiments were performed based on the standard protocol that includes removal of the corneal epithelium to facilitate diffusion of riboflavin into the stroma. Corneal stromal thickness and group refractive index (GRI) were measured by a 1310-nm FD-OCT imaging system at three critical points of the procedure: 1) immediately after epithelial removal; 2) after 30-minute riboflavin instillation; 3) after 30-minute UVA irradiation with continuing instillation. We found that

the refractive index of the bovine cornea changed significantly from epithelial removal to riboflavin instillation and UVA irradiation, increasing from 1.377 ± 0.005 (mean \pm standard deviation) after de-epithelization to 1.387 ± 0.003 after 30-min instillation and 1.388 ± 0.008 after subsequent irradiation. The corneas also underwent considerable decrease (10-20%) in stromal thickness, with thinning of $95 \pm 29 \mu\text{m}$ (mean \pm standard deviation) after riboflavin instillation and further decrease (~5%) with thinning of $42 \pm 19 \mu\text{m}$ after UVA irradiation. Our study highlights the importance of corneal thickness monitoring during CXL especially after riboflavin instillation when the decrease is largest, to avoid delivering endothelial cytotoxic doses. Increase in refractive index heightens the concern for corneal thinning and the need for careful monitoring as a safety precaution.

22. Office of Applied Research and Safety Assessment's Approach to Public Health with Advanced Technology and Predictive Tools

Authors: Torrence, Mary CFSAN/OARSA; Solomotis, Marianne CFSAN/OARSA; OARSA

Plain Language Synopsis: OARSA's revised strategic roadmap focuses microbiologic and toxicologic research around 6 critical areas. OARSA's research involves using advanced technology and predictive tools to uniquely address: toxicology, *Campylobacter*, *Cyclospora cayetanensis*, foodborne viruses, probiotics, and produce food safety.

Abstract:

The Office of Applied Research and Safety Assessment (OARSA) developed a strategic plan to articulate and direct our critical contribution to CFSAN's mission to protect public health. While the strategic plan provides the roadmap for OARSA, our research agenda sets the final goal. As a regulatory agency, CFSAN oversees food safety, nutrition, dietary supplements, food additives, cosmetics, and color additives. This necessitates regulations, guidance documents, and policy decisions based on

science. As one of the leading research offices, it is imperative that OARSA charts their research agenda according to its research and organizational strengths. This agenda recognizes OARSA's distinctive research expertise and ability to contribute CFSAN's mission. By focusing on our unique contribution, OARSA is able to complement essential programmatic and research priorities, while leveraging our resources to protect public health and enhance food safety.

This presentation describes the current OARSA research agenda focused around the use of advanced technology and predictive tools to address critical areas in the protection of public health: toxicology, *Campylobacter* spp., *Cyclospora cayetanensis*, foodborne viruses, probiotics, and produce food safety. Although research in these areas is not exclusive to other offices, OARSA's expertise is demonstrated with advanced technologies and predictive tools in new and expanded products. For example, OARSA has established new genomic databases for foodborne viruses, *Campylobacter*, *Cyclospora*, and probiotics. This complements CFSAN's GenomeTrakr, which helps identify and predict foodborne outbreaks. To address emerging produce food safety needs, OARSA established a research framework that takes advantage of unique laboratory facilities and research contracts with academic centers. This framework allows other offices the ability to address identified data gaps. In parallel to OARSA's microbiological research, our Toxicology Division is using new and advanced technologies (e.g. 3D bioprinting and microfluidic chips) to compare new methods with established approaches (in vitro, in vivo, in silico modeling). This toxicologic work provides the opportunity to develop a concordant database for the use in predictive approaches for regulatory toxicology.

23. An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics

Authors: Wang, Deyun Wang, FDA/ORR/ORS/NMPL; Park, Junyong, University of Maryland,

Baltimore County; Leazer Jr., John L., FDA/ORA/ORS/NFFL; Keire, David A., FDA/CDER/OPQ/DPA; Chen, Kang, FDA/CDER/OPQ/DPA

Plain Language Synopsis: Protein Higher Order Structure (HOS) is a critical quality for drug products. Nuclear Magnetic Resonance (NMR) has been a sensitive analytical method to measure and compare protein HOS from different manufacturers. The quantification of spectral differences using Mahalanobis distances (DM) is achieved, serving as reference metrics for future studies.

Abstract:

Higher order structure (HOS) is a critical quality attribute (CQA) for safety and efficacy of protein or peptide therapeutics. The HOS similarity between a generic, biosimilar, or follow-on product and a reference listed drug (RLD) should be demonstrated as part of the regulatory approval process. Quantitative HOS similarity evaluation of the same protein drug substance from different manufacturers can be challenging, especially when protein HOS might be sensitive to process and formulation differences from firm to firm. In this poster, we present an NMR spectroscopy method for quantitative assessment of HOS differences of US marketed insulin therapeutics from different manufacturers. Standard 1D ¹H NMR spectra were collected on insulin drug samples using a 600-MHz spectrometer equipped with a room-temperature probe; the spectra were subject to principle component analysis (PCA). The unitless Mahalanobis distances (DM) in PCA space were calculated between products that contained the same insulin drug substance but were formulated differently by different manufacturers. The DM between insulin lispro RLD Humalog® and its 2017 approved follow-on product Admelog®, which matched the RLD formulation closely, was ~100. And the DM between the two independently formulated insulin regular products approved in 1980s, Humulin® R and Novolin® R, was larger, ~200. While the NMR-spectra-derived DM value of 200 between insulin regular drug products was larger than the DM value of 100 between

insulin lispro drug products, the contribution of difference in insulin drug formulation was not known. Assessments of insulin drug substance spectra were performed with mass-balanced and reversible dialysis. Both HumulinR® and NovolinR® were dialyzed to the same buffers of pH 4.0 or pH 7.4. The DM was reduced to less than 1, suggesting insulin regular drug substances were convertible upon formulation difference. In summary, the observed dynamic range of the DM value proved to be a robust, sensitive, and convenient metric for HOS similarity assessments, and the resulting DM values can be used as a bar for quantitative HOS similarity evaluation and surveillance of intact insulin formulations and buffer exchanged drug substance if mass balance was well controlled.

24. Quantitative and objective evaluation of upper limb function: A comparison of motion analysis systems

Authors: Wang, Sophie, University of Maryland, FDA/CDRH; Kontson, Kimberly, FDA/CDRH

Plain Language Synopsis: Quantitative, objective evaluation of movement during use of advanced upper limb prosthetic technology will inform regulatory decisions. However, tools to evaluate movement can be costly and limiting. This work compares motion analysis systems varying in cost and complexity to determine adequacy of consumer-grade motion analysis systems in evaluating movement quality.

Abstract:

The evaluation of upper limb function is important when making benefit and effectiveness determinations for medical products designed to provide rehabilitative, therapeutic, or assistive benefits to individuals with upper limb impairment or disability. Upper limb prosthetic devices are an example of one such medical product. Research programs in the Department of Defense are developing advanced, upper-limb prostheses with simultaneous control of multiple degrees of freedom (DOFs) and

sensory feedback mechanisms via peripheral nerve and muscle implants. The increased risk associated with implanted componentry elevates the need for robust scientific evidence of benefit and effectiveness; yet methods to evaluate upper limb function are currently lacking. New methods to determine risk-to-benefit ratios are necessary for regulatory review of these devices.

Given the high incidence of musculoskeletal pain among upper limb prosthesis users, likely due to compensatory movements used to account for low dexterity and loss of distal DOFs, movement quality is an important factor to consider during review. Motion capture is a promising approach for benefit assessment, as it can provide objective, quantitative measurements of body kinematics. However, state of the art optoelectric motion analysis systems are costly and require technical knowledge and dedicated recording environments. Simple, affordable consumer-level motion-capture systems are highly attractive.

To examine whether consumer-level motion analysis systems can detect departures from the normative range of variation, three motion analysis systems will be compared: Vicon Motion Capture System (gold standard), Microsoft Kinect, and Inertial Measurement Units. Ten able-bodied participants will undergo a within-subject experimental design to assess movement under normal conditions and an induced disability condition that will restrict the upper limb DOFs. Participants will perform selected tasks from the validated outcome measure, Jamar Hand Function Test, as well as a novel object transport task, targeted Box and Blocks Tests. Three trials of each task will be performed while being recorded by the three motion capture systems. Root mean square errors relative to Vicon values and ANOVA comparisons of peak joint angles, mean joint angles, standard deviations, and other values will be performed to examine system performance.

25. Evaluation of drug-to-excipient ratio effects on the drug release profile in drug coated balloons

Authors: Wickramasekara, Samantha, FDA/CDRH; Tran, Mandy, FDA/CDRH; Woolford, Steven, FDA/CDRH; Yoda, Coumbe, FDA/CDRH; Oktem, Berk, FDA/CDRH; NguyenPho, Agnes, FDA/CDER; McDermott, Martin, FDA/CDRH

Plain Language Synopsis: Drug-coated balloons (DCB) are a popular treatment for blocked arteries. Drug to excipient (D:E) ratio of DCB is important in transferring the drug to the target site. We studied drug release profiles of DCB with different (D:E) ratios to predict the efficiency of drug delivery at the target site.

Abstract:

Approximately 8.5 million people in the United States have peripheral arterial disease (PAD), which is the narrowing of vessels that carry blood from the heart to the body's extremities and is primarily caused by the build-up of fatty plaque in the arteries (stenosis). Balloon angioplasty and bare metal stents were initially used for the treatment of stenosis, but restenosis (re-blockage) became the rate limiting factor for these procedures. Drug-coated balloons (DCB) have emerged as the alternative procedure for restenosis because of their ability to treat a variety of occlusion types with a uniform dose of anti-proliferative drugs. There are several types of coating matrices used to produce DCBs. In this study, the relationship between coating composition and drug release under physiologically relevant conditions was examined to understand how differences in coating composition impact the drug transfer from the balloon surface to the simulated body fluids. To conduct the experiments, the balloons were coated using an in-house developed micro-pipetting method. Paclitaxel was used as the anti-tumor drug because it reduces cell proliferation and Iopromide was used as the excipient because it allows the drug to adhere to the target site longer. Balloons were coated with different drug-to-excipient ratios (3:1, 3:2 and 1:2 drug-to-

excipient ratio respectively). In vitro drug release kinetics were studied with Vention balloons. A track and deploy method was performed using a “mock” artery, using coated POWERFLEX Extreme balloon catheters to simulate an in vitro environment. The amount of drug released to the medium at each time point was quantified by high performance liquid chromatography (HPLC). In vitro drug release kinetics studies showed that the coating conditions used for micro-pipetting affects the drug release rate into the simulated fluid. Scanning electron microscopy (SEM) images showed that 3:1 ratio produced more uniform and thin coating throughout the balloon surface compared to the other drug-to-excipient ratios. Track and deploy studies showed that 3:1 drug-to-excipient ratio is the most effective coating ratio for this procedure, since it produces the highest drug recovery. This data provides useful information for understanding how coating composition influences the drug release profile under simulated conditions.

26. Low-Frequency Raman Mapping and Multivariant Image Analysis for Characterization of Complex Pharmaceuticals

Authors: Willett, Daniel FDA/CDER; Yilmaz, Huzeyfe, FDA/CDER; Wokovich, Anna, FDA/CDER; Zidan, Ahmed, FDA/CDER; Rodriguez, Jason, FDA/CDER

Plain Language Synopsis: Low-frequency Raman mapping was used in conjunction with multivariate image analysis techniques to provide characterization of various pharmaceutical dosage forms. This combined approach allows for characterization of API distribution and size, as well as polymorph detection, that can be visualized across the dosage form.

Abstract:

The low-frequency Raman (THz-Raman) region (10 cm^{-1} to $200\text{ cm}^{-1}</math>) provides access to lattice vibrations of molecular crystals and can be used to investigate intermolecular interactions in the solid state. In the pharmaceutical laboratory, THz-Raman$

can provide polymorph detection as well as identification, both of which can be critical quality attributes in pharmaceutical materials that must be assessed and monitored during or after manufacturing. The combination of multivariate statistical analysis with THz-Raman mapping data can further enable scientists to detect changes in intermolecular interactions occurring in the pharmaceutical products.

The combined approach was used to study three different dosage forms: a combination tablet containing multiple active ingredients, a transdermal drug delivery systems (TDDSs), and a topical formulation. The results showed that the combined approach was useful in mapping the active pharmaceutical ingredient (API) distribution and crystal sizes in the tablet with multiple APIs. For the TDDS system studied, the combined approach disclosed unintended polymorph formation within the TDDS. Finally, the combined approach demonstrated the ability of THz-Raman spectroscopy to probe crystal orientation. Orientation identification was useful to establish when spectral variances in crystalline APIs from different orientations could be misinterpreted as the presence of a different polymorphic form. Overall, the potential of THz-Raman mapping with multivariate statistical analysis was demonstrated here through analysis of a variety of drug products. This initial study was the first step in a series of planned studies to establish protocols for combining spectroscopic and chemometric approaches as a tool to discern the quality of complex drug products.

27. Tip Enhanced Raman Spectroscopy as a Novel Method for Supporting Physicochemical Sameness of Ophthalmic Emulsions

Authors: Wood, Erin L. FDA/CDER/OPQ/IO/SRS; Tyner, Katherine FDA/CDER/OPQ/IO/SRS

Plain Language Synopsis: We employed a novel technique enabling the evaluation of physical and chemical characteristics of

a locally acting ophthalmic drug product in order to evaluate similarity between a reference listed drug and a generic.

Abstract:

Locally acting complex formulations, such as ophthalmic emulsions, are challenging to evaluate for bioequivalence, due to reliance on clinical endpoints that may lack sufficient sensitivity. While Q3 similarity (similar physical and structural properties) may be used to support establishing bioequivalence, this becomes increasingly difficult for formulas consisting of nanoscale features that challenges the development of generic formulations. In this work, methods to characterize ophthalmic emulsions through tip-enhanced Raman spectroscopy (TERS) are described. TERS is uniquely suited to provide physicochemical information: it combines nanoscale spatial resolution and physical imaging of atomic force microscopy with the chemical specificity of Raman spectroscopy. This combination enables sub-diffraction chemical mapping of nanoscale features, such as the globules and micelles present in the emulsion product of interest. Results show that TERS is a superior technique for providing physicochemical information when compared to more traditional Raman chemical mapping.

28. Application of multiple attribute method (MAM) in the analysis of Big Protein Project (BPP) rituximab samples

Authors: Wu, Di, CDER/OPQ/OTR/DPA; Oyugi, Mercy, CDER/OPQ/OTR/IO; Rogstad, Sarah, CDER/OPQ/OTR/IO

Plain Language Synopsis: The MAM is a LC-MS based peptide mapping method that has been proposed to ETT as a replacement for conventional QC methods. This study of BPP has applied MAM to identify and quantify the modifications in rituximab and support ETT assessment of this novel technology.

Abstract:

Background: The multi-attribute method (MAM) is a liquid chromatography-mass spectrometry-(LC-MS) based peptide

mapping method that has been proposed to the Emerging Technology Team (ETT) as a replacement for conventional quality control (QC) methods.

Purpose: MAM can be used to identify and quantify multiple product quality attributes (PQAs) in therapeutic proteins within a single method. To evaluate the reproducibility and robustness of MAM, our lab has conducted a series of studies using rituximab.

Methodology: The method was developed and validated previously and we applied the workflow to samples from OTR's Big Protein Project (BPP). This study addressed the use of MAM to analyze nine rituximab drug product lots with various sources and expiration dates. A 70-minute liquid chromatography gradient coupled with a Thermo Q-Exactive mass spectrometer was used to study the modifications.

Results: The data were analyzed using the Chromeleon software platform. We successfully analyzed the abundance of 21 PQAs in each of the lots, compared lot-to-lot variation, and analyzed newly detected peaks between drug lots with reference.

Conclusion: Within this study, we were able to distinguish between lots, differentiate products from approved or unapproved manufactures, and evaluate PQA levels across expiration dates. These data will be compared with other analytical results for the BPP studies. OTR's MAM research supports the ETT assessment of this novel technology.

29. A Near-Infrared (NIR) Spectroscopy-based Process Analytical Technology (PAT) System for A Narrow Therapeutic Index Drug Blend Process Monitoring and End-Point Detection: From Development to Scale up

Authors: Talwar, Sameer , Duquesne University/Duquesne Center for Pharmaceutical Technology; Pawar, Pallavi, Rutgers University/ Department of Chemical and Biochemical Engineering; Wu, Huiquan, FDA/CDER; Sowrirajan, Koushik , FDA/CDER; Friedman, Richard, FDA/CDER

Plain Language Synopsis: Deployment of

near-infrared spectroscopy to enable real-time monitoring powder blending process for a narrow therapeutic index drug product was tested. Proposed hybrid model using data from different process scales is beneficial in simplifying and reducing the cost of model building relative to model creation with data from only pilot/manufacturing scale.

Abstract:

The deployment of near-infrared (NIR) spectroscopy to enable real-time monitoring of the powder blending process for a narrow therapeutic index (NTI) drug product (phenytoin sodium) as a platform approach was tested. The study used a robust experimental design and multiple NIR sensors to investigate model development, scale-up, and the effect of various blend end-point algorithms on the predicted mixing end time. The study incorporated a global calibration strategy, wherein spectral data collected using two NIR spectrometers at two process scales (150 g and 25 kilos) was combined in a single calibration method. The authors demonstrated that unique end points were determined using the different algorithms derived from standard deviation, average, and distributions of concentration predictions for all major components. The selection of a suitable algorithm must involve consideration of critical quality attributes of the final dosage form, such as the active content for a NTI drug product, as considered for the current study. The control over the distribution of phenytoin sodium was the most critical aspect, considering its high potency and a narrow therapeutic index, since over- or under-blending of an NTI formulation may trigger efficacy and safety concerns. Hence, the algorithms based on testing its bias from target concentration (moving window averages and prediction residuals) offer the most straightforward interpretation and consistent trends for both sensors. In contrast, the algorithms based on testing global homogeneity for all components (active and excipients) yielded the longest blending end point, but potentially more sensitivity to subtle variations in

blend uniformity. The algorithms based on qualitative analysis of spectral variance (such as PCA) yielded the lowest end points. This implies that a low deviation doesn't necessary mean homogeneity; rather a modification of the powder mixture attributes. The proposed hybrid modeling approach based on combining data from different process scales can be beneficial in simplifying and reducing the cost of model building relative to model creation, with data from only pilot/manufacturing scale. However, it is critical to recognize that success of such an approach depends on the spectroscopic variability captured at different scales and its relative contributions in the final NIR model.

30. Use of Whole Genome Sequencing-Based *in silico* Serotyping for Improved Accuracy of Shigella Identification

Authors: Wu, Yun, FDA/OC/OSPD/CFP; Lau, Henry, FDA/ORA/SANFL; Lau, David, FDA/ORA/SANFL; Lee, Teresa, FDA/ORA/SANFL; Payne, Justin, FDA/CFSAN

Plain Language Synopsis: Shigellosis is a potentially life-threatening disease caused by bacteria from the Shigella, the conventional identification of which is laborious, inaccurate, and expensive. We developed a click-button, whole genome sequencing-based Shigella identification application that is more accurate, simpler, and can be integrated into existing an FDA surveillance program without additional cost.

Abstract:

Shigella spp. cause diarrheal disease with serious public health implications. Conventional Shigella identification methods are laborious, time-consuming, and can be erroneous due to cross-reactivity between serotyping antisera. It is also difficult to differentiate Shigella from enteroinvasive Escherichia coli (EIEC), a separate clade of pathogenic E. coli that is highly similar to Shigella. Further, serotype interpretation is complicated for inexperienced users. An easier, more accurate method is needed. We systematically inspected 48 large assemblies and 221 whole genome

sequencing (WGS) data of *Shigella* isolates. For 6 serotypes that were not previously characterized genetically, respective serotype determinants were identified. Genes commonly perceived as characteristic hallmarks in *Shigella* were examined to establish rules for EIEC differentiation. Low concordance rate between conventional designation and molecular serotyping was observed: 86.4% and 80.5% at species and serotype level, respectively. The differentiation gene markers showed high variability among different serotypes. Using the information obtained from the systematic examination of *Shigella* genomic information described above, we developed an automatic *Shigella* identification pipeline, ShigaTyper, that takes an assembly-free approach to rapidly predict *Shigella* serotype by aligning raw WGS reads to an in-house curated reference sequence database. A serotype can be unambiguously predicted from 59 different *Shigella* serotypes by ShigaTyper at a data processing speed of 538 MB/min with a 98% overall accuracy from a regular laptop. Once installed, training in bioinformatics analysis and prior knowledge of *Shigella* genetics is not required. ShigaTyper was validated using WGS from a separate set of 344 *Shigella* and 36 non-*Shigella* isolates. This pipeline is the first step toward building a comprehensive WGS-based analysis pipeline of *Shigella* spp. in a field laboratory setting, where speed is essential and resources need to be more cost-effectively dedicated. Characterization of the newly identified, episomally located serotype determinant of *S. boydii* 20 will also be presented.

31. Sample size calculation through simulations using probit regression for countermeasure treatment development under Animal Rule

Authors: Xi, Mingyu, FDA/CDER/OB/DBII

Plain Language Synopsis: This project will demonstrate how to determine the minimum sample size in conducting a natural history study, dose range study, and the pivotal study in countermeasure drug development under the Animal Rule, where the primary endpoint is death rate through simulations under probit

regression

Abstract:

When drugs are developed to prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic substances, human efficacy studies are usually not ethical, and field trials may not be feasible. In such situations, FDA may grant approval based on adequate and well-controlled animal efficacy studies, provided that these results are likely to produce clinical benefit in humans, as per the Animal Rule. To conduct such an efficacy study, animals are first exposed to the toxic substance and then treated with the study drug. Due to the severity of toxicity, animals are sacrificed at the end of the study. One of the challenges in the study is to minimize the number of animals used, especially when large animals are used. This project will demonstrate how to determine through simulations the minimum number of animals needed.

Lethal Dose 50% (LD50) is the amount of the substance required to kill 50% of the test population. The percent mortality in the probit scale and the log-scale of concentration of toxic gas will be assumed to be linear, thus giving a straight-line plot. If a straight line with natural history shifts to the right after treatment, then the LD50 is increased. The ratio of LD50 under treatment to LD50 under control is termed dose modification factor (DRF). A DRF of 1.2 represents an approximately 20% reduction in gas lethality. DRF will be used as a measurement to choose the best dose of the study treatment. The simulation will demonstrate how to determine the minimum number of doses of gas and minimum number of animals in each gas dose group to produce a reliable natural history mortality curve. It will compare confidence intervals of various LDs in different scenarios. Then it will simulate different death curves under different DRFs to illustrate how to choose the best treatment dose. The project will also study the relationship between DRF and the survival benefit. Finally, the project will provide some information on the sample

size of the pivotal animal efficacy study to detect different clinically meaningful survival benefits.

32. Leveraging Real-World Data for Regulatory Decision-Making: A Novel Statistical Approach for Augmenting Patient Cohort in the Investigational Clinical Studies

Authors: Yue, Lilly, FDA/CDRH; Lu, Nelson, FDA/CDRH; Chen, Wei-chen, FDA/CDRH; Li, Heng, FDA/CDRH; Tiwari Ram, FDA/CDRH; Wang, Chenguang, JHU; Xu, Yunling, FDA/CDRH

Plain Language Synopsis: A novel statistical approach for identifying real-world similar patients to augment the patient cohort in investigational clinical studies of medical products to help regulatory decision-making

Abstract:

In medical product development, there has been an increased interest in utilizing real-world data that have become abundant with recent advances in biomedical science, information technology, and engineering. High-quality real-world data may be used to generate real-world evidence for regulatory or healthcare decision-making. This poster will focus on a novel statistical method that we have just developed to augment the primary patient cohort enrolled in a clinical study with patients selected from a real-world data source containing both clinical outcome and covariate data at the patient-level. The proposed approach uses the propensity score methodology to identify real-world patients who are similar to those in the primary patient cohort in terms of the patient baseline characteristics. Either frequentist or Bayesian method can then be applied to the augmented patient cohort for outcome data analysis, with down-weighting of the information from the real-world data source. The performance of the proposed approach is evaluated via simulation studies. Examples based on our pre-market review experience are provided to illustrate the implementation of the proposed approach. The proposed method can be applied to both pre-market and post-market clinical studies for the safety and effectiveness evaluation of medical products.

33. Raman spectroscopy for screening tubing for phthalates

Authors: Yakes, Betsy Jean, FDA/CFSAN; Carlos, Katherine, FDA/CFSAN; Begley, Timothy, FDA/CFSAN

Plain Language Synopsis: There has been scrutiny around the use of some plasticizer classes, especially in milk processing tubing, and the associated potential of transfer into food. The goal of this work is to develop a spectroscopy screening method for plasticizers and evaluate feasibility of use in field analysis and industrial surveys.

Abstract:

Phthalate and non-phthalate plasticizers are used in a wide variety of food contact materials in order to increase the flexibility of the materials. There has recently been scrutiny of the use of plasticizers in milk processing tubing and the potential for transfer of these compounds into this commodity. To understand the prevalence of phthalate use versus non-phthalate use in tubing in the food industry, there is a need for a robust, rapid, portable analytical method. FDA has recently begun acquiring and evaluating portable devices for field and or inspection use, including Raman and infrared spectroscopy, that potentially could detect and identify plasticizers in tubing. This presentation will highlight our initial research using one Raman device and illustrate the potential of this technology as a field screening technique for industry surveys.

34. Detection of abnormal prion protein in blood of mice during the incubation period of experimental variant Creutzfeldt-Jakob disease

Authors: Yakovleva, Oksana, CBER/OBRR/DETTD/LBTSEA; Pillant, Teresa, CBER/OBRR/DETTD/LBTSEA; Asher, David M., CBER/OBRR/DETTD/LBTSEA; Gregori, Luisa, CBER/OBRR/DETTD/LBTSEA;

Plain Language Synopsis: Human variant Creutzfeldt-Jakob disease (vCJD) is a fatal infection transmissible by blood transfusion. We developed a test for PrPTSE, a disease biomarker, in the blood of vCJD-infected mice.

PrPTSE appeared in mouse blood several months before clinical signs, suggesting our test might be applicable to screen human blood for PrPTSE.

Abstract:

Background

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease of humans infected with the agent of bovine spongiform encephalopathy (BSE). Amounts of abnormal prion protein (PrPTSE, marker of all transmissible spongiform encephalopathies [TSEs, prion diseases]) in blood of vCJD patients are extremely low. However, blood transfusions have transmitted vCJD. One in 2000 people in UK might be asymptomatic vCJD carriers, so early detection of PrPTSE in blood is important to protect the safety of the blood supply. Using a test called protein misfolding cyclic amplification (PMCA) we detected PrPTSE in blood samples collected from vCJD-infected mice at the clinical phase.

Aim

To determine the kinetics of appearance of PrPTSE in the blood of vCJD-infected mice. In addition, assuming a direct correlation between PrPTSE and infectivity, to establish the earliest time during experimental vCJD infection when blood first becomes infectious.

Methods

We inoculated 8-10 CJD-susceptible mice or PrP-knockout mice with macaque-adapted vCJD brain homogenate by intracerebral, intraperitoneal (ip), intravenous (iv), or combined ip + iv routes. vCJD brain homogenate contained high levels of PrPTSE. We collected blood at intervals after injection starting at 24 hr and during two years of incubation. Each blood sample was assayed for PrPTSE by PMCA. However, we were concerned that, especially at early time points, PrPTSE detected in blood might result from residual inoculum rather than de novo generated PrPTSE. To address this point, we inoculated PrP-knockout mice (completely resistant to vCJD and other TSE infections) and conducted a similar experiment to

determine how long the inoculated brain-derived PrPTSE remained in circulation.

Results

We detected PrPTSE in the blood of mice in all groups on the day after inoculation and during the first month. Later we observed differences in PrPTSE kinetics in mice injected by different routes. PrPTSE from the inoculum was detected in the blood of PrP-knockout mice for one month post-inoculation but not thereafter.

Conclusions

We detected PrPTSE in mouse blood for one month after inoculation, most likely from residual inoculum. PrPTSE generated de novo appeared in blood of vCJD mice several months before signs of disease, depending on route of inoculation and concentration of inoculum.

35. Development of in vitro methods to determine skin permeation and retention of UV filters in sunscreen products

Authors: Yang, Yang, OPQ/OTR/DPQR; Ako-Adounvo, Ann-Marie, OPQ/OTR/DPQR; Willett, Daniel, OPQ/OTR/DPA; Zhang, Jinhui, OPQ/OTR/DPQR; Wang, Jiang, OPQ/OTR/DPQR; Hsu, Hao-jui, OPQ/OTR/DPQR; Korang-Yeboah, Maxwell, OPQ/OTR/DPQR; Wang, Jian, OND/ODEIV/IO; Adah, Steven,

Plain Language Synopsis: Methods were developed to comprehensively assess skin absorption of marketed sunscreen products. The outcomes of this study may provide guidance for designing human absorption studies to evaluate sunscreen products, potentially assist in future product development, and facilitate approval of novel UV filters in the US.

Abstract:

Sunscreen products are preventative medicine for sunburn and sun (UV)-related skin cancer. These products are available as over-the-counter medicine (OTC) and regulated under the OTC monograph. The systemic exposure of the active ingredients (or UV filters) in sunscreen products after dermal application presents critical safety

concerns. FDA recommends conducting pharmacokinetic (PK) trials under maximal-use conditions (maximal-use PK trial, or MUSt) to evaluate the safety of sunscreen products available in the U.S. Per the recently published MUSt guidance by FDA, in vitro skin permeation test (IVPT) is recommended for product selection prior to PK trials. IVPT has the potential to successfully screen a large number of sunscreen products to help select the formulations with the highest potential of skin absorption for further in vivo safety evaluations. For this purpose, an IVPT method was developed to evaluate the absorption potential of various ingredients generally present in sunscreen products, using human cadaver skin. Using this method, five out of seven hundred commercially available sunscreen products (including cream, lotions, and sprays) that fit the selection criteria were tested for skin permeation potential of UV filters (avobenzone, ecamsule, oxybenzone and octocrylene) and preservatives (parabens). Emulsion types (o/w or w/o) of semi-solid formulations were determined using Raman microscopy. Rheological properties of the semi-solid formulations were also tested to understand the effect of product viscosity on skin permeation. Since UV filters are considered to have low skin permeation, sensitive and robust analytical methods were indispensable. A high-throughput method for the quantification of UV filters and excipients in skin permeation samples using an advanced robotics system coupled with tandem mass spectrometry (RapidFire-MS/MS), was successfully developed and validated. A selective and robust UPLC method was also developed and validated for simultaneous quantitation of all UV filters and parabens extracted from sunscreens and skin samples post-IVPT. The outcomes of this study may provide helpful guidance for designing human absorption studies for the evaluation of safety of sunscreen products and may potentially assist in future product developments and facilitate approval of novel UV filters in the US.

36. Development of next-generation sequencing and metagenomics for detection of foodborne viruses within oysters

Authors: Yang, Zhihui, FDA/CFSAN; Meade, Gloria K., USDA/ARS; Mammel, Mark, FDA/CFSAN; Kingsley, David, USDA/ARS

Plain Language Synopsis: Detection of viruses in foods is a major public health concern. This joint FDA/USDA study will help to provide a scientific basis for regulations ensuring the safety and security of our nation's food supply.

Abstract:

Introduction: Shellfish are filter feeders that concentrate various viruses present in surrounding water within their tissues. Thus, viral contamination of shellfish poses a risk for foodborne illnesses. Metagenomics offers new opportunities for detection, identification of viruses, and investigating of human enteric virus profiles in shellfish, in an unbiased way. However, the protocols of the sample preparation, next-generation sequencing (NGS), and data analysis required are complicated and must be developed and optimized.

Purpose: The purpose of this study was to develop NGS and metagenomic approaches for investigating foodborne virus profiles present in oyster samples.

Methods: Seeding samples with approximately 107 pfu of Tulane virus, two separate strategies were used to optimize the sample preparation protocol: 1) partial viral particle purification from homogenized digestive diverticula by differential ultracentrifugation; 2) virus extraction from whole homogenized oysters with the GPTT protocol. Treatments with ribonuclease and deoxyribonuclease before RNA isolation were also evaluated.

Viral RNA was isolated, followed by RNA-based library generation. Libraries were sequenced on the MiSeq platform (Illumina) generating paired-end reads. CLC Genomics Workbench, CosmosID and in-house tools were used for metagenomics analysis on the NGS data. The performance of each strategy was assessed based on the reads number,

the percentage of total viral reads, and the percentage of the positive control Tulane virus reads.

Results: 1) The highest percentage of viral reads and Tulane virus reads were obtained from the samples prepared with ultracentrifugation strategy; 2) after the viral particle enrichment, DNase and RNase treatment didn't significantly increase the percentage of viral reads; 3) virus identification and abundance profiles present in oyster samples were successfully obtained with our protocols.

37. Specificity of Cytosine Base Editors in Human Pluripotent Stem Cells

Authors: McGrath, Erica, FDA/CBER; Shin, Hyunsu, FDA/CBER; Zhang, Linyi, FDA/CBER; Phue, Je-Nie, FDA/CBER; Wu, Wells, FDA/CBER; Jang, Yoon-Young, JHU/Oncology; Javier Revollo, Javier, FDA/NCTR; Ye, Zhaohui, FDA/CBER

Plain Language Synopsis: This study examines the effect of genome editing tools on genomic integrity in human stem cells. By sequencing analysis of genetically modified iPSCs, it finds that editing by certain cytosine base editors may result in unintended genetic modifications with a sequence pattern distinct from that of the traditional CRISPR/Cas nucleases.

Abstract:

Genome editing tools, such as meganucleases, ZFNs, TALENs and CRISPR, are transforming research and medicine with unprecedented efficiency in altering genomic sequences in living cells. These engineered endonucleases generally initiate editing by creating site-specific DNA double strand breaks (DSBs) in the genome. More recently, Base Editors, fusion of catalytically impaired CRISPR/Cas with DNA modifying enzymes such as nucleoside deaminases, have been developed. Unlike traditional genome editing nucleases, Base Editors do not create DNA DSBs but instead induce base changes by direct chemical modifications. Although the editing efficiencies of both cytidine base editors and adenine base

editors have been demonstrated in a variety of animal and plant species, specificity of these editors has not been fully addressed. A limitation of most base editor specificity studies is the biased analysis involving only in silico predicted CRISPR off-target site analysis. Given the extensive evidence on the role of APOBEC family cytidine deaminases in causing mutations in human cancers, it is important to investigate the mutagenic potential of APOBEC-based cytosine base editors in clinically relevant cell types using unbiased genomic analysis approaches. In this study, we examined the mutation landscapes of cloned base-edited human induced pluripotent stem cells (iPSCs) using whole genome sequencing (WGS). Among iPSC clones with confirmed on-target editing, our analyses identified clones with genomic variation levels comparable to control iPSC clones, indicating high specificity genomic modification by the editor in these cells. However, clones with significantly increased (up to ~10 fold higher) mutation loads were also identified. Strikingly, the majority of the increased point mutations were C to T transitions outside of conventional CRISPR off-targets. Further analysis of the sequences around these mutation sites has identified a conserved sequence motif previously reported as a signature pattern of APOBEC mutagenesis in human cancers, thus implicating a role of the ectopically expressed APOBEC-based base editors in the formation of these mutations. This study demonstrates that cytosine base editor-mediated base editing may result in distinct patterns of unintended genetic modifications from those of the traditional CRISPR/Cas nucleases. Additional studies are needed to identify factors affecting the balance of base editor efficiency and specificity.

38. High throughput glycan profiling for improved quality control of therapeutic glycoproteins

Authors: Zhang, Baolin, FDA/CDER; Zhang, Lei, FDA/CDER; Luo, Shen, FDA/CDER

Plain Language Synopsis: Glycosylation of therapeutic proteins has a profound impact

on their safety and efficacy; therefore, they must be adequately analyzed and controlled throughout the product lifecycle. This project aims to develop innovative glycosylation analysis techniques for quality control applications.

Abstract:

The quality of therapeutic glycoproteins, including monoclonal antibodies (mAbs) and other biologics, is dictated by their carbohydrate-- or glycan--profiles. However, the complexity of protein glycosylation poses a daunting analytical challenge. The most widely used methods, including HPLC coupled with mass spectrometry (MS), involve laborious enzymatic digestion procedures and low-throughput analyses of the resulting free glycans. Lectin microarray technology has the potential to address the challenge of glycan analysis by harnessing lectins (natural carbohydrate-binding proteins) to decipher glycan structures. This presentation describes a lectin microarray platform that directly measures glycans attached to intact proteins, without the need to clip glycans from the protein backbone. We tested a variety of samples from therapeutic mAbs to plasma proteins using lectin chips printed with a set of 45 different lectins that selectively recognize glycan epitopes found in recombinant glycoproteins (e.g., fucose, sialic acids, mannose, and N-acetylglucosamine). For all the samples tested, the glycan profiles derived from lectin-binding signals are consistent with their known glycan profiles. Of interest, the lectin microarray is sensitive enough to detect alterations in the terminal glycan species, such as galactose versus sialic acid epitopes. Upon optimization of lectin chips, the lectin microarray platform could be adopted as a complementary tool for high-throughput screening of glycan profiles of therapeutic glycoproteins.

39. Validation of a novel loop-mediated isothermal amplification (LAMP) method for the detection of *Salmonella ser. Enteritidis* in shell eggs

Authors: Hu, Lijun, FDA/CFSAN;Butler,

Melanie, FDA/CFSAN; Li M. Ma, OSU/DEPP; Hammack, Thomas, FDA/CFSAN; Brown, Eric, FDA/CFSAN; Zhang, Guodong*, FDA/CFSAN

Plain Language Synopsis: The study was aimed to validate a prot6E gene-based LAMP method developed in our laboratory for detecting Salmonella ser. Enteritidis in shell egg samples in comparison with the FDA BAM culture method and a real-time PCR method. It could be another effective tool for enforcing the FDA egg rule.

Abstract:

Background: Salmonella ser. Enteritidis (SE) is a major public health concern worldwide. Rapid and accurate detection of SE in eggs and egg products, which are the major sources of SE, is imperative for surveillance and outbreak investigation. Most molecular detection methods are aimed at the genus Salmonella. An SE-specific LAMP method has been developed in our laboratory.

Purpose: To validate a prot6E gene-based LAMP method developed in our laboratory for detecting SE in shell egg samples in comparison with the FDA BAM culture method and a real-time PCR method.

Methodology: We conducted 4 separate trials with 4 SE isolates of different phage types. Shell eggs were surface disinfected and cracked aseptically. Each trial consisted of 20 samples (inoculated at ~5 cells/L), 5 positive controls (inoculated at ~50 cells/L), and 5 negative controls (un-inoculated). Each sample/control contained 1L liquid eggs. Preparation of egg samples followed FDA BAM method. DNA prepared from pre-enrichment cultures were used for LAMP and real-time PCR assays. LAMP assay was carried on the Genie III device. PCR (TaqMan™ Salmonella Enteritidis Detection Kit) was performed on with ABI 7500 Fast real-time PCR instrument.

Results: There were 15, 13, 17, and 15 positives for SE among 20 samples tested for each trial with FDA BAM culture methods. All 20 positive control samples were positive and all 20 negative control samples were negative

for SE. LAMP and PCR results matched the BAM culture results.

Conclusion: Our newly designed prot6E gene-based LAMP method was equally effective in detecting SE from shell eggs compared to real-time PCR and BAM culture methods. It could be used as another effective tool for the detection of SE from shell eggs for FDA in an outbreak investigation and for enforcing the Egg Rule.

40. The development of chemo-enzymatic method for profiling N- and O-glycans on fusion therapeutic glycoproteins

Authors: Zou, Guozhang, FDA/OBP; Ju, Tongzhong, FDA/OBP

Plain Language Synopsis: A chemo-enzymatic method for profiling N- and O-glycans on fusion therapeutic glycoproteins

Abstract:

N-Glycosylation is a critical quality attribute (CQA) of therapeutic proteins, such as monoclonal antibodies (mAbs), due to its impact on their efficacy, half-life, and stability. Many therapeutic proteins are also O-glycosylated in addition to its N-glycosylation; yet, the role of O-glycosylation in the quality and safety of protein drugs remains elusive. Much effort has been expended to characterize glycosylations and to understand their impacts on drug quality and safety. However, characterization of O-glycans is still very challenging due to the lack of a universal enzyme to release O-glycans from glycoproteins. In this study, we developed a chemo-enzymatic approach to simultaneously analyze both N- and O-glycan profiles of protein drugs. Using Fetuin, which is a glycoprotein, and abatacept, which is a therapeutic Fc-fusion glycoprotein, we were able to analyze both N-glycans and O-glycans quantitatively and qualitatively in a single setting by mass spectrometry, MALDI-TOF-MS. Besides the reported glycan structures, our data also disclosed the presence of trace amounts of bisecting GlcNAc and non-human glycan structures, e.g. the terminal galactose- α -1,3-galactose (α -Gal) and

N-glycolylneuraminic Acid (Neu5Gc), in both N- and O- glycans of abatacept. Our novel method is feasible and reproducible. We are currently testing and validating the method with more therapeutic fusion proteins, and will eventually standardize the experimental procedures for manufacturers to assess glycosylations of their protein drugs. This method will not only facilitate manufacturing and developing high quality protein drugs in industries, but will also enable the reviewers to adequately assess the quality and safety of therapeutic proteins with regard to their glycosylations.

Poster Session 2

Topic: Product Accessibility, Integrity, and Security (Day 1, P.M.)

41. Thrombin Generation Assay for IgG Products

Authors: Aaron, Leslyn S, FDA/CBER; Tobin, Grainne A, FDA/CBER; Ovanesov, Mikhail V, FDA/CBER; Francis, Kori M, FDA/CBER; Bhattacharyya, Lokesh, FDA/CBER

Plain Language Synopsis: DBSQC's Thrombin Generation Assay allowed direct comparison of FXIa activity in approved IG products and is suitable for routine lot release testing of IG products, ensuring distributed batches contain safe levels of FXIa-like activity.

Abstract:

In 2010 an increased number of thrombotic events were associated with the administration of intravenous immune globulin (IGIV) products. Investigations identified plasma-derived coagulation factor XIa (FXIa) as the causative factor. As a result, FDA's DBSQC developed and validated a robust method for the quantitation of FXIa-like activity in IG products using the WHO International Standard for Activated Blood Coagulation Factor XI (FXIa), with human as the standard. The method measures FXIa-like activity by monitoring the fluorescence emission caused by cleavage of a fluorogenic substrate by FXIa in factor-XI-deficient plasma. The results of FXIa-like activity show low variability (RSD \leq 15%) in the assay range (0.3 - 10.0 mIU/mL). The LOQ of the assay, 0.3 mIU/mL, is well below the approved specifications limits for IGIV products. The results indicate that low amounts of FXIa activity can be measured reliably in immune globulin (IG) products.

Between February 2016 and March 2019, representative samples from 693 batches of 22 different IG products were evaluated in our laboratory to determine the level of FXIa-like activity. The results show less than 5 mIU/mL activity for IGIV products, which is generally considered as the safe level for these products. For IGIM and IGSC products, the FXIa-like activities are in the range of 0.3 to 10.0 mIU/mL; however, some are found to contain activities greater than 10 mIU/mL. The amount of FXIa-like activity in IGIM and IGSC products are not a safety concern, given

the low doses in which they are administered. The Thrombin Generation Assay allowed direct comparison of FXIa activity in approved IG products and is suitable for routine lot release testing of IG products, ensuring distributed batches contain safe levels of FXIa-like activity.

42. Processing removes bovine spongiform encephalopathy agent from heparin

Authors: Andrews, Omozusi, FDA/CBER; Bett, Cyrus, FDA/CBER; Shu, Qin, FDA/CDER; Kaelber, Nadine, FDA/CBER; Asher, David, FDA/CBER; Keire, David, FDA/CDER; Gregori, Luisa, FDA/CBER

Plain Language Synopsis: Heparin, a leading intravenous anticoagulant, is sourced exclusively from porcine intestinal mucosa. Years ago, concerned about theoretical risk of bovine spongiform encephalopathy (BSE) causing variant Creutzfeldt-Jakob disease, manufacturers withdrew bovine heparin from the US market. A 3-step heparin manufacturing process substantially removed spiked BSE agent contaminating crude bovine heparin.

Abstract:

Background: U.S.-marketed heparin, an important anticoagulant, is sourced from porcine intestinal mucosa. Bovine lung was once used, but heparin manufacturers, concerned about contamination with the agent of bovine spongiform encephalopathy (BSE—a transmissible spongiform encephalopathy spread to humans as variant Creutzfeldt-Jakob disease), withdrew that product. We previously showed that a scaled-down manufacturing process removed substantial amounts of scrapie (model) and BSE agents spiked into crude heparin, from final products. We assessed the ability of a bench-scale typical heparin manufacturing process to remove BSE and scrapie agents spiked into bovine mucosa (now considered by U.S. manufacturers as better raw material than lung) from crude heparin.

Purpose: To develop a reproducible three-step heparin manufacturing process from bovine mucosa to crude heparin and evaluate

the total process from crude to final pure heparin.

Methodology: Typical heparin manufacturing from mucosa to crude heparin consists of three core steps: enzyme digestion, resin capture, and methanol precipitation. We extracted crude heparin from bovine intestinal mucosa and compared its structure and function with commercial crude heparin, using nuclear magnetic resonance and anti-factor Xa/IIa potency assays. To assess removal of BSE—a biosafety-level-3 agent—we first used scrapie (biosafety-level-2 agent often used as surrogate for BSE agent). We spiked 1% scrapie-infected hamster brain homogenate into 10 mL of bovine mucosa. Later we spiked actual 1% BSE-infected cow brain into bovine mucosa. We then extracted crude heparin. We collected samples at each step of the process to analyze by a sensitive Real-Time Quaking-Induced Conversion (RT-QuIC) assay that measures abnormal prion protein, PrPTSE, a surrogate biomarker for infectious agents of both scrapie and BSE.

Results: Our crude bovine heparin displayed similar structure and functional potency to commercial crude heparin. The manufacturing process removed ~5 log₁₀ of scrapie PrPTSE in 3 independent experiments and >3 log₁₀ of BSE PrPTSE (to the limit of detection).

Conclusion: Processing bovine mucosa removed substantial BSE- and scrapie-associated PrPTSE from crude heparin. These studies, plus our previous finding that purification of pharmaceutical heparin from its crude intermediate also removed substantial PrPTSE and infectivity, provide reassurance that bovine heparin might be safely reintroduced to the US market.

43. Applications of High Throughput Dynamic Light Scattering in the field of Therapeutic Protein Drug Product Quality

Authors: Bhirde, Ashwinkumar, FDA/OPQ/OBP; Agarabi, Cyrus, FDA/OPQ/OBP

Plain Language Synopsis: Novel methods to evaluate quality of therapeutic protein drug products under relevant scenarios

Abstract:

Dynamic light scattering has been around for a number of years in the field of protein characterization. High throughput dynamic light scattering (HT-DLS) has led to speedy, low-volume characterization of protein drugs. However, HT-DLS has been used primarily as a formulation-screening tool until now. We are developing methods that have the potential to be used for drug product quality assessments in the pharmaceutical industry. Stability of therapeutic proteins (TPs) is a critical quality attribute that affects both the safety and efficacy of the drug. Size stability is routinely performed during and after biomanufacturing. We have developed novel methods to evaluate in-use and thermal stress stability of TPs using algorithm-driven HT-DLS. The TPs were evaluated at relevant temperature conditions, as well as under dilution and thermal stress for size stability. A combined assessment of autocorrelation function and photos of sample well images could be useful in formulation screening. Our experiments indicate that dilution of TPs have an impact on the HD size. Thermal stress experiments showed the importance of using different data processing methods to access size distribution. Polydispersity index was useful in evaluating sample heterogeneity. Herein we show that algorithm-driven HTS-DLS can provide additional supportive information during and after bio-manufacturing of complex drug formulations.

44. Patient Exposure During Pre-market Trials And Relationship With Post-market Safety Outcome

Authors: Cherkaoui, Sanae, FDA/CDER/OSE/RSS; Pinnow, Ellen, FDA/CDER/OSE/RSS; Bulatao, Ilynn, FDA/CDER/OSE/RSS; Day, Brendan, University of Maryland/School of Medicine; Kalaria, Manish, FDA/CDER/OSE/RSS; Brajovic, Sonja, FDA/CDER/OSE/RSS; Dal Pan, Gerald, FDA/CDER/

Plain Language Synopsis: The number of patients exposed to a drug or biologic increases after FDA approval. This increased exposure enables identification of new risks.

We studied the relationship between the number of patients exposed before FDA approval and safety-related label changes or withdrawal for drugs and biologics after approval.

Abstract:

Background: Drug exposure substantially increases after approval, enabling identification of new risks. The relationship between patient exposure in pre-market clinical trials and post-approval safety label changes is not well understood.

Objective: To characterize pre-market exposure for new molecular entity (NME) and new therapeutic biologic (NTB) and its relationship to post-market safety outcomes.

Methods: We evaluated pre-market exposure and regulatory characteristics for NMEs and NTBs approved by FDA between 10/1/02 and 12/31/14 using publicly available FDA documents. We recorded the number of patients in the clinical development program included in the FDA-defined safety population. Post-market safety outcome was defined as a safety-related withdrawal or safety update to the boxed warning, contraindications, warnings and precautions, adverse reactions or drug interactions sections of the label through 6/30/18. We evaluated the relationship of quartiles of the safety population, regulatory pathways, and post-market safety outcome, using chi square and logistic regression. Continuous variables were analyzed using Wilcoxon test. Kaplan-Meier analyses were done to examine association with time to first safety outcome.

Results: Our study included 339 products (278 NMEs, 61 NTBs). The median size of the safety population was 947 with no change over time. NMEs had more pre-market exposure than NTBs (median: 1057 vs 880, $p=0.04$). 111 (33%) were orphan products. The median exposure was lower in orphan compared to non-orphan products (524 vs 1806, $p<0.001$). The median size of the safety population was significantly smaller for fast track designation (599 vs 1396, $p<0.001$), priority review (612 vs 1662, $p<0.001$), and accelerated approval

(505 vs 1148, $p<0.001$). Expedited programs had a smaller safety population size for non-orphan, but not for orphan products, compared to non-expedited programs. Smaller safety population size was associated with decreased risk of post-market safety outcomes, when compared to the highest quartile (Q1: OR=0.31, 95% CI 0.14-0.69; Q2: OR=0.47, 95% CI 0.20-1.08; Q3: OR=0.82, 95% CI 0.33-2.02).

Conclusion: The median size of pre-market exposure has not changed over time. Orphan drugs and expedited programs are associated with varying pre-market exposures. Larger safety population size was associated with an increase in post-market safety outcomes.

45. Characterizing Safety Issues in Post-marketing Label Changes for New Molecular Entity Drugs and New Therapeutic Biologics Approved by the U.S. Food and Drug Administration Between 2002 and 2014

Authors: Day, Brendan, University of Maryland/School of Medicine; Pinnow, Ellen, FDA/CDER/OSE/RSS, Bulatao, Ilynn, FDA/CDER/OSE/RSS; Cherkaoui, Sanae, FDA/CDER/OSE/RSS; Kalaria, Manish, FDA/CDER/OSE/RSS; Brajovic, Sonja, FDA/CDER/OSE/RSS; Dal Pan, Gerald, FDA/CDER

Plain Language Synopsis: Safety issues (e.g., side effects) that are discovered after a drug is already FDA-approved are often added to the drug's label (or 'package insert'). We determined the most common new safety issues that are added to the label for a group of FDA-approved drugs.

Abstract:

Background: Although pre-marketing studies identify common adverse effects of new products, post-marketing surveillance data often identify additional safety issues, which are then incorporated into label changes. Limited data exist regarding the nature of the actual new safety issues incorporated into label changes.

Purpose: To characterize the frequency, type, and trends in new safety issues incorporated into label changes for new products.

Methods: Using publicly available sources (Drugs@FDA and the Drug Safety-related Labeling Changes database), we created a retrospective cohort of products (new molecular entities [NMEs] and new therapeutic biologics [NTBs]) approved by FDA between 10/1/02 and 12/31/14. We identified new safety issues in label changes from approval through 6/30/18 in the following label sections: Boxed Warning (BW), Contraindications (C), Warnings and Precautions (WP), Adverse Reactions (AR), and Drug Interactions (DI). New safety issues were MedDRA-coded into preferred term (PT) and system, organ, class (SOC). We determined the frequency of the most common safety issues overall and stratified by NME vs. NTB, label section (BW, WP, and AR), and timing since approval. Lastly, we used Kaplan-Meier estimates to evaluate for differences in time-to-first safety issue for NME vs. NTB.

Results: There were 339 products in the cohort (278 NMEs and 61 NTBs), with a median follow up time of 8.8 years. There were 3672 new safety issues overall (75% in NMEs, 25% in NTBs). The five most common safety issues overall were: angioedema, anaphylactic reaction, Stevens-Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity. Each issue occurred in roughly 10% or more products for both NMEs and NTBs. NTBs had more infections and neoplasms whereas NMEs had more hepatic issues. There was no significant difference in time-to-first safety issues for NMEs vs NTBs.

Conclusions: This study provides valuable insight into the safety issues incorporated into post-marketing safety-related labeling changes for FDA-approved therapeutic products. Serious immune reactions made up the five most common safety issues, with other issues more prominent in stratified analyses. These findings provide valuable insight into post-marketing safety issues with relevance to prescribers and regulators alike.

46. **Marker free live attenuated *Leishmania major* (LmCen-/-) induces strong host protective immune response against vector bite transmitted Visceral Leishmaniasis**

Authors: Ranadhir Dey, FDA/CBER; Subir Karmakar, FDA/CBER; Fabiano Oliveira, NIAID/NIH; Nevien Ismail, FDA/CBER; Wenwei Zhang, McGill University/Canada; Shinjiro Hamano, Nagasaki University/Japan; Greg Matlashewski, McGill University/Canada; Shaden Kamhawi, NIAID/J

Plain Language Synopsis: Leishmaniasis is a spectrum of diseases caused by the protozoan parasites *Leishmania*, blood borne pathogens transmitted by insect vector. In our lab we generated a live, attenuated *Leishmania* vaccine strain using a genetic method. In preclinical animal models, our findings suggest that attenuated dermatotropic *Leishmania* could be a promising vaccine against leishmaniasis.

Abstract:

Leishmaniasis are vector-borne parasitic diseases for which there is no licensed vaccine available. A low dose of dermatotropic *Leishmania* infection (leishmanization) confers protection against cutaneous leishmaniasis (CL), a process called leishmanization. However, the form of immunization is not practical because of the greater risk of infection in a naïve population. However, genetically modified, live, attenuated, non-pathogenic parasites might induce the same protective immunity as leishmanization. We have developed centrin-gene deficient *Leishmania major* (LmCen-/-) using CRISPR-Cas methodology and evaluated the safety, immunogenicity, and cross-protective efficacy against *L. donovani* challenge in a hamster model. Although, intradermal immunization of golden Syrian hamsters with LmCen-/- did not develop visible lesions, it induced a strong pro-inflammatory immune response compared to wild type *L. major* infection, as measured by real time quantitative PCR. Immunized hamsters were challenged with *L. donovani* either by intradermal needle injection or by infected sand flies as

natural mode of infection. In both sets of experiments, twelve months post-challenge, non-immunized challenged hamsters developed severe pathology of VL, while immunized hamsters were protected. We also evaluated the cellular immune response in immunized hamsters after challenge with the wild type parasites and compared it with non-immunized and challenged hamsters. Spleen cells from LmCen^{-/-} immunized and challenged hamsters produced significantly more Th1-associated cytokines, including IFN- γ and TNF- α , and significantly reduced expression of the anti-inflammatory cytokines IL-10 and IL-21, compared to non-immunized and challenged animals. The enhanced pro-inflammatory immune response correlates with the control of parasitemia in immunized animals. Our studies demonstrate that the LmCen^{-/-} mutant parasite is a safe immunogen that has the potential to be an effective vaccine against VL, and thus can be tested in humans.

47. Development and application of an HPLC method to isolate and identify poloxamers in large molecule drug manufacturing

Authors: Faison, Talia, FDA/CDER; Angart, Phil, FDA/CDER; Agarabi, Cyrus, FDA/CDER

Plain Language Synopsis: Poloxamers are non-ionic copolymers that are frequently used in biopharmaceutical manufacturing to produce drug substance or stabilize drug product. However, they may also behave as an impurity that can affect drug quality and safety. We are developing a chromatographic method to identify and quantify poloxamers to evaluate the potential risk in antibody drug manufacturing.

Abstract:

Poloxamers are non-ionic tri-block copolymers composed of polyethylene oxide chains attached to a central polypropylene oxide chain. These molecules have traditionally been used in pharmaceutical formulations as surfactants, emulsifying agents, solubilizing agents, dispersing agents, and in vivo absorbance enhancers, due to their amphiphilic properties. They also

provide cell cushioning effects by reducing shear stress in bioreactors, making them a popular component of cell culture media in the bioprocessing sector. However, there is little published literature on the chemical analysis of poloxamers in biologic drug products, and they are difficult to detect using common spectroscopic techniques, due to poor UV absorbance. As drug product submissions increasingly include poloxamers in processing or final formulation, we sought to develop an HPLC method to reliably separate and identify poloxamers from large molecule drug formulations and raw materials, in order to support the regulatory challenges presented by this potential impurity. In this study, we show initial investigations using gel permeation chromatography with refractive Index detection, to determine poloxamer presence, mass, and concentration from commercial standards and samples of a purified antibody drug substance.

48. Evaluation of protective immunity induced by LdCen^{-/-} in presence of pre-existing Plasmodium yoelii infection

Authors: Gannavaram, Sreenivas, FDA/CBER/OBRR; Thomas, Charles, FDA/CBER/OBRR; Muneem, Abraar, FDA/CBER/OBRR; Zheng, Hong, FDA/CBER/OBRR; Kumar, Sanjai, FDA/CBER/OBRR; Nakhasi, Hira FDA/CBER/OBRR.

Plain Language Synopsis: Co-infections of blood-borne pathogens such as Plasmodium and Leishmania are common in endemic areas. Biomarkers of protection induced by anti-parasitic vaccines are most commonly evaluated in naïve animal models that may not reflect the endemic conditions. We used a plasmodium pre-exposed animal model to study anti-leishmania vaccine immunity.

Abstract:

Visceral leishmaniasis and malaria are transmitted in overlapping geographic areas in many parts of the world, which may result in concurrent or prior exposure to both parasites in some individuals. Studies of co-infection of virulent Plasmodium

and Leishmania have shown activation of distinct immunological pathways that might lead to either exacerbation or control of pathogenesis, depending on the parasite species. In order to develop a prophylactic vaccine against visceral leishmaniasis, we tested centrin-deleted, live, attenuated Leishmania donovani parasites (LdCen^{-/-}) as potential vaccines. However, vaccination studies using LdCen^{-/-} were performed mainly in naïve rodent models; therefore, immunological correlates of protection may not reflect the endemic conditions, where simultaneous exposure to multiple pathogen infections is prevalent. In this study, we observed the effect of a prior exposure to Plasmodium yoelii 17XNL (PyNL, nonlethal) on the immune response and protective efficacy of the LdCen^{-/-} vaccine. We performed LdCen^{-/-} immunization in C57Bl/6 mice that were either actively infected with PyNL and/or had cleared their infection. The PyNL infection showed no impact on LdCen^{-/-}-specific CD4⁺ T cell responses, including memory responses and polyfunctional responses. However, polyfunctional CD8⁺ T cell responses were impaired in the presence of PyNL. Upon challenge with virulent L. donovani, reduction in splenic parasite burdens was impaired in mice pre-exposed to PyNL, compared to those that received LdCen^{-/-} alone. This suggests that prior exposure to PyNL affects the protective immunity induced by LdCen^{-/-} parasites. The possible immune mechanism affecting the protective immunity induced by LdCen^{-/-} parasites due to PyNL will be discussed.

49. Baseline Adjustment in Concentration-QTc Modeling: Impact on Assay Sensitivity

Authors: Huang, Dalong, FDA/CDER; Chen, Janell FDA/CDER; Tsong, Yi, FDA/CDER

Plain Language Synopsis: This poster discusses three types of baseline adjustments (time-matched, pre-dose, and day-averaged) used in concentration-QTc analysis regarding assay sensitivity, using real and simulated moxifloxacin data, and makes recommendations.

Abstract:

The ICH E14 guidance acknowledges that there is no best approach for baseline adjustment. Time-matched baseline and pre-dose baseline have been commonly used as a covariate in concentration-QTc (C-QTc) modeling for parallel and crossover studies, respectively. Huang et al. (2017) showed that the C-QTc model using time-matched baseline has a low chance of showing assay sensitivity in parallel study.

This poster discusses three types of baseline adjustments (time-matched, pre-dose, and day-averaged) regarding assay sensitivity using real and simulated moxifloxacin data, and makes recommendations.

50. Attempts to transmit chronic wasting disease of deer to mice expressing human prion protein

Authors: Kaelber, Nadine, DETTD/LBTSEA; Pilant, Teresa, DETTD/LBTSEA; Cervenak, Juraj, DETTD/LBTSEA; Morozova, Olena, DETTD/LBTSEA; Asher, David, DETTD/LBTSEA; Gregori, Luisa, DETTD/LBTSEA

Plain Language Synopsis: Chronic Wasting Disease (CWD) is a fatal, contagious disease affecting deer, elk, moose, and caribou. CWD is found in many parts of North America. We tested mice expressing human prion protein—a protein that influences human susceptibility to several human spongiform encephalopathies—to determine whether CWD might spread to humans.

Abstract:

Chronic wasting disease (CWD) is a contagious, fatal transmissible spongiform encephalopathy (TSE), or prion disease, that affects deer, elk, moose, and caribou (collectively called cervids). CWD is characterized by emaciation and progressive neurological dysfunction, with neuronal vacuolation and accumulations of abnormal prion protein (PrP) in brain. CWD, first recognized in Colorado in the 1960s, subsequently spread through much of North America. CWD has already been confirmed in 26 U.S. states and 3 Canadian provinces;

cases have also occurred in South Korea and Northern Europe. So far, there have been no confirmed reports of human TSE attributed to eating or processing meat of infected cervids. These observations are encouraging, but the zoonotic risk of CWD cannot be totally discounted, because known acquired human TSEs often have very long asymptomatic incubation periods. Furthermore, CWD might resemble bovine spongiform encephalopathy, a TSE of cattle transmitted to humans mainly by the oral route, with long incubation periods and an extremely low attack rate.

We investigated the risk that CWD might infect humans by using transgenic mice (Tg66) overexpressing human prion protein as a possible surrogate for humans. We first inoculated Tg66 mice with brain suspensions from deer with confirmed CWD. Brains of mice in the study were tested for abnormal prion protein to establish transmission of CWD infection. For initial screening, we used a commercial ELISA test (Idexx HerdCheck®). We detected no abnormal prion protein in any brain, which suggested no CWD transmission. However, a few mice showed substantial vacuolation in brains, suggestive of TSE. Next, we re-screened all mouse brains using Real-Time Quaking-Induced Conversion (RT-QuIC), a more sensitive *in vitro* test that detects abnormal prion protein. Results indicated that brains of some mice inoculated with deer brain isolates contained abnormal PrP. This result suggested that CWD infection might have been transmitted to Tg66 mice without causing overt neurological illness. To confirm this interpretation, we passaged the brains of the RT-QuIC-positive mice into additional mice and tested brains of all those animals by both ELISA and RT-QuIC. Neither test detected abnormal PrP in any brain. These results remain puzzling and warrant further investigation.

51. Differentiating the effects of different oxidative stress conditions on therapeutic monoclonal antibodies

Authors: Lai, Lo, FDA/OBP; Heinzl, Geoffrey A, FDA/OBP; Rao, Ashutosh, FDA/OBP

Plain Language Synopsis: Our study concluded that the use of multiple oxidizing conditions, including those that mimic metal-catalyzed oxidation, may be essential during stability studies for characterizing the oxidative stress response in biopharmaceuticals.

Abstract:

Protein oxidation is a critical quality attribute for therapeutic proteins, including monoclonal antibodies. Oxidative stress can occur during routine manufacturing, processing, storage, or use. Multiple methods are currently used as stress tests to induce oxidative damage. In this study, we compared molecular modifications induced by two different oxidizers: Copper (II) (MCO) and 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) in therapeutic monoclonal antibodies (mAb). Our study included rituximab, trastuzumab, obinutuzumab, and ofatumumab. At the molecular level, we performed LC/MS (methionine oxidation), carbonyl formation ELISA, and dihydroxyphenylalanine (DOPA) modifications. The results of LC/MS indicated that in trastuzumab and obinutuzumab, AAPH induced more methionine in Fc subunits than MCO did. Oxidative carbonyl formation, which occurs in lysine, arginine, threonine, and proline, showed that rituximab, trastuzumab, and obinutuzumab were more sensitive to MCO than AAPH. In the case of higher-order structures, circular dichroism (CD), MFI, hydrophobicity, SDS-PAGE, and amyloid-like formations were analyzed. Preliminary analysis by SDS-PAGE disclosed cross-linked species as well as fragmentation, resulting in substantially decreased native protein content after MCO treatment. AAPH decreased native protein after 6 hours. Analysis of secondary structure by CD suggested that MCO treatment for 24 hours substantially disrupted the alpha-helices and beta-sheets in IgG folds, whereas AAPH treatment showed minimal impact on secondary structural elements. Ofatumumab and rituximab suffered changes to secondary structure from MCO, while trastuzumab and obinutuzumab retained much of their folded state. Tertiary

structure was assessed using 4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid (bis-ANS) to identify exposed and potentially solvent-accessible hydrophobic regions. Bis-ANS fluorescence increased continually during MCO treatment, with AAPH treatment again showing little structural impact, even after 24 hours. We further tested enzyme activity or ADCC activity in these drug products. The result indicated that MCO decreased ADCC activity in trastuzumab as soon as 30min after treatment, but not in the other two mAbs. These data confirm that since different mAbs respond uniquely to either oxidizing conditions, performing orthogonal oxidation experiments might be important for characterizing the stability and degradation profile of therapeutic proteins.

52. Development of a Policy Analysis Framework for Evaluating Recommendations to Amend the FDA Food Code

Authors: Liggins, Girvin, FDA/CFSSAN; Otto, Jessica, FDA/CFSSAN

Plain Language Synopsis: This poster outlines the development of a retail food policy analysis framework for evaluating the validity, practicality, and relative impact of proposed changes to the Food and Drug Administration (FDA) Food Code and other retail food policy.

Abstract:

Policy analysis is a multifaceted endeavor focused on problem solving. This poster outlines the development of a retail food policy analysis framework for evaluating the validity, practicality, and relative impact of proposed changes to the Food and Drug Administration (FDA) Food Code and other retail food policy. By illustrating the phases and key considerations involved in developing and evaluating retail food policy recommendations, this framework guides informed decision-making, while not by itself making a decision. The framework considers the interplay between facts, technical and scientific evidence, values, and desired actions and enables systematic focusing on the most salient aspects of policy

proposals. Analysts tasked with developing and evaluating policy recommendations will benefit from the framework simplifying the complex nature of their work and enabling a normalized historical record of the reasoning, rationale, and considerations involved in the policy debate and decision-making process.

53. Evaluation of milk powder authenticity with a portable mid-infrared spectrometer and a non-targeted chemometric approach.

Authors: Limm, William, FDA/CFSSAN; Karunathilaka, Sanjeewa, FDA/CFSSAN; Yakes, Betsy, FDA/CFSSAN; and Mossoba, Magdi, FDA/CFSSAN.

Plain Language Synopsis: A portable, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) device in conjunction with chemometrics is potentially an ideal screening tool for the in situ, rapid, and routine, non-targeted assessment of milk powder authenticity.

Abstract:

A portable, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) device in conjunction with chemometrics were evaluated as rapid screening tools for the non-targeted detection of milk powder (MP) adulteration, using melamine (MEL) as a surrogate contaminant. A single-class, soft independent modelling of class analogy (SIMCA) model was developed for each of two spectral ranges. Model development was based on a diverse set of MPs and was used to classify both wet-blended (WB) and dry-blended (DB) MEL and a set of MP control test samples. Satisfactory prediction of MP authenticity was obtained along with 100% correct classification rates for concentrations as low as $\geq 0.30\%$ for WB and $\geq 1.0\%$ for DB MEL test samples, when the spectra in the 850-750 cm^{-1} range were used for SIMCA. This portable infrared device, in conjunction with SIMCA classification, is potentially an ideal screening tool for the in situ, rapid, and routine non-targeted assessment of milk powder authenticity.

54. Defining the right diluent for intravenous infusion of therapeutic monoclonal antibodies

Authors: Luo, Shen, FDA/CDER; McSweeney, K. Melodi, FDA/CDRH; Zhang, Baolin, FDA/CDER

Plain Language Synopsis: Intravenous infusion of therapeutic monoclonal antibodies (mAbs) involves diluting the medication into a product-specific diluent. Using the wrong diluent can result in product instability during infusion, potentially compromising patient safety. This project aims to define the key factors that dictate compatibility of mAbs with diluent(s) and human plasma.

Abstract:

The majority (~63%) of FDA-approved therapeutic monoclonal antibody (mAb) products are administered via intravenous (IV) infusions. This involves diluting the medication into an infusion bag containing a diluent solution (e.g., saline or 5% dextrose). According to FDA-approved product labels, normal saline is acceptable for use with all therapeutic mAbs; however, certain mAb product labels (~30%) indicate, "Do Not Use Dextrose." While much emphasis has been placed on product stability in the infusion bag, little published evidence or standardized best practices are associated with assessing compatibility with plasma components. To fill this knowledge gap, we initiated this study to assess compatibility of therapeutic mAbs with diluents and human plasma. We developed in vitro models to simulate the IV infusion interface in which a diluted mAb is mixed with human plasma or serum.

Using this model, we tested a panel of 11 FDA-approved therapeutic mAbs with distinct formulations and IgG isotypes (IgG1, IgG2, IgG4, and a Fc fusion protein). Our results show that formulation pH and ionic strength are critical determinants of product stability at the interface with 5% dextrose and plasma. Six mAbs with a formulation pH ~6.0 exclusively formed insoluble aggregates when mixed with dextrose and plasma or serum. The resulting

pH remained essentially unchanged due to the lack of buffering capacity of dextrose solution. Using mass spectrometry, we identified several abundant plasma proteins in the insoluble protein aggregates, including complements C3 and C4. Notably, these plasma proteins are characterized by an isoelectric point at ~ pH 6.0, which may lose solubility when encountering dextrose solution with an approximate pH. Such aggregation was not observed when mAbs were dissolved in normal saline. The dextrose mediated aggregation was effectively disrupted by raising pH or salt concentration in the dextrose solution. Despite in vitro observations, our data suggest that such a phenomenon may occur only transiently at the interface of IV infusion. Our research advances regulatory science and warrants additional studies and discussions among stakeholders about selecting the right IV diluent for therapeutic mAbs.

55. FDA-NIBSC-WHO collaborative research to develop the 1st International Bioactivity Standard for Adalimumab

Authors: Nalli, Ancy, FDA-NCI/CDER; Wadhwa, Meenu, NIBSC/Biotherapeutics Group; Bird, Christopher, NIBSC/Biotherapeutics Group; Twomey, Julianne, FDA/CDER; Zhang, Baolin, FDA/CDER

Plain Language Synopsis: Our research is part of an international collaborative project between FDA, National Institute for Biological Standards and Control (NIBSC), and World Health Organization (WHO), to develop the 1st WHO international standard for adalimumab which can be used by manufacturers for quality control testing during the development of adalimumab products

Abstract:

FDA regulates an increasing number of biologic products, including therapeutic monoclonal antibodies (mAbs) and their biosimilar counterparts. Adalimumab is a fully-humanized antibody against tumor necrosis factor-alpha (TNF- α), approved for the treatment of inflammatory diseases. The expiration of the innovator product patent

led to a rapid development of adalimumab biosimilars. The World Health Organization (WHO) has recognized a global need for international standards (IS) as reference materials for quality control testing of relevant products. The National Institute of Biological Standards and Control (NIBSC), on behalf of WHO, operates a program for developing standards for therapeutic mAbs, including adalimumab. This study includes participation of 28 organizations worldwide, including the FDA laboratory (PI: Dr. Baolin Zhang) that has regulatory review and research expertise in this matter.

Ampouled adalimumab samples were prepared using bulk adalimumab drug substance donated by the manufacturers of commercial products. Preparations containing approximately 50 µg of adalimumab protein were developed at NIBSC and supplied to participating laboratories. To determine the relative bioactivity of these samples, we developed cell-based TNF-α neutralization assays using L929 and WEHI-164 cell-lines, which are sensitive to TNF-α induced killing. A pilot assay to elicit a dose-response curve for TNF-α induced cytotoxicity was performed to determine a TNF-α concentration that induced a slightly submaximal cytotoxic effect in both cell-lines. A suboptimal dose of 10 IU/mL was used to titrate increasing doses of adalimumab in TNF-α neutralization assays. The resultant dose-response curves were analyzed using a four-parameter logistic (sigmoid curve) model with GraphPad Prism Software to generate EC50 values that indicate TNF-α neutralizing activity.

The study results are provided to NIBSC and will be evaluated with results from other participating laboratories. The final results will be used to assign international units for TNF-α neutralization bioactivity of adalimumab. The IS will potentially serve as a bioactivity benchmark and support manufacturer reference standards and in vitro bioassay validation. This will help to ensure consistency in product quality and harmonization of bioactivity of adalimumab

products globally. These projects directly address CDER's strategic initiatives to elevate awareness and importance of product quality, as well as to strengthen partnerships and engage stakeholders.

56. A Novel Multiplex-Competitive ELISA and Western Blot Method for the Detection and Characterization of Gluten in Fermented-Hydrolyzed Foods

Authors: Panda, Rakhi, FDA/CFSAN; Garber, Eric A.E., FDA/CFSAN

Plain Language Synopsis: This research introduces a novel, multiplex-competitive ELISA and a western blot method for recognizing gluten proteolysis. The two complimentary methods can be used to identify appropriate hydrolyzed gluten calibrants of comparable digestion, which is essential for accurate gluten quantification in fermented-hydrolyzed foods.

Abstract:

Background: In 13, FDA issued the gluten-free final rule, defining "gluten-free" for food labeling. The rule also addressed uncertainties in interpreting the results of current gluten detection methods for fermented-hydrolyzed foods. Commercially available methods cannot distinguish between different hydrolytic patterns, a severe limitation that makes accurate quantification impossible.

Purpose In: To ensure accurate gluten quantification in fermented-hydrolyzed foods, a novel, multiplex-competitive ELISA and a western blot method was developed using multiple antibodies that recognize different gluten epitopes (gliadin, deamidated gliadin, and glutenin).

Methodology: Five gluten-specific antibodies (G12, R5, 2D4, MloBS, and Skerritt) from nine commercial ELISA test kits were used to develop the multiplex-competitive ELISA. The assay was used to evaluate 87 different fermented-hydrolyzed foods from six food groups (wheat beer, barley beers, gluten-reduced barley beers, soy-based sauces, vinegars, and sourdough breads). Western

blot analysis, using the same nine gluten specific antibodies and a subset (65 samples) of the same fermented-hydrolyzed foods was performed. Hierarchical clustering of the apparent gluten concentration profiles, obtained by the multiplex-competitive ELISA and the western blot, was performed using the Ward's Minimum clustering method.

Results: The multiplex-competitive ELISA recognized the protein/peptide-profile differences among the different categories of fermented-hydrolyzed foods and clustered foods, based on the type and the degree of fermentation. The western blot analyses, by separating the antigenic proteins by size, provided novel information regarding differences in the proteolytic processes associated with the different fermentation processes, while confirming the clusters indicated by the multiplex-competitive ELISA. Further, unlike the multiplex-competitive ELISA, the western blot analyses distinguished between the presence of antigenic proteinaceous materials and false positives due to the presence of binding inhibitors (observed with four soy-based sauces and one vinegar).

Conclusion: The novel, multiplex-competitive ELISA and the western blot method provide insight into the extent of proteolysis from various fermentation processes, which is essential for accurate gluten quantification in fermented-hydrolyzed foods. Further, the two complementary approaches have the potential to provide information about the immunopathogenicity of different fermented-hydrolyzed foods.

57. Effects of polysorbate composition and heterogeneity on biotechnology drug product quality

Authors: Pegues, Melissa A., FDA/CDER; Szczepaniak, Karol, FDA/CDER; Rao, V. Ashutosh, FDA/CDER

Plain Language Synopsis: Polysorbates are commonly used to prevent damage to protein drugs. We tested how variation in fatty acid content of polysorbates affects their ability to stabilize protein drug formulations under

conditions that degrade the polysorbate. Both the polysorbate and the therapeutic protein were monitored for changes important for safety and quality.

Abstract:

Polysorbates are common excipients in drug formulations, stabilizing therapeutic proteins by preventing adsorption at surface interfaces. Degradation of these excipients could lead to instability of the drug formulation and subsequently cause quality, safety and efficacy concerns, including aggregation and immunogenicity of the therapeutic protein. Additionally, degradation of polysorbates has been associated with sub-visible and visible particle formation. The formation of fatty acid particles depends on the length of the fatty acid chain. Therefore, we investigated whether the fatty acid ester composition of polysorbate 80 affects particle formation and therapeutic protein stability when subjected to accelerated degradation conditions. Polysorbate-80-containing drug formulations were reformulated with either polysorbate 80 that has a "pure" fatty acid ester composition or with compendial polysorbate 80 containing heterogeneous fatty acid esters. Accelerated stress conditions were then applied and changes to both the polysorbate and the therapeutic protein were monitored for stability, purity, and potency. Specific conditions, including the spiking of host cell lipases/esterases, were found to lead to significant degradation of the polysorbate and increases in particle formation for both polysorbates tested. We are currently studying specific changes in protein stability and critical quality attributes using orthogonal methods, including potency assays and chromatography methods, to characterize the protein aggregates. Our goal is to correlate the composition and quality of the surfactants with final protein drug product quality, and identify key risk assessment criteria for excipient quality.

58. Impact of PEGylation in pharmacokinetic (PK) assessment of pegfilgrastim using alternate assays

Authors: Shi, Da, FDA/CDER; Shah, Ankit, FDA/CDER; Schrieber, Sarah, FDA/CDER; and Howard, Kristina, FDA/CDER.

Plain Language Synopsis: This study compared detection of filgrastim and pegfilgrastim using commercially available assays to better understand variability in free drug detection in patient samples. Assay performance varied considerably between vendor products and drugs, suggesting that assays designed for filgrastim detection may not be ideal for pegfilgrastim detection.

Abstract:

Background: Polyethylene glycol (PEG), a flexible, uncharged and highly hydrophilic polymer, is used to prolong the half-life and improve efficacy of therapeutic proteins and nanoparticles. Despite these key advantages of PEG conjugation, biosimilars developed from their respective originator products may fail to meet requirements for FDA approval. This could be broadly attributed to the presence of PEG, which can present challenges in the bioanalytical methods to assess serum concentrations of the PEGylated compound for PK studies. In addition, aggregate formation, steric hindrance, chemical properties of PEG (source, length, conjugation site) and immunogenicity in physiologic conditions could also contribute to PK variability.

Purpose: Since there are over a dozen PEGylated products currently marketed, there is an urgent need to understand potential causes for the PK variability of these products. There has been intense development of pegfilgrastim biosimilar products, so this product and its non-PEGylated counterpart, filgrastim, were chosen as the initial products to evaluate PK assays and inherent variability.

Methodology: Three different commercially available ligand binding assays (LBA) were evaluated following FDA's Bioanalytical Method Validation Guidance for initial validation. Parameters assessed included sample stability at different temperatures, effect of matrix, determination of standard

analytes, recovery of quality controls, accuracy, and precision. Using the optimized and qualified assay methods, inter-donor and sex-specific variability in serum from 50 healthy donors were evaluated for both pegfilgrastim and filgrastim.

Results: Two of three assays demonstrated performance within guidance recommendations for filgrastim and pegfilgrastim for both fresh and frozen samples. The third assay was able to be fully validated only for filgrastim, but not pegfilgrastim, due to differences in both fresh and frozen sample results. In addition, no differences were found during assessment of 50 individual serum samples. The paired comparison of LBA methods demonstrated key differences, which are critical for their applicability in PK assessment.

Conclusion: The variability observed in the calibrator, assay matrix, and storage conditions contributed to inconsistent PK measurement of pegfilgrastim, but less so for filgrastim. Therefore, the commercially available assays that detect filgrastim may not be appropriate to detect pegfilgrastim, except in specific conditions.

59. Implementation of FDA developed test methodologies for quantification of light scattering from vacuoles in new technology intraocular lens (NTIOL) implants

Authors: Spiezio, Vincent, FDA/CDRH; Walker, Bennett, FDA/CDRH; Calogero, Don, FDA/CDRH; Ilev, Ilko, FDA/CDRH

Plain Language Synopsis: We developed and implemented test methodologies to quantitatively evaluate light scattering from vacuoles within intraocular lenses (IOLs) and to predict dominant vacuole characteristics affecting light scatter. A multimodality approach including high-resolution digital microscopy, novel scanning light scattering profiler, and computer simulations, was used to correlate experimental and analytical vacuole light scattering properties.

Abstract:

In recent years, intraocular lens (IOL)

material has changed from hard polymethyl-methacrylate (PMMA) to more flexible acrylic material. These new technology intraocular lens (NTIOL) materials simplify implantation, but also tend to form small, fluid filled vacuoles, commonly known as glistenings. These vacuoles have been known to induce significant unwanted light scattering that causes glare and reduced visual acuity, and often leads to IOL explantation and replacement. Current vacuole evaluation methods largely assess backward light scattering effects when forward light scattering is the primary cause of visual problems for the patient. We developed and implemented a multimodal test methodology to quantify vacuole characteristics within a lens, to measure the forward scattered light, and to determine dominant vacuole properties that affect light scatter. The first test platform was a high-magnification digital microscope, which provided high-resolution submicron images of all IOL samples tested, and allowed determination of each vacuole's size, orientation, and total number present in each lens. The second evaluation platform was a novel scanning light scattering profiler (SLSP), which provided quantitative evaluation of forward and backward light scatter from IOLs, using goniophotometry principles. This platform was adapted to simulate in-situ measurement conditions using a model cornea and saline bath. When the quantitatively measured scattered light was compared to measured vacuole characteristics, there was a high correlation between the amount of scattered light and vacuole size, number, and orientation. The third approach includes a computer simulation of the SLSP platform using Zemax ray tracing software. Vacuoles were constructed within simulated IOLs, and ray traces were performed to simulate SLSP bench tests. Zemax simulations yielded results similar to SLSP experiments, demonstrating the ability to predict scatter from vacuoles using computer modeling. The multimodal test methodology addresses unmet regulatory and public health needs to evaluate and predict critical IOL scatter

before IOL implants enter clinical trials. This minimizes the risk that highly scattering IOLs will enter the market and cause safety concerns for patients.

60. Effect of whole blood storage on *Treponema pallidum* infectivity

Authors: Tamrakar, Pratistha, OBRR/DETTD/LBTSEA ; Bett, Cyrus, OBRR/DETTD/LBTSEA; Molano, Ruth Damaris, CBER/DVS; Asher, David M, OBRR/DETTD/LBTSEA; Gregori Luisa, OBRR/DETTD/LBTSEA

Plain Language Synopsis: The last recognized case of transfusion transmitted syphilis in the U.S. occurred in 1966. One reason for this might be refrigeration of blood, which inactivates *Treponema pallidum*, the spirochete bacterium causing syphilis. We evaluated the effect of refrigeration on treponemal survival by conducting rabbit infectivity studies.

Abstract:

Background: Before 1938, when universal testing of blood donations began, 138 cases of transfusion-transmitted syphilis (TTS) had been reported in the U.S. The last recognized case of TTS in the U.S. occurred in 1966. In addition to testing, another factor might have reduced the risk of TTS: Whole blood and red blood cells are refrigerated until use. Cold temperatures inactivate *Treponema pallidum* (NTp), the spirochete bacterium causing syphilis. Taken together, universal donor testing, cold inactivation, and the low incidence of syphilis in blood donors, have reduced the risk of TTS to undetectable levels. Some in the blood industry conclude that syphilis testing is no longer necessary and propose its elimination. In this study, we address industry concerns and evaluate the effect of cold storage on treponemal survival.

Objective: To reassess whether routine storage of blood and components reliably reduces risk of TTS, we are studying infectivity of stored whole blood and blood components spiked with NTp, by conducting infectivity studies in rabbits. (NTp cannot be cultured.)

Results: We propagated the Nichols strain of NTp in rabbit testes to create archival and working glycerolized stocks, characterizing them using dark-field microscopy and PCR. We spiked fresh human whole blood with NTp (Day 0) and, each day through Day 9 post-spike, we sampled stored blood and injected aliquots into each of two rabbits to track survival of spirochetes. We also injected heat-inactivated NTp and normal unspiked human whole blood as negative controls. We monitor the rabbits by weekly serology testing for the presence of antibodies against treponemes and check frequently for overt orchitis, performing a non-treponemal serological test to confirm syphilitic orchitis. We plan to collect lymph nodes from necropsies of apparently healthy animals to blind passage into naïve rabbits. The overall goal is to establish the day of cold storage beyond which infectivity is no longer detected.

Impact: The results of this project should establish the kinetics of inactivation of treponemes in blood stored cold, which will help FDA evaluate whether universal testing of blood donors for syphilis continues to contribute to safety.

61. Detection of Microbial Adulteration in Probiotics.

Authors: Tartera, Carmen, FDA/CFSAN; Gangiredla, Jayanthi, FDA/CFSAN; Barnaba, Tammy, FDA/CFSAN; Patel, Isha, FDA/CFSAN; Mammel, Mark, FDA/CFSAN; Gebru, Solomon, FDA/CFSAN.

Plain Language Synopsis: Consumers rely on product labels to accurately reflect contents. Our aim is to use next-generation sequencing technology as an analytical tool to determine potential adulteration of dietary supplements available in the U.S. that contain live microbes.

Abstract:

Many products available to the consumer, such as dietary supplements and foods, contain intentionally added live microorganisms that may provide a human health benefit. This has led to an increased production of these commodities to meet

the demand for these new health-related supplements. However, identification and characterization of the microbes in these products is lacking, and pre-market requirements are limited to general safety and identity concerns. Consumers rely on product labels that report identity and viability, to be accurate and true. High-throughput, next generation sequencing (NGS) can support metagenomic investigations as a feasible means to analyze these products and eliminate any bias of culture-based sampling or the inability to isolate all microbes present. In addition, 'fingerprints' of all identified microbes can be used in future regulatory applications, e.g. strain identification. This analysis depends on the use of public and newly created in-house sequence Kmer databases to identify the microbial components and establish phylogenetic relationships among these microbes, based on core gene multi-locus sequence typing (MLST). Sensitive detection of low-concentration microbial constituents and contaminants in these products is a challenge, due to the high numbers of intentionally added beneficial microbes belonging to the genus *Lactobacillus* and *Bifidobacterium*. The aim of this project is to determine the potential effects of specific phages on decreasing the product's indigenous microorganism's levels by improving the sensitivity of detection of low constituents and contaminants. Preliminary studies using *Lactobacillus* phages have shown promising results, with significant increase on the sensitivity of detection of low concentration of pathogens i.e. *E. coli*. FDA requires that manufacture of dietary supplements follow Good Manufacturing Practices; but unlike drugs, products can be sold without the agency's approval. However, FDA can act if a dietary supplement is adulterated. The impact of this research will increase FDA's regulatory tools in dietary supplements and will help protect public health.

62. Lot release panels for donor screening assays improve safety of the blood supply

Authors: Swati Verma, Sherwin Lee, Mohan Haleyurgirisetty, Ragupathy Vishwanath, Krishnakumar Devadas, Indira Hewlett and David M Asher

Plain Language Synopsis: CBER regulates U.S. supplies of blood and plasma. CBER formulates release panels for eight transfusion-transmissible pathogens provided to manufacturers of donor screening assays. If test results comply with established reactivity, CBER approves distribution of test kits. CBER also prepares reference reagent panels to support development of new and improved tests.

Abstract:

CBER regulates tests to screen blood and plasma donors for major transfusion-transmitted pathogens. Licensed biologicals are subject to lot release per 21 CFR 610.1.A (General Biological Products Standards): "... No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product." Manufacturers submit lot release test results to CBER for review and release kits only after approval.

The Division of Emerging and Transfusion Transmitted Diseases (DETTD) in the Office of Blood, CBER, formulates panels for lot release testing of donor screening tests and in vitro retroviral diagnostic tests. Each panel issues with a memorandum describing the panel with expected reactivity for each member. CBER maintains reference and lot release panels for tests that detect infections with eight pathogens: 1) Hepatitis B and C, HIV, HTLV, West Nile, and Zika viruses or antibodies to them; 2) Babesia and Trypanosoma cruzi parasites and antibodies. Each panel contains six to eight members, with varying concentrations of analyte (nucleic acid or antibody) serially diluted with analyte-negative human plasma ("Basematrix"). A panel has at least two reactive members (designated +), two non-reactive members (designated -), and members containing analyte close to the limit

of detection of the assay (designated +/-). DETTD also provides reference panels to developers validating candidate assays. After assay licensure, its reference panel becomes a lot release panel to test kit lots; results must comply with established reactivity.

DETTD recently formulated an HIV-1 p24 antigen panel for lot release of HIV-1 antigen-antibody combination donor screening assays. This panel might also serve as reference panel for new diagnostic assays. Panel members are serially diluted HIV-1 Group M, clade B virus, and Group O virus. FDA sent samples to manufacturers and assigned consensus values.

Panel formulation is labor-intensive, requires meticulous handling, and was formerly contracted to private companies. DETTD now formulates panels in-house, which is more efficient and cost-effective. DETTD's development of reference and lot release panels supports public health by ensuring consistent and reliable donor screening assays that protect the blood supply and facilitate industry's efforts to improve testing.

63. Failure mode identification of Insulin drug products – Impact of relevant stress conditions on the quality of drug

Authors: Vijaya Chikkaveeraiah, Bhaskara, FDA/CDER/OPQ/OBP; Bhirde, Ashwinkumar, FDA/CDER/OPQ/OBP; Agarabi, Cyrus, FDA/CDER/OPQ/OBP

Plain Language Synopsis: Insulin and its analogs are used to lower blood glucose levels in diabetic patients. Formation of aggregates and other particulates negatively affect their uses. We are evaluating the impact of aggregates and particulates on the quality of Insulin drug products under relevant, real-world conditions.

Abstract:

Insulin and its analogs are used to regulate glucose metabolism in diabetic patients. These products lower blood glucose levels by stimulating peripheral glucose uptake, especially by skeletal muscle and fat, and by inhibiting hepatic glucose production.

Currently, insulin analogs are most widely used in diabetic patients. These analogs have weaker monomer-monomer interactions to promote quicker dissociation on administration from the oligomeric state to the biologically active monomer. However, the formation of aggregates or other particulates in the analogs may negatively affect product quality. Unlike many protein drugs that are stored refrigerated and administered by health care professionals, insulin drugs can be self-administered by patients using auto-injectors or pens. Some patients may need insulin throughout the day, just prior to eating, which often requires them to carry these drugs with them. We are evaluating potential real-world scenarios that may negatively affect insulin product quality. Shifts in pH, exposure to elevated temperatures, agitation, and/or contact with hydrophobic surfaces, can all induce conformational changes to insulin and its analogs, promoting precipitation or chemical degradation. Marketed insulin analogs were studied for their stability and purity under various real-world conditions using industry standard techniques, such as dynamic light scattering (DLS), size exclusion chromatography (SEC), and micro-flow imaging (MFI). This study will improve understanding of potential failure modes encountered while handling insulin analog products.

64. Arsenic species in edible seaweeds commercialized in the United States

Authors: Wolle, Mesay, FDA/CFSSAN; Conklin, Sean, FDA/CFSSAN

Plain Language Synopsis: The distribution of arsenic species in commercially available seaweed products was determined using methods recently developed and single-lab validated at FDA

Abstract:

Seaweeds are increasingly being cultivated for food, as they are rich in nutrients, such as amino acids, Vitamin K, and iodine. However, seaweeds are known to accumulate arsenic in dozens of chemical forms, some of which are of known toxicities and others of which

haven't been fully studied for potential toxicity. Most risk assessment practices associated with dietary arsenic are based on monitoring inorganic arsenic, which is a Class I carcinogen. Such an approach is generally adequate, as most products are known to accumulate arsenic in forms of defined properties. However, the approach may leave species of potential or unknown toxicities unidentified when applied to seaweeds, where arsenic has a complex and variable distribution of species. Comprehensive speciation analysis that aims at capturing a complete picture of the distribution of arsenicals is recommended. The poster presents a wide-ranging speciation analysis of arsenic in edible seaweeds commercialized in the United States. Samples were purchased from local supermarkets and online and analyzed by methods recently developed [1] and single-lab validated [2] at FDA. The accuracy of the analytical results was substantiated by analyzing certified reference materials and using spike recovery tests.

[1] M. M. Wolle, S. D. Conklin; *Anal Bioanal Chem* 410 (2018) 5675

[2] M. M. Wolle, S. D. Conklin; *Anal Bioanal Chem* 410 (2018) 5689

Poster Session 2

Topic: Predictive Tools (Day 1, P.M.)

65. Identification of Biomarkers of *Trypanosoma cruzi* Infected Cells for Imaging and Targeted Drug Delivery Applications

Authors: Acharyya, Nirmallya, FDA/CBER/OBRR; Serna, Carylinda, NIH/NCI; Silberstein, Erica, FDA/CBER/OBRR; Acosta, David, FDA/CBER/OBRR; Nagarkatti, Rana, FDA/CBER/OBRR; Debrabant, Alain, FDA/CBER/OBRR.

Plain Language Synopsis: The blood-borne parasite *Trypanosoma cruzi* is the causal agent of Chagas disease. Our studies suggest that parasite proteins are present at the surface of infected cells in tissues of the infected host. These proteins could be exploited to develop either drugs to improve treatment, or probes for imaging-based diagnosis.

Abstract:

Background: Chagas disease is caused by the blood-borne parasite *Trypanosoma cruzi*. This disease is endemic in Latin America and affects other countries, including the U.S., due to population migration. One third of infected individuals will develop severe cardiac or gastrointestinal symptoms, while others will never develop pathologies. *T. cruzi* parasites infect and grow within nucleated cells in various tissues. Using a proteomics approach, we identified previously seven candidate parasite proteins expressed at the surface of infected MK2 cells in vitro.

Purpose: The goal of this study is to provide additional experimental evidence that these candidate proteins are indeed expressed at the surface of infected host cells in vitro.

Methodology: Three of these proteins, HSP70, GRP78, and mucin, which showed low sequence homology with their host homologs, were recombinantly expressed in bacteria, and specific polyclonal antibodies were made against them. These antibodies were tested by ELISA and by Western blot against their respective recombinant proteins and against parasite cell lysates. They were also tested by immunofluorescence assays (IFA) under fixed and native conditions.

Results: The anti-GRP78, anti-HSP70, and anti-mucin antibodies showed high antibody

titers by ELISA and single band reactivity by Western blot, with *T. cruzi* trypomastigote and epimastigote parasite lysates. These antibodies also reacted with proteins associated with the cytoplasm and/or the endoplasmic reticulum of the parasite by IFA, using fixed and permeabilized preparations of both extracellular parasites (trypomastigotes and epimastigotes) and *T. cruzi* infected-MK2 cells (amastigotes). Of significance, IFA performed under "live" conditions, i.e. with non-fixed, non-permeabilized cells, showed labeling of the infected host cell surface membrane.

Conclusion: These "live IFA" results are consistent with our proteomics data and suggest that the parasite's HSP70, GRP-78, and mucin proteins are indeed present at the surface of infected MK2 cells in vitro. The transport of these proteins from the parasite to the host-cell surface membrane is being investigated. These three parasite proteins represent ideal targets for probes (e.g. antibodies or aptamers) used in imaging and drug targeting studies to either identify new sites of parasite persistence in the host or to improve treatment of Chagas disease by targeted drug delivery.

66. Evaluation of 3D-Cultured Human Skin as a Potentially Less Variable Alternative to Excised Human Skin for In Vitro Permeation Tests (IVPT)

Authors: Ako-Adounvo, Ann-Marie, CDER/OPQ/DPQR; Hamad, Ghaled, CDER/OPQ/DPQR; Zidan, Ahmed, CDER/OPQ/DPQR; Raney, Sam G., CDER/OGD/ORS/DTP; Strasinger, Caroline, CDER/OPQ/ONDP/DNDPII/NDPBV; Ghosh, Priyanka, CDER/OGD/ORS/DTP; Dave, Kaushalkumar, CDER/OPQ/ONDP/DB/BB

Plain Language Synopsis: In vitro permeation test (IVPT) studies evaluate cutaneous pharmacokinetics, but results are highly variable. Here we evaluated the potential of using lab-cultured skin as a replacement for human skin and compared results using different diffusion cells. The lab-cultured skin was less variable but overestimated drug

permeation compared to human skin.

Abstract:

Background and Purpose: An in vitro permeation test (IVPT) evaluates the rate and extent of drug permeation from topical or transdermal formulations into and through skin mounted in diffusion cells. While IVPT studies can support regulatory decisions, there is currently no compendial diffusion cell apparatus or skin type specified for use in IVPT studies. Notably, there can be substantial differences in the magnitude of drug permeation observed through the skin from different individuals. To evaluate whether 3D-cultured (grown) human skin might reduce the variability observed with excised human (cadaver) skin from different individuals, the cutaneous pharmacokinetics (PK) of transdermal testosterone was compared for different types of cultured and excised skin preparations, and the influence of different diffusion cell apparatus was also assessed.

Methods: The permeation of testosterone from a transdermal gel through excised human skin (either heat-separated epidermis (HSE) or dermatomed skin) was compared with EpiDermTM and EpiDermFTTMTM cultured skin, as well as with a TeflonTM filter membrane. These were studied on PermeGear flow-through cells as well as Hanson and PermeGear Franz diffusion cells (N=3). Skin thickness and trans-epidermal water loss (TEWL) were measured prior to dosing. Receiver samples were collected at designated timepoints and analyzed by a validated high-performance liquid chromatography (HPLC) method. Experimental mass balance included an accounting of any testosterone remaining in or on the skin/membrane.

Results: The results indicated that cultured human skin preparations (EpiDermTM and EpiDermFTTMTM) were typically less variable but more permeable than excised human (cadaver) skin preparations. As expected (based upon barrier function) the rank order for total drug permeated was: TeflonTM

membrane > EpiDermTM > EpiDermFTTMTM > HSE > dermatomed skin. Additionally, the diffusion cell apparatus itself appeared to influence the amount of drug permeating a given skin/membrane, potentially by altering dose administration or sampling effects.

Conclusion: Although cultured human skin preparations may provide lower sample-to-sample variability compared to excised human (cadaver) skin, the compromised barrier function of cultured skin (to testosterone permeation) may limit its usefulness for IVPT studies. Other drugs with different hydrophobicities should be evaluated to further evaluate the potential of cultured human skin for IVPT.

67. Evaluation of ROKA Atlas Based Assay for Major Foodborne Pathogens in Food and Environmental Samples

Authors: Brown, Eric, FDA/CFSAN; Zheng, Jie, FDA/CFSAN; Reed, Elizabeth, FDA/CFSAN; Chen, Yi, FDA/CFSAN; Kase, Julie, FDA/CFSAN; Hammack, Thomas, FDA/CFSAN; Ali, Laila, FDA/CFSAN*

Plain Language Synopsis: The ROKA Atlas Assay is a molecular method that uses ribosomal RNA (rRNA) as the target for detection, since each bacterial cell can have 500-10,000 copies of rRNA, allowing the release of many analytes after lysis.

Abstract:

Introduction: Foodborne pathogens including Salmonella, Listeria monocytogenes, and Shiga toxin-producing Escherichia coli (STEC), account for nearly 48 million foodborne illnesses in the U.S. each year, including 128,000 hospitalizations and 3,000 deaths. Rapid, sensitive, and specific detection methods are needed to identify major foodborne pathogens at various points in the food supply chain.

Purpose: To evaluate the ability of the Roka Atlas Assay to detect major foodborne pathogens in food and environmental samples.

Methods: A 24-hr pre-enrichment or enrichment for food and environmental

samples was used. Samples were added into a Roka G1 Modified sample transfer tube for bacterial lysis, template-specific sample extraction, amplification, and probe detection per fully automated assay protocol in the Atlas instrument. Each assay reagent kit was validated with a set of Atlas Salmonella, E. coli, or Listeria calibrators (positive and negative) provided by the manufacturer. Results were confirmed with the FDA Bacteriological Analytical Manual (BAM) culture method in tandem.

Results: A total of six food and two environmental matrices were evaluated in this study for Salmonella, STEC, or L. monocytogenes detection. Three pre-enrichment broths were also evaluated for Salmonella detection using Roka Atlas Salmonella Assay (SEN). The results from the assay were equivalent in most cases to the BAM culture method results for Salmonella, STEC, or L. monocytogenes, respectively. Universal pre-enrichment broth (UPB) was the best pre-enrichment broth for detecting Salmonella in sprout-spent irrigation water on the Atlas System.

Significance: The ROKA Atlas system provided a more rapid, sensitive, and specific molecular method for detecting major foodborne pathogens from a variety of food and environmental samples.

68. The Effects of Formulation Factors and Actuator Design on Mometasone Furoate Metered Dose Inhaler (MDI) Performance

Authors: Bielski, Elizabeth, ORS/OGD/CDER/FDA; Conti, Denise S. ORS/OGD/CDER/FDA; Oguntimein, Oluwamurewa ORS/OGD/CDER/FDA; Sheth, Poonam, Recipharm, Present Address: AstraZeneca; Hallinger, Madeline Recipharm, Present Address: AstraZeneca; Svensson, Mårten Emmace

Plain Language Synopsis: The influence of formulation on metered dose inhaler (MDI) performance is not well understood. Here we varied the amount of excipients and drug particle size in MDI formulations and actuator parameters to assess factors that would influence MDI product performance. This

work facilitates improvement in drug product development for MDIs.

Abstract:

Introduction: Orally inhaled drug products (OIDPs), including metered dose inhalers (MDIs), have been used to treat a variety of lung disorders. [1] The major influence on product performance for MDIs include some key factors, such as the physiochemical properties of the drug, the amount and type of excipients, and device properties. [2] The influence of formulation factors on MDI performance is not well understood. Therefore, the purpose of this work is to investigate how formulation factors along with actuator parameters influence key in vitro performance parameters for mometasone furoate (MF).

Methods: Three MDI suspension formulations of MF were manufactured with changes in active pharmaceutical ingredient (API) size, oleic acid (surfactant), and ethanol content (cosolvent), relative to the levels of the commercial MDI product Dulera®. The MF MDIs were characterized and evaluated by a variety of in vitro tests to evaluate product performance. Four actuator variants differing in orifice diameter, orifice jet length, and sump depth were included in the analysis to evaluate formulation-actuator interactions. Data were statistically evaluated to determine the effects by formulation factors, actuator design, and formulation-actuator interactions.

Results: Statistically significant effects on some key in vitro performance parameters were seen in these three MF MDI formulations and four different actuators. The formulation with lowest API particle size, and highest oleic acid and ethanol content demonstrated a significant increase (65-97%) in fine particle mass (FPM<2µm), enabling increased deposition at lower stages of a Next Generation Impactor (NGI). Actuator variants that had smaller orifice diameter demonstrated a 15-24% increase in FPM<5µm and a 20-27% increase in ex-anatomical throat dose, demonstrating orifice diameter

as a key parameter for actuator design and enhanced MDI performance. No significant interactions were seen between different MF formulations and actuator variants.

Conclusions: The amount and type of excipients and API size used in suspension MDI formulations of MF have been shown to influence in vitro product performance independent of actuator design. Results from this work enable enhancement in quality by design (QbD) approaches to streamline drug product development for MDIs and provide insights into modulating drug product parameters to achieve a desired performance profile.

References:

1. Gupta, Abhishek, Stephen W. Stein, and Paul B. Myrdal. "Balancing ethanol cosolvent concentration with product performance in 134a-based pressurized metered dose inhalers." *Journal of aerosol medicine* 16.2 (2003): 167-174.
2. Sheth, Poonam, et al. "Influence of formulation factors on the aerosol performance of suspension and solution metered dose inhalers: a systematic approach." *The AAPS journal* 19.5 (2017): 1396-1410.

69. Investigation of Pharmacokinetic Sensitivity to Lung Deposition of Locally-Acting Orally Inhaled Drug Products

Authors: Boc, Susan, FDA/CDER/OGD/ORS; Conti, Denise S., FDA/CDER/OGD/ORS; Chen, Mong-Jen, University of Florida/College of Pharmacy/Department of Pharmaceutics; Jiao, Yuanyuan, University of Florida/College of Pharmacy/Department of Pharmaceutics; Kurumaddali, Ab

Plain Language Synopsis: We conducted a pharmacokinetic investigation of dry powder inhaler formulations engineered for different lung depositions, to determine whether pharmacokinetic studies can be used to differentiate regional lung deposition of different formulations. The results showed that pharmacokinetic studies can help support generic development and

bioequivalence evaluation of OIDs.

Abstract:

Introduction: FDA recommends demonstrating bioequivalence (BE) of orally inhaled drug products (OIDPs) using the weight-of-evidence approach: evidence of qualitative and quantitative formulation sameness (same ingredients within 95-105% of the reference product concentration), equivalence of in vitro drug delivery performance, in vivo systemic exposure, and drug delivery to the local sites of action (lungs) [1]. The challenge is establishing equivalence in the lungs; specifically, equivalence in the amount, residence time, and regional deposition of drug in the lungs. Given the incomplete understanding of how pharmacokinetics (PK) relates to drug concentrations in the lungs, and uncertainties with in vitro correlations between lung deposition and clinical efficacy, comparative clinical endpoint BE studies are currently recommended. Hypothetically, PK studies can provide this information when oral bioavailability of the drug is negligible (e.g., fluticasone propionate, FP) or prevented through charcoal co-treatment. The area under the concentration-time curve (AUC) indicates the lung dose, while peak concentration (C_{max}) suggests airway deposition: faster absorption and higher C_{max} suggests more peripheral deposition [2]. This study investigated PK sensitivity to dry powder inhaler (DPI) formulations engineered to differ in central-to-peripheral lung deposition.

Methods: DPI formulations (A, B, C) of FP, with the same FP particle size distribution (PSD), were engineered to target similar emitted dose (ED), but different regional lung deposition, by varying the amount of carrier (lactose) fines. Formulations were characterized through in vitro ED, aerodynamic PSD, and dissolution. A double-blind, 4-way-crossover (formulation C was repeated for assessing intra-subject variability), single-dose, randomized PK study was performed in 24 healthy subjects.

Results: Following dose normalization (because formulation A showed lower ED), formulations B and C (similar mass median aerodynamic diameter, MMAD) revealed similar PK profiles, indicating BE. The C_{max} of formulation A (largest MMAD) was significantly lower, indicating higher central lung deposition. No statistical difference was observed in AUC among formulations.

Conclusion: PK was sensitive to DPI formulation factors; a trend indicated that PK may help differentiate central-to-peripheral lung deposition. The results from this work suggest that PK may provide supporting evidence on drug concentrations in the lungs, which could help generic development and BE evaluation of OIDs.

References:

- [1] Adams, WP., et al., Demonstrating Bioequivalence of Locally Acting Orally Inhaled Drug Products (OIPs): Workshop Summary Report. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, 2010; 23(1):1-29.
- [2] Hochhaus, G., et al., Pharmacokinetics of Orally Inhaled Drug Products. *The AAPS Journal*, 2014; 17(3): 769-775.

70. Hepatotoxicity assessment in hepatic humanized mice

Authors: Cai, Yan, FDA/CDER; Connerney Mona, Taconic Biosciences; Knapton, Alan, FDA/CDER; Stewart, Sharron, FDA/CDER; Gandhi, Adarsh, FDA/CDER; Patel, Vikram, FDA/CDER and Howard, Kristina, FDA/CDER

Plain Language Synopsis: This study evaluated a chimeric mouse that was developed to have a humanized liver to determine if this model can demonstrate human-specific hepatotoxicity *in vivo* and compared it to non-humanized and genetically humanized mouse models.

Abstract:

Background: Interspecies differences have limited the predictive utility of hepatic toxicity studies performed using standard rodent models. Differences in cytochrome P450 enzymes and function can lead to erroneous

conclusions on the safety, or lack thereof, for human use. Therefore, it is important to develop and validate animal models to better identify drugs that may be hepatotoxic, and allow those that do not pose a risk to continue through development.

Purpose: The goal of this study was to evaluate a chimeric mouse developed to have a humanized liver to determine if this model can demonstrate human-specific hepatotoxicity *in vivo*, compared to non-humanized and genetically humanized mouse models.

Methodology: These studies tested floxuridine (10 mg/kg, IP), flutamide (100 mg/kg, PO), and trovafloxacin (50 mg/kg, PO), which are approved drugs with reported cases of hepatotoxicity not predicted by rodent studies. Four groups of mice (hepatic humanized TK-NOG, control TK-NOG, h3A4 or h2D6 knock-in, and C57Bl/6) were divided into groups of five, for a total of 20 mice per drug study for 28 days.

Results: Mouse weights and survival were monitored and blood samples taken weekly to evaluate drug levels. At study end we evaluated CBC/serum chemistry, leukocyte subpopulations via flow cytometry, and histopathology. Hepatic humanized mice treated with floxuridine showed decreased survival along with decreased hematocrit, and elevated total bilirubin level, ALP, and ALT compared to other groups. Trovafloxacin-treated hepatic humanized mice also had earlier study death than the other mouse strains. They also showed reduced leukocyte counts and increased total bilirubin level compared to other strains. All hepatic humanized mice treated with flutamide developed severe illness at day 7 and required euthanasia, while the other strains were unaffected. Flutamide-treated hepatic humanized mice showed increased ALP levels compared to other strains.

Conclusions: These results suggest that hepatic-humanized TK-NOG mice, but not other strains, developed clinical and serologic evidence of hepatotoxicity when treated with floxuridine, trovafloxacin, or flutamide. The

results also demonstrated that hepatic-humanized mice may improve our ability to detect drug-induced liver injury and enhance pharmaceutical safety in pre-clinical and post-marketing phases of development.

71. Transmissible Spongiform Encephalopathy in Squirrel Monkeys: a Unique Complex Proteinopathy

Authors: Cervenak, Juraj, FDA/CBER/OBRR/DETTD/LBTSEA; Pomeroy, Kitty, FDA/CBER/OBRR/DETTD/LBTSEA; Asher, David, FDA/CBER/OBRR/DETTD; Piccardo, Pedro, FDA/CBER/OBRR/DETTD/LBTSEA/Neurobiology Division, The Roslin Institute, University of Edinburgh, Midlothian, UK

Plain Language Synopsis: Squirrel monkeys with experimental bovine spongiform encephalopathy had markedly different incubation periods but a similar transmissible spongiform encephalopathy (TSE). In addition to TSE, their brains showed complex proteinopathy involving proteins found in non-transmissible encephalopathies but not A β protein. Brains of macaques and humans with CJD had similar TSE but without complex proteinopathy.

Abstract:

Background: Transmissible spongiform encephalopathy (TSE) agents (“prions”) have contaminated human medical products, blood components, and animal vaccines. We determined susceptibility to infection with TSE agents of cell lines used or proposed to manufacture biologics. Cells were exposed to the agents of sporadic and variant Creutzfeldt-Jakob diseases (sCJD, vCJD) and bovine spongiform encephalopathy (BSE). We tested passaged cultures for abnormal prion protein (PrPTSE) and for infectivity by assays in animals. No cell line became infected. However, a control titration of the BSE agent in monkeys yielded interesting results.

Purpose: To compare TSE in squirrel monkeys (SQs) infected with classic BSE (C-BSE) with TSEs of macaques with experimental vCJD and humans with sCJD.

Methods: We inoculated SQs with serial

dilutions of a suspension of brain from a cow with C-BSE (SQ.BSE) as a positive control and monitored them over ten years. Tissue sections of brains from 48 SQs with and without overt TSE were stained with hematoxylin-eosin and immunohistochemistry (IHC). H&E-stained sections showed vacuolation (spongiosis) typical of TSEs. For IHC, sections were probed with antibodies directed against PrP. Astrogliosis was detected with antibody to glial fibrillary acidic protein (GFAP). Sections were also probed with antibodies directed against β -amyloid and phosphorylated tau (A β and p-tau, present in Alzheimer’s disease), α -synuclein (seen in Parkinson’s disease), and ubiquitin (seen in several neurodegenerative diseases).

Results: SQs inoculated with BSE brain (diluted up to 100-fold) developed TSE. Incubation times of two animals inoculated with the same dilution and volumes of the BSE brain extract varied markedly (3y and 8y). Brains of all SQ.BSE had typical changes of TSEs: vacuolation, astrogliosis, and PrPTSE; the same brains also contained p-tau, ubiquitin, and α -synuclein, but not A β . The brain of a macaque with vCJD and a patient with sCJD had TSE without additional proteinopathies.

Conclusions: SQ.BSE had markedly different incubation periods but a similar encephalopathy. In addition to TSE, their brains showed complex proteinopathy involving proteins found in non-transmissible encephalopathies but not A β protein. Brains of macaques with vCJD and humans with sCJD had TSE without complex proteinopathy, highlighting an important role the host must play in manifesting disease.

72. LC-MS/MS Determination of Gyromitrin in Mushrooms as the method to Identify False Morel Mushrooms

Authors: Chamkasem, Narong, FDA/ORR/SFFL

Plain Language Synopsis: A new analytical method was developed to identify false morel mushroom by detecting the presence

of gyromitrin, a toxic chemical found in the mushroom. The new method will replace the physical examination method currently used at FDA.

Abstract:

False morel mushrooms have been responsible for severe intoxication and even death by vomiting, diarrhea, jaundice, convulsions, and coma. Gyromitrin is the major toxin contained in these fresh mushrooms. The current method to determine gyromitrin in false morels is by physical examination. This method needs extensive technical training and there is no longer an expert available at FDA. FDA needs a more modern chemical testing method to accurately determine gyromitrin and its metabolite in the false morels, in support of its regulatory program. Acetonitrile extraction and salting-out sample cleanup method were used to extract gyromitrin spiked into three different blank mushrooms. The sample extract was directly injected to an LC-MS/MS instrument to determine gyromitrin in the sample. Retention time against the authentic standard and at least two MRM transitions were monitored to achieve true identification of gyromitrin in the sample. The average recovery for gyromitrin at 0.4, 4, and 40 $\mu\text{g/g}$ ($n = 18$) ranged from 81-106%, with a relative standard deviation of $\leq 8\%$. This method may be used to replace the physical examination technique to identify false morel mushrooms.

73. Additive phase considerations in exposure modeling for biocompatibility assessment of medical device polymers

Authors: Chandrasekar, Vaishnavi, FDA/CDRH; Zheng, Jiwen, FDA/CDRH; Isayeva, Irada, FDA/CDRH; Saylor, David, FDA/CDRH

Plain Language Synopsis: The goal of this work is to develop a simple exposure model that is valid for additives in medical device polymers present both below their matrix saturation limit (soluble) or phase separated (agglomerates). The exposure estimates can subsequently be used in toxicological risk assessments to evaluate device

biocompatibility.

Abstract:

Polymers used in medical devices may contain many additives, such as plasticizers, color additives, etc. that can result in health hazards if leached into the body at toxicologically significant levels. Historically, animal testing is used to evaluate potential health hazards due to the release of these additives; but toxicological risk assessments are becoming increasingly popular. Risk assessment involves understanding the potential of these additives to migrate out of the polymer, and comparing their exposure to a toxicological threshold value. Exposure modeling approaches can be valuable tools for predicting patient exposure, and are highly dependent on the physical state of the additive in the polymer, which in turn depends on the compatibility of the additive with the polymer, as well as on processing conditions (solubility, dispersion and distribution). In this talk, a simple diffusive transport model will be proposed that can be used to provide conservative exposure estimates for additives present in polymers. Model considerations will be described for additives that are present both below their matrix saturation limit (soluble) or phase separated additives that are typically used at concentrations above their matrix saturation (agglomerates), such as several pigments and fillers. Results from this study indicate that a diffusion-model-based approach to predict exposure has potential for use as a rapid, screening tool to assess the risk of small molecule additives in medical device polymers.

74. Development of a Software Platform Integrating Physiological Models and Toxicity Data to Inform Health Risk Evaluation of ENDS Constituents

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Plain Language Synopsis: Comprehensive physiological models of the oral airway and respiratory tract are being developed to assess how ENDS aerosol constituents are deposited and absorbed. These models will be integrated into a software platform that incorporates toxicological data and modeling results, to inform evaluation of ENDS aerosol dosimetry and potential health risks.

Abstract:

Background: Electronic nicotine delivery systems (ENDS) heat e-liquids containing nicotine, water, propylene glycol, glycerin, and various flavorings to deliver an aerosol mixture that is inhaled by the user. Potential health risks for ENDS users may depend on many factors, such as the concentrations of individual constituents, ENDS characteristics, and user behavior, all of which affect deposition of ENDS aerosols in the respiratory tract. Health risks can be evaluated by comparing internal dosimetry profiles following ENDS usage with available toxicity data of ENDS constituents.

Methods: Dosimetry models were developed to describe the evolution and deposition of ENDS aerosols in the respiratory tract, using a computational fluid dynamics model of the oral airways and a multiple-path particle dosimetry model of the lung airways. Aerosol dynamics included in the dosimetry models consisted of droplet coagulation, constituent evaporation, and water vapor condensation, along with droplet deposition and vapor uptake. Additionally, a physiologically-based pharmacokinetic (PBPK) model was implemented to simulate nicotine disposition following lung deposition. These models were packaged into a Java-based software platform.

Results: The software platform accepts inputs for user topography, ENDS profile, and constituent properties, to simulate an ENDS user session and compute the respiratory deposition of ENDS constituents and nicotine PBPK profiles. The software platform will be presented to demonstrate how dosimetry modeling results can be used in the risk

assessment process for ENDS constituents.

Conclusions: The software platform packages, together with advanced aerosol dosimetry models, a nicotine PBPK model, and toxicological data for ENDS, constituent a user-friendly interface that serves as a tool for regulatory scientists and informs the health risk evaluation of ENDS constituents.

Acknowledgment: This work was supported by the FDA Center for Tobacco Products. This is not a formal dissemination of information by FDA and does not represent agency position or policy.

Poster Session 3

Topic: Predictive Tools (Day 2, A.M.)

1. **PATH Study Wave 1 Biomarkers of Inflammation and Oxidative Stress Markers Among Adult E-Cigarette and Cigarette Users**

Authors: Carol H. Christensen, FDA/CTP; Joanne Chang, FDA/CTP; Brian Rostron, FDA/CTP; Hoda Hammad, FDA/CTP; Dana van Bommel, FDA/CTP; Arseima Y. Del Valle-Pinero, FDA/CTP; Baoguang Wang, FDA/CTP; Elena Mishina, FDA/CTP; Lisa Faulcon, FDA/CTP; Ana DePina, FDA/CTP;

Plain Language Synopsis: We examined biomarkers of potential harm, including high sensitivity C-reactive protein [hs-CRP], interleukin-6, fibrinogen activity, soluble intercellular adhesion molecule-1, and a marker of oxidative stress, 8-isoprostane, in e-cigarette users and compared these levels to other tobacco user groups.

Abstract:

Background: In 2013-14, 7.8% of U.S. adults reported current e-cigarette use. Inflammation and oxidative stress can be induced by smoking and play roles in smoking-related diseases. We evaluated the cross-sectional association between these biomarkers of potential harm with e-cigarette and cigarette use.

Methods: Adult blood and urine samples collected from 3,625 Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013-2014) participants were analyzed for biomarkers of inflammation (high sensitivity C-reactive protein [hs-CRP], interleukin-6, fibrinogen activity, soluble intercellular adhesion molecule-1) and a marker of oxidative stress (8-isoprostane). We evaluated five tobacco user groups: e-cigarette-only users, cigarette-only users, dual users, recent former smokers (<12 months), and never tobacco users. We estimated geometric mean ratios (GMRs) by tobacco user group compared with former smokers, adjusting for age, sex, race/ethnicity, education level, cardiovascular disease (CVD) and CVD risk factors, cancer, respiratory diseases, pack-years, and time since cessation. We compared biomarker concentrations using

never tobacco users and cigarette users as referents.

Results: The median age of e-cigarette-only users is 41.2 years, and 95% are former smokers who average 2.3 years of e-cigarette use. We did not observe differences in biomarker concentration between e-cigarette-only users and former smokers. Compared to cigarette-only users, e-cigarette-only users have lower concentration of all biomarkers. Dual users have significantly greater concentration of 8-isoprostane than smokers (GMR: 1.09 [95%CI 1.03, 1.16]). Compared to former smokers, we observed greater concentrations of all biomarkers in cigarette-only users and dual users, including a greater concentration of hsCRP in dual users (GMR: 1.76 [95%CI 1.15, 2.69]). Biomarker values were similar for former smokers and never tobacco users.

Conclusions: E-cigarette-only users have similar levels of biomarkers of inflammation and oxidative stress as recent former smokers without e-cigarette use and lower levels than smokers and dual users. Dual users have a greater level of oxidative stress than smokers.

2. **Characterization of Factor VIII (FVIII) Binding Sites on its clearance receptor Low-Density Lipoprotein Receptor-Related Protein 1 (LRP)**

Authors: Chun, Haarin, FDA/CBER; Kurasawa, James H, FDA/CBER; Uceda, Cortez G, FDA/CBER; Shestopal, Svetlana A, FDA/CBER; Karnaukhova, Elena, FDA/CBER; Lee, Timothy K, FDA/CBER; Sarafanov, Andrey G, FDA/CBER

Plain Language Synopsis: To better understand the FVIII clearance mechanism limiting replacement therapy of patients with hemophilia A, using various in vitro approaches, we determined the main FVIII-binding fragments of LRP, a major FVIII clearance receptor. This research facilitates generation and review of new FVIII variants with increased half-life.

Abstract:

Introduction: Factor VIII (FVIII) is an essential blood coagulation factor, and its deficiency results in a coagulation disorder, hemophilia A. Current replacement therapy of hemophilia A is limited by a relatively short plasma half-life of FVIII or its modified variants (12-19 h). The clearance of FVIII is mediated largely by low density lipoprotein receptor-related protein 1 (LRP). Previous studies demonstrated FVIII-binding regions on LRP and proposed a bivalent mode for this interaction. In the present study, we further investigated in detail the binding interfaces by mapping the LRP sites for binding FVIII using several in vitro approaches.

Methods: Various recombinant LRP fragments were produced in a baculovirus expression system and purified by chromatography. The fragments were tested for interactions with FVIII by surface plasmon resonance (SPR) and cell-based flow cytometry assay for LRP-mediated uptake of FVIII by human hepatic Huh-7 cells. Correctness of the overall folding of selected LRP fragments was verified by circular dichroism spectroscopy.

Results: Several LRP fragments comprising specific complement-type repeats (CRs) were found to bind FVIII with affinity similar to that of LRP (KD = 50-100 nM) by SPR. A majority of the same fragments consistently inhibited LRP-mediated internalization of FVIII in the tissue culture model. Specificity of these interactions was confirmed by silencing LRP expression and using, as competitors, 1) an anti-FVIII antibody fragment, 2) receptor-associated protein (RAP), and 3) mutated variants of the LRP fragments.

Conclusion: We identified specific CR-doublets of LRP-forming primary sites for interaction with a specific region on FVIII. These results support the bivalent nature of interaction between FVIII and LRP and indicate that the bivalent sites of the receptor interact with FVIII alternatively in a dynamic mode. These data further contribute to understanding FVIII clearance mechanisms and are useful for generating new FVIII variants with extended half-life in the circulation and for developing a method to

assess the quality of FVIII products by testing their binding to fragments of LRP.

Disclaimer

This is an informal communication and it represents the authors' own best judgment. These comments do not bind or obligate FDA.

3. Digoxin causes increased inner retinal scatter which accounts for the loss of outer microglia fluorescence when imaged using simultaneous confocal and optical coherence microscopy

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Plain Language Synopsis: We developed a microscope that enables simultaneous imaging of the retinal layers and fluorescent cells sensitive to injury, to evaluate the effect of the neurotoxic drug digoxin.

Abstract:

The mouse retina contains 2 layers of microglia whose processes ramify in two zones in the inner and outer retina. Using a transgenic mouse where the retinal microglia were labeled with a green fluorescent protein (GFP), we examined how the $\alpha 2/\alpha 3$ -subunit selective Na-K ATPase blocker digoxin caused a loss of the microglial fluorescent signal from the outer retinal layers. To examine how digoxin altered the microglia morphology and the retinal structure in real time, we developed a new mouse eyecup imaging preparation that allowed simultaneous confocal and optical coherence microscopy (OCM). Mouse retinæ were imaged using a miniature eyecup chamber and superfused with oxygenated Ames Ringer at 35°C. To examine for cell death, the nuclear binding dye 7-amino-actinomycin D (7AAD) was used to label dead cells. Digoxin (3 or 10uM) was bath-applied to the retina for 10 minutes, followed by a 30 min washout period and nuclear dye labeling for cell death.

In each case examined, the first effect of digoxin (3 μ M) was a 57 \pm 11% (mean \pm std. dev.) decline of the confocal fluorescence signal in the processes of the outer retinal microglia from their pre- drug levels, while the inner microglia fluorescence declined only 14 \pm 12% (n=7retinas) (P<0.001). Digoxin at 10 μ M produced a more severe loss of microglial fluorescence (n=3 retinas). Histological data suggested this inner/outer fluorescence difference in digoxin may be due to increased optical scatter by the swollen processes of inner retinal cells. This was confirmed by OCM, where 10 μ M digoxin also caused an increase in swelling and hyperreflectivity of the inner plexiform layer. We conclude that following digoxin exposure, light scatter by the swollen inner plexiform layer causes a loss of the fluorescent image of the outer microglia due to its tissue screening effects.

4. Microstructural Mapping of Dry Powder Inhaler (DPI) Formulations using Morphologically Directed Raman Spectroscopy (MDRS): A novel analytical tool for DPI product characterization

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Plain Language Synopsis: Commonly-used in vitro testing for DPI products do not provide insight on formulation microstructure. Using MDRS and in vitro dissolution testing, we found that the dissolution performance of DPI products may correlate with formulation microstructure. This work discloses the potential of MDRS in providing information on DPI formulation differences.

Abstract:

Introduction: Dry powder inhaler (DPI) formulations typically consist of fine micronized drug particles blended with coarse carrier particles. Cascade impactors are commonly used to characterize the

aerodynamic particle size distribution of DPIs; but they do not provide insight into the microstructure of the aerodynamically classified particles, which may affect their dissolution performance. Morphologically directed raman spectroscopy (MDRS) is a novel, in vitro technology for elucidating morphological and chemical features of blends of drugs and excipients [1]. This study explores the use of MDRS and in vitro dissolution to improve understanding of the microstructure of DPI formulations.

Materials and Methods: Advair® Diskus® [100/50, 250/50, and 500/50 μ g Fluticasone Propionate (FP) / Salmeterol Xinafoate (SX)], Seretide® Accuhaler® (100/50 μ g FP/SX), Flovent® Diskus® (100 μ g FP) and Flixotide® Accuhaler® (100 μ g FP) were the commercial DPIs selected. The impactor-sized mass (ISM) was collected on a large-surface-area filter membrane using the Unidose® Aerosol Collection system [2] via a USP inlet port (60 L/min, 4 seconds) mounted on a Morphologi G3-ID (Malvern Instrument). In vitro dissolution studies were conducted in a modified USP Apparatus V with phosphate buffer saline (PBS) and 0.2% w/v sodium dodecyl sulfate (SDS) [3], using the ISM collected from five actuations excepted for Advair® Diskus® 500/50 and 250/50 μ g FP/SX (one and two actuations, respectively).

Results: The % FP dissolved from Advair® Diskus® 100/50 μ g FP/SX was higher than all other DPIs. The MDRS data demonstrated that the fraction of FP agglomerated with lactose and/or SX in Advair® Diskus® 100/50 μ g FP/SX was higher than all other DPIs. This finding in the microstructure of Advair® Diskus® 100/50 μ g FP/SX may help to explain its faster dissolution: the highly soluble lactose particles dissolve rapidly from FP-lactose agglomerates, exposing the surfaces of FP particles, thus enabling faster dissolution.

Conclusion: The collected ISM of commercial DPIs was analyzed using MDRS and found to have different microstructures, which may help to explain differences in dissolution performance. Therefore, MDRS has the potential to be a new analytical tool to

provide information on DPI formulation differences, which may contribute to generic product development.

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5. Screening of 3-MCPD metabolites in human hepatocytes in vitro

Authors: Araujo, Magali, FDA/CFSAN/OARSA/DT; Eckstrum, Kirsten, FDA/CFSAN/OARSA/DT; Mapa, Mapa, S.,T., FDA/CFSAN/OARSA/DT; Zhao, Yang, FDA/CFSAN/OARSA/DT; Mossoba, Miriam, FDA/CFSAN/OARSA/DT; Sprando, Robert, L., FDA/CFSAN/OARSA/DT

Plain Language Synopsis:

3-Monochloropropane-1,2-diol (3-MCPD) is a food contaminant that may be harmful to the kidneys and reproductive system. We used human liver cells in vitro to search for products of 3-MCPD breakdown that could participate in the toxic effects of 3-MCPD.

Abstract:

3-Monochloropropane-1,2-diol (3-MCPD) is a food contaminant formed under high temperatures in processed foods containing fats, oils, emulsifiers. Studies in animals have shown that 3-MCPD may be toxic to the kidneys and reproductive system, but the mechanisms involved are not yet fully elucidated. In mammals, 3-MCPD metabolism in the liver generates intermediate compounds and metabolites that could contribute to 3-MCPD toxicity. We searched for 11 intermediate compounds

and metabolites in supernatants of human hepatocytes continuously exposed in vitro to 3-MCPD (0 mM, 1mM, 2.5 mM, 5 mM) for 6h, 24h, 48h, 72h. We found that 3-MCPD was not toxic for the hepatocytes at the treatment doses and time of exposure. A screening by liquid chromatography/mass spectrometry (UHPLC-QTOF-MS/MS) disclosed beta-chlorolactic acid (b-CLA), a major 3-MCPD metabolite, in supernatants of hepatocytes exposed to 3-MCPD. A quantitative assessment by liquid chromatography/mass spectrometry (UHPLC-QqQ-MS/MS) showed increased b-CLA concentrations with 3-MCPD doses (from $5.0 \pm 0.13 \mu\text{M}$ after 6h of 1mM 3-MCPD to $177 \pm 26.3 \mu\text{M}$ after 72h of 5mM 3-MCPD, $p < 0.01$ $n = 3-7$). 3-MCPD concentrations in hepatocytes supernatants did not change throughout the experiment. Our results demonstrate that 3-MCPD is metabolized into b-CLA by human hepatocytes in vitro, providing a physiological relevance of this model to predict liver metabolites formation in vivo. These results also provide a basis for future studies to investigate a potential role of b-CLA on the toxic effects of 3-MCPD.

6. Evolution of video-based automated assessment of activity measures and behavior of caged mice: Improving scientific assessments and animal welfare

Authors: Dennis, John, FDA CBER OM DVS; Pu, Alex, FDA CBER ORISE; Salem, Ghadi NIH CIT; Pohida, Tom, NIH CIT

Plain Language Synopsis: Algorithms designed to automate assessment of research animals in their home cages allow rapid quantitative comparisons between study groups. FDA and NIH have developed a unique and inexpensive system to assess living rodents, useful for the evaluation of health, welfare, therapy effectiveness, and adverse effects that modify animal behavior.

Abstract:

Rodents are used in large numbers to assess the toxicity of medical products during development. However, the available tools to assess rodent health through behavior

are limited for many reasons. Recently, an increased interest in automated assessments of caged rodent behavior has been recognized in the pharmaceutical industry and in the field of laboratory animal science, made possible by technological innovations and faster computer processing techniques. A multi-year collaboration between FDA and NIH resulted in a new, customizable system that integrates into existing, high-capacity ventilated rodent racks. Our design uses mechanical fabrication of some components by 3-D printing and inexpensive high-resolution depth cameras with near-infrared illumination for night-time monitoring. Algorithms are being developed to provide detailed analysis of rodent activity, captured by video, which reflect both animal health and animal welfare. Animal behavior data collection tools may provide useful information, which supplements other physiological and histopathological measures. The benefits of such systems include unbiased, quantitative data that far exceeds what is typically evident from random, daytime observations of animals in their home cages.

7. Development and validation of plasma miRNA biomarker signature panel for the detection of early HIV-1 infection

Authors: Biswas, Santanu, CBER/FDA; Haleyurgirisetty, Mohan, CBER/FDA; Lee, Sherwin, CBER/FDA; Devadas, Krishnakumar, CBER/FDA and Hewlett, Indira, CBER/FDA

Plain Language Synopsis: Circulating miRNAs are potential biomarkers for the diagnosis of early stage HIV-1 infection when viral markers may or may not be detected. This would facilitate early detection of HIV-1 and initiation of therapy by reducing the window period. Initiation of ART treatment during this stage would greatly reduce HIV-1 transmission.

Abstract:

Background: Accurate laboratory diagnosis of HIV-1 is essential to reduce the risk of HIV-positive individuals transmitting HIV-1 infection.

Purpose: The goal of this study is to identify and assess a panel of host derived plasma miRNAs that could serve as a prognostic and predictive biomarker to diagnose early/acute HIV-1 infection.

Methods: A total of 372 microRNAs were analyzed in nine plasma samples from HIV-1 infected individuals in the early phase of infection and from three healthy controls, using the miRNA PCR-array. Seventeen microRNAs were selected and validated in 80 plasma samples from HIV-1 infected individuals in the early phase of infection (20 samples each from the eclipse stage, RNA+ stage, Ag+ stage, and Ag+Ab+ stage) and 25 healthy controls. Using the validation study results a plasma miRNA panel was developed and evaluated to detect early/acute HIV-1 infection in 49 blinded samples.

Results: We identified an miRNA panel (PeHIV-1) containing four differentially expressed miRNAs (miR-16-5p, miR-20b-5p, miR-195-5p, and miR-223-3p) that could distinguish early HIV-1 infection from healthy controls with high AUC [1.000[1.00-1.00]], sensitivity (100 %), and specificity (100%). We also found that miR-223-3p demonstrates 100% sensitivity and specificity (AUC 1.00[1.00-1.00]) and could distinguish the eclipse stage of HIV-1 infection from healthy controls. To detect the eclipse stage of HIV-1 infection we also developed a four-miRNA-based (miR-16-5p, miR-206, let-7g-3p, and miR-181c-3p) panel (PE) with AUC 0.999 [0.995-1.000], 100 % sensitivity and 95.8% specificity.

Conclusion: This work supports FDA's mission of protecting public health. We have identified a miRNA panel (PeHIV-1), that could serve as a potential biomarker for detecting early/acute stage of HIV-1 infection and could help initiate early antiretroviral treatment, thus preventing the spread of HIV-1 infection. In addition, these non-viral biomarkers could be used as surrogate biomarkers to detect HIV-1 infection when viral markers may not be detected.

Disclaimer:

This abstract reflects the views of the authors and should not be construed to represent FDA's views or policies.

8. A Framework for determining the Most Challenging Simulated Use Pathway: Applicability towards Bench Tracking for Medical Devices

Authors: Soneson, Joshua, FDA/CDRH/OSEL; Duraiswamy, Nandini, FDA/CDRH/OSEL

Plain Language Synopsis: In vitro simulated use pathways are often used for tracking medical devices to evaluate device performance. However, it is challenging to determine the worst case anatomical pathway for the purpose of bench testing devices. The overall goal of this work is to provide a framework for determining the most challenging simulated use pathway, with the aim of using it for bench tracking of medical devices.

Abstract:

The physical and chemical properties of various coatings on the surface of catheters, guidewires, and other intravascular delivery systems have been studied to improve lubricity and tracking through patient anatomies. However, little research has been done considering the impact of the physical characteristics of the anatomical pathways through which catheters are frequently threaded to characterize bench performance. In this study, the three-dimensional curvature of numerous arterial pathways, stemming from the base of the femoral artery to the Circle of Willis (CW), were analyzed to quantitatively characterize the tortuosity of arterial pathways, with a goal of creating a criterion for identifying the most tortuous pathway for use in simulated use testing by manufacturers.

The arterial pathway model was designed by combining computed tomography (CT) scans of three separate patients who possessed challenging arterial anatomies at the femoral, aortic, and neurovascular end. The model was assembled in medical 3D printing software (3-matics), where the model was then trimmed to consist of a single pathway

for four total iterations, generating four unique pathways. The Four Pathways consist of: 1) right femoral artery, abdominal and thoracic aorta, right vertebral artery, Circle of Willis; 2) right femoral artery, abdominal and thoracic aorta, left vertebral artery, Circle of Willis; 3) right femoral artery, abdominal and thoracic aorta, right carotid artery, Circle of Willis; 4) right femoral artery, abdominal and thoracic aorta, left carotid artery, Circle of Willis. Curvatures were calculated from the centerlines of each pathway and further analyzed the proximal and distal sections of all pathways. The right vertebral artery had the highest overall mean curvature of its entire pathway, as well as the highest curvature of its distinguishing region.

A framework was established for determining challenging pathways based on curvature metrics, and could be further developed in the future using other techniques.

9. Payload of T-DM1 binds to cell surface cytoskeleton-associated protein 5 to mediate cytotoxicity of hepatocytes

Authors: Endo, Yukinori, FDA/CDER; Takeda, Kazuyo, FDA/CBER; Mohan, Nishant, FDA/CDER; Shen, Yi, FDA/CDER; Jiang, Jiagsong, FDA/CDER; Rotstein, David, FDA/CVM; Wu, Wen Jin, FDA/CDER

Plain Language Synopsis: Ado-trastuzumab emtansine (T-DM1) is an antibody-drug conjugate (ADC) approved for the treatment of HER2-positive metastatic breast cancer. T-DM1 consists of trastuzumab, a microtubule inhibitor DM1, and a thioether linker. In this study, we used the cellular and murine models to investigate the mechanisms by which T-DM1 induced hepatotoxicity.

Abstract:

Off-target toxicity is a major cause of dose-limiting toxicity for antibody-drug conjugates (ADCs), the mechanism of which is poorly understood. We demonstrated that cytoskeleton-associated protein 5 (CKAP5) is a cell surface target for T-DM1 and that binding of T-DM1 to CKAP5 is mediated by payload (DM1). This study introduced a novel molecular mechanism of ADC payload-

mediated interaction with cell surface molecules to induce cytotoxicity. Upon binding to CKAP5, T-DM1 causes cell membrane damage and leads to calcium influx into the cells, resulting in disorganized a microtubule network and apoptosis. While binding of T-DM1 with HER2 is critical for killing HER2-positive tumor cells, our data suggest that cytotoxicity induced by T-DM1 interaction with CKAP5 may preferentially damage normal cells and tissues where HER2 expression is low or missing, causing off-target toxicity. This study provides molecular basis of ADC-induced off-target cytotoxicity and opens a new avenue for developing next generation of ADCs.

10. Cytokine and chemokine production by HIV-2 infected human macrophages: potential role for CCL-2 in reduced pathogenicity

Authors: Gao, Chunling Gao, FDA/CDER; Ouyang, Weiming, FDA/CDER; Stantchev, Tzanko, FDA/CDER; Tiffany, Linda, FDA/CDER; Clouse, Kathleen A, FDA/CDER

Plain Language Synopsis: HIV-2-infected MDM shows decreased CCL-2 expression compared with HIV-1-infected MDM

Abstract:

Human monocyte-derived macrophages (MDM) infected in vitro with HIV-1 and/or HIV-2, secrete macrophage colony-stimulating factor (M-CSF) and chemokines, such as CCL-2, CCL-3, and CCL-4. Both HIV-1 and HIV-2 infection induces M-CSF production in MDM. However, CCL-2 production by HIV-2-infected MDM was significantly reduced compared to uninfected controls, whereas HIV-1 infection consistently enhanced CCL-2 secretion.

The β chemokine suppression after infection of MDM with select HIV-2 isolates occurred at both protein and RNA levels, based on RT-PCR, ELISA, Western blot, and microarray analyses. In general, HIV-2 is less pathogenic and exhibits lower viremia and reduced mortality relative to patients infected with HIV-1. Inhibition of β chemokine production may be serving as a deterrent to HIV-2 infection and progression in both macrophages and T cells by limiting cell

recruitment and impairing the establishment of virus reservoirs in both cell lineages. This may account for the reduced pathogenicity and delayed disease progression commonly observed with HIV-2.

We found that CCL-2 expression and activity is modulated by STAT-1. CCL-2 expression was suppressed by the STAT-1 inhibitor fludarabine and CCL-2 secretion was increased when exogenous STAT-1 was added to the cell cultures. In summary, our findings may provide insight into novel therapeutic was to treat HIV-infected patients that could delay disease progression and interfere with establishment of viral reservoirs.

11. Application of Physiologically Based Pharmacokinetic Modeling for Evaluation of Maternal Antidepressant Exposure

Authors: George, Blessy, FDA/CDER; Lumen, Annie, FDA/NCTR; Nguyen, Christine, FDA/CDER; Wesley, Barbara, FDA/CDER; Wang, Jian, FDA/CDER; Crentsil, Victor, FDA/CDER

Plain Language Synopsis: Physiological changes that occur during pregnancy can affect maternal drug efficacy and increase risk to the unborn child. We developed a model that takes into account pregnancy-related physiological changes to predict the plasma concentration of sertraline (an antidepressant) during pregnancy. The model's satisfactory performance suggests it has potential use for sertraline dose adjustment during pregnancy.

Abstract:

Pregnancy is a high-risk period for women with psychiatric illness, such as depression. Therefore, treatment with antidepressants may be unavoidable when indicated. Physiological changes that occur during pregnancy can affect the pharmacokinetics (PK) of many drugs including antidepressants. PK variations may translate into undesirable pharmacodynamic (PD) consequences, such as decreased maternal drug efficacy, and present unknown risk to the unborn child. Hence, the ability to quantify maternal and fetal exposure to antidepressants can provide an evidence-based approach to improving

drug efficacy and safety during pregnancy. Sertraline is among the most frequently used antidepressants during pregnancy. We used a deterministic physiologically based pharmacokinetic (PBPK) model to characterize changes in exposure to sertraline during pregnancy. We used knowledge of sertraline disposition in non-pregnant women, physiological changes during pregnancy, and data from in vitro metabolism studies. Simulations were performed to predict sertraline dosimetry in non-pregnant women and during the second and third trimesters of pregnancy. The average (min - max range) predicted-to-observed sertraline area-under-the-curve (AUC) ratio was 1.7 (0.7 - 2.6) in second trimester, and 1.7 (0.8 - 3.7) in third trimester of pregnancy. The average (min - max range) predicted-to-observed sertraline maximum plasma concentration (C_{max}) ratio was 1.07 (0.5 - 1.8) in second trimester and 0.97 (0.5 - 1.4) in third trimester of pregnancy. For the model performance in non-pregnant women, the predicted-to-observed ratio for AUC and C_{max} averaged 0.98. The model we developed can be used for maternal sertraline dose adjustment during pregnancy. Ongoing research includes extending the current model to predict fetal exposure to maternal sertraline.

12. Development of a Virtual HIV Pregnant Population for Use in PBPK Modeling

Authors: Gockenbach, Mary, FDA/CDER/OND/DPMH; Momper, Jeremiah, UC San Diego; Grimstein, Manuela, FDA/CDER/OCP/DPM; Mirochnick, Mark, Boston Medical Center; Capparelli, Edmund, UC San Diego; Struble, Kim, FDA/CDER/OAP/DAVP; Johnson, Tamara, FDA/CDER/OND/DPMH; Sach

Plain Language Synopsis: A model was built to predict maternal exposure to rilpivirine during pregnancy. Adjusting the model based on laboratory data (including hematocrit, albumin, and creatinine) in pregnant women living with HIV improved predictions when compared to observed clinical data.

Abstract:

Background: Physiological changes during pregnancy affect the pharmacokinetics (PK) of antiretrovirals (ARV). Physiologically-based pharmacokinetic (PBPK) modeling can predict drug PK during pregnancy; however, existing pregnancy PBPK models for ARVs use laboratory values from reference populations that may differ from those in pregnant women living with HIV (PWLH).

Purpose: The IMPAACT Network Protocol P1026s is an international, multicenter, prospective, opportunistic study of ARV drugs in PWLH. Laboratory data from 500 women in P1026s were analyzed and compared with parameters in the Simcyp (v17) pregnancy model. A total of 1091 hematocrit (HCT) values, 1096 albumin (ALB) values, and 1284 serum creatinine (SCR) values were compared. The purpose was two-fold: to investigate whether these parameters vary between the populations, and determine whether incorporating these differences into the PBPK model would improve prediction of maternal exposure to ARVs.

Methodology: Laboratory values were grouped together and averaged at predetermined timepoints. Means were compared to those from literature using Welch's T-test. Polynomial regression was used to generate time-dependent functions for each parameter. The Simcyp pregnancy model was then modified with these functions and tested using a PBPK model for rilpivirine (RPV).

Results: HCT, ALB, and SCR in P1026s pregnant women were significantly lower than in reference pregnant women during 2nd and 3rd trimesters. Polynomial equations were generated as follows (GA = gestational age in weeks): HCT (%) = $35.8191 - 0.2683 * GA + 0.005383 * GA^2$, ALB (g/L) = $43.3224 - 0.5651 * GA + 0.007917 * GA^2$, and SCR (mg/dL) = $0.6671 - 0.01067 * GA + 0.0002175 * GA^2$. Updating the model with these functions resulted in a predicted decrease in RPV exposure of 11-16% for C_{max}, 12-18% for AUC, and 13-18% for C_{min}, reducing slight overprediction of exposure in the previous model.

Conclusion: Based on clinical data from P1026s, HCT, ALB, and SCR in PWLH are significantly different from the Simcyp reference population of pregnant women. Incorporating these differences into a PBPK model improved prediction of PK parameters for RPV. Future work will investigate other parameters that may influence drug disposition, such as alpha1-acid glycoprotein. Understanding the influence of differences in critical physiological parameters between populations of pregnant women is important for modeling and simulation and for clinical care.

13. Evolution of the DLAPI (Q)SAR Database. Development and Implementation of a New Web-Based Platform

Authors: Green, David CDER/OPQ/ONDP/DLAPI; Scott, Barbara CDER/OPQ/ONDP/DLAPI; Kim, Marlene CDER/OTS/OCP/DARS; Roy, Kirk CDER/OTS/OCP; Kruhlak, Naomi CDER/OTS/OCP/DARS; Skanchy, David CDER/OPQ/ONDP/DLAPI

Plain Language Synopsis: A new DLAPI (Q)SAR database was developed and implemented. The new, web-based database provides improved search functionality and greater access to (Q)SAR results across CDER. The database facilitates the DLAPI triage mechanism for drug impurities, ensures a seamless workflow, and efficient review process.

Abstract:

ICH M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk states that negative (Quantitative) Structure Activity Relationship, or (Q)SAR, predictions for impurities with alerting structures, if generated with M7 compliant methodologies, can be used to justify control of the impurity at ICH Q3A levels in lieu of submission of Ames data. Since the publication of ICH M7, the Division of Lifecycle API (DLAPI) in the Office of Pharmaceutical Quality (OPQ), in collaboration with the Computational Toxicology Consultation Service (CTCS) in the

Office of Translational Sciences (OTS)/Office of Clinical Pharmacology (OCP)/Division of Applied Regulatory Science (DARS), implemented a triage mechanism for drug impurities containing alerting structures that have not been controlled by the Drug Master File (DMF) holder. A significant part of this triage mechanism is a database established to archive (Q)SAR results generated by the CTCS, which enables DLAPI assessors to search for previously evaluated compounds. This reduces the need to resubmit these compounds to CTCS for evaluation. The original DLAPI (Q)SAR Database was an Excel database that worked well for the first few years of the implementation of the M7 triage process. However, due to the volume of DMF review and success of the collaboration, the database has grown to over several thousand structures and its limitations have become readily apparent. Requests for increased functionality to include substructure search capability from the assessors and Excel stability issues prompted change. After over two years of work, a new, stable, web-based database built on a commercial platform went live in Spring 2019. This database provides improved search functionality and greater access to (Q)SAR results across the center. The new database facilitates the DLAPI triage mechanism for drug impurities and ensures a seamless workflow and efficient review process. This effort is consistent with DLAPI's effort to increase review efficiency.

14. In Vitro Models to Evaluate Wear Particles from Total Joint Arthroplasty Materials

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Plain Language Synopsis: Implant loosening remains a major orthopedic problem, despite medical and material advancements. Wearing of the implant components over time can trigger unfavorable biological responses, resulting in device failure. We developed models to predict physiological responses

to new materials and better understand the relationship between material selection and clinical outcomes.

Abstract:

Orthopedic devices for total joint arthroplasty have played a remarkable role in restoring the health and motion of millions of patients. The polymeric biomaterials used in these devices wear in the body, releasing nanometer to micrometer size particles that elicit localized chronic inflammatory responses that lead to osteolysis and implant loosening. With the advent of wear-resistant polymers, such as crosslinked ultra-high molecular weight polyethylene (UHMWPE), polyether ether ketone (PEEK), and polycarbonate urethane (PCU), the rate of wear generation could be reduced, but long-term biological responses have not been thoroughly investigated. The ASTM standards recognize a simple 2D co-culture of the critical-sized wear particles (< 10 micron may be phagocytosed) with macrophages or osteoblasts to evaluate the biological responses. This testing fails to capture the complexity of the in vivo microenvironment, potentially resulting in inaccurate predictions, and is not suitable for long-term evaluation. These limitations can be overcome by using 3D in vitro models that use hydrogels to encapsulate the cells and particles, making the system more analogous to in vivo conditions. In this context, extracellular matrix-based collagen hydrogels are known to simulate the physiological microenvironment. This model will enable the evaluation of critical-sized particles in contact with macrophages and osteoblasts by improving the cell-particle interaction and long-term stability.

Model polymers were used to establish and optimize the 3D system. Wear particles similar to those reported clinically were generated, isolated, and collected. Critical-sized particles were characterized based on their morphology and size distribution. Control cultures were performed with 2D monolayer atop tissue culture polystyrene, with or without particles.

Different concentrations of 3D collagen hydrogels encapsulated with particles and macrophages were assessed for cell viability, cell and particle distribution, morphology, phagocytosis, and inflammatory markers.

This 3D model is intended to 1) offer a more straightforward investigational tool to provide a least-burdensome approach to preliminary evaluation of long-term performance, and 2) enable FDA to better evaluate the suitability of newly-emerging materials, decreasing regulatory burden and accelerating their path to the market.

15. Salmonella Serotyping using Whole Genome Sequencing

Authors: Ibrahim, George, FDA/ORR; Morin, Paul, FDA/ORR

Plain Language Synopsis: Using whole genome sequencing and the online in silico SeqSero tool to predict Salmonella serotypes in 1,041 Salmonella isolates, we compared both traditional and WGS/SeqSero Salmonella serotyping results to determine the possibility of replacing traditional Salmonella serotyping with WGS and SeqSero Salmonella serotyping.

Abstract:

Until recently, traditional serology and the Kauffmann White Scheme (KWS) have been the gold standards for Salmonella serotyping. Whole genome sequencing (WGS) is now an alternative in this field. Serovar prediction based on WGS can be performed using in silico data analysis tools. The SeqSero in silico tool enables analysis and prediction of serotype results directly from WGS raw reads. The other molecular Salmonella serotyping assays developed on the Luminex platform do not detect all serotype antigens, as they focus primarily on the most common serotypes reported for human clinical specimens.

In this study, we report a retrospective analysis of our laboratory inventory of 1,041 Salmonella isolates collected between 1999 and 2017. These isolates are of public health significance, since they all came from either

food, feed or environmental swabs. They were all serotyped by both traditional serology and WGS using an in silico "SeqSeo" v1.0 for serovar prediction. Paired reads of WGS raw data (fastq files) of the 1,041 Salmonella isolates were uploaded to the online SeqSero tool version 1.0. The SeqSero database predicted the Salmonella serotype of the requested isolate within a few minutes. Both predicted identical Salmonella serotypes in 899 isolates (86.4% of the 1,041 Salmonella isolates). SeqSero assignments differed from traditional serological testing in 80 isolates (7.7%) and no serotype prediction was ascertained from 62 isolates (5.9%). It is anticipated that results will improve as more whole genomes are added to the Salmonella national database (NCBI).

This retrospective study is an excellent example of using WGS and SeqSero as a data analysis tool to predict Salmonella serotypes that provide numerous advantages, including molecular and genetic details regarding the characteristics of the Salmonella isolates (antimicrobial resistance and virulence), compared to traditional KWS serotyping, reduce labor requirements, and increase budgetary savings, which can reach well above \$20,000 or more per year for our laboratory.

In conclusion, it is evident that using WGS and in silico tools for Salmonella serotyping might someday replace traditional serotyping.

16. Circulating Biomarkers of Neurotoxicity: Identifying fluidic endpoints correlating with central nervous system toxicity in a rodent model of neurotoxicity.

Authors: Syed Z. Imam, FDA/NCTR; Zhen He, FDA/NCTR; Susan M. Lantz, FDA/NCTR; James Raymick, FDA/NCTR; Bonnie Robinson, FDA/NCTR; Elvis Cuevas, FDA/NCTR; Sumit Sarkar, FDA/NCTR; Charles Law, FDA/NCTR; Joseph P. Hanig, FDA/CDER; David Herr, US EPA; Denise MacMillan

Plain Language Synopsis: Predictive and minimally-invasive methods to detect brain toxicity

Abstract:

Neurotoxicity has been linked to exposure to a number of common drugs and chemicals; yet efficient, predictive, and minimally-invasive methods are lacking. Fluid-based biomarkers, such as those found in serum, plasma, urine, and cerebrospinal fluid (CSF), have great potential due to the relative ease of sampling. However, data on their expression and translation are lacking or inconsistent. Here, we present data on biomolecules that have some promise for detecting and characterizing neurotoxicity induced by a single intraperitoneal injection of the known neurotoxic agent, trimethyltin (TMT). A single dose of TMT led to significant alterations in total oxidative stress markers, changes in lipid homeostasis, circulating interleukins and related factors, and markers of neuroinflammation. These findings provide an opportunity to explore the correlation of these fluid biomarkers with traditional neuropathology and magnetic resonance imaging (MRI) that serve to define TMT-induced neurotoxicity. Our data demonstrate a comprehensive correlation of TMT-induced neuropathology with several potential neurotoxicity biomarkers and MRI-based endpoints, findings suggestive of an involvement of specific pathways that can be assessed using peripheral fluids.

17. Development of an In Vitro Blood Flow Loop System for Thrombogenicity Evaluation of Medical Devices and Biomaterials

Authors: Jamiolkowski, Megan A., FDA/CDRH/OSEL; Golding, Madelyn D., FDA/CDRH/OSEL; Hartung, Matthew C., FDA/CDRH/OSEL; Malinauskas, Richard A., FDA/CDRH/OSEL; Lu, Qijin, FDA/CDRH/OSEL

Plain Language Synopsis: This project involves developing a reliable benchtop test method to assess the potential of biomaterials to induce blood clot formation under flow conditions. Such a method would be very useful for improving the design and evaluating the safety of blood-contacting medical devices, while reducing the need for animal studies.

Abstract:

A robust in vitro test to evaluate the thrombogenicity of biomaterials and medical devices under dynamic flow conditions would be very useful for improving the design and evaluating the safety of blood-contacting medical devices, while reducing the need for animal studies. Results of thrombogenicity testing can be significantly affected by key test conditions, such as blood species and sample preparation. We developed an in vitro blood flow loop test system and investigated the effects of blood source, sample length, and test loop configuration on the thrombogenicity results.

Four sources of animal blood were examined: abattoir pig blood and live-donor blood from pigs, sheep, and cows. Immediately before starting each dynamic flow test, the blood was recalcified and heparinized to a donor-specific heparin concentration. The target heparin level was based on a static pre-test, whereby latex tubes were incubated in recalcified blood to assess thrombus coverage under a series of heparin concentrations. The anticoagulated whole blood was recirculated at 200 mL/min through a polyvinyl chloride tubing loop for 1 hour at room temperature. Six materials with varying thrombogenicity traits were investigated: a negative control polytetrafluoroethylene (PTFE), a positive control latex, and four commonly used biomaterials. Additionally, two controls and two biomaterial samples were examined in three loop configurations (one 12 cm long sample/loop, one 18 cm sample/loop, and two 12 cm samples/loop), using donor pig blood. At the end of the tests, the percentage of thrombus surface area coverage, thrombus weight, and reduction in platelet count (%) were measured.

The results showed that the test loop system was able to effectively differentiate materials with different thrombogenic potentials (latex > biomaterials ≥ PTFE), regardless of the blood used. However, the anticoagulation level and the test sensitivity were dependent on the blood used, with the donor sheep and cow

blood providing slightly better differentiation among the materials. The test sample length (12 cm and 18 cm) did not significantly impact the relative thrombogenicity results of different materials. The addition of a second material sample to the loop did not increase the test sensitivity.

18. Development of a novel in vitro model to predict risk of skin-to-skin drug transfer during transdermal hormonal replacement therapy

Authors: Kamal Nahid, OTR/DPQR; Zidan Ahmed, OTR/DPQR; Ibrahim Sarah, OPQ/ONDP, Cruz N Celia, OTR/DPQR; Ashraf Muhammad, OTR/DPQR

Plain Language Synopsis: The risk of hormonal transfer from treated patients to non-dosed subjects during transdermal hormonal therapy can be evaluated only by pharmacokinetic studies. Here, a novel in vitro method was developed to assess this risk. This method can quantitate the amounts of the hormones that are retained and that permeate the fresh skin.

Abstract:

Transdermal hormonal therapy (THT) delivers exogenous hormones directly into the systemic circulation via skin. Transdermal estrogen, progesterone, or testosterone products are most commonly available as a drug-in-adhesive transdermal delivery systems (TDS), emulsions, or gels. The recommended application sites for these topical products are the abdomen, thigh, and/or the shoulders and upper arms. It has been reported that residual hormones on the skin surface have the potential of transfer to healthy individuals via skin contact with the application site for up to 8 hours post application. This hormone transfer to healthy individuals may cause clinically significant hormonal imbalance and adverse effects. Currently, there are no in vitro methods to assess the risk of skin-to-skin drug transfer. The current study aimed at developing a novel in vitro permeation method using vertical diffusion cells to evaluate this risk. A model testosterone transdermal gel was

employed. The risk of skin-to-skin transfer was evaluated after various residence times on the skin. The dosed skin samples were then transferred over the surface of fresh skin samples for permeation and mass balance testing. The results demonstrated that the amount of the hormone recovered from dosed skin increased by increasing the residence time of the gel on the skin surface. The extent of drug transfer and permeation from the dosed to fresh skin samples was directly proportional to the amount of drug retained by the dosed skin. Therefore, this study provided a novel *in vitro* method that can be used to predict the risk of skin-to-skin transfer during THT.

19. Phenotype-Based Mechanistic Studies for the Assessment of Drug Safety and Drug-Drug Interactions in Zebrafish: Efficacy of Dietary Supplements

Authors: Kanungo, Jyotshna, FDA/NCTR; Gu, Qiang, FDA/NCTR; Ali, Syed, FDA/NCTR; Paule, Merle, FDA/NCTR; Robinson, Bonnie, FDA/NCTR

Plain Language Synopsis: Zebrafish embryos can be used as alternate animal models to predict drug safety/toxicity and drug-drug interaction effects. They express the drug-metabolizing enzymes. The embryos can be treated with many drugs in combination at once and the drug-drug interaction effects (e.g., cardiotoxicity, developmental toxicity, and neurotoxicity) monitored *in vivo*.

Abstract:

Zebrafish embryos are routinely used in chemical toxicity assessments and are considered excellent pre-clinical models. For mechanistic studies, zebrafish embryos are better suited than higher order vertebrates, since the embryos can be treated with a number of drugs at once and the phenotypic changes in response are quick and visible. In our studies, in addition to organ-specific toxicities, manifestations of drug-drug interactions arising from the use of combination drugs can be monitored *in vivo* in zebrafish. Live monitoring of multiple organ/tissue toxicities, such as cardiotoxicity

and neurotoxicity, is an advantage, since the embryos/larvae are transparent. Analyses of key enzymes involved in drug metabolism, such as the cytochrome P450 family (CYP) members, help further the understanding of the role of drug metabolism in the expression of various toxicities. Several drugs, such as ketamine, verapamil, and cyclosporine A, show effects (alone or in combination with other drugs) in these embryos that are similar to those in humans. Importantly, the modes of action using phenotypic, biochemical, and genomic approaches can be elucidated using these embryos. Subsequently, such findings have enabled the prevention of adverse effects by co-treatment with dietary supplements (e.g., acetyl l-carnitine and N-acetylcysteine). Our studies provide clues as to how the adverse outcomes of these drugs might occur in humans, thus leading to better risk characterization and assessment. Furthermore, potential therapeutic intervention points are also disclosed, as the effector molecules of their actions are precisely identified.

20. The FDA/CDER Computational Toxicology Consultation Service's Chemical Dictionary

Authors: Cross, Kevin, FDA/CDER; Kim, Marlene, FDA/CDER; Kruhlak, Naomi FDA/CDER

Plain Language Synopsis: An internal repository of chemical structures (CDER Chemical Dictionary) has been established to help organize and evaluate consultation data and results. This presentation discusses the types of chemicals registered, how the chemicals are standardized, how consultation documents are archived, and how this information is used to support regulatory decisions.

Abstract:

Over the past 15 years FDA/CDER's Chemical Informatics Program has collected data on thousands of proprietary and non-proprietary chemicals (drugs, drug impurities, and other substances) through its regulatory research projects and internal Computational Toxicology Consultation Service. The data

is used to develop and validate quantitative structure-activity relationship ((Q)SAR) models for a range of toxicological endpoints of regulatory significance. As such, CDER reviewers from any office can submit requests for (Q)SAR evaluation using these models, or request chemical informatics-based analyses to support the evaluation of chemicals of unknown toxicity. To track the requests of chemicals, an internal repository of chemical structures, known as the CDER Chemical Dictionary, has been established to help organize and evaluate these data and results. Most recently, the CDER Chemical Dictionary was modernized using Leadscape Enterprise structure registration and document management software, which allows chemical structures to be registered and linked to QSAR consultation reports and experimental data. To date over 30,000 chemicals have been registered from both new and generic drug applications, external databases, and the published literature. Consequently, it is common to encounter the same chemical drawn with different chemical structure conventions (e.g., for stereochemistry, charges, and salts) and assigned non-standard names or other various identifiers. Furthermore, multiple consultation requests may be received for the same chemical from different parts of FDA/CDER. To reduce redundancy and ensure consistency in responses, an internal registration workflow has been developed using the Chemical Dictionary whereby all incoming structures are standardized to a specific format. This allows the identification of existing chemical structures that are linked to archived (Q)SAR reports and experimental data, and verifies that new structures added to the database are unique. This presentation discusses the types of chemicals submitted to the Computational Toxicology Consultation Service, what information is stored, how the chemicals are standardized, how consultation documents are archived, and how this information is used to support regulatory decisions.

21. Predictive Human Testing for Skin Irritancy and Contact Sensitization in Risk Assessment for Topical Drug Products: Historical Use in the Division of Dermatology and Dental Products (DDDP), CDER and Potential Evolution with New Scientific Developments

Authors: Hon-Sum Ko, FDA/CDER; Jill Lindstrom, FDA/CDER; Nancy Xu, FDA/CDER

Plain Language Synopsis: The use of predictive human testing for skin irritancy and sensitization in support of topical drug product applications is presented. Additional approaches to risk assessment for these toxicities with long-term use studies and newer technologies, such as in vitro testing with human cells and tissues may be useful in future.

Abstract:

Background: In vivo testing on humans has been used for decades to complement dermatotoxicity information acquired in nonclinical studies for topical drug products. Predictive patch tests for skin irritation and contact sensitization are among such studies.

Purpose: To evaluate the historical use of predictive human patch tests for skin irritancy and contact sensitization in support of topical drug products and explore alternative approaches to the risk assessment of these skin reactions

Methodology: CDER's database was reviewed for topical drug product approvals in DDDP over a 15-year period to look for the human studies on irritancy and contact sensitization with the to-be-marketed formulations, prior to product approval. A public workshop was held to obtain input on future use of these human studies and other non-animal tests in evaluating dermal toxicity of topical drug products.

Results: Among 56 New Drug Applications for topical drug products approved between 2003 and 2017, there were 32 studies with the to-be-marketed formulation being studied in phase 3 clinical trials for irritancy potential, 28 for contact sensitization potential, and 27 designed with combined testing for both.

The great majority involved exposure for up to 3 weeks in cumulative irritancy testing (for irritation) and an additional 2 days in repeat-insult patch testing (for sensitization), using occluded patches containing the drug product applied to healthy adult volunteer skin. Documentation of the product as irritant or sensitizer was uncommon, and their data were rarely included in product labeling. Expert input at the public workshop included 1) dedicated data collection on irritancy and sensitization in clinical trials and post-marketing studies with long-term follow-up for real-world evidence, and 2) development of in vitro methodologies with human cells and tissues. Work is in progress with other federal agencies and OECD to explore and establish standards on these novel in vitro human tests.

Conclusion: DDDP's experience in the use of predictive human testing for skin irritancy and sensitization over a 15-year period in support of topical drug product applications has been presented. Additional approaches to risk assessment for these toxicities including newer technologies may be useful in future.

22. In Vitro Buccal Membrane Absorption (IVBMA) Model for Tobacco Constituent Testing: Membrane Integrity Assays.

Authors: Kraeling, Margaret, FDA/CFSAN; Justiniano, Rebecca, FDA/CFSAN; Vaught, Cory, FDA/CFSAN; Lauterstein, Dana, FDA/CTP; Crespo-Barreto, Juan, FDA/CTP; Sprando, Robert, FDA/CFSAN; Weil, Roxana, FDA/CTP; Yeager, Raymond, FDA/CTP; Yourick, Jeffrey, FDA/CFSAN

Plain Language Synopsis: Membrane integrity assays were used to evaluate an in vitro buccal membrane absorption (IVBMA) model.

Abstract:

Assays that provide clear information regarding the influence of harmful and potentially harmful tobacco constituents (HPHCs) on buccal permeability and absorption via the buccal pathway are sparse in published, peer-review literature. Therefore, an in vitro buccal membrane absorption (IVBMA) model was developed by

adapting in vitro dermal absorption system methods, using porcine buccal mucosa as a tissue surrogate for human buccal mucosa, due to similarities in morphology and permeability. Membrane integrity assays were compared for use in validating test tissues. Porcine buccal mucosa was isolated from connective tissue and cut with a dermatome to a thickness of 400-500 microns. Buccal mucosa was mounted in flow-through diffusion cells and saliva permeability coefficients (Kp) were measured using tritiated artificial saliva, pH 7.7. The average baseline Kp value for porcine buccal mucosa using tritiated saliva was 3.15×10^{-2} cm/h (n = 4). Buccal Kp values were compared to porcine and human dermal Kp values. The average baseline Kp value for porcine and human skin using tritiated saliva was 3.7×10^{-3} cm/h (n = 3) and 2.7×10^{-3} cm/h (n=3), respectively.

Saliva Kp values were compared to transepidermal water loss (TEWL) and transepithelial electrical resistance (TEER) measurements to determine baseline criteria for the IVBMA and dermal tissues. Buccal mucosa mounted on transwell plates exhibited average normalized TEER levels of 86.60 Ohms cm², compared to 78.35 Ohms cm² for porcine skin and 78.58 Ohms cm² for human skin. TEWL values for the buccal mucosa averaged 85.16 g/m²/h, compared to 40.9 and 6.14 g/m²/h for porcine and human skin, respectively. Saliva Kp values were about 10 times higher in buccal mucosa compared to skin (porcine and human). TEWL values were about two times higher in buccal mucosa compared to porcine skin and ten times higher than human skin. TEER values did not seem to correlate with changes in Kp values across tissues. Therefore, TEWL may be a better measure of membrane integrity than TEER for this model and tissues. (Supported in part by the Research Participation Program at FDA, administered by the Oak Ridge Institute for Science and Education.)

23. Role of Cell-Biomaterial Interactions on the Immunosuppressive Potency of Mesenchymal Stromal Cells

Authors: Kwee, Brian, FDA/CBER; Lam, Johnny, FDA/CBER; Sung, Kyung, FDA/CBER

Plain Language Synopsis: Tissue engineering biomaterials are often combined with mesenchymal stromal cells (MSCs) in clinical trials of tissue regeneration to enhance MSC survival and function. Commonly used biomaterials used in these clinical trials were evaluated for their ability to regulate the ability of MSCs to suppress inflammation.

Abstract:

Mesenchymal stromal cells (MSCs) are widely studied for their ability to suppress immune responses in vitro and in pre-clinical animal models. In particular, the immunosuppressive capacity of these cells have shown promising results in pre-clinical models of wound healing, where MSCs have been shown to dampen chronic inflammatory responses that contribute to tissue degeneration. In clinical models of wound healing, however, these cells have shown mixed results, likely due both to the donor-to-donor variability of MSCs and the poor survival of the implanted cells. Biomedical engineers and clinicians have recently sought to enhance the survival of these cells for these applications by seeding these cells onto biomaterial scaffolds. However, there are currently no studies that have evaluated the immunosuppressive capacity of the MSCs on these biomaterials. We evaluated the immunosuppressive capacity of two MSC cell lines on fibrin and collagen hydrogels. We showed that IFN- γ -stimulated MSCs seeded on 2D fibrin reduce CD4+ and CD8+ T-cell proliferation to a greater extent than MSCs seeded on 2D collagen. This result was shown to be similar on fibrin and collagen matrices of varying concentrations, which are known to exhibit varying bulk physical properties. These results may be due to the fact that cells engage different integrins on fibrin and collagen matrices. The findings of this work may help clinicians and biomedical engineers design biomaterials that will enhance the

immunosuppressive capacity of various manufactured MSCs for pre-clinical and clinical studies.

24. Development of a microphysiological system to evaluate the trophic effect of mesenchymal stromal cell preparations on endothelial cell vasculogenic network formation – A platform for assessing paracrine therapeutic potential of MSC preparations

Authors: Lam, Johnny, FDA/CBER; Kwee, Brian, FDA/CBER; Sung, Kyung, FDA/CBER

Plain Language Synopsis: We adapted a multi-channel microphysiological platform amenable to the co-culture of blood-vessel cells and various preparations of adult multipotent stromal cells in a format that enables evaluation of indirect cell-to-cell stimulation by stromal cells of blood vessel network formation in vitro.

Abstract:

Bone-marrow-derived multipotent stromal cells (MSCs) are increasingly recognized for their ability to indirectly stimulate tissue repair via the secretion of trophic and anti-inflammatory factors. Many developing regenerative medicine strategies take advantage of this trophic phenomenon rather than the actual grafting of implanted MSCs as the primary mechanism for stimulating tissue repair. The observation that MSCs naturally reside in the perivascular niche of the bone marrow suggests that there may be significant crosstalk between MSCs and vascular endothelial cells. Furthermore, recent evidence has demonstrated that MSCs secrete multiple pro-angiogenic paracrine factors, such as vascular endothelial growth factor, stromal cell-derived factor 1, and hepatocyte growth factor. We adapted a multi-channel, microphysiological platform to co-culture endothelial cells and various preparations of stromal cells in a format that enables evaluation of trophic stimulation by stromal cells of endothelial vasculogenic network formation. The system also enables whole-chip, high-resolution imaging and the computational

quantification of vasculogenesis. We hypothesize that the ability of stromal cells to stimulate vasculogenic network formation of endothelial cells via paracrine factors will be influenced by manufacturing parameters, such as cell passage.

25. Transitioning to Composite Bacterial Models in ICH M7 (Q)SAR Analyses

Authors: Landry, Curran, FDA/CDER; Kim, Marlene, FDA/CDER; Kruhlak, Naomi, FDA/CDER; Cross, Kevin, Leadscope Inc; Saiakhov, Roustem, MultiCASE Inc; Chakravarti, Suman, MultiCASE Inc; Stavitskaya, Lidiya, FDA/CDER

Plain Language Synopsis: By combining all available bacterial reverse mutation assay data into a composite model, the predictive power and coverage can be increased and the model is still usable under ICH M7 guidelines.

Abstract:

The International Council on Harmonisation (ICH) M7(R1) guideline describes the use of complementary (quantitative) structure-activity relationship ((Q)SAR) models to assess the mutagenic potential of drug substance impurities in new and generic drug products. Historically, two statistics-based models have been used to predict mutations at G-C (guanine-cytosine) and A-T (adenine-thymine) sites, to comprehensively assess bacterial mutagenesis. In this study, composite bacterial mutagenicity models covering multiple mutation types were developed using two commercial statistical software platforms. These new models contain more than double the number of chemicals (n=9,341 and n=13,514) than the corresponding non-composite models, with data harvested from the published literature, FDA approval packages for drugs approved between 2009 and 2017, Center for Food Safety and Applied Nutrition public databases, online repositories, and through data sharing efforts. Additionally, the use of composite bacterial mutagenicity models simplifies impurity analysis in an ICH M7 (Q)SAR workflow by reducing the number of model outputs requiring review. Cross-validation performance statistics for the new

models range from 84% to 91% in sensitivity and 81% to 89% in negative predictivity. Additionally, an external validation set of 398 drug impurities representing proprietary pharmaceutical chemical space showed performance statistics ranging from 67% to 79% in sensitivity, 91% to 94% in negative predictivity, and 94% to 96% in coverage. This data set was used in part to confirm that gaps in the applicability domain of the previous models were filled, while high predictive performance was maintained. This effort represents a major enhancement to (Q)SAR models that are recommended for use under ICH M7(R1), leading to improved patient safety through greater predictive accuracy, applicability, and efficiency when assessing the mutagenic potential of drug impurities.

26. Compilation and Estimation of Levels of Volatile N-nitrosamines in Processed Meat and Poultry Products

Authors: Lee, Hyoung, FDA/CFSAN/OFAS

Plain Language Synopsis: This paper provides an update on the levels of volatile N-nitrosamine in processed meat and poultry products compiled from recent studies. Historically, the presence of these substances in food and chronic dietary exposure at low levels is considered to be of potential health concern to humans.

Abstract:

Analyses of N-nitroso compounds in food has been a subject of intense international study over the past several decades. However, much of the literature was published prior to 1980 and may not reflect the technological advances in current analytical methodology and food preservation techniques. In processed meat and poultry products in particular, the use of both nitrate and nitrite has decreased considerably since these early reports were published, resulting in a lower potential for N-nitrosamine formation. Nitrate and nitrite in processed meat and poultry products are precursors of endogenously formed N-nitroso compounds, which are known carcinogens. Therefore, it is important to ensure that the N-nitrosamine

concentration present in processed meat and poultry products is below a level that poses a potential health concern to consumers.

A database of N-nitrosamine levels in processed (e.g., cured, canned, smoked) meat and poultry products is presented. The database is compiled from the literature based on 25 references published between 1985 and 2018 from 14 countries. Over 1800 samples of processed meat and poultry products, including bacon, ham, salami, sausage, and various other processed meat and poultry products were examined for the presence of eight volatile N-nitrosamines. A weighted mean of the published N-nitrosamine levels was calculated for each such meat product identified.

Results showed that N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR) are the most frequently identified volatile N-nitrosamines occurring in processed meat products. N-nitrosodiethylamine (NDEA), and N-nitrosodibutylamine (NDBA) are also frequently observed, but to a lesser extent. Relatively high levels of N-nitrosamines were found in pork (fried), poultry (spiced, grilled), and bacon (fried).

This database will be of interest to nutritionists, meat and poultry processors, and regulatory officials, as it provides current information on the N-nitrosamine concentration in processed meat and poultry products, which will allow the dietary intake of N-nitrosamines by various consumer subgroups to be evaluated. This is particularly useful for risk assessors and risk managers in exploring the relationship between processed meat and poultry consumption and adverse health outcomes.

27. Drug Safety Analysis Hackathon

Authors: Lee, Peter, FDA/OCP/OTS

Plain Language Synopsis: A safety analysis tool (eSafety) was implemented to execute a standard analysis plan against the integrated summary of safety data of 32 drug applications. The automated analysis results were comparable to the safety conclusions in

80% of the final review documents.

Abstract:

We completed a 12-hour hackathon to automate the integrated summary of safety (ISS) dose-response (ER) analyses of 32 new molecular entities (NME), essentially executing a standard ISS analysis plan for these NMEs using eSafety, an ISS analysis tool. The objective was to evaluate the reliability and efficiency of eSafety platform and the impact of the standardized analysis plan. The evaluation showed that the automated analysis plan executed on eSafety could adequately provide safety analysis results concluded in roughly 80% of the final review documents. This finding (80% coverage) is similar to the office experience learned from the 2018 Frontload reviews.

A total of 40 formal Briefing Meetings took place in Office of Clinical Pharmacology during the period from January 2018 to March 2019. From these NMEs, 32 with adequate ISS data archived in the electronic document room (EDR) were selected for the hackathon. A standard analysis plan was followed for every NME. The analyses were executed back to back using eSafety, adhering to the standard plan without much tweaking. The analysis outputs were compared to the safety ER called for in the briefing documents, based on various criteria, such as proper safety study pool, critical safety endpoints, adequate dosing levels/regimens, and encompassing analysis methods.

A high level comparison between the automated ISS analysis results and the briefing documents of the 32 NMEs is summarized. The finding is not surprising, since eSafety was designed to provide the complete analyses of ISS of all possible variations, and has been shown to cover most of sponsor's Clinical Safety Summary in the past submissions (>60 NMEs). The standard analysis plan helps to narrow the scope of the analyses, and specifically, to address the relevant safety issues for clinical pharmacology reviews.

28. Use of human monocytes to evaluate safety of vaccine adjuvants: production of pyrogenic and proinflammatory mediators by human monocytes activated with MDP adjuvant is amplified by T cell derived Glycoprotein Iba (GPIba)

Authors: Liu, Fengjie, FDA/CBER; Romantseva, Tatiana, FDA/CBER; Wu, Wells, FDA/CBER; Shen, Rong-Fong, FDA/CBER; Golding, Hana, FDA/CBER; Zaitseva, Marina, FDA/CBER

Plain Language Synopsis: Production of pyrogenic mediator Prostaglandin E2 (PGE2), and proinflammatory cytokines in human monocytes in response to MDP adjuvant requires a second signal, which is delivered by T-cell-derived Glycoprotein Ib alpha. Our study assists in the development of in vitro based cell assays to evaluate safety of novel adjuvants.

Abstract:

Background: Muramyl dipeptide (MDP) is a very potent adjuvant that activates the NOD2 receptor in antigen presenting cells (APC). Studies in animal models have shown that MDP included in vaccine formulations greatly facilitates immune response to vaccine antigen. However, human clinical trials of MDP-adjuvanted vaccines revealed adjuvant-induced pyrogenic and inflammatory responses.

Purpose: Human monocytes express NOD2 receptor. In vivo, monocytes are the major source of Prostaglandin E2 (PGE2), a proximal mediator of fever. In the study, MDP adjuvant was used to investigate the mechanism of PGE2 production in primary human monocytes.

Methodology: PGE2 FRET assay, ELISA, qRT-PCR, Western blotting, Mass Spectrometry.

Results: MDP-induced production of PGE2 in human peripheral blood mononuclear cells (PBMCs) but not in purified monocytes suggests the need for a second signal. Conditioned medium prepared from CD3 bead- purified human T cells (Tc CM) but not from negatively selected T cells, greatly enhanced production of PGE2 and IL-1b and

IL-6 proinflammatory cytokines in MDP treated monocytes. The co-stimulatory factor in Tc CM was identified as glycoprotein Ib alpha (GPIba) protein by mass spectrometry and was confirmed by Western blotting of conditioned medium. Antibody mediated blocking of GPIba or of the GPIba receptor Mac-1 integrin on monocytes, inhibited secretion of PGE2 and IL-1b/IL-6 in monocytes activated with MDP and Tc CM. Importantly, recombinant GPIba protein also increased PGE2 as well as IL-1b and IL-6 production in human monocytes activated with MDP.

Conclusion: Cross-talk between GPIba/Mac-1 and MDP/NOD2 signaling pathways is required for production of PGE2 and pro-inflammatory cytokines in monocytes activated with MDP.

Impact for public health: For safety evaluation, proinflammatory activity of novel adjuvants and adjuvant formulations could be evaluated using in vitro assays that mimic in vivo vaccine-induced cell activation and include both monocytes and T cells.

29. Damage to red blood cells from medical devices: Computational predictions of plasma hemoglobin levels in patients of different sizes

Authors: Malinauskas, Richard, FDA/CDRH; Saylor, David, FDA/CDRH; Buehler, Paul, FDA/CBER; Brown, Ronald, FDA/CDRH

Plain Language Synopsis: Damage to red blood cells as they flow through medical devices can release hemoglobin into the plasma, which can lead to adverse patient events. To assist in the safety evaluation of devices, we developed a computational model to assess time-varying levels of plasma hemoglobin in patients of different sizes.

Abstract:

Blood passage through medical devices can cause red blood cell injury (i.e. hemolysis) and increased plasma free hemoglobin (pfH) levels, which may lead to adverse effects, such as renal injury. As a new blood-contacting device is developed, its hemolytic potential is assessed in vitro under clinical-

use conditions by measuring the rate of pfH generated in a recirculating flow loop using animal blood. To help determine device safety and to spur innovation, it would be beneficial to directly relate measured in vitro hemolysis levels to actual clinical performance. To assist in this process, we developed a biokinetic model linking in vivo hemolysis rates to time-dependent pfH concentrations, while accounting for plasma haptoglobin (Hpt) that can bind and safely eliminate pfH. The model was parameterized using studies that characterized the evolution of pfH and Hpt following the introduction of pfH in humans, and evaluated by predicting hemolysis rates, pfH, and Hpt levels, in three patient groups during and after cardiopulmonary bypass surgery. The congruity of the computational model with the clinical data suggests that it can infer in vivo hemolysis rates and provide insight into pfH concentrations that may cause concern. The model was subsequently used to assess acceptance threshold hemolysis values proposed in the literature for circulatory assist blood pumps (i.e. normalized index of hemolysis (NIH) = 0.01 and 0.1 g/100L, and pfH > 20 mg/dL) and the impact of patient weight on pfH accumulation. Simulating a typical adult patient with a ventricular assist device exposed to the threshold hemolysis rates, total and unbound pfH reached steady-state levels within 24-48 hr. Using physiological scaling, the model predicted that the standard criterion of clinical hemolysis (pfH > 20 mg/dL) would be breached in an 80 kg adult at a value of NIH in vivo = 0.07 g/100L; more importantly, the pfH level would be nearly threefold greater in a 10 kg pediatric patient at this same NIH level. Pending further clinical verification, the model may assist in the development of new blood-contacting medical devices for different patient populations by helping to define acceptance limits for damage to red blood cells.

30. Application of dissolution profile comparison for gastric pH-dependent drug-drug interaction prediction

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Plain Language Synopsis: This study assessed how dissolution profile comparisons generated at pH 1.2, 4.5, and 6.8, could be incorporated to predict gastric pH-dependent drug-drug interactions for weak-base drugs with pH-dependent solubility, when co-administered with acid reducing agents.

Abstract:

Purpose: Absorption of orally administered weak base drugs (WBDs) with pH-dependent solubility may be reduced when co-administered with acid-reducing agents (ARAs), leading to clinically significant drug-drug interactions (DDIs). A preliminary framework for WBDs based on their solubility and clinical dose was proposed to evaluate in vivo DDI potentials in our previous publication (Zhang et al, *Clinical Pharmacology & Therapeutics*, 2014). This framework does not include DDI potential evaluation using in vitro dissolution data, which could be important for DDI predictions of drug products with formulation modifications (e.g. use of acidulants, surfactants, or solid dispersion to enhance drug solubility). The objective of this study is to assess how dissolution profile comparisons under different pH conditions may be incorporated to predict gastric pH-dependent DDIs.

Methods: We collected information for new molecular entities (NMEs) approved from 2003 to 2018 by FDA that included dedicated DDI studies with ARAs. 28 NMEs with available dissolution profiles generated in pH 1.2, 4.5, and 6.8 media were summarized. These media were selected to mimic gastric conditions under fasted (pH 1.2), fed states (pH 4.5), or when ARAs are co-administered

(pH 6.8). Similarity factor (f_2) was used to compare dissolution profiles for possible pH-dependent DDI prediction (i.e., pH 1.2 vs pH 6.8 and pH 4.5 vs pH 6.8 were compared to predict DDIs at fasting and fed condition). We defined $f_2 < 50$ indicating non-similar dissolution profiles as predicted positive DDI. ΔAUC or ΔC_{max} of NMEs $\geq 25\%$ when co-administered with ARAs are recorded as positive observed DDI. Prediction accuracy was calculated based on the correlation between predicted and observed DDIs.

Results: Gastric pH-dependent DDI was predicted for 28 NMEs (including 24 WBDs, 2 weak acids and 2 neutral drugs) with 92.8% prediction accuracy. 8 DDI studies (different NMEs) under fed conditions were predicted as true positives or true negatives.

Conclusions: With similar prediction accuracy as when using solubility and clinical dose, dissolution profile comparisons generated at pH 1.2, 4.5, and 6.8 may be used to predict gastric pH-dependent DDI potentials and help to evaluate the need for conducting clinical DDI studies, thus improving the efficiency of drug development.

31. Atezolizumab potentiates T-cell-mediated cytotoxicity and coordinates with FAK to suppress cell invasion and motility in PD-L1+ triple negative breast cancer cells

Authors: Mohan, Nishant, FDA/CDER; Hosain, Salman, FDA/CDER; Zhao, Jun, FDA/CDER; Shen, Yi, FDA/CDER; Jiang, Jiansong, FDA/CDRH; Endo, Yukinori, FDA/CDER; Wu, Wen Jin; FDA/CDER;

Plain Language Synopsis: Triple negative breast cancers (TNBC) are highly aggressive subtype of breast cancers. Atezolizumab is an FDA-approved immune checkpoint inhibitor-therapeutic antibody that demonstrated clinical success for treatment of human malignancies. This study is focused on investigating the therapeutic efficacy of atezolizumab in treating TNBC in cell culture models.

Abstract:

Immune check point inhibitors targeting

programmed cell death protein-1 (PD-1) and its ligand (PD-L1) have shown clinical success in treating human malignancies. Triple negative breast cancer (TNBC), which is primarily characterized by high heterogeneity and presence of tumor infiltrating lymphocytes, remains a therapeutic challenge due to unavailability of approved targeted therapy. Therapeutic potential of immune check point inhibitors for TNBC patients is under active clinical investigation. In this study, we show that FDA-approved anti-PD-L1 antibody, atezolizumab (ATE), potentiates T-cell-mediated cytotoxicity and apoptosis of TNBC cells that express higher levels of PD-L1, but does not have significant effect on TNBC cells expressing low levels of PD-L1. PD-L1 knockdown further confirmed that the ability of ATE to promote T-cell-induced cytotoxicity is PD-L1 expression dependent. Combination of ATE with PD-L1 upregulating agents, such as HDAC and proteasomal and lysosomal inhibitors, further augmented cytotoxic activity of T-cells toward TNBC cells. Based on analysis of breast cancer tissue samples deposited in The Cancer Genome Atlas (TCGA), we found a positive correlation between PD-L1 and focal adhesion kinase (FAK) mRNA expression in PD-L1-positive (PD-L1+) TNBC, suggesting a functional association of FAK and immune checkpoints. We further demonstrated that ATE dramatically downregulates the phosphorylation status of focal adhesion kinase (FAK), an important regulator of cell invasion and migration, and significantly enhances FAK inhibitor mediated inhibition of cell motility and invasion of PD-L1+ TNBC cells, independent of T cells. Taken together, our data suggest that ATE shows promising anti-tumor activity in PD-L1+ TNBC, via both T cell dependent and independent mechanisms.

32. Applying Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation for Quality Risk Assessment: Case Study – Setting Clinically Relevant in vitro dissolution “safe space”/specifications for a BCS Class II drug product.

Authors: Moldthan, Huong, FDA/CDER; Xu,

Da, FDA/CDER; Raines, Kimberly, FDA/CDER; Seo, Paul, FDA/CDER; Zhao, Ping, Bill and Gates Foundation; Ding, Hong, FDA/CDER; Lee, Sau, FDA/CDER; Wu, Fang, FDA/CDER

Plain Language Synopsis: PBPK modeling; risk assessment; Particle size; dissolution profile; critical quality attributes; absorption model.

Abstract:

Introduction: Drug X is indicated for the treatment of a highly contagious viral disease with a mortality rate of 30%. There is a concern for the availability of Drug X for immediate treatment, in the event of a bioterrorism attack. Drug X, a BCS class II drug substance with potent anti-viral activities (demonstrated via in vitro virology studies and an animal disease model), was developed under Animal Rule (FDA Animal Rule: 21 CFR 314.600), relying heavily on animal efficacy findings and having limited human clinical data. In the absence of significant human data, PBPK modeling and simulation can provide clinically meaningful input into drug product quality control. We established a human PBPK model based on monkey data and demonstrated the model could provide a quantitative basis for setting a clinically relevant drug substance particle size specification for the drug product (Poster, AAPS 2018). In this study we further expanded the model application by incorporating dissolution profiles into the PBPK model to predict/simulate clinically relevant “safe space”/specifications for dissolution.

Methods: Verify the previously established PBPK human model using in vitro dissolution profiles and corresponding clinical data of four different doses under fasted or fed conditions. Parameter sensitivity analysis was performed to identify the critical quality attributes/parameters. Also, virtual bioequivalence (BE) simulation was performed to set dissolution profile safe space/clinically relevant dissolution specifications, using the target clinical batch as reference.

Results: The human PBPK model for Drug

X predicts drug exposure (i.e., AUC_t, AUC_{inf} and C_{max}) from Phase 1 and 3 clinical studies with a prediction error within $\pm 20\%$. The sensitivity analysis indicated that drug substance solubility and dissolution are the critical parameters that could impact drug exposure. Virtual BE analysis demonstrated that a dissolution profile with a 33% slower release from the target profile could maintain bioequivalence to the bio-batch. Thus, the clinically relevant “safe space”/specifications for dissolution could be set accordingly.

Conclusion: PBPK M&S could serve as a useful tool for setting the clinically relevant dissolution “safe space”/specifications for drug product and reduce the risk of having unnecessary human drug exposure during drug development.

33. Toxicological Assessment of Free 3-MCPD and Select 3-MCPD Esters on Human Proximal Tubule Cells In Vitro

Authors: Mossoba, Miriam, FDA/CFSAN; Mapa, S. T. Mapa, FDA/CFSAN; de Araujo, Magali, FDA/CFSAN; Zhao, Yang, FDA/CFSAN; Flannery, Brenna, FDA/CFSAN; Flynn, Thomas, FDA/CFSAN; Sprando, Jessica, FDA/CFSAN, Wiesenfeld, Paddy, FDA/CFSAN; Sprando, Robert FDA/CFSAN

Plain Language Synopsis: Infant formula manufactured in the U.S. may contain food contaminants called chloropropanols. Examples include free 3-monochloro-1,2-propanediol and its fatty acid esters. Toxicology data from rats has raised questions about their safety in humans. Using an in vitro model of human kidney cells, the potential toxicity of several chloropropanols was evaluated.

Abstract:

Chloropropanol chemical compounds are contaminants that can be formed during industrial processing of foods, such as lipids used in commercially available infant and toddler formula in the United States. Many studies have demonstrated that the most common chloropropanol contaminant, 3-monochloropropane-1,2-diol (3-MCPD), as well as its lipid ester derivatives, may

have the capacity to induce kidney injury and other negative health effects in animal models. To investigate the safety of free 3-MCPD and nine of its common esters in commercial formula, we investigated whether the proximal tubule cells of the kidney would be vulnerable to their effects in a direct exposure model *in vitro*. Using the established human kidney proximal tubule cell line, HK-2, we performed 24-hour treatments using 3-MCPD and nine mono- or di-esters derived from palmitate, oleate, and linoleate. By directly exposing HK-2 cells at treatment doses ranging from 0 to 100 μM , we could evaluate their effects on cell viability, mitochondrial health, reactive oxygen species (ROS) production, and metabolic endpoints of oxidative phosphorylation and glycolysis rates. Overall, we found limited evidence of 3-MCPD ester toxicity towards HK-2 cells under the conditions tested. Additional research is underway to further investigate these effects.

34. Proteomic Analysis of Swine Serum Following LPS Stimulation

Authors: Olumee-Shabon, Zohra, FDA/CVM; Chattopadhyaya, Chaitali, FDA/CVM; Myers, Michael J., FDA/CVM

Plain Language Synopsis: We profiled changes in swine serum samples at different time points following stimulation by lipopolysaccharide (LPS), using gel-based assays and nanoflow liquid chromatography mass spectrometry. We identified several previously unreported proteins in swine. Our results serve a basis for future studies seeking to qualify swine proteomic biomarkers of inflammation.

Abstract:

Serum samples were collected from a group of 4 pigs prior to (baseline), and 24 and 48 h following lipopolysaccharide (LPS) stimulation to reveal proteomic changes during inflammation. Two other pigs served as untreated controls. We identified 165 proteins using SDS-PAGE, of which 47 proteins were also detected by 2-dimensional gel electrophoresis, prior to

nanoflow liquid chromatography coupled tandem mass spectrometry. LPS stimulation modulated more than half (72%) of all characterized proteins, many of which are known to be involved with innate and adaptive immunity. Pig serum samples obtained 24 h after LPS initiation of inflammation showed protein modulations of serum albumin, serotransferrin, light and heavy immunoglobulin chains (IGs), and major acute phase proteins, including haptoglobin (HPT), serum amyloid A2 (SAA2), C-reactive protein (CRP), β -2-glycoprotein 1 (B-2GP1), α -2-HS-glycoprotein (A2HS), α -1-antitrypsin (A1AT), and α -1-acid glycoprotein (A1AG). SAA2 was distinguished from the other SAA isoforms by its unique peptide sequence. The proteomic analysis of swine serum following LPS stimulation indicated the importance of SAA2, which appears to be unique and may be regarded as a potential diagnostic biomarker of inflammation in swine.

35. Effects of Electrical Stimulation on hiPSC-CMs Responses to Classic Ion Channel Blockers

Authors: Pang, LI, FDA/NCTR/DSB; Wei, Feng, FDA/NCTR/DSB; Strauss, David G., FDA/CDER/OTS/OCP/DARS; and Stockbridge, Norman, FDA/CDER/OND/DCRP

Plain Language Synopsis: Human-heart-muscle-like cells can now be prepared from individual donors and hold great potential for personalized drug safety prediction. We tested whether controlling beating rates with electrical stimulation can help evaluate responses of these cells to drugs that alter heart rhythm.

Abstract:

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely used to assess cardiac safety profiles of new drug candidates and hold great potential for personalized cardiac safety prediction, particularly for drug-induced proarrhythmia. However, hiPSC-CMs fire spontaneously and the variable beating rates among wells and replicate assay plates can be a confounding factor that

interferes with data interpretation. Moreover, batch variations of hiPSC-CMs hamper their application for precision medicine. Controlling beating rates with electrical stimulation (E-pacing) may reduce batch and assay variations and enable evaluation of rate/frequency-dependent drug effects. However, E-pacing on hiPSC-CMs has not yet been validated with high-throughput assays. In this study, we compared responses of hiPSC-CMs to the challenges of classic cardiac ion channel blockers under spontaneous beating and E-pacing conditions, using the Axion Maestro Microelectrode Array, a popular high-throughput assay platform. The rate-dependent drug effects and assay variations were examined. Effects of E-pacing on different batches of hiPSC-CMs responses to IKr channel blockades were also evaluated. We found that, compared to spontaneous beating hiPSC-CMs, E-pacing 1) reduced well-to-well, plate-to-plate, and batch-to-batch variabilities; 2) impaired repolarization prolongations induced by IKr channel blockers and revealed reverse use dependency; 3) rate-dependently attenuated the sensitivity to an IKs channel blocker; 4) eliminated effects of INa blockades on depolarization spike amplitudes; 5) exhibited limited effects on hiPSC-CMs responses to ICa-L and If channel blockers. In conclusion, E-pacing reduced batch and assay variations. Analyzing the responses of hiPSC-CMs in both spontaneous beating and E-pacing conditions may help better assess the effects of test compounds on cardiac electrophysiology.

36. FDA Probabilistic Quantitative Assessment of Coronary Heart Disease Risk of Industrially-Produced Trans Fatty Acids from Partially Hydrogenated Oils in Human Foods.

Authors: Park, Jin-Young K., FDA/CFSAN/OFAS; Koehler, Kathleen M., Consultant; Whiteside, Catherine, FDA/CFSAN/OFAS (Retired); Anderson, Ellen, FDA/CFSAN/OFAS; Honigfort, Mical, FDA/CFSAN/OFAS; and Zajac, Andrew, FDA/CFSAN/OFAS.

Plain Language Synopsis: Coronary heart disease is a major cause of death and illness

in the U.S. This probabilistic risk assessment demonstrated substantial coronary heart disease burden in U.S. adults associated with consuming as little as 0.05% of daily dietary energy from industrially-produced trans fatty acids in partially hydrogenated oils.

Abstract:

Partially hydrogenated oils (PHOs) are the main dietary source of industrially-produced trans fatty acids (IP-TFA or trans fats). In 2015, FDA determined there is no consensus among qualified experts that PHOs are generally recognized as safe substances for human consumption. In 2018, FDA denied a food additive petition requesting approval for the use of PHOs in certain human food applications. To support these decisions, FDA conducted comprehensive scientific reviews of epidemiological and toxicological data on the association of trans fats and adverse health outcomes. This study applied probabilistic quantitative risk assessments to human study findings to estimate coronary heart disease (CHD) risk and public health burden, using four methods. Method 1 used changes in serum low-density lipoprotein cholesterol (LDL-C); method 2 used changes in both LDL-C and serum high-density lipoprotein cholesterol (HDL-C); method 3 used a combination of emerging CHD risk factor biomarkers; method 4 used prospective observational studies of CHD events associated with trans fat intake. The probabilistic analyses considered the variability in risk parameters and uncertainty associated with IP-TFA exposure and estimated mean changes in CHD risk with lower and upper 95% uncertainty intervals (95% UI). In one scenario, the analysis found that consuming 0.05% of total daily dietary energy (% of energy) of IP-TFA instead of cis-monounsaturated fatty acids can cause mean increases in annual CHD cases of 814 (95% UI 510-1,151, method 1), 1,502 (990-2,043, method 2), 3,145 (1,580-4,778, method 3) or 6,877 (3,611-10,694, method 4) in U.S. adults. These estimates include annual CHD deaths of 290 (182-410, method 1), 535 (353-728, method 2), 1,121 (563-1,703, method 3),

and 2,450 (1,287-3,811, method 4). Sensitivity analyses using alternate risk parameters or an alternate exposure scenario resulted in minor changes in results. The FDA risk assessment of IP-TFA intake of 0.05% of energy from PHO uses in human foods demonstrates a substantial increase in CHD risk for U.S. adults. This study also shows the successful application of a probabilistic risk assessment tool and biomarker studies to public health burden estimates for the U.S. population.

37. Comprehensive Evaluation of MesoScale Discovery PR2 1800 Rapid Assay for Abrin and Ricin Detection

Authors: Pillai, Christine, FDA/CFSAN; Manickam, Gowri, FDA/CFSAN; Thirunavukkarasu, Nagarajan, FDA/CFSAN; Pillai, Segaran, FDA/OC; Hodge, David, DHS/S&T; Anderson, Kevin DHS/S&T; Hammack, Thomas, FDA/CFSAN; Brown, Eric, FDA/CFSAN; Sharma, Shashi, FDA/CFSAN

Plain Language Synopsis: Intentional release of, or contamination with, abrin or ricin toxin can pose a severe threat to public health, resulting in high mortality and morbidity. Rapid and accurate detection of these toxins are critical for timely public health actions and decisions for reducing public health and economic impacts in a biothreat situation.

Abstract:

Rapid and robust assays for accurate detection of abrin and/or ricin toxins in clinical, food, or environmental samples is vital for enhancing our national preparedness and response capabilities against bioterrorism. Deliberate aerosolization of, or contamination with, abrin or ricin in food or other sources poses a severe threat to public health and national security. Rapid and reliable detection of these toxins in suspicious food matrices or environmental materials is an unmet necessity for rapid response to a public health emergency. In this study, we evaluated an electrochemiluminescence (ECL)- based assay platform built on an MSD PR2 instrument for detecting ricin and abrin toxins, using the Public Health Actionable

Assay (PHAA) criteria as established by an interagency working group. The PHAA criteria comprised seven phases, which included an inclusivity panel (comprised of diverse cultivars) to understand assay sensitivity and an exclusivity panel (comprised of near neighbor plant materials, lectins, white powders, environmental background materials, and BioWatch filter extracts) to determine the assay specificity. To determine the robustness of this technology, we performed repeatability, limit of detection, and dynamic range of quantitation studies for the presence of ricin or abrin in a sample. The positive aspects of this technology are the innovative approach for multiplexing (ability to detect multiple analytes in a single well) with desired sensitivity and specificity. The negative aspects of this platform were the limited dynamic range for quantitation and severe hook effect that can potentially lead to a false negative result in the presence of high toxin concentration. We also observed signal bleed-over at high concentrations of toxins, resulting in false positive results for the other target analyte that is in close proximity to the true positive analyte.

38. Development of an in vitro Bio-assay using Human Intestinal and Immune Cell-lines to Measure the Immuno-pathogenicity of Food Allergens

Authors: Cho, Chung Y, FDA/CFSAN; MacMahon, Shaun, FDA/CFSAN; Garber, Eric A.E., FDA/CFSAN

Plain Language Synopsis: Food allergies affect >15 million Americans. FDA currently uses ELISA methods to analyze allergens in foods. But these methods do not measure the allergic reactions caused by allergens in complex food matrices. An in-vitro bioassay using human intestinal cell lines can fill in these knowledge gaps by measuring the biological effects.

Abstract:

Food allergies are a rapidly growing public health problem that affect >15 million Americans. The Food Allergen Labeling and Consumer Protection Act (FALCPA) requires

that allergens contained in eight major food products be declared in plain English. As strict avoidance is the only option for allergic consumers, accurate methods are needed to ensure correct labeling. The currently used Immunochemical methods (e.g. ELISA) detect IgG antigenic epitopes, and not allergenic elements. Hence, immunochemical methods may not detect antigenic epitopes that are transformed during food processing, while the immuno-pathogenicity could continue to persist. To address this gap in analytics, an in-vitro bioassay that uses human intestinal epithelial and immune cell lines to measure the biological effects caused by food allergens was developed. This novel biological activity-based assay compares the allergen-induced immuno-biological responses in Caco-2, HT-29 & T84 intestinal epithelial cells individually, as well as each co-cultured together with THP-1 cells. The goals of this project are to compare the cell signaling and immune modulation induced by different food allergens in a dose-dependent manner, and to further develop an in vitro bioassay using these cell lines.

39. Utilization of a human in vitro airway epithelial tissue model to evaluate the respiratory toxicity of ortho-phthalaldehyde

Authors: Wang, Yiyang, FDA/NCTR; Wu, Qiangen, FDA/NCTR; Muskhelishvili, Levan, FDA/NCTR; Davis, Kelly, FDA/NCTR; Tripathi, Priya, FDA/NCTR; Xiong, Rui, FDA/NCTR; Rua, Diego, FDA/CDRH; Mayhall, Elaine, FDA/CDRH; Weeks, Jon, FDA/CDRH; Bryant, Matthew, FDA/NCTR; Cao,

Plain Language Synopsis: Robust non-animal assays for pulmonary toxicology are needed to make competent product development and risk assessments for new materials requiring safety testing. This study reports a proof-of-concept for use of an in vitro testing approach to evaluate medical device substances that come in contact with respiratory tissues.

Abstract:

Ortho-phthalaldehyde (OPA) is a liquid chemical sterilant used for high-level disinfection of heat-sensitive medical devices,

such as endoscopes and microsurgical instruments. Although viewed as a safer alternative to glutaraldehyde, a known skin and respiratory sensitizer, an OPA exposure limit to control associated health risks from inhalation exposure has not been established. To simulate human OPA inhalation exposure, we exposed a human air-liquid-interface (ALI) in vitro airway epithelial tissue model to OPA aerosols at the air interface with a single dose of OPA aerosols prepared from solutions containing 0 (control), 0.2, 0.5 and 1.0 mg/ml OPA; tissue responses were evaluated at 20 min, 24 h, and up to 5 days later. Treatments with 0.2 and 0.5 mg/ml OPA were non-cytotoxic and significantly increased trans-epithelial electrical resistance (TEER). One mg/ml OPA, however, was cytotoxic, based on the lactate dehydrogenase (LDH) release assay and TEER measurement. Except for the apical LDH release, membrane leakage from the basolateral side and TEER returned to baseline following a 5-day recovery. Single OPA exposures decreased the levels of reduced (GSH) and oxidized glutathione (GSSG), lowered GSH/GSSG ratios, and upregulated the expression of heme oxygenase-1, indicating induction of oxidative stress. Dose-dependent functional disruptions, such as inhibition of cilia beating frequency (CBF), decreases in MUC5AC secretion, and aberrant cytokine secretions, were observed 24 h following single exposures. Measurements made up to 5 days following the exposures indicated that the alterations in CBF, MUC5AC secretion, and cytokine secretion only partially recovered from the treatments, while MUC5B secretion and induction of aldo-keto reductase 1B10 persistently increased over the same time frame. No morphological changes were observed following a single exposure. Taken together, these findings suggest that OPA exposure induces respiratory irritation. Our findings will help support setting a limit to inhalation exposure for this chemical as a high-level disinfectant.

40. Growth Potential of *Listeria monocytogenes* in Apple Flesh and Juice.

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Center for Food Safety and Applied Nutrition, U.S. FDA, MOD-1, Laurel, MD20708.

Plain Language Synopsis: Understanding the survival and growth of *Listeria monocytogenes* in different apple varieties and possible virulence mechanism implications.

Abstract:

Background: Recent outbreaks of listeriosis and associated recalls raised questions regarding survival and growth potential of *Listeria monocytogenes* (Lm) in acidic fruits like apples.

Purpose: Understanding Lm survival/growth in apple varieties and possible virulence mechanism implications.

Methods: The growth potential of Lm (a rifampicin resistant serotype 1/2b strain) on artificially inoculated cut chunks and in juices from seven apple varieties [Braeburn, Fuji, Gala, Golden Delicious(GD), Granny Smith(GS), McIntosh and Red Delicious(RD)] was tested following incubation at 10°C. The bacterial populations were measured using plate counts. The transcriptional profiles of inoculated Lm in different apple juices were compared by a *Listeria*-specific DNA microarray. Biochemical parameters (pH, acidity, sugar, polyphenol, and antioxidant) of apple varieties were also measured by standard methods.

Results: Lm grew ~1-2 log in the chunks of Braeburn, Fuji, Gala, and GD, but not in GS, McIntosh and RD. Inoculated Lm did not grow in any apple juices; rather, the Lm population decreased ~4-5 log in 48 hours in GS, McIntosh and RD. The pH of different apples varied between 3.2-4.5. Lm, inoculated in apple juices adjusted to pH 6.8, decreased ~1-2 log in 48 hours in GS and McIntosh, but maintained their inoculated numbers in other apple juices. The polyphenol, antioxidant, and acidity of GS were significantly higher

than those of other apple varieties ($p < 0.05$). Microarray analyses disclosed 163 Lm genes (cut-off value 2) were either up- or down-regulated in various apple juices. Of interest, the *gadB* and *inlA* genes were over-expressed in Lm when exposed to GS and McIntosh.

Significance: Lm-exposed GS or McIntosh apples may acquire an advantage over other apple varieties through increased expression of *gadB* (acid tolerance-related) and *inlA* (invasion-related) genes. Understanding the growth/survival of Lm in apple and its role in virulence gene expression may have important implications in risk assessment.

41. Mouse models of drug induced squamous cell carcinomas

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Plain Language Synopsis: Cutaneous squamous cell carcinoma is a commonly reported adverse event in patients who are immunosuppressed or treated with RAF inhibitors, and has been associated with expression of oncogenic Ras. Murine models have been optimized to investigate the role of oncogenic human H-, K-, and N-Ras isoforms in tumorigenesis.

Abstract:

Skin toxicities that arise as adverse drug events (AEs) include cutaneous squamous cell carcinomas (cSCC), which are observed following treatment with RAF inhibitors for metastatic melanoma or in immunosuppressed patients following solid organ transplantation. Notably, among the patients treated with BRAF inhibitors, up to 31% of those exhibiting AEs related to cutaneous toxicity present with cSCC (Reviewed in Wu JH et al. 2017 PMID:28129674).

A subset of cSCC that arise following RAF inhibitors have been reported to harbor

mutations in H-Ras. Mutations in all Ras isoforms, H-, K-, and N-Ras have been detected in solid malignancies in humans, where K-Ras is the predominantly mutated isoform followed by N-Ras. We have used a well-established orthotopic grafting model of primary murine epidermal keratinocytes transduced with a H-Ras-encoding retrovirus to evaluate cooperative effects between oncogenic H-Ras and other genetic alterations that may convert tumors to malignancy. Presently, we are using lentiviral vectors to express oncogenic human H-, K-, and N-Ras isoforms to evaluate their phenotypic contribution alone and in combination with other genetic alterations observed in human cancers. These studies are designed to clarify whether other Ras isoforms yield the same phenotype as H-Ras.

As expected, mice grafted with H-Ras-transduced primary keratinocytes formed benign tumors on the grafted site. Introduction of a K-Ras mutation also yielded a benign phenotype (confirmed histologically), whereas no tumors were seen with N-Ras through 4 weeks (time of tumor harvest). Ongoing In vitro experiments are investigating the Ras-isoform-dependent regulation of downstream signaling pathways in epidermal keratinocytes, and monitoring cell morphology and proliferation rates to identify potential differences between the isoforms.

This mouse model allows both in vivo and in vitro assessments and can be used to evaluate the mechanisms of drug-induced tumor cell outgrowth driven by other oncogenic mutations. In addition, the grafting model has been adapted to immune competent mice to evaluate the role of the immune system in mediating AEs.

42. Analysis of Endpoints used in CDER's Novel Drug Approval

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Knowledge Management Team, Office of Translational Science-Immediate Office,

CDER

Plain Language Synopsis: Novel therapeutics approved by FDA between 2010 and 2018 were analyzed to find out the type and characteristics of the endpoints used for the approval of NMEs by different review divisions in CDER. All data were collected using the DASH database and CDER Novel Drug Approval Dashboard.

Abstract:

Background: Between 2010 and 2018, FDA approved 330 novel therapeutics. Using data from pivotal clinical trials of the newly approved novel therapeutics over the years, the types of endpoints were analyzed, and the endpoints were classified into 3 different groups.

Purpose: The purpose of the analysis is to find regulatory considerations regarding various endpoints, specifically, surrogate endpoints used by different divisions for the approval process.

Methodology: New drug and biologic product marketing applications (NDAs and BLAs) approved by FDA's Center for Drug Evaluation and Research (CDER) between 2010 and 2018 were analyzed to find out the type and characteristics of the endpoints used for the approval of new molecular entities (NMEs). All data were collected using the Data Analysis Search Host (DASH) database and CDER Novel Drug Approval Dashboard.

Results: The analysis shows the numbers and type of endpoints used in pivotal efficacy trials as the basis for drug approval in different review divisions. The other attributes such as accelerated approvals, breakthrough or orphan designations by each division were analyzed in parallel.

43. Color Hazard and RiSk Calculator (CHRIS)

Authors: Saylor, David, FDA/CDRH; Chandrasekar, Vaishnavi, FDA/CDRH; Simon, David, FDA/CDRH; Turner, Paul, FDA/CDRH; Markley, Laura FDA/CDRH; Hood, Alan, FDA/CDRH

Plain Language Synopsis: The Color Hazard and RiSk calculator (CHRIS) is a web-based

tool that conducts screening level risk assessments to aid in the biocompatibility evaluation of medical devices that contain color additives. CHRIS provides instantaneous feedback on whether color additives in a device require additional testing to demonstrate acceptable biological risk.

Abstract:

The Color Hazard and RISk calculator (CHRIS) is a web-based tool that conducts rapid screening level risk assessments to aid in the biocompatibility evaluation of medical devices containing color additives (CAs). Based on a small set of user inputs, CHRIS uses a conservative mass transport model to estimate exposure and compares the result to a tolerable exposure (TE) value to compute a margin of safety (MOS). Based on the MOS value and toxicological profile, CHRIS provides the user with a list of biocompatibility endpoints that are determined not to be of concern.

The mass transport model used to estimate exposure is based on assumptions that are valid or conservative for most CA-containing devices. The result is a simple model equation in which the only unknown is the diffusion coefficient (D) of the CA in the polymer. While diffusion data on CAs are not widely available, we aggregated data from the literature on solute diffusion in commodity plastics, food packaging, and drug delivery systems. Based on these data, we established upper bounds (worst-case) for D as a function of solute molecular weight in 20 polymer matrices commonly used in device applications. TE values were derived for eight commonly used CAs, based on the lowest point-of-departure (POD) reported in the literature and application of uncertainty factors (UFs) assuming the most invasive category of medical device contact. When toxicological data was inadequate to identify a POD, a threshold of toxicological concern (TTC) value was used as the TE.

Using this approach, CHRIS can address systems comprised of any combination of the eight CAs and 20 polymer matrices. However,

CHRIS also has the ability to evaluate arbitrary CA-containing systems, albeit under more conservative assumptions. A pilot study suggests that the screening level approach employed by CHRIS can drastically reduce the need for biocompatibility or chemical characterization testing to evaluate the risk associated with CA used in medical devices. Finally, we emphasize that the framework underlying CHRIS is not strictly limited to CA and can be readily extended to address biocompatibility concerns associated with other additives or impurities in medical device polymers.

44. Two-stage sample size re-estimation for bioequivalence crossover studies

Authors: Meiyu Shen, CDER/FDA, Estelle Russek-Cohen, CDER/FDA, Eric V Slud, Statistical program/University of Maryland, CSRM/ U.S. Census Bureau

Plain Language Synopsis: We propose a pilot study for updating final sample sizes based on the pilot study's sample variance. We analyze the combined data from both Stage 1 and Stage 2 with a new test statistic using the pooled variance of two stages. The exact critical value for the new test statistic considers the distribution of the pooled variance and the desired experimentwise type I error rate (usually .05)

Abstract:

Bioequivalence (BE) studies are an essential part of the evaluation of generic drugs. The most common in vivo BE study design is the two-period, two-treatment, open-label, crossover design. Under normality of log(AUC) or log(C_{max}), the sample size for BE studies is a function of the assumed mean difference, the assumed variance, equivalence margins, type I error rate, and type II error rate. Since the studies for BE are often smaller than the studies typically seen in superiority studies, sample size re-estimation to achieve specified experimentwise type I error rate can still be a complicated issue when the new sample size is updated only based on the observed sample variance, and no interim analysis

is conducted. In our proposed, unblinded sample size re-estimation strategy, the new total sample size is calculated from the exact power function for the one stage using the estimated variance from the observed data of an internal pilot study (Stage 1) as the true variance. If the number of additional subjects is less than 4, we stop here and analyze the Stage 1 data with the standard t-quantile. Otherwise, we collect data from additional subjects (Stage 2). We then analyze the combined data from both Stage 1 and Stage 2 with a new test statistic using the pooled variance of two stages. The exact critical value for the new test statistic considers the distribution of the pooled variance and the desired experimentwise type I error rate (usually .05).

45. **In vitro studies to predict hepatotoxicity of kinase inhibitors lead to the identification of key mechanisms for regorafenib toxicity**

Authors: Shi, Qiang, FDA/NCTR; Ren, Lijun, FDA/NCTR; Greenhaw, James, FDA/NCTR; Yang, Xi, FDA/CDER; Mattes, William, FDA/NCTR

Plain Language Synopsis: Over 60 percent of FDA-approved protein kinase inhibitors (KI) can damage the liver. Three cell-based models were evaluated and found useful in identifying KIs with harmful liver effects. Regorafenib (brand name Stivarga) was the most toxic KI in liver and heart cells, but its metabolites were remarkably less harmful.

Abstract:

Of the 47 FDA-approved small molecule kinase inhibitors (KIs), 31 (66%) have hepatotoxicity warnings in drug labels, with six of them being box warnings (BWs). The mechanisms and predictions of KI hepatotoxicity are poorly understood. This study aimed to assess if in vitro models are useful in predicting KI hepatotoxicity. Freshly isolated rat liver mitochondria and primary cultured rat and human hepatocytes were treated with 44 KIs at concentrations normalized to human peak blood levels (C_{max}) achieved at therapeutic doses. Mitochondrial functions, mode of cell

death, and adenosine triphosphate (ATP) levels were determined. Human hepatocyte cytotoxicity at 100-fold C_{max} showed an accuracy of 0.66 in predicting KI clinical hepatotoxicity, with sensitivity and specificity of 77% and 47%, respectively. In contrast, the accuracy for rat hepatocytes and isolated mitochondria were 0.60 and 0.56, respectively. Regorafenib, a KI with BWs for hepatotoxicity, was identified to be the most cytotoxic and mitotoxic drug among 44 KIs tested, because it caused significant toxicity at clinically relevant concentrations (1 to 2.5-fold C_{max}). Regorafenib is metabolized by liver CYP3A4 and UGT1A9, producing two pharmacologically active metabolites, M2 and M5, whose C_{max} (~8 μM) are the same as regorafenib. In human hepatocytes, regorafenib caused nearly complete ATP depletion at 2.5-fold C_{max}, M2 injured only 60% of the cells, and M5 was essentially non-toxic, even at 5-fold C_{max}, the highest testable concentration. A similar trend was observed in rat primary hepatocytes, human induced pluripotent stem cell derived hepatocytes (iPSC-Hepatocytes), and cardiomyocytes (iPSC-CMs). In human hepatocytes pretreated with the CYP3A4 inhibitor ketoconazole, the cytotoxicity of regorafenib was significantly increased. The remarkable difference in cytotoxicity is at least partially due to the direct mitochondrial liability, as regorafenib caused complete uncoupling of oxidative phosphorylation at C_{max}, while M2 and M5 showed similar effects only at much higher concentrations (>16 μM). Our findings suggest that 1) isolated liver mitochondria and primary hepatocytes can aid the identification of hepatotoxic KIs, and 2) regorafenib hepatotoxicity is associated with its mitochondrial liability, and its pharmacologically active metabolites M2/M5 are remarkably less toxic and might be further explored as safer new drugs.

46. **A three-dimensional cell culture model of placental trophoblasts to study the mechanisms of Trypanosoma cruzi vertical transmission**

Authors: Silberstein, Erica, FDA/CBER/

OBRR; Kim, Kwang Sik, The Johns Hopkins University; Debrabant, Alain, FDA/CBER/OBRR

Plain Language Synopsis: The placenta constitutes the primary barrier preventing transmission of pathogens from mother-to-baby. A three-dimensional (3D) culture of human placental trophoblasts provides an excellent system that resembles the architecture and placental function. We use 3D-based JEG-3 trophoblast cultures to study *Trypanosoma cruzi* congenital infection.

Abstract:

Background: *Trypanosoma cruzi* (*T. cruzi*) is the etiological agent of Chagas disease (CD), which affects 6-8 million people in Latin America. Congenital transmission is an important route of parasite infection and occurs in 5-10% of births from infected mothers. CDC estimates that 40,000 *T. cruzi*-infected women of childbearing age live in the U.S. Pregnant women are more susceptible to infection due to the transient depression in cell-mediated immunity during pregnancy. According to the PAHO, vertical transmission accounts for more than 25% of new cases of CD worldwide. Twenty to 30% of *T. cruzi*-infected babies are born with low birth weight and are at risk for developing chronic CD. The placenta achieves essential functions during pregnancy and creates a physical barrier composed of trophoblast cells, which play a crucial role in fetal protection from pathogens.

Purpose: Develop a three-dimensional (3D) culture system of human trophoblasts that mimics placental function to study *T. cruzi* congenital infection.

Methodology: Using the human placental derived JEG-3 cell line co-cultured with human brain microvascular endothelial cells (HBMEC), we established a 3D-based culture system in a rotating bioreactor. In this system, Cytodex beads are first coated with HBMEC cells for 3 days. JEG-3 cells are subsequently added and co-cultured for 21 days.

Results: We found that 3D-cultured JEG-3 (3D JEG-3) cells released significantly higher

levels of the pregnancy-associated hormone beta-human chorionic gonadotrophin in the culture media, compared to cells cultured in standard flasks (2D JEG-3). Additionally, we detected the presence of multinucleated 3D JEG-3 cells, a hallmark of placental trophoblasts fusion. These results indicate that JEG-3 cells grown in the bioreactor express markers of primary human trophoblasts. Experiments are underway to compare parasite infectivity and growth in 3D and 2D JEG-3 cells using GFP-expressing *T. cruzi* parasites. We expect parasite infection/invasion and growth will be hampered in 3D JEG-3 cells as observed for other pathogens.

Conclusion: Our culture system represents a new platform to investigate the mechanisms by which *T. cruzi* bypasses the placental barrier and may be used to evaluate the safety and effectiveness of novel anti-parasitic drugs for their potential use to treat women during pregnancy.

47. Predicting Exposure and Toxicity to Nickel Released from Cardiovascular Devices using Multi-scale Modeling

Authors: Simon, David, FDA/CDRH; Saylor, David, FDA/CDRH; Chandrasekar, Vaishnavi, FDA/CDRH; Skoog, Shelby, FDA/CDRH; Turner, Paul, FDA/CDRH; Sussman, Eric, FDA/CDRH

Plain Language Synopsis: Many cardiovascular devices contain nickel, which can lead to adverse health effects if released in sufficient quantities. However, patient exposure to nickel from these implants is not well established. We developed models to help assess risk by predicting the release and accumulation of nickel in patients with these devices.

Abstract:

Many cardiovascular device alloys contain nickel, which if released in sufficient quantities, could cause adverse health effects. In vivo nickel release from implanted devices and subsequent biodistribution of nickel ions to local tissues and systemic circulation are not well understood. To determine the extent of in vivo nickel release

by these devices, we developed a multi-scale (material, tissue, and system) biokinetic model. The model correlates nickel release from an implanted cardiovascular device to concentrations in serum and urine, which can be readily monitored, as well as in peri-implant tissue. The model was parameterized for a specific cardiovascular implant type, nitinol septal occluders, using in vitro nickel release test results, studies of ex vivo uptake into heart tissue, and in vivo and clinical measurements from the literature. Our results show that the model accurately predicts nickel concentrations in peri-implant tissue in an animal model and in serum and urine of septal occluder patients. The congruity of the model with these data suggests it may provide useful insights for establishing nickel exposure limits and interpretation of bio-monitoring data. Additionally, we use the model to predict local and systemic nickel exposures due to passive release from nitinol devices produced using a wide range of manufacturing processes. These predictions are used to develop relationships between release rate and exposure. The predictions indicate peri-implant tissue and serum levels of nickel will remain below 5 $\mu\text{g/g}$ and 10 $\mu\text{g/L}$, respectively, in patients who receive implanted nitinol cardiovascular devices, provided the rate of nickel release per device surface area does not exceed 0.074 $\mu\text{g}/(\text{cm}^2 \text{ d})$, and total amount released is less than 32 $\mu\text{g/d}$. Finally, the predicted local nickel exposure estimates can be used with in vitro cytotoxicity to characterize in vivo nickel-mediated cytotoxicity.

48. Long-term neural and vascular structural and functional changes in response to implanted cortical microelectrodes

Authors: Solarana, Krystyna, FDA/CDRH; Ye, Meijun, FDA/CDRH; Gao, Yurong, Univ. of Rochester Medical Center; Rafi, Harmain, FDA/CDRH; Hammer, Daniel X, FDA/CDRH

Plain Language Synopsis: Brain-implanted electrodes are used to restore motor function and sensory feedback to patients with paralysis; but these devices experience

high rates of signal degradation and device failure over time. We used imaging techniques and electrical signal recordings to assess tissue damage and functional changes in chronically-implanted mice.

Abstract:

Cortically-implanted microelectrode arrays provide a direct interface with neuronal populations in the brain and are used to restore movement capabilities and provide sensory feedback to patients with paralysis or amputation. Initially after implantation, penetrating electrodes produce higher neural signal fidelity and spatial resolution than surface electrodes; but within the first year, they experience high rates of signal degradation that limit effectiveness and lead to device failure. Here, we used a multimodal approach combining in vivo electrophysiology and optical imaging to assess vascular and cellular changes over a year in animals with implanted electrodes, and to examine the contribution of the brain tissue response to electrode performance. At acute timescales, we observed structural damage in some animals from the mechanical trauma of electrode insertion, evidenced by severed dendrites in the electrode path and local hypofluorescence. We also saw superficial vessel growth and remodeling associated with the window surgery within the first few weeks in both implanted and control animals, while the deeper capillary network remained stable over the first six months. After longer periods of implantation, there was evidence of degeneration of the severed dendrites superficial to the electrode path and localized cell loss, along with deep vascular changes near the electrode tip. Single cell spike recording amplitude decreased after the first month, likely a result of gliosis. The local field potential remained relatively constant up to 6 months, particularly in the high-gamma band, indicating long-term electrode viability and neuronal functioning at further distances from the electrode. This multifaceted approach provides a more comprehensive picture of the ongoing biological response at the brain-electrode interface than can be achieved with postmortem histology alone.

49. The Application of an Updated Cramer et al. Decision Tree and Threshold of Toxicological Concern to Safety Assessment

Authors: Stice, Szabina, FDA/CFSAN; Adams, Timothy, FDA/CFSAN; Kolanos, Renata, FDA/CFSAN

Plain Language Synopsis: The Cramer et al. Decision Tree (CDT) and the concept of the Threshold of Toxicological Concern (TTC) are used together to screen chemicals at low levels of exposure for prioritization of follow-up testing. This poster presents updates to the CDT and the TTC.

Abstract:

The Cramer et al. (1978) Decision Tree (CDT) prioritizes chemicals according to their toxic potential using a sequence of 33 mainly structure-based yes or no questions, to which the answer either refers the user to another question or assigns the substance to one of three structural classes of toxic potential. Each question was designed based on information on chemical structure, reactivity, metabolism, toxicokinetics, biochemistry, and animal toxicology existing over 40 years ago.

The concept of the Threshold of Toxicological Concern (TTC) refers to the establishment of a level of exposure for chemicals below which there would be no appreciable risk to human health. The TTC approach historically relied upon grouping chemicals using the CDT. Each of the three CDT classes has a corresponding TTC level. Once a chemical is grouped, its consumption is assumed to be safe as long as its intake is below its class TTC.

Given the scientific knowledge accumulated since 1978, the CDT has been overdue for an update. More than 18,000 scientific studies were reviewed to determine the effects of species, strain, sex, and target organ on toxicity and metabolic fate. These studies provided no-observed-effect-levels for 1,900+ substances that were then organized according to their structure, metabolic fate, and toxic potential. Analysis of this database resulted in the development of more refined questions, leading to an increased number of classes of toxic concern. The toxic potential

of each of the six classes was quantified by determining the class TTCs.

These classes of toxic potential can be used for food ingredients, food contact substances, herbal dietary supplements, pesticide residues, and pharmaceutical excipients for safety assessment, prioritization for future evaluation, and post-market surveillance to assess safety based on current intake levels. By screening and prioritizing chemically-defined substances, the Expanded Decision Tree (EDT) will help focus resources on the safety assessments of substances with greater potential for public health risk.

50. Assessment of a non-clinical animal model to predict therapeutic protein and biological drug product immunogenicity

Authors: Sung, Jungeun, FDA/CDER; San Emeterio, Cheryl, FDA/CDER; Hosain, Salman, FDA/CDER; Knapton, Alan, FDA/CDER; and Howard, Kristina, FDA/CDER

Plain Language Synopsis: Biological drugs can elicit unwanted immune responses affecting patient safety and drug efficacy. As the biological drug market continually expands, few models exist to effectively improve our understanding of these adverse human responses. This work shows how immune humanized mice may improve understanding of immunogenicity and adverse events in patients.

Abstract:

Background: Immunogenicity from biological drug products is associated with stimulation of the immune system and production of anti-drug antibodies that can either reduce the efficacy of the administered drug, or in some cases, target an endogenous protein counterpart. Due to the specificity of these responses to the human immune system, traditional animal models have not been effective in identifying this risk. We have evaluated humanized mice to determine if they can replicate known human immune responses to biological drug products

Purpose: The goal of this study was to compare strains of immune -humanized mice

to determine which, if any, could produce robust antibody responses and other common signals of immune system activation.

Methods: This study used BLT-immune humanized mice that develop a fully engrafted human immune system, including human thymus. Four unique strains of immune compromised mice (NOG, NOG/hGM-CSF/hIL-3, NOG/hIL-6, and NCG) had immune humanization surgery, with five mice of each strain tested through surgery. A total of four surgeries were completed, each using a unique human donor tissue. Following surgery and hematopoietic stem cell transplant, BLT-humanized mice were evaluated every three – four weeks for development of all major immune cell types and all stages of B cells, via flow cytometry. Antibody levels were evaluated using ELISA and flow cytometry.

Results: NOG/hGM-CSF/hIL-3 and NOG/hIL-6 strains supported improved B- and T-cell production, compared to the NOG and NCG strains that lack human cytokine genes. Further, in contrast to previously published data, NOG/hGM-CSF/hIL-3 and NOG/hIL-6 strains appear capable of supporting typical B-cell subsets and antibody production.

Conclusion: Selection of the mouse strain used to create human immune system mice can affect the ability of the mouse to model human immune responses. We identified two mouse strains that provide a more complete representation of B- and T-cell function. These mouse strains both have human cytokines associated with adaptive immune responses inserted into the mouse's genome. Future studies will use these models to investigate adaptive immune response to biosimilars as compared to originator biologics, generic protein drugs, and combinatorial drug therapies.

51. Nickel release from nitinol under physiological conditions

Authors: Shi, Huiyu, FDA/CDRH; Turner, Paul FDA/CDRH; Shin, Hainsworth, FDA/CDRH; Saylor, David, FDA/CDRH; Takmakov, Pasha, FDA/CDRH; Godar, Dianne, FDA/CDRH;

Sivan, Shiril, FDA/CDRH; Di Prima, Matthew, FDA/CDRH; Weaver, Jason D., FDA/CDRH; Sussman, Eric M., FDA/C

Plain Language Synopsis: Nitinol is a nickel and titanium alloy that is widely used in medical devices. Due to the risk of adverse health effects from nickel, we are studying how the chemistry of the body can influence the amount of nickel that could be released from nitinol into a patient's body.

Abstract:

Nitinol, an alloy of nickel (Ni) and titanium (Ti), is widely used in medical devices (e.g., stents) because of its unique shape memory and pseudo-elastic properties. However, if the nitinol (NiTi) surface is not passivated or finished adequately, Ni can be released into the patient's body, posing health risks of toxicity and carcinogenicity. NiTi devices located throughout the body can be exposed to extremes in pH and reactive oxygen species produced during inflammatory reactions. Using three different physiological conditions, pH, hydrogen peroxide (H₂O₂), and hypochlorite/hypochlorous acid (ClO⁻), we measured Ni release from three different finishes varying in TiO₂ thickness: black oxide (BO, ~1900 Å), electropolish (EP, ~240 Å), and chemical etch (CE, ~140 Å). NiTi wires were immersed in various solutions for 7 to 14 days, then collected and replaced daily and analyzed for Ni using inductively coupled plasma mass spectrometry (ICP-MS). In vivo, the pH range is ~1.5-8.5; CE wires released four times and BO wires released 25 times the amount of Ni at pH 2.2 as at pH 7.2. Compared to controls, BO and CE wires released two times the amount of Ni at 0.1 mM and 1 mM H₂O₂, respectively. In vivo, ClO⁻ can reach ~50 mM; at 0.19 mM, BO wires released two times the amount of Ni at 77.3 mM CE, and EP wires released six times the amount of Ni, compared to controls. Thus, different NiTi surface finishes combined with various physiological environments in the body can affect the rate of Ni release from NiTi.

52. Antibody dependent enhancement of Influenza disease promoted by increased virus fusion kinetics: Implications for evaluation of Safety and Efficacy of next generation influenza vaccines and therapeutic antibodies

Authors: Juanjie Tang, Katie Winarski, Laura Klenow, Jeehyun Lee, Elizabeth M. Coyle, Kazuyo Takeda, Hana Golding and Surender Khurana

Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Silver Spring, MD USA

Plain Language Synopsis: Influenza monoclonal antibodies 69/1 and 78/2 targeting different sites in the hemagglutinin of H3N2 virus induced increased lung pathology and altered Th2/Th1 cytokines following virus challenge. This mouse model of enhanced respiratory disease combined with In vitro assays will help to evaluate universal influenza vaccines and therapeutic antibodies.

Abstract:

Background: Multiple, next-generation (universal) influenza vaccines and broadly neutralizing antibodies (bNAbs) in clinical development inhibit virus replication at post-entry stage or use Fc-dependent mechanisms. However, these antibodies have the potential to mediate enhancement of viral infection and/or disease. There is no in vivo animal model available to assess the potential antibody-dependent enhancement (ADE) effect of influenza A virus vaccines or antibodies.

Purpose: In this study, we used two previously characterized murine monoclonal antibodies (MAbs) 69/1 and 78/2, to investigate the mechanisms of ADE effects of influenza antibodies, and to develop in vivo and in vitro models/assays to facilitate the safety evaluation of antibody and vaccine products against influenza.

Methodology: Mouse study, histopathology, immunohistochemistry, confocal microscopy, negative stain electron microscopy.

Results: Mice treated with MAbs 69/1 or 78/1,

followed by H3N2 virus challenge, showed increased lung pathology and changes in lung Th2/Th1 cytokine and chemokine levels, demonstrating an enhanced respiratory disease (ERD) model. MAb 69/1 showed no protection, with no increase in viral loads in lung homogenates. While MAb 78/2 was protective at the high dose, it enhanced viral loads at the low dose, indicating MAb 78/2 exhibited a dose-dependent effect on viral load. Both 69/1 and 78/2 induced increased sensitivity to trypsin cleavage at higher than normal pH ranges. In a novel assay, pHrodo-labeled virus particles were tracked within endosomes during infection. Shorter endosomal residence time was found with MAb 69/1- and 78/2-treated virus, suggesting that these antibodies promoted faster viral fusion kinetics. Structurally, MAb 69/1 and 78/2 Fab bound the HA globular head or base of the head, respectively, and induced destabilization of the HA stem domain.

Conclusion: Our studies assessed the enhanced respiratory disease in MAbs 69/1- or 78/2-treated mice following influenza virus infection and disclosed that these antibodies promote fast viral fusion kinetics, which may contribute to the ADE effects.

Impact: Our findings highlight the need to carefully evaluate next generation influenza vaccines and antibody-based therapeutics for both protection and enhanced disease following influenza virus challenge. We have established in vitro assays and an in vivo model to assess antibody-mediated enhanced infection or disease, which could improve safety evaluation of universal influenza vaccines and antibodies that do not block influenza virus-receptor interaction.

53. Model Informed Drug Development Approaches for Cell and Gene Therapies

Authors: Artur Belov, FDA/CBER; Kimberly Schultz, FDA/CBER; Richard Forshee1, FDA/CBER; Million A Tegenge

Plain Language Synopsis: Leveraging insight from modeling and simulation techniques can be crucial for informing decisions. Here we share some of CBER's perspectives on the

opportunities and challenges for using MIDD approaches for product review, as well as recent applications and exploratory analyses for cell and gene therapies.

Abstract:

As part of FDA's PDUFA VI commitments, the Center for Biologics Evaluation and Research (CBER) and Center for Drug Evaluation and Research (CDER) are conducting a Model-Informed Drug Development (MIDD) pilot program. Sponsor(s) who apply and are selected will be granted meetings that aim to facilitate the application of MIDD approaches throughout the product development and regulatory processes. Due to their multifaceted and complex mechanisms of action, cell and gene therapies seem particularly suitable for MIDD approaches, as safety and efficacy assessments incorporate numerous input and data sources. Integration of these various data items into a systematic process and generation of quantitative data to inform regulatory decisions remain challenging. Leveraging insight from quantitative modeling and simulation techniques, such as through the MIDD program, can be crucial for informing decisions for both the sponsor(s) and the regulatory review teams. Here we share some of CBER's perspectives on the opportunities and challenges for using MIDD approaches for product review, as well as recent applications and exploratory analyses for cell and gene therapies.

54. Product Formulation can Impact the Detection of Innate Immune Response Modulating Impurities in Therapeutic Proteins and Peptides

Authors: Thacker, Seth, FDA/CDER/OPB/DBRRIII/laboratory of Immunology/COE-Infectious Disease and Inflammation; Baker, Logan, FDA/CDER/OPB/DBRRIII/laboratory of Immunology/COE-Infectious Disease and Inflammation; Rachuri, Swaksha FDA/CDER/OPB/DBRRIII/laboratory of

Plain Language Synopsis: We demonstrated in an assay to detect levels of impurities, that drug formulation can have a strong impact on

assay sensitivity. The study disclosed several limitations in both the cell-based assay and the LAL assay in detecting impurities in fully formulated products.

Abstract:

Therapeutic proteins can contain trace levels of innate, immune response modulating impurities (IIRMI) consisting of variants of the product, host cell components, or manufacturing impurities. By modulating the innate immune response, these impurities have the potential to affect the therapeutics' immunogenicity. Increased product immunogenicity can result in toxicities or loss of efficacy. Thus, it is important to accurately assess the presence of IIRMI in therapeutic products before they are administered to patients or healthy volunteers. Our group developed a cell-based platform to detect minute quantities of multiple IIRMI in therapeutic proteins. We explored the impact of eight frequently used formulation excipients on the system's capacity to sense IIRMI, and their ability to affect the LAL assay. We demonstrated that several excipients can modify the sensitivity of cells to respond to a variety of purified TLR ligands, while other commonly used excipients do not appear to affect the assay. We observed a similar modulation in the LAL assay in response to LPS. These results show that product formulation needs to be considered when using cell-based assays to assess innate immune modulating impurities in therapeutic proteins, and that testing of drug product may be the most appropriate step to test for impurities.

55. Exploring Factors Affecting Nifedipine Biphase Dissolution Profile Using an inForm Platform

Authors: Tian, Li, FDA/CDER/OPQ/OTR/DPA; Rodriguez, Jason, FDA/CDER/OPQ/OTR/DPA; Tang, Fuxing, FDA/CDER/OGD/ORS/; Lin, Ho-Pi, FDA/CDER/OPQ/ONDP/DB; Jiang, Wenlei, FDA/CDER/OGD/ORS; Gao, Zongming, FDA/CDER/OPQ/OTR/DPA

Plain Language Synopsis: In our biphasic dissolution study, an organic phase is

added on top of the water phase to mimic permeation of a drug with poor water solubility. Factors affecting the biphasic results and their bio-relevance are studied on an inForm platform.

Abstract:

Background: Biphasic dissolution testing is an important in vitro tool for predicting bioavailability and establishing in vitro-in vivo correlation (IVIVC) for biopharmaceutics classification system (BCS) II and IV drugs. Nifedipine is a BCS II drug that may lead to severe side effects if its plasma concentrations are too high. An established IVIVC with biphasic dissolution testing may help predict the plasma concentrations in vivo. Literature on biphasic studies did not attempt to correlate the in vitro factors with physiological parameters. There is a need to develop more clinically relevant biphasic methods to investigate the relationship between in vitro factors and physiological parameters.

Purpose: To evaluate the effect of in vitro factors on biphasic dissolution profile of nifedipine

Methodology: Nifedipine drug substance was used to eliminate the interference from excipients in this exploratory study. Effects on solubility and the octanol-water partition coefficient were tested by shake flask method at 37°C. Effects on the octanol-water interface tension were tested on a Sigma 701 tensiometer. Biphasic dissolution studies were performed on Pion inForm, an automated platform that can perform biphasic dissolution experiments in low media volume.

Results: Water phase of 500 – 750 mL, octanol of 250 – 400 mL, and agitation rate of 50 – 100 rpm were used in a USP 1 or 2 vessel. Faster agitation gave a better mixing at the interphase and more nifedipine was transferred into octanol. The amount of nifedipine transferred at 100 rpm was used to obtain the correlation with in vivo results. In our study, inForm is used with a total of 90 mL solution and maintains a stable interface

area, resulting in a stable in vitro interface area/water phase volume (A/V) ratio. How in vitro factors such as surfactant, octanol saturation, and stir rate affect the biphasic results with various A/V ratios was studied and related to the literature on intestinal A/V ratio.

Conclusion: The inForm platform has the potential to reduce the time and solvent volume. These results will be used to facilitate establishing a methodology for biphasic and IVIVC studies and to compare to the future biphasic study coupling USP 2 and USP 4 apparatuses.

56. Investigating the Mode of Action for DNA Damage by Flavors in ENDS Using In Vitro and In Silico Screening

Authors: Luis G. Valerio, Jr, FDA/CTP; Pei-Hsuan (Chris) Hung, FDA/CTP; Mamata De, FDA/CTP; Matthew Savidge, FDA/CTP

Plain Language Synopsis: Use of predictive tools is part of FDA's Predictive Road Map. This study used high throughput predictive techniques with an in vitro assay for genetic toxicity and in silico structure-based models to better understand the genotoxic liability of 150 flavors relevant to tobacco products.

Abstract:

Flavors have been widely used as ingredients in tobacco products, including e-liquids of electronic nicotine delivery systems (ENDS). Understanding their risk for inducing chromosome damage is important for public health. A dataset of flavors was screened with an in vitro assay to assess the mode of action for inducing DNA damage in human cells (TK6). A multiplexed flow cytometric assay (+/- metabolic S9 fraction) with biomarkers for DNA damage response pathways (p53 nuclear translocation, γ H2AX to detect double-strand breaks, phospho-histone H3 to detect mitotic cells) was used. Exposures that resulted in > 80% cytotoxicity were not included in the genotoxic potential and MoA analyses. MoA calls of aneugenicity, clastogenicity, or nongenotoxicity were based on analyzing biomarker responses using an ensemble of previously validated

machine learning models (logistic regression, artificial neural network, random forest). Positive and negative controls for each MoA were included. The flavors were screened in silico with a series of chemical structure-based computational models for chromosomal damage and carcinogenicity. The in vitro results showed that 26 (17%) of the tested flavors were determined positive for genotoxicity: 8 flavors had aneugenic MoAs, 16 flavors had clastogenic MoAs, and 2 cinnamyl derivatives exhibited mixed aneugenic and clastogenic MoAs. Unsaturated ketones required metabolic activation. The remaining data set did not exhibit aneugenic or clastogenic MoAs, but the in silico analysis predicted a higher rate of flavors as genotoxic compared to in vitro results. Long-chain aldehydes, alkyl diols, and terpenoids that were positive in vitro were structural classes difficult to predict via in silico. We conclude that a number of flavors documented to be found in ENDS aerosol or e-liquids were genotoxic in the in vitro assay, and when in silico prediction is used in tandem it generally strengthened the evidence for positive results. We conclude that the in vitro and in silico assessments this study used as rapid tools to screen flavor ingredients for clastogenic and aneugenic MoAs warrant further investigation. Understanding the underlying MoA of genotoxicants will facilitate an efficient translation of hazard to human risk and help to prioritize testing to confirm genotoxicity.

57. Open Format for Ion Channel Datasets from Cardiac Electrophysiology In Vitro Assays under CiPA

Authors: Ghasemian, Maryam, FDA/CDER; Dybdahl, Niels, Sophion Bioscience A/S; Stiehler, Johannes, Nanion Technologies GmbH; Mirams, Gary, University of Nottingham; Pierson, Jennifer, HESI; Li, Zhihua, FDA/CDER; Wu, Min, FDA/CDRH; Wu, Wendy, CDER/FDA; Vicente, Jose

Plain Language Synopsis: The potential of new drugs to cause abnormal heart rates can be assessed in laboratory experiments quantifying drug effects on multiple ionic

currents active during the heart-beat. We developed an open format to facilitate data sharing and analysis of ion channel datasets used for proarrhythmic potential assessment of drugs.

Abstract:

Background: To predict torsade risk of new drugs, the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative proposes to use a mechanistic model of the human ventricular myocyte that integrates multiple cardiac ionic currents. The effects of the drug on the ion channel currents are characterized using standardized in vitro assays. Currently, data from ion channel assays are stored using proprietary formats and exported to formats specified ad-hoc for sharing with others (e.g., spreadsheets with specific layout).

Purpose: To develop an open data format for results of ion channel experiments under CiPA. The format will foster sharing results in research environments and will facilitate the review of these datasets when included in regulatory submissions.

Methodology: Gathering of format requirements included 1) review and assessment of data formats used by commercially available manual patch clamp and high throughput systems (HTS) and 2) prototyping of files using actual data from in vitro multi-ion channel patch clamp experiments. Because of the similarities with ECG datasets, the annotated ECG XML HL7 format was used as reference.

Results: A format specification describing one master XML file with links and metadata to access raw waveform recordings stored in external files was developed. The XML file contains information about the device and software used, cell line, vehicle-control solutions, drug concentrations, assay protocols, experimental conditions, and links to the waveform files, together with their corresponding measurements (i.e., cursors locations and measured current or voltage values). An R package with an example dataset and functions to read the format and

produce tabulated analysis datasets was also developed.

Conclusion: Coordinated by the Health and Environmental Sciences Institute (HESI), a group of researchers from industry, academia, and FDA, developed an open data format for CiPA's ion channel in vitro experiments. This format could serve as an interface into CiPA's in silico model to predict torsade risk. In addition, an R package to facilitate other ion channel data analysis was developed. Together with standardized voltage protocols, the new open data format will streamline the submission and review process of nonclinical, in vitro data as part of the proarrhythmic assessment of new drugs.

58. Predictive classification of traumatic brain injury using EEG data-driven machine learning

Authors: Vivaldi, Nicolas, FDA/CDRH; Solarana, Krystyna, FDA/CDRH; Ye, Meijun, FDA/CDRH

Plain Language Synopsis: The current gold standard for diagnosing traumatic brain injury is a clinical assessment and CT scan. However, this is costly and requires patients to visit properly equipped facilities. Here, we evaluate the potential to use machine learning on EEG data as an inexpensive, portable, diagnostic alternative.

Abstract:

Traumatic brain injury (TBI), such as from a head impact or jolt, disrupts brain function. Short term effects include impaired thinking, sensation, and emotions, while long term complications include risk of epilepsy and chronic traumatic encephalopathy. Diagnosis relies on clinical neurological assessment and CT scan. The variability of injuries and risk of misdiagnosis make it important to identify objective, quantitative, and computationally efficient biomarkers. With the development of portable EEG devices and advancement of analytic tools, EEG-based discriminative algorithms have been under development for the detection of TBI. The aim here was to evaluate the classification performance of EEG algorithms developed

through supervised machine learning (ML) on a randomly collected dataset, from a diverse patient population. Raw EEG data was obtained from the Temple University Hospital EEG Corpus repository (100 Healthy, 100 TBI, 15 minutes per subject), assuming random distribution of artifacts across all recordings. Including demographics, the features calculated were: phase-amplitude coupling, average power spectral density, spectral entropy, and inter-channel coherence, resulting in 1048 features. The support vector model was trained using quadratic kernels on the full feature set, and separately a subset of features chosen by forward sequential feature selection (FSFS). K-fold cross validation ($k = 10$) was performed to measure model performance. FSFS identified gender, average spectral density, and coherence at specific frequency and channel combinations, as the subset minimizing misclassification. The model performed with 81% sensitivity and 54% specificity. The receiver operating characteristic area under the curve was 0.7469, which is higher than reported CT algorithms alone, but lower than other ML-based on CT images. Quantitative data from EEG recordings may provide objective biomarkers for TBI; however, more work is needed to optimize model performance. Kernel type, feature selection method, and learning algorithm can all be modified, leading to countless interpretations of raw data. ML with EEG versus CT is more cost effective and deployable if predictive accuracy can be improved to match or exceed image-based learning. Deep learning may provide an unbiased alternative that could maximize the potential of EEG based biomarkers of TBI.

59. Performance evaluation of photoacoustic breast imaging systems using flow phantoms with adjustable blood oxygenation

Authors: Vogt, William, FDA/CDRH; Zhou Xuewen, FDA/CDRH; Andriani, Rudy FDA/CDRH; Wear, Keith, FDA/CDRH; Garra, Brian, FDA/CDRH; Joshua Pfefer, FDA/CDRH

Plain Language Synopsis: Photoacoustic imaging (PAI) is an emerging technology for breast cancer imaging and oximetry.

To facilitate PAI device development and regulatory evaluation, we developed a performance test method using a breast-mimicking phantom containing flowing blood with tunable oxygen saturation. Results quantified how tissue properties and device design affect oximetry measurement accuracy.

Abstract:

Photoacoustic imaging (PAI) is a rapidly emerging hybrid modality that combines optical excitation with acoustic detection to achieve deep tissue imaging of light-absorbing molecules, such as hemoglobin in blood. The ability of multispectral PAI devices to detect vasculature and map tissue blood oxygen saturation (SO₂) enables many potential clinical applications, especially breast cancer detection, tumor margining, and treatment monitoring. PAI systems are commercially available for animal imaging and exploratory clinical use, and FDA has received several photoacoustics-based device submissions. Due to the complexity and high cost of clinical studies, bench testing in tissue-simulating phantoms offers a reproducible, low-cost approach for characterizing imaging device performance under well-controlled conditions. To facilitate PAI device development and streamline regulatory evaluation, we developed a performance test method based on a phantom made from our previously developed PVC plastisol material, which has breast-mimicking optical and acoustic properties. The phantom contained fluid channels at depths of 5-35 mm connected to a flow circuit filled with bovine whole blood. An inline membrane oxygenator was used to adjust and stabilize blood oxygen concentration. Imaging was performed using FDA's custom PAI system, which allows for using different ultrasound transducers and laser wavelengths to simulate different device designs. Phantoms were imaged at blood SO₂ levels from 30-99%, with SO₂ measurements compared against gold-standard CO-oximetry. Results indicated that SO₂ measurements are highly susceptible to

spectral artifacts produced by spatially- and spectrally-dependent light distribution in turbid tissues. The severity of these artifacts depends strongly on tissue optical properties, which can vary greatly within breast tissue and between patients. Image processing algorithms used to correct for these effects, which often must make assumptions regarding tissue properties and morphology, are thus a critical aspect of PAI device design and performance evaluation. Our test method may be used to support device optimization, generate high-quality performance data to support regulatory decision-making, and guide standardization of PAI devices.

60. An ex vivo model of medical device-mediated bacterial skin translocation

Authors: Wang, Hao, FDA/CDRH/OSEL/DBCMS; Wang, Yi, FDA/CDRH/OSEL/DBCMS; Shin, Hainsworth, FDA/CDRH/OSEL/DBCMS; Phillips, Kenneth S., FDA/CDRH/OSEL/DBCMS

Plain Language Synopsis: In this project, we made a new porcine skin model to study the translocation of bacterial cells across the different antimicrobial catheter-skin interfaces. This ex vivo model could be helpful for the high-throughput evaluation of antimicrobial catheter devices in a more realistic condition than in vitro.

Abstract:

The skin serves as a barrier and part of the immune system protecting us from harmful bacteria. When the skin is compromised through medical device injection, insertion, or continuous indwelling presence, infection risk increases. Historically, in vitro biofilm models were developed to create highly reproducible bacterial lawns and test antimicrobial or antibiofilm strategies against large bioburdens. In recent years our understanding of medical device contamination during skin penetration has increased, along with the knowledge of biofilm persister cells and viable but non-culturable bacteria. These two features of pathogenesis point to the importance of medical device contamination from the

skin microbiome as a first step in many infections, and imply that we may need functionally different models that better replicate skin-device interactions in the pathogenesis process. In this work we developed a reproducible and sensitive skin translocation model to study how antimicrobial and anti-biofilm interventions may delay or prevent bacterial translocation across a device-skin interface. We compare the delay time to translocation for untreated interfaces and antimicrobial interfaces. Antimicrobial-matched contact killing coating and drug eluting coatings are compared. The differences seen using this model are then evaluated for the potential to explain current gaps between in vitro testing and clinical outcomes for antimicrobial devices. This ex vivo model takes biofilm testing one step closer to in vivo conditions, provides a high-throughput approach to studying anti-biofilm technologies, and has significant potential for application in regulatory science.

61. Dynamics of Microglial Morphology as an Acute Biomarker of Ketamine Neurotoxicity

Authors: Wong, Elissa, FDA/CDRH; Solarana, Krystyna, FDA/CDRH; Liu, Zhoulin, FDA/CDRH; Hammer, Daniel, FDA/CDRH; Ye, Meijun, FDA/CDRH

Plain Language Synopsis: Microglia are the brain's resident immune cells and they respond to neurotoxic events by changing morphology and function. This study aims to be a 'proof of concept' that real-time changes in microglial morphology could serve as a sensitive biomarker of neurotoxicity, thus improving tools for identifying potentially neurotoxic medical products.

Abstract:

Neurotoxicity studies provided to FDA by industry for regulatory review of medical products currently focus on animal behavior and cellular abnormalities visible within fixed tissue. While essential, data obtained with these methods may lack sensitivity. A live imaging-based assay could potentially increase sensitivity by tracking the dynamics of neural responses to neurotoxicants at

the cellular level. Our research focused on determining whether the dynamics of the resident immune cell, microglia, in the mouse cortex could serve as a biomarker of neurotoxicity. In vivo two-photon microscopy (TPM) was used to track the morphological changes in microglia acutely following local ketamine (600 μ M in saline) application through a thinned skull in both adolescent and adult Cx3Cr1 GFP+/- mice in which microglia express green fluorescent protein. Single, high-dose or chronic low-dose ketamine exposure has been shown to induce changes to neuronal architecture and trigger neuronal cell death. In our study, the 3D morphology of microglia in each animal was repeatedly imaged every 5 minutes from 1 hour pre- to 2 hours post-continuous ketamine application. Control animals received only saline application for 3 hours. In FIJI, the Simple Neurite Tracer and Sholl Analysis plugins were used to quantify the ramification and coverage of microglial processes over time. Results show that within 1 hour of ketamine exposure, microglial branching complexity slightly decreases in adult but not adolescent mice. These subtle morphological changes are indicative of immune reactivity and were not observed in control animals. Further studies are planned to determine the progression of microglial responses through 24 hours. Dynamic changes in microglial morphology shortly following neurotoxicant exposure may provide a promising avenue for rapidly detecting early neurotoxic events.

62. Integration of Omics-based Analysis with Pathophysiological Endpoints to Evaluate the Mode of Action for Cigarette Smoke Toxicity in an in vitro Human Airway Tissue Model

Authors: Xiong, Rui, FDA/NCTR/DGMT; Wu, Qiangen, FDA/NCTR/DBT; Tripathi, Priya, FDA/NCTR/DGMT; Muskhelishvili, Levan, Toxicologic Pathology Associates; Davis, Kelly, Toxicologic Pathology Associates; Bryant, Matthew, FDA/NCTR/DBT; Rosenfeldt, Hans, FDA/CTP; Healy,

Plain Language Synopsis: A novel integrative approach is proposed for assessing cigarette

smoke toxicity in a human air-liquid-interface airway tissue model. Findings from this study suggest that such an approach may be a useful tool for identifying dysregulated genes and adverse cellular events caused by tobacco products.

Abstract:

Cigarette smoke (CS) contains over 7000 chemicals, including toxicants that have been implicated in the pathogenesis of disease caused by smoking, such as chronic obstructive pulmonary disease and lung cancer. We previously reported on a test battery that measures disease-related endpoints, such as changes in tissue integrity, cilia beating frequency (CBF), mucin production, oxidative stress, cytokine release, and tissue remodeling, in a human in vitro air-liquid-interface (ALI) airway tissue model. In this study, we integrated transcriptome analysis into the existing test platform and assessed the mode of action for CS toxicity in the ALI cultures of fully differentiated bronchial epithelial cells. Cells were repeatedly exposed to up to 32% of whole smoke generated by smoking five 3R4F reference cigarettes under the International Organization for Standardization (ISO) machine smoking regimen. The integrative analysis of molecular changes, tissue responses, and transcriptomics regulation revealed CS-dependent effects on protein handling systems (proteasome, autophagy, and ER stress response), glutathione metabolism, xenobiotic metabolism (cytochrome P450 enzymes), and cilia biogenesis and function. Pathway networks, such as those involving glutathione and xenobiotic metabolism, may contribute synergistically to oxidative stress and inflammatory responses after repeated CS exposures. These responses, in turn, may trigger aberrant tissue remodeling, eventually leading to the onset of respiratory diseases. Particularly, a subset of cilia-associated genes was downregulated by CS, a finding that correlates with the reduction in CBF observed in CS-exposed cultures. By integrating the omics-based approach with

in vitro measurements, we demonstrated that the CS-induced alterations observed in the in vitro ALI cultures are similar to those reported in the bronchial epithelium from smokers. Such an approach may be a useful tool for identifying dysregulated genes and adverse cellular events caused by inhaled toxicants, such as CS.

63. Developing an Animal Model of Drug-Induced Respiratory Depression to Assess Co-Administration of an Opioid with Other Sedative Psychotropic Drugs

Authors: Xu, Lin, FDA/CDER; Stewart, Sharron, FDA/CDER; Shea, Katherine, FDA/CDER; Chockalingam, Ashok, FDA/CDER; Weaver, James, FDA/CDER; Patel, Vikram, FDA/CDER; Matta, Murali, FDA/CDER; Davis, Michael, FDA/CDER; Zhu, Hao, FDA/CDER; Rouse, Rodney, FDA/CDER

Plain Language Synopsis: Developing an Animal Model of Drug-Induced Respiratory Depression to Assess Co-Administration of an Opioid with Other Sedative Psychotropic Drugs

Abstract:

Opioids and benzodiazepines are frequently co-prescribed to patients with both pain and psychiatric or neurological disorders. However, co-prescription of these drugs increases the risk for severe respiratory depression and death. In 2016, FDA required the addition of boxed warnings describing this risk to labeling for all prescription opioids and benzodiazepines. It is anticipated that sedating psychotropic drugs (SPDs) with differing mechanisms of action (e.g., antipsychotics, antidepressants, non-benzodiazepine sedative-hypnotics, etc.) may be increasingly prescribed in place of benzodiazepines. Despite being marketed for many years, many SPDs have neither human nor animal data to quantify or qualify the potential for causing respiratory depression either alone or in combination with an opioid. In this work, an animal model was established to address this question and generate data to inform regulatory decision-making. Arterial partial pressure of oxygen and carbon dioxide

were selected as practical and sensitive measures of respiratory depression. An oral exposure route was selected for all drugs to approximate clinical use. Diazepam was selected as the benzodiazepine positive control to demonstrate that the model was sufficiently sensitive to detect an additive or synergistic effect on respiratory depression with the opioid, oxycodone. Pharmacokinetic studies were conducted at three dosing concentrations with oxycodone (opioid; 6.25, 60, 150 mg/kg) and diazepam (benzodiazepine; 2, 20, 200 mg/kg). Partial pressures were collected at all the PK timepoints. A dose-dependent decrease in arterial oxygen partial pressure and increase in carbon dioxide partial pressure were observed with oxycodone. Diazepam caused similar changes at only the highest dosing concentration (200 mg/kg). Based on area under the curve assessment, oxycodone 150 mg/kg and diazepam 20 mg/kg were approximate clinical single-dose exposures in humans. Diazepam was administered 30 minutes after oxycodone to deliver peak serum concentrations of both drugs at nearly the same time. Decreases in partial pressure of oxygen and increases in partial pressure of carbon dioxide consistent with additional respiratory depression were observed in rats co-administered oxycodone and diazepam, compared to oxycodone alone. These findings support the utility of this animal model for assessing opioid-induced respiratory depression and its potential exacerbation by other SPDs.

64. Unique futures of IL-6 mediated neonatal germinal center reaction

Authors: Jiyeon Yang; Jiro Sakai; Shafiuddin Siddiqui; Robert C Lee; Derek DC Ireland; Daniela Verthely*ⁱ; and Mustafa Akkoyunlu**

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Plain Language Synopsis: Diminished host response to vaccines during the neonatal term is well-established in humans and in animal models. We studied differences in

the innate and adaptive immune systems of infants and adults to provide clues for the delay in the development of protective immune response in vaccinated infants.

Abstract:

The inability of infants to mount proper T follicular helper (TFH) cell response contributes to the weak vaccine responses in this age group. Regulatory T (Treg) cells control germinal center (GC) reaction by differentiating into regulatory TFH (TFR) cells and limiting TFH cell-mediated antibody responses. We found higher TFR : TFH ratio in neonatal mice than adult mice, despite the fact that their Treg expansion was limited over the course of immunization. Following immunization, Treg cells remained abundant in the interfollicular niches in adult mouse spleen, while residing in follicles of neonatal spleen. In immunized adults, IL-6 is critically important in the onset of TFH differentiation, and vaccines containing IL-6 improve antibody responses in adult mice by increasing IL-21 production. In sharp contrast to adults, we measured diminished IgG and IgA responses when IL-6 was co-injected with a pneumococcal conjugate vaccine in neonatal mice. The suppression of antibody responses was accompanied by an increase in TFR differentiation and a reciprocal decrease in TFH population. In vitro analysis of IL-6-stimulated CD4⁺ T cells from immunized mice provided a plausible explanation of the suppressive effect of IL-6 in neonatal mice. After IL-6 stimulation, phospho-STAT-3⁺ TFR cells expanded more than the TFH cells in neonatal mice. In adult mice however, TFH cells benefitted more from IL-6 stimulation than TFR cells. The expression levels for IL-6R likely played a role in IL-6 activity because neonatal TFR cells expressed higher IL-6Ra than the TFH cells, while the expression level of adult IL-6R was higher on TFH cells than TFR cells. Underscoring the role of higher IL-6R-expressing TFR cells in controlling neonatal GC responses, inclusion of IL-21 or the adjuvant, CpG in the pneumococcal conjugate vaccine led to a decrease in IL-6R⁺ TFR population, increased IL-21 producing

TFH cells, expanded GC B cells and boosted anti-vaccine antibody responses. These findings disclose a mechanism for the ablated TFH development in immunized neonatal mice and provide insights into adjuvant-mediated immune response improvements in neonates.

65. Transcranial high-intensity focused ultrasound produces long-term neurologic changes in mice

Authors: Ye, Meijun, FDA/CDRH/OSEL; Solarana, Krystyna, FDA/CDRH/OSEL; Rafi, Harmain, FDA/CDRH/OSEL; Patel, Shyama, FDA/CDRH/ODE; Nabili, Marjan, FDA/CDRH/OIR; Liu, Yunbo, FDA/CDRH/OSEL; Huang, Stanley, FDA/CDRH/OSEL; Fisher, Jonathan A.N., New York Medical College

Plain Language Synopsis: Focused ultrasound (FUS) is being investigated for a variety of neurotherapeutic applications; yet little is known about the interaction between FUS waves and neural tissues, and potential long-term side effects. To address this, we assessed neuroinflammatory, behavioral, and electrical signal changes following transcranial FUS exposure in mice.

Abstract:

Focused ultrasound (FUS) is increasingly evaluated as a potential non-invasive therapy for medical conditions for which current treatments have low efficacy or high risks. FUS is FDA-approved to treat patients with essential tremor and is being investigated for new applications including brain tumors, Parkinson's disease, and psychiatric disorders by thermal ablation of effected tissue. FUS is also increasingly studied as a method of blood brain barrier disruption to facilitate drug delivery into the brain, and for targeted neuromodulation. With the expansion of FUS applications under development, little is known about the mechanisms underlying the interactions between FUS waves and neural tissues, as well as the potential long-term side effects. Thus, an evaluation of the safety and precision of this technique is warranted. To address these gaps in knowledge relating to ultrasound exposure to the brain, we

assessed the neuroinflammatory response, evaluated behavioral effects, and recorded long-term brain activity using surface micro-electrocorticographic (μ ECoG) signals following transcranial, high-intensity, focused ultrasound (HIFU) exposure in adult mice. Rotarod testing demonstrated a decline in performance across all animals with HIFU treatment, as well as reduced exploratory behavior in an open field task. Histological analysis revealed mild astrogliosis and elevated microglial reactivity, both proximal to the HIFU-targeted region but also distally in the hypothalamus, hippocampus, thalamus, and caudate putamen, apparent as early as 24 hours post-HIFU and persisting at one month. Furthermore, low frequency (δ) and high frequency (β , γ) oscillations recorded by ECoG, were altered at acute and chronic time points following HIFU application. ECoG signal changes on the hemisphere ipsilateral to HIFU exposure were of greater magnitude than the contralateral hemisphere and persisted for up to three months. These results demonstrate that therapeutic FUS may have far-reaching biological effects resembling observations in mild traumatic brain injury conditions that should be considered when investigating the safety and spatial resolution of this technique for clinical applications.

66. Developing Quantitative Methods to Compare Exposure-Response Relationships Between Pediatrics and Adults to Support Pediatric Extrapolation

Authors: Qunshu Zhang, James Travis, Rebecca Rothwell, Yaning Wang, Jian Wang

Plain Language Synopsis: Similar exposure-response (E-R) relationship is one of the pre-requisites for pediatric extrapolation of efficacy. Historically, the assessment of E-R similarity was based on visual inspection of two E-R curves. The objective of this study was to develop a quantitative approach to assess E-R similarity between pediatric and adult population to inform decision making of pediatric extrapolation.

Abstract:

Objectives: FDA has allowed extrapolation of efficacy from adults and an abbreviated pediatric development program when there is expectation of a similar exposure-response (E-R) relationship, in addition to a similar disease progression and treatment response in children, when compared to adults. Historically, the assessment of E-R similarity was based on visual inspection of two E-R curves. The objective of this study was to develop a more quantitative approach to compare E-R relationships between pediatric and adult populations, to inform decision making in pediatric drug development.

Methods: The methods were developed for both linear and logistic PK/PD models. The estimated treatment response in pediatrics and adults was calculated at three points along the E-R curve (at the 10%, 50% and 90% adult exposure quantiles), and the distribution of the estimated differences (pediatric – adult) at these points were calculated using both bootstrap and Bayesian methods. The E-R relationships were considered to sufficiently support efficacy extrapolation if the estimated probability of exceeding a non-inferiority margin based on the adult and pediatric data at all three points was greater than a pre-defined, non-inferiority threshold.

Results: Clinical trials of nine drugs were identified in which E-R similarity was concluded using visual inspection, five drugs with linear E-R relationships (levetiracetam, oxcarbazepine, topiramate, lamotrigine, perampanel) and four drugs with logistic E-R relationships (infliximab, golimumab, darunavir, esomeprazole). The bootstrap and Bayesian posterior probability of exceeding the non-inferiority margin ranged from 53% to 100%.

Conclusions: This study developed and presented examples of a quantitative approach to assess the magnitude of difference in the exposure-response relationship between pediatric and adult patients. This method clarified that non-inferiority instead of similarity is required

to extrapolate adult efficacy to pediatric patients. It also provided reliable objective criteria for non-inferiority assessment, and informed pediatric trial design and decision making for efficacy extrapolation in pediatric population.

Poster Session 3

Topic: Advancing Digital Health and Artificial Intelligence (Day 2, A.M.)

67. **QUICK: Quality and Usability Investigation and Control Kit for Mass Spectrometric Data in Detection of Persistent Organic Pollutants**

Authors: Guo, Wenjing, FDA/NCTR; Archer, Jeffrey, FDA/ORA; Moore, Morgan, FDA/ORA; Bruce, Jeffrey, NCTR/ORA; McLain, Michelle, FDA/ORA; Shojaee, Sina, FDA/ORA; Zou, Wen, FDA/NCTR; Fairchild, Russel, FDA/ORA; Hong, Huixiao, FDA/NCTR

Plain Language Synopsis: Quality control is very time consuming for the detection of persistent organic pollutants in the laboratory. To facilitate the process, we developed a software kit that is proved to greatly improve the quality control efficiency and enhance FDA regulation of persistent organic pollutants in human and animal foods.

Abstract:

Persistent organic pollutants (POPs) are a significant public and environmental health concern due to their toxicity, long-range transportability, persistence, and bioaccumulation in animals. Exposure to these pollutants may cause adverse health effects, such as cancers, diabetes, birth defects, dysfunctional immune and reproductive systems. Consequently, to help protect and promote human health, it is important to monitor POPs in food and the environment. Gas Chromatography (GC) coupled with mass spectrometry (MS) has been widely applied for determining trace concentrations of organics in food and environmental matrices. In the U.S. Food and Drug Administration (FDA), GC/MS has been used for quantitative determination of POPs in human and animal foods. Stringent quality control procedures are needed to ensure reliability and accuracy of POPs detected using GC/MS. However, data processing in the quality control procedures is very time consuming. To improve efficiency, we developed a software kit QUICK (Quality and Usability Investigation and Control Kit) to automate and execute FDA's quality control protocol. QUICK ensures instrument optimization and data usability. We tested QUICK on a diverse set of target samples by

comparing quality control measurements determined using the software with those evaluated by human expert analysts. The tests confirmed that QUICK meets FDA requirements and provides reliable quality investigation and control of POPs' GC/MS analytical data. In conclusion, the software improved the efficiency of the analytical process by reducing the need for user intervention and the time for data analysis of POPs in human and animal foods.

68. **Utilizing FDALabel to Investigate Adverse Drug Reaction Patterns in Antidepressant Drug Labeling**

Authors: Ingle, Taylor, FDA/NCTR; Hill, Nathaniel, FDA/NCTR, UALR; Liu, Zhichao Liu FDA/NCTR; Wu, Leihong, FDA/NCTR; Yang, Junshuang, FDA/NCTR; Zhou, Guangxu, FDA/NCTR; Yang, Mary, UALR; Tong, Weida, FDA/NCTR; Fang, Hong, FDA/NCTR

Plain Language Synopsis: FDALabel provides access to valuable information in FDA-approved drug labeling documents. For example, data queried from antidepressant drug labeling can be used for ADR analysis and pattern discovery, to assess risks and to promote safe and effective treatments for patients with depression, to promote drug safety and public health.

Abstract:

Introduction and Purpose: Depression affects millions of Americans and is one of the most common mental illnesses in the United States. Depression increases the risk of suicide, which is the tenth overall leading cause-of-death and second-leading cause-of-death among adolescents and young adults. As depression rates increase, so does the use of prescribed antidepressants, which can have potentially severe and sometimes life-threatening adverse drug reactions (ADRs), including suicide. FDA-approved drug labeling includes ADR information (e.g., Boxed Warning, Warnings and Precautions, and Adverse Reactions) to help ensure the safe and effective use of prescribed antidepressants. This reliable source of information can be used to investigate and

research potential ADRs to promote drug safety and public health.

Methodology: DrugBank was used to identify a total of 32 single active-ingredient drugs classified as antidepressants. A unique ingredient identifier for each drug was used to query the FDALabel database for updated drug product labeling documents. Oracle SQL was used to extract ADR terms from different sections of the labeling documents. An ADR profile of the most frequently occurring terms was constructed to identify distribution patterns across the different labeling sections. Two-way hierarchical cluster analysis was performed on antidepressants and their respective ADRs to identify patterns.

Results: Five Established Pharmacologic Classes (EPC) were associated with antidepressant drug products. All 32 antidepressant drugs identified had BOXED WARNINGS associated with the increased risk of suicidality in teens and young adults. Seven unique ADRs (Depression, Completed suicide, Obsessive-compulsive disorder, Dizziness, Agitation, Mania, Seizure) were identified among BOXED WARNING, WARNINGS AND PRECAUTIONS, PEDIATRIC USE, and ADVERSE REACTIONS sections of the labeling documents. Multiple ADR patterns were identified within the WARNINGS AND PRECAUTIONS sections. For example, Hepatic ADRs Liver injury, Jaundice, Hepatotoxicity, and Hepatitis occurred among two serotonin and norepinephrine reuptake inhibitor drugs, duloxetine hydrochloride (Cymbalta) and milnacipran hydrochloride (Savella).

Conclusion: ADR patterns in antidepressant drugs could provide valuable information to assess risks and to promote safe and effective treatments for patients with depression. Here, information queried from FDALabel provided data for ADR analysis and pattern discovery in support of drug-safety research and public awareness of antidepressant drugs.

69. Codon and Codon-Pair Usage Tables (CoCoPUTs): facilitating genetic variation analyses and recombinant gene design

Authors: Holcomb, David, FDA/CBER/OTAT/DPPT; Kames, Jacob, FDA/CBER/OTAT/DPPT; Alexaki, Aikaterini, FDA/CBER/OTAT/DPPT; Athey, John, FDA/CBER/OTAT/DPPT; Santana-Quintero, Luis V., FDA/CBER/HIVE; Lam, Phuc Vihn Nguyen, FDA/CBER/HIVE; Hamasaki-Katagiri, Nobuko, FDA/

Plain Language Synopsis: Codon-pairs are sequential codons in a coding sequence. Codon-pair usage is non-random, specific to each species, and cannot be predicted by single codon usage alone. Because codon-pair usage affects translation efficiency and fidelity, we have designed a web interface to provide codon, codon-pair, and dinucleotide usage statistics for all species.

Abstract:

Many recombinant protein therapeutics that are regulated by DA have a significantly altered DNA sequence, containing multiple synonymous mutations to improve production yields. This is possible because the genetic code is degenerate, which means that multiple codons may encode the same amino acid. This results in codon usage bias: synonymous codons may be used at different rates. Codon usage has been shown to affect translation rate and fidelity, and for this reason, many of the biologics we regulate undergo codon optimization to increase protein expression yields or accommodate other needs such as high bioavailability.

Codon-pairs refer to sequential codons in a coding sequence. Codon-pair usage is non-random, specific to each species, and cannot be predicted by single codon usage alone. Furthermore, changes in codon-pair utilization may affect translational fidelity and efficiency more significantly than changes in single codons. Codon-pair deoptimization has been used in viral genes such as Influenza virus and Zika virus for vaccine development. Codon-pair optimization has been shown to significantly improve protein expression yields. In addition, comparing codon-

pair usage profiles is helpful for disease prediction and evolutionary studies.

Despite these applications, there is no publicly accessible tool for species-specific codon-pair usage data and metrics. To address this need, we have developed a web interface in partnership with HIVE-FDA for accessing and visualizing codon-pair usage data, by parsing available and correctly annotated CDSes for all species in GenBank, including all full RefSeq assemblies. Codon, codon-pair and dinucleotide usage data were calculated on HIVE-FDA's server, and is accessible on HIVE-GWU's public portal.

This has resulted in codon-pair usage tables for 973,985 species from GenBank and 53,104 species with RefSeq assemblies. Tables include genomic, mitochondrial and plastid codon, codon-pair and dinucleotide usage. As with our previous web tool (HIVE-CUTs), codon usage data are displayed graphically. The new interface features codon-pair frequency heatmaps, and dinucleotide frequency bar graphs. Additionally, the interface provides metrics such as effective number of codons and codon-pairs, GC% content and a taxonomic tree when multiple species are searched. Finally, codon, codon-pair, and dinucleotide usage raw data are downloadable.

Because of the importance of codon-pair bias in translation, and the potential uses in recombinant gene design and optimization, we believe that the present tool will be an invaluable resource for academia and industry.

70. Structure and Target Based Statistical Tools for Safety Analysis

Authors: Lababidi, Samir, FDA/OC; Callahan Lawrence, FDA/OC;

Plain Language Synopsis: The Global Substance Registration System uses R package with Shiny application to enable analysis of safety data based on a variety of structural parameters, classification systems and both on-target and off-target interactions.

Abstract:

The FDA Adverse Event Reporting System (FAERS) database is designed to support FDA's postmarket safety surveillance program for drug and therapeutic biologic products. FAERS is routinely used for the identification of new safety concerns. Integration of FAERS data and clinical trial safety data with FDA's Global Substance Registration System (GSRS) enables both molecular structure and target-based analysis. GSRS also uses R package with Shiny application to allow analysis of safety data based on a variety of structural parameters, classification systems and both on-target and off-target interactions. Several case studies using FAERS and Clinical Trials data with the GSRS are being performed. The GSRS is a freely distributed substance, product and application registration system compliant with the ISO IDMP standards that was co-developed by FDA and NIH's National Center for Advancing Translational Sciences (NCATS) and is distributed to both regulators and industry throughout the world. The statistical tools, incorporating regression models and machine learning algorithms, being developed for the GSRS, will also be freely distributed. Integration of publicly available FAERS data with GSRS and statistical tools drastically expands the scope and access to the evaluation of safety signals.

71. Constructing a mitochondrial sequence classifier (mitoKmer) for identification of eukaryotic species in shotgun metagenomic samples.

Authors: Mammel, Mark, FDA/CFSAN; Ramachandran, Padmini, FDA/CFSAN; Pava-Ripoll, Monica, FDA/CFSAN; Ottesen, Andrea, FDA/CFSAN; Leonard, Susan, FDA/CFSAN; Gangiredla, Jayanthi, FDA/CFSAN

Plain Language Synopsis: Sequences of mitochondrial genomes specific to different species of animals, plants, fungus, and protists were used to identify reads of whole genome shotgun sequencing of mixed samples. The relative abundance of these species in environmental or foods samples

can be determined with this tool.

Abstract:

Mitochondrial sequences have demonstrated usefulness for phylogenetic resolution of eukaryotic species and provide high sensitivity for detection when applied to metagenomic datasets due to multiple copies of mitochondrial DNA (mtDNA) in eukaryotic cells. Here we use k-mers (k=30) derived from selected sequence fragments of mitochondrial genomes to identify species present in metagenomic samples. K-mers from reference genomes of 9200 species (including plants, mammals, and arthropods among others) were stored in a hash table and classified as species- or genus-specific. The k-mers were filtered against chromosomal 18S and 28S rRNA sequences to reduce non-specific and confounding signals. Reads from shotgun metagenomic sequence datasets were classified by a custom profiling tool that finds exact matches of k-mers in the reference database to the reads. For normalization of read counts, all 200 base-pair substrings from all reference genomes were tested against the database to calculate coverage of the mitochondrial genomes by the stored k-mers. The ability to correctly determine the relative abundance of eukaryotic species composition was tested by generating synthetic reads in known proportions from reference genomes followed by quantification using the profiling tool. Important applications for food safety include detection and identification of insects in foods and correct identification of animal intrusions in fresh produce-growing regions, including water sources used for irrigation. This mtDNA k-mer database and profiling tool is named as mitoKmer and will be the appropriate tool for these applications. Finally, mitoKmer will be incorporated into the Galaxy platform for public use.

72. Acute radiation syndrome and telomere length: updates on an MCMi project

Authors: Mascia, Francesca, FDA/CDER; Pegues, Melissa, FDA/CDER; Rosen, Elliot, FDA/CDER; Biel, Thomas, FDA/CDER; Rao,

Ashutosh

Plain Language Synopsis: In the event of a nuclear accident, there are no precise tools to measure the severity of impact from radiation on the population. We are using a relevant rat model to measure telomere length and understand if it can be used to distinguish between lethal and non-lethal radiation damage on a whole animal system level.

Abstract:

Protection from acute radiation syndromes (ARS) remains an unmet medical need. ARS is a collection of symptoms that occur after accidental or intentional exposure to gamma radiations. One of the rate-limiting steps to the development of appropriate medical countermeasures is the absence of accurate and rapidly deployable bio-dosimeters. Telomeres, tandem DNA-repeats at the ends of chromosomes, are hypersensitive to oxidant and UV irradiation-induced DNA damage. The kinetics of telomere shortening, relationship to dose, cause of cell death, and opportunities to treat telomere shortening and improve cell survival are not known. We investigated if changes in telomere length could be used as a mechanistically-and clinically-relevant biomarker of ARS using a rat animal model. Wistar-Kyoto rats were exposed to sub-lethal and lethal doses of gamma radiations and then tissues (skin, intestine, bone marrow and blood) were collected immediately after (2-72 hours) and up to two weeks to determine the early and late dynamics of telomere length, DNA damage extent (levels of g-H2AX) and cell death. Telomere length was measured by FISH and FACS analysis using a telomere-specific probe fluorescent probe. We are investigating the correlation between telomere length with the radiation dose, DNA damage, accumulation of reactive oxygen species, and viability of the irradiated subjects/tissues. We propose that the analysis of telomeric DNA can provide a mechanistically sound biodosimeter endpoint and allow for pragmatic medical countermeasure development.

73. EEG Spectral Connectivity Analysis in a Large Clinical Population

Authors: Nahmias, David, FDA/CDRH; Kontson, Kimberly, FDA/CDRH

Plain Language Synopsis: This study explores neural connectivity differences between clinically normal and abnormal individuals using a large, publicly available, electroencephalography (EEG) database. Applying mathematical methods to 4,170 individuals' EEG, we found several differences across populations. These results can inform evaluation of devices using neural connectivity as a clinical endpoint or diagnostic aid.

Abstract:

This study explores neural connectivity in resting state through coherence and spectral graph-based methods across large populations with electroencephalography (EEG). Using the Neural Engineering Data Consortium (NEDC) EEG Corpus, we extract EEG data in a 10-20 montage and accompanying patient characteristics. Non-medicated subjects with clinically normal EEG are used as the normative population (n=1,167) while a group with a similar age distribution of medicated subjects with clinically abnormal EEG are used as the abnormal population (n=2,940). Parameters and properties of spectral coherence connectivity graphs are computed across frequency bands. We establish default mode networks (DMN) for the different populations on several frequency bands. We find that frequency bands differ across the populations more than specific graph properties. However, we find that there is an increased level of connectivity in the abnormal population. These results can inform evaluation of devices using neural connectivity as a clinical endpoint or diagnostic aid.

74. A natural language processing approach for structuring and analysis of FDA Meeting Minutes documents

Authors: Shen, Michelle, FDA/CDER; Voqui, Jessica, FDA/CDER; Subramani, Suresh, FDA/NCTR; Florian, Jeffrey, FDA/CDER;

Zhao, Weizhong, FDA/NCTR; Meehan, Joe, FDA/NCTR; Tong, Weida, FDA/NCTR; Popat, Vaishali, FDA/CDER

Plain Language Synopsis: The Center for Drug Evaluation and Research (CDER) conducts approximately 2,500 to 3,000 formal industry meetings with drug sponsors each year. Knowledge extracted from the minutes recorded from these meetings is essential for ensuring consistent advice to industry. Using natural language processing, we attempt to leverage information contained within minutes.

Abstract:

The Center for Drug Evaluation and Research (CDER) in the Food and Drug Administration (FDA) conducts approximately 2,500 to 3,000 formal industry meetings with drug sponsors each year. In these meetings, reviewers are asked to provide advice and comment on impactful issues related to the clinical development program, such as new clinical trial protocol design, adequacy of evidence for safety and efficacy, and Agency policies and procedures. To ensure consistency in responses across regulatory submissions, reviewers must review prior communications for similar products, drug classes, and indications. The Meeting Minutes Project aims to evaluate the use of natural language processing (NLP) to organize and consolidate previous Agency responses into a format that facilitates aggregating and displaying both sponsor questions and FDA responses for a comprehensive analysis.

To extract existing metadata within semi-structured fields of documents and to identify and extract the free-text Question and Answer (Q&A) section of a Meeting Minutes document, pattern matching was used to create possibility patterns lists for fields of interest. To extract the free-text Discussion section, pattern matching was used to overcome the lack of delineation of sections.

The Discussion section of Meeting Minutes were extracted into a spreadsheet with the following headers: Application Number, Filename, Unique Identifier, Indication,

Division, Heading, Document Section, Discipline, Question, Response, Discussion, and Comments. The collection of these fields, extracted from both the document metadata and the Discussion section, rendered it possible to view individual Questions in a spreadsheet ,while allowing filtering and sorting of Questions by Application Number, Division, and Discipline.

We developed a proof-of-concept for extracting the Q&A sections of Meeting Minutes, using rule-based pattern matching and applied this to all 230 of our pilot pre-NDA/BLA meeting documents to synthesize institutional knowledge and inform development of standard pre-NDA meeting communications. Currently, questions categorized as “Clinical” are being evaluated to identify common themes to help regulators understand and address frequently encountered questions. This will become part of a broader effort to provide sponsors clarifying information that will aide in preparing for the Pre-NDA/BLA meetings.

Poster Session 4

Topic: Advancing Digital Health and Artificial Intelligence (Day 2, P.M.)

1. A Data Anomaly Detection Tool for Site Selection at FDA

Authors: Wang, Xiaofeng (Tina), FDA/CDER/OTS/OB/IO; Schuette, Paul, FDA/CDER/OTS/OB/IO; Kam, Matilde, FDA/CDER/OTS/OB/IO;

Plain Language Synopsis: This poster describes the application of a data anomaly detection tool for site selection and use with sensitivity analyses, along with challenges of implementation at FDA.

Abstract:

On-site inspections are important to ensure the quality of the trial data and the reliability of the trial results submitted to the U.S. Food and Drug Administration (FDA). With the increasing size and complexity of the trials, statistical tools are needed to assist the site selection process and identify potentially problematic sites. We describe our experience with a centralized statistical monitoring platform as part of a Cooperative Research and Development Agreement (CRADA) between CluePoints and FDA. The approach employed in the CRADA to centralized statistical monitoring is based on a large number of statistical tests performed on all subject level data submitted, to identify sites that differ from the others. An overall data inconsistency score is calculated from a high-dimensional p-value matrix to assess the inconsistency of the data between one site and the data from all sites. Sites are ranked by the data inconsistency score ($-\log(p)$, where p is an aggregated p-value). Operationally, only sites with highest ranks and larger sizes are recommended for inspections. Results from one de-identified application are provided to demonstrate the typical data anomaly findings through the Statistical Monitoring Applied to Research Trials (SMART) analysis. Sensitivity analysis are performed after excluding laboratory data and questionnaires data. Graphics from de-identified subject-level trial data are provided to illustrate abnormal data patterns. Possible causes of data anomalies are discussed. This data-driven approach can be effective and efficient in selecting sites that exhibit data anomalies and it also provides insights to the

statistical reviewers for conducting sensitivity analyses, subgroup analyses and site by treatment effect explorations. However, challenges exist with messy data and with the lack of conformance to data standards.

2. Development of Detailed Transfusion Exposure Information from EHR Nursing Notes Using Natural Language Processing

Authors: Natarajan, Karthik, Columbia U Medical Center; Duke, Jon, Georgia Tech Research Institute (GTRI); Boyd, Richard, GTRI; Williams, Alan, CBER/OBE, Whitaker, Barbee, CBER/OBE; Anderson, Steven, CBER/OBE

Plain Language Synopsis: A major goal of the CBER Biologics Efficacy and Safety initiative is to identify biologic product exposures and outcomes not available from previously coded patient health data. Artificial Intelligence techniques (natural language processing) were used to precisely extract key characteristics of patient blood transfusions from EHR nursing notes.

Abstract:

Background: The CBER Biologics Efficacy and Safety (BEST) Program applies innovative methods to improve and automate the identification and reporting of adverse events for CBER-regulated biologics. For blood transfusion, ISBT-128 product description codes provide valuable identification of blood component patient exposures, however information about event timing and patient vital signs is needed to characterize post-transfusion adverse events.

Aims: Formal observation of transfusions by nursing staff is standard practice in the U.S. We applied natural language processing (NLP) to mine unstructured data from relevant nursing notes.

Methods: Columbia University Medical Center data analysts leveraged a Python-based NLP platform, ClarityNLP, developed by Georgia Tech Research Institute. Before deploying the tool within Columbia's environment, a representative set of 15 de-identified transfusion nursing notes were

used by Georgia Tech as training cases for the NLP extraction tool. After the tool was trained, it was installed within the Columbia environment and tested on a separate set of transfusion nursing notes.

Results: ClarityNLP was able to accurately extract information from 34,000 semi-structured notes, including: blood products ordered, transfusion start/end-times, patient reactions, temperature, oxygen level, blood pressure and heart rate during and after transfusion. Validation conducted by chart review of 100 patient records was used to assess the accuracy of the mined data regarding blood product ordered, reaction during transfusion and start and end times of the transfusion. For each of these fields the ClarityNLP tool reproduced the data with 100 percent accuracy and supplied transfusion end times for structured records that were missing this key data point.

Summary: ClarityNLP can efficiently digest many transfusion nursing notes simultaneously and precisely extract the main characteristics of a transfusion. This capability can be used in conjunction with structured data analysis to produce a more accurate and complete picture of patient transfusions. This advance represents key progress in the capability of the CBER BEST program to identify biologic product exposures and outcomes not available from structured EHR data.

(This abstract has also been accepted for oral presentation at The 29th Regional Congress of the ISBT, Basel, Switzerland, 22 - 26 June, 2019)

3. **A Deep Learning Model to Recognize Food Contaminating Beetle Species based on Elytra Fragments**

Authors: Wu, Leihong, FDA/NCTR; Liu, Zhichao, FDA/NCTR; Bera, Tanmay, FDA/NCTR; Ding, Hongjian, FDA/ORA; Jenkins-Barnes, Amy, FDA/ORA; Furlanello, Cesare, FBK; Maggio, Valerio, FBK; Tong, Weida, FDA/NCTR; Xu, Joshua, FDA/NCTR

Plain Language Synopsis: Adapt deep learning and convolutional neural network in

food contaminating beetle recognition.

Abstract:

Insect pests are often associated with food contamination and public health risks. Accurate and timely species-specific identification of pests is a key step to scale impacts, trace back the contamination process, and promptly set intervention measures, which usually have serious economic impact. The current procedure involves visual inspection by human analysts of pest fragments recovered from food samples, a time-consuming and error-prone process. Deep Learning architectures have been widely applied for image recognition, outperforming traditional other machine learning algorithms; however only few studies have applied deep learning for food contamination detection. This poster describes one solution for automatic identification of 15 storage product beetle species frequently detected in food inspection. This approach is based on a convolutional neural network trained on a dataset of 6,900 microscopic images of elytra fragments, obtaining an overall accuracy of 83.8% in cross validation. Notably, the classification performance is obtained without the need of designing and selecting domain specific image features, thus demonstrating the promising prospects of Deep Learning models in detecting food contamination.

4. **ARGOS-QC: A Comparative Quality Assessment Tool for Genome Assemblies**

Authors: Yi Yan, Heike Sichtig

Plain Language Synopsis: ARGOS-QC is a software that assesses genome assembly quality through a comparative approach. ARGOS-QC calculates common assembly quality metrics (N50, L50, ANI, et al.) and compares these metrics to user-defined backgrounds, such as all GenBank microbial assemblies or GenBank microbial assemblies of specific GC content.

Abstract:

Despite rapid advances in sequencing and assembly technologies, it remains unclear how to best assess the quality of genome assemblies. Currently, multiple metrics are needed for the assessment of genome assembly quality, and the interpretation of these metrics depends on the intended use of the assemblies. Here we present ARGOS-QC, a software that provides generalized assessment of genome assembly quality through a comparative approach. ARGOS-QC calculates commonly used assembly quality metrics (N50, L50, ANI, et al.) of target assemblies and compares these metrics to user-defined backgrounds, such as all GenBank microbial assemblies. ARGOS-QC also provides meta-data comparison of target assemblies based on user-defined parameters. The results of ARGOS-QC are captured in both static and interactive reports to aid in the interpretation of the assembly assessment result. In this study, we present an overview of the ARGOS-QC workflow and a demonstration.

5. **BLISS Tool for Site Selection: A Scientific Approach**

Authors: Yao, Zhihao FDA/CDRH; Xu, Jianjin FDA/CDRH; Huang, Lan FDA/CDRH; Xu, Zhiheng FDA/CDRH; Zalkikar, Jyoti FDA/CDER; Tiwari, Ram FDA/CDRH;

Plain Language Synopsis: This poster provides likelihood ratio test (LRT) based methods for site selection in premarket device datasets.

Abstract:

Multi-site clinical trials provide timely patient enrollment. However, the conduct of clinical trials may not be homogeneous across multiple sites. It is important to inspect the study sites that seem different compared with other sites in clinical trials, and investigate problems related to the sites. For example, sites with unusually large number of protocol deviations or serious adverse events may be found worthy for site inspection or monitoring. FDA conducts site inspections and data audits through the

Bioresearch Monitoring (BIMO) program to ensure that the data submitted are of good quality and are not prone to any anomaly. Usually one cannot inspect all the sites in a study due to limited resources and there is a need to select a few sites for inspection. Selecting sites based on manual check and/or eyeballing on descriptive site statistics is very inefficient when there are many sites and variables. Therefore, scientific tools are needed to help in selecting the sites that are aberrations for inspection. We have developed a technical framework that includes two novel statistical approaches, namely the Fisher combination approach and the likelihood ratio test (LRT) approach, and BIMO LRT inspection Statistical Software (BLISS) to identify the sites as signals that are different compared with other sites in a clinical trial. These approaches have good power and sensitivity for identifying site signals while controlling false discovery rate. BLISS provides an interactive interface, including data loading, variable selection, parameter option, and final report (ranking tables plus visualization). This Software (BLISS) was programmed using R and JavaScript and was displayed using the Shiny dashboard by RStudio. The combination of R and JavaScript provides freedom to generate more dynamic and interactive features, in both the back-end and the front-end. All these dynamic features offer end users more flexibilities to understand and analyze site selection data.

Poster Session 4

Topic: Outbreak! (Day 2, P.M.)

6. Evaluation of Antiviral Activity of the Neuraminidase Inhibitor, Laninamivir, Combined with Interferon Against Pandemic A(H1N1) Influenza Virus

Authors: Adams, Simone, FDA/CDER; Donnelly, Raymond, FDA/CDER; Ilyushina, Natalia, FDA/CDER

Plain Language Synopsis: Evaluation of Antiviral Activity of the Neuraminidase Inhibitor, Laninamivir, Combined with Interferon Against Pandemic A(H1N1) Influenza Virus

Abstract:

Each year, 5-20% of the population of the United States becomes infected with influenza A virus. Influenza viruses primarily use two surface proteins, hemagglutinin (HA) and neuraminidase (NA), to attach and detach from host cells and facilitate the spread of infection. NA can be targeted by antiviral agents known as NA inhibitors to prevent detachment and spread of the virus. Laninamivir, a novel NA inhibitor, is a long-lasting and effective treatment for influenza. The goal of this project was to examine if combination treatment with two classes of anti-influenza drugs, laninamivir and interferon (IFN)- λ 1, can alter the emergence of resistant variants in vitro. We serially passaged pandemic A/California/04/09 (H1N1) influenza virus in human epithelial cells (Calu-3) in the presence or absence of increasing concentrations of laninamivir or laninamivir plus IFN- λ 1. After ten passages in the presence of laninamivir, an amino acid mutation, E119G, was found in the H1N1 NA protein. Acquisition of this mutation resulted in significant increase (\uparrow 339-fold) in the IC50 value for laninamivir by this mutant virus compared to the parental virus. After ten passages in the absence of selective pressure, HA1 mutations D127E/G155E were observed in our model H1N1 virus. These HA1 changes resulted in significantly decreased (\downarrow ~3.5-fold) receptor binding affinity for YDS, 6'SU-6'SLN, and 6'SL sialyl ligands and an increase in viral growth at 24, 48, and 72 h post-infection compared to the wild-type virus. Notably, when laninamivir was used

in combination with IFN- λ 1, the E119G NA mutation was present in a pure population as early as passage five, and acquisition of this mutation correlated with significantly reduced sensitivity to laninamivir (\uparrow 260-fold) as compared to the parental virus. Our results showed that treatment with laninamivir, used alone or in combination with IFN- λ 1, can lead to the emergence of drug-resistant influenza virus variants. Furthermore, addition of IFN- λ 1 in combination with laninamivir may promote mutations in N1 NA more rapidly than therapy with laninamivir alone.

7. Strengthening laboratory approaches for the detection of *Cyclospora cayetanensis* in prepared dishes: Addressing future outbreak investigations

Authors: Almeria, Sonia, FDA/CFSAN/OARSA*; Murphy, Helen, FDA/CFSAN/OARSA; Assurian, Angela, JIFSAN, University of Maryland; Cinar, Hediye Nese, FDA/CFSAN/OARSA; Ewing, Laura, FDA/CFSAN/OARSA; Durigan, Mauricio, FDA/CFSAN/OARSA; Gopinath, Gopal, FDA/CFSAN/OARSA; d

Plain Language Synopsis: There were no standards for the detection of *Cyclospora cayetanensis* in prepared dishes (coleslaw, pico de gallo, guacamole and green salsa) linked to outbreaks. FDA validated method was evaluated and optimized for *C. cayetanensis* detection in such complex dishes, which will strengthen laboratory applications for *Cyclospora* outbreak investigations.

Abstract:

Cyclospora cayetanensis is an important foodborne parasite responsible for outbreaks frequently linked to fresh produce, including prepared food dishes. In 2016, a regulatory method was established by the Food and Drug Administration (FDA) for the detection of *C. cayetanensis* in cilantro and raspberries. Additional fresh produce items have been implicated in recent outbreaks including restaurant coleslaw, a variety of salads from McDonald's restaurants in the Midwest, supermarket trays containing mixed vegetables and a dip, and to unrelated

Mexican-style restaurants, affecting 2299 people in 2018. There are no standards for the detection of *C. cayetanensis* in dishes containing produce combined with multiple ingredients. The purpose of the present study was to evaluate specific modifications of the FDA Bacteriological Analytical Manual (BAM) Chapter 19B method for optimal detection of *C. cayetanensis* in prepared dishes (coleslaw, pico de gallo-salsa, guacamole, and green salsa). The FDA method entails produce washing, DNA extraction, and a TaqMan real-time PCR assay targeting the 18S rDNA gene of *C. cayetanensis*. In the prepared dish of coleslaw with dressing, the method was optimized to detect 5 oocysts in a 25 g sample by using 1.0% Alconox® in the wash solution instead of 0.1% as originally described. Following this modification, detection rates in coleslaw were 80%, 90% and 100%, in samples seeded with 5 oocysts (n=10), 10 oocysts (n=10) and 200 oocysts (n=10), respectively. When testing pico de gallo-salsa, very gentle washing was essential to detect as low as 5 oocysts in 25 g samples (81.8%, n=11). Very dense samples such as green sauce and guacamole produced large pellets after the washing concentration step, therefore a modification of the DNA extraction step was required to process and homogenize those larger pellet samples of the matrix. The method was robust and reproducible in every multi-ingredient food analyzed, detecting as few as 5 oocysts in 25 g samples. In conclusion, the method modifications improved the FDA method for the detection of *C. cayetanensis* in complex food matrices. Evaluation, optimization, and validation of laboratory methods for the detection of *C. cayetanensis* in a variety of matrices, including such complex dishes, will strengthen the use of laboratory applications for Cyclospora outbreak investigations.

8. Comparison of thermal resistance of wild-type and mutant *Listeria monocytogenes*

Authors: Halik, Lindsay A., IFSH/IIT; Suehr, Quincy J., FDA/CFSAN; Salazar, Joelle K., FDA/CFSAN; Grasso-kelley, Elizabeth M. Grasso-Kelley, IFSH/IIT; Keller, Susanne E.,

FDA/CFSAN; Anderson, Nathan M., FDA/CFSAN

Plain Language Synopsis: *Listeria monocytogenes* contamination resulted in recalls of numerous baked foods. Thermal resistance of *Listeria monocytogenes* is not well characterized. The purpose of this study was to compare the thermal resistance of wild-type and mutant *Listeria monocytogenes* strains subjected to baking conditions. Thermal resistance of *Listeria monocytogenes* is strain-dependent.

Abstract:

Listeria monocytogenes contamination resulted in recalls of numerous baked foods. The thermal resistance of *Listeria monocytogenes* under low-moisture conditions is not well characterized. In addition, differences found in serotypes may allow identification of genes related to desiccation and thermal resistance. The purpose of this study was to compare the thermal resistance of different desiccated *Listeria monocytogenes* strains subjected to baking conditions and compare genetic differences which may be related to thermal resistance. Cellulose filter membranes (0.22 µm pore size) were inoculated individually with one of seven *Listeria monocytogenes* strains (Scott A, ATCC 13932, ATCC 49594, NZRM 4242, NZRM 4237, FRRB 02542, and LM-004, and mutants derived from LM-004 at a level of 9.5-log CFU/filter. Strains were chosen based on their association with foodborne recalls and/or outbreaks. The inoculated membranes were dried for 24 h and then subjected to hot-air roasting at 129°C (265°F) for 0, 15, 30, or 45 min. Each study was conducted in triplicate. The filter membranes were vortexed in buffered peptone water to recover *Listeria monocytogenes* from the membrane surface. Viable organisms were enumerated on tryptic soy agar with 0.6% yeast extract and incubated at 37°C for 48 h. Colonies were confirmed as *Listeria* via streaking on Modified Oxford Medium. By pairwise comparison, the thermal inactivation curve for LM-004 was statistically different from

curves of all other strains ($p < 0.05$). With 15 min roasting, LM-004 reached the limit of detection (2 log CFU/mL) with a >6.86 -log CFU/mL reduction whereas an average 4.68 ± 1.45 -log reduction was achieved for the other six strains. Thus, thermal resistance of *Listeria monocytogenes* is strain-dependent. On the basis of these findings, WGS was conducted on Scott A and LM004, and results compared. Mutants were developed based on suspect genes present in Scott A and missing in LM-004. There were no significant differences in inactivation rates among LM-004 and the mutant strains tested. Thus, the absent genes do not appear to affect thermal resistance of *Listeria monocytogenes*. This project provided data to fill knowledge gaps with respect to regulatory evaluation of pathogen destruction during baking processes in response to outbreak events and FSMA requirements.

9. Assessment of chloramphenicol, chloramphenicol-base, and chloramphenicol-alcohol in crawfish following waterborne exposure

Authors: Jester, Edward L.E., FDA/CFSAN; Baltzer, Katherine, FDA/CFSAN; El Said, Kathleen R., FDA/CFSAN; Benner, Jr, Ronald A., FDA/CFSAN

Plain Language Synopsis: Chloramphenicol (CAP) is a broad-spectrum antibiotic used in aquaculture that has been banned in many countries due to the potential to cause health issues, including fatal aplastic anemia. This study aims to identify possible biomarkers of CAP residue in crawfish exposure studies.

Abstract:

Chloramphenicol (CAP) is a highly effective, broad-spectrum antibiotic, with a history of usage in aquaculture species. Use of CAP in food animals has been banned in many countries, including the United States, due to health concerns. It has been associated with numerous toxic effects in humans and can cause potentially fatal aplastic anemia. The presence of CAP residues in aquaculture products is a major concern for the U.S. Food and Drug Administration, since it continues

to be used for food animal disease treatment in some countries. CAP is rapidly eliminated following exposure suggesting the need for an alternative marker residue. Very few depletion and metabolism studies of CAP in seafood have been conducted. Currently, validated confirmatory analytical methods are available for monitoring CAP, but not its biomarkers. Previously, we identified two CAP metabolites, CAP-base (CAP-B) and CAP-alcohol (CAP-OH), in crab and shrimp following CAP exposure. We also assessed the persistence of CAP, CAP-B, and CAP-OH in crab. In this study, we exposed crawfish to CAP to identify if CAP-B and CAP-OH are also present in crawfish and could be possible marker residues for CAP exposure. The persistence of CAP, CAP-B, and CAP-OH was also evaluated following waterborne exposures. CAP residue levels were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) on a Waters Acquity UPLC system coupled to a XEVO TQ-MS fitted with an electrospray ionization (ESI) source. Separation of analytes was achieved in reverse phase mode on a Phenomenex Kinetex 1.7 μ m PFP column (50 x 2.1 mm) in 5 min.

In 100 ppm (24 hour) exposed crawfish, concentrations of CAP (depletion time) ranged from 139 (0 h) to 0.245 ng/g (24 h), CAP-B ranged from 31.0 (8 h) to 0.129 ng/g (192 h), and CAP-OH ranged from 1.24 (8 h) to 0.150 ng/g (168 h).

10. Geographic differences in enrollment and microbial etiology in clinical trials of antibacterial drugs, 2001-2017

Authors: Bart, Stephen, ORISE & FDA/CDER/OND/OAP; Farley, John, FDA/CDER/OND/OAP; Bala, Shukal, FDA/CDER/OND/OAP; Amini, Thushi, FDA/CDER/OND/OAP; Cox, Edward, FDA/CDER/OND/OAP

Plain Language Synopsis: Recent clinical trials for antibacterial drugs were analyzed to examine geographic trends in enrollment and microbiology. Eastern European enrollment increased in many trials, though skin trials remained mainly focused in North America.

Regional similarities and differences in microbiology may support generalizability of trials held elsewhere and inform trial site selection.

Abstract:

Background: Despite increasing antimicrobial resistance, scientific and economic challenges have hindered new antibiotic development. Regional differences in microbial epidemiology among clinical trial participants worldwide have not been well characterized, despite being an important consideration for planning clinical trials and assessing the generalizability of trial findings.

Purpose: The study goal was to analyze recent antibacterial drug clinical trial datasets to determine how regional enrollment has changed over time and how microbial epidemiology differs among regions.

Methods: A total of 42 phase 3 trials previously submitted to the FDA for selected bacterial infections initiated after 2001, totaling 29,282 subjects, were retrospectively analyzed. Enrollment characteristics across the study period were compared to identify trends in recruitment by geographic region and the prevalence of different bacterial species and resistance phenotypes.

Results: In cUTI, cIAI, and CABP trials, Eastern European enrollment increased to $\geq 70\%$ of subjects, while for ABSSSI trials, North American enrollment increased to 66.3%. Higher-enrolling regions generally enrolled more subjects per study site. Increased recruitment was correlated with enrollment of subjects with a history of IV drug use in ABSSSI trials and inversely correlated with subjects' prior antibacterial drug treatment in cIAI trials. Microbiological patterns were broadly similar; selected differences relative to North America include increased *K. pneumoniae* in Asian cIAI subjects (20.3% of isolates/9.0%, $p=0.0057$), increased cephalosporin resistance in South American Enterobacteriaceae cUTI isolates (26.8%/15.7%, $p=0.044$), and decreased prevalence of *S. aureus* (and

methicillin-resistant *S. aureus*) in Eastern European ABSSSI isolates (43.7%/61.9% and 5.3%/53.9% respectively, $p<0.0001$). ABSSSI isolates from IV drug users were more likely to include oral *Streptococcus* species compared to non-IV drug users.

Conclusions: Antibacterial clinical trials have exhibited shifts in geographic enrollment likely driven by factors that impact recruitment. Epidemiological similarities across regions could support the generalizability of trials while differences may facilitate appropriate site selection for drugs targeting certain organisms or resistance mechanisms.

11. Interaction of live attenuated Leishmania parasites infected neutrophils with dendritic cells augments CD4+Th1 cell priming in C57BL/6 mouse

Authors: Bhattacharya, Parna, FDA/CBER; Ismail, Nevien, FDA/CBER; Karamakar, Subir, FDA/CBER; Takeda, Kazuyo, FDA/CBER; Dey, Ranadhir, FDA/CBER; Nakhasi, Hira, FDA/CBER

Plain Language Synopsis: Visceral Leishmaniasis is a neglected tropical disease caused by protozoan parasite *Leishmania donovani*. To develop successful vaccine, we have reported previously about *Leishmania donovani* Centrin knock out parasites (LdCen^{-/-}). Here we have reported cross talk between neutrophil and dendritic cell in LdCen^{-/-} infected mice which initiates protective immune response.

Abstract:

Visceral leishmaniasis (VL), is a vector-borne disease with no available vaccine. We have previously reported the protective role of live attenuated centrin gene-deleted *L. donovani* (LdCen^{-/-}) parasite vaccine in animal models. LdCen^{-/-} induces strong innate immunity, which leads towards protective Th1 response. Neutrophils are involved in initial steps of most responses to pathogens and can also enhance various T cell responses either directly or indirectly through activation of dendritic cells (DCs). Recently we have demonstrated neutrophil's

direct antigen presenting potential in generating early host protective immune response to LdCen^{-/-}. However, neutrophil mediated immunomodulation of DC function in response to live attenuated Leishmania vaccine has not been studied yet. Hence, we evaluated the interaction between neutrophils and DCs during LdCen^{-/-} infection and compared with LdWT both in vitro and in vivo. Robust chemo attractive activity for DCs was detected in the supernatants of neutrophils exposed to LdCen^{-/-} compared to LdWT in vitro. Additionally, uptake of LdCen^{-/-}-mCherry-infected neutrophils by DCs augmented their expression of costimulatory molecules and heightened CD4⁺T cell priming in comparison with DCs with LdWTRFP infected neutrophils in vitro. Intradermal immunization in the ear with LdCen^{-/-} induces synchronized higher neutrophil and DC recruitment compared to LdWT parasites. Furthermore, to investigate neutrophil-DCs interactions in vivo, we evaluated dermal DCs recovered from mice 24h after infection with LdWTRFP / LdCen^{-/-}-mCherry parasites. We observed, DCs which have LdCen^{-/-}-parasitized neutrophils have higher CD4⁺T cell priming capacity thereby promoting heightened Th1 response compared to LdWT. Also, depletion of neutrophil significantly abrogates antigen presenting potential of DC in LdCen^{-/-} infected mice. This study suggests that interaction of infected neutrophils with DCs plays pivotal role in shaping the vaccine induced host protective adaptive immune response.

12. Investigating the immunogenicity of a meningococcal serogroup A Factor H binding protein cholera holotoxin-like chimera glycoconjugate vaccine

Authors: Bizzell, Erica, FDA/CBER; Price, Gregory A., FDA/CBER; Lee, Che-Hung, FDA/CBER; Reveille, Alexandra M., FDA/CBER; Bash, Margaret C., FDA/CBER

Plain Language Synopsis: Attachment of serogroup A meningococcal polysaccharide to serogroup B meningococcal Factor H binding protein (FHbp), or to an FHbp cholera holotoxin-like chimera enhances antibody

responses to FHbp in a mouse immunization model.

Abstract:

The surface-expressed lipoprotein Factor H binding protein (FHbp) of *Neisseria meningitidis* (Nm) is a key virulence factor that binds the immune system's regulator of the complement cascade, Factor H. FHbp can also induce robust bactericidal antibody responses, and recombinant forms of FHbp are components of both serogroup B meningococcal (MenB) vaccines available in the U.S Previous work has shown that FHbp serves as an effective carrier protein when conjugated to MenA polysaccharide (Ps), as this construct induced robust bactericidal antibody responses against MenA. Limited anti-MenB responses, however, suggest that FHbp conjugation to MenA-Ps (MAP) modifies or masks antigenic epitopes of FHbp. We explored the use of FHbp expressed as a cholera holotoxin-like chimera (FHbp-A2-CTB), which is immunogenic in mice, as a carrier protein. Genetic fusion of FHbp to the A2 domain of cholera toxin allows for non-covalent conjugation of the FHbp-A2 construct to the cholera toxin B (CTB) subunit in the bacterial periplasm upon expression in *Escherichia coli*. Using reductive amination, MAP was conjugated to the FHbp-A2-CTB construct, FHbp alone, or CTB alone. The immunogenicity of these groups was assessed via immunization of mice with equimolar amounts of these constructs and MAP alone, MAP-tetanus toxin conjugate (MAP-TT), and the FHbp-A2-CTB chimera. Each FHbp construct served as an effective carrier protein, inducing high anti-MenA bactericidal titers. While MAP conjugation to FHbp dampened anti-MenB bactericidal titers, conjugation to the FHbp-A2-CTB chimera preserved anti-MenB bactericidal responses in immunized mice. FHbp-specific IgG responses were comparable between FHbp-conjugate immunized groups. However, using one-way analysis of variance (ANOVA) correcting for multiple comparisons to determine statistical significance, we observed significantly lower

anti-FHbp IgG2b responses in the FHbp-CTB chimera immunized group compared with the FHbp-MAP ($p < 0.0002$) or FHbp-A2-CTB-MAP ($p < 0.0001$) immunized mice. As anti-polysaccharide responses are mainly IgG2-driven, these data suggest that conjugation of FHbp or the FHbp-A2-CTB chimera to MAP enhances FHbp-specific IgG2 responses through a polysaccharide-dependent mechanism.

13. Enterotoxigenic profile characterization of *Bacillus cereus* by using targeted RNA sequencing

Authors: Cao, Guojie, FDA/CFSAN; Hait, Jennifer, FDA/CFSAN; Kastanis, George, FDA/CFSAN; Tallent, Sandra, FDA/CFSAN

Plain Language Synopsis: Toxin-producing *Bacillus cereus* is an environmentally ubiquitous pathogen causing diarrheal syndrome and emesis with spores that can persist in harsh conditions. It is clinically important to detect the toxins causing human illness.

Abstract:

Purpose: The purpose of this study was to develop an accurate and effective multiplex probe panel to detect and characterize toxin profiles and expression in *Bacillus cereus* using sequencing data.

Methods: Twelve *Bacillus cereus* strains originating from foods and patients were selected. Total RNA was isolated by using the RiboPure Bacteria Purification kit. A novel work flow was employed that combined the Illumina TruSeq RNA Exome kit and Bioscience Custom Panel protocol. RNA was converted to cDNA. The amplified cDNA was then hybridized to customized probes for genes encoding targeted toxins, including hemolytic enterotoxin hemolysin BL (hbl), nonhemolytic enterotoxin (nhe), cytotoxin K (cytK), and emetic toxin (ces). The post-capture library was amplified and sequenced using a MiniSeq sequencer. Analysis of the raw reads was performed using CLC Genomics Workbench 10 with PCR results being used as a reference.

Results: RNA-seq data of ces, hbl, and nhe matched PCR results. All strains contained gene cytK, whereas the number of samples was five in the PCR assay. Expression of nheB was higher than nheA and nheC in all 12 samples. Expressions of hblA and hblD were higher than hblB and hblC in the four samples with positive results of hbl. Expression of cytK ranged from 0.21% to 54.19% in each tested strain.

Significance: We initiated a novel targeted RNA-seq workflow using a customized multiplex probe panel to characterize toxin profiles in *B. cereus*. The work flow is capable of specifically detecting target toxins and elucidating protein expression, providing an accurate and valuable assay to identify *B. cereus* and its toxins in food safety programs.

14. Gamma irradiation of antioxidants tris(nonylphenyl) phosphite (TNPP) and Irganox 1076 in polyethylene food contact material

Authors: Celiz, Mary Dawn, FDA/CFSAN; Morehouse, Kim, FDA/CFSAN; deJager, Lowri FDA/CFSAN; Begley, Timothy, FDA/CFSAN

Plain Language Synopsis: When polymer additives in food contact materials are exposed to irradiation doses applicable for food produce, radiolysis products may be produced. We investigated the effect of irradiation doses of 0.5-20 kGy on the concentration of the polymer antioxidants used in food contact materials and the radiolysis products formed.

Abstract:

Pre-packaged food such as bagged lettuce that is pre-cut, and ready-to-eat are convenient to consumers. Demand for pre-packaged food is expected to increase, along with consumer expectations of obtaining safer food. Use of food irradiation technology may increase to meet the market demands. Before marketing irradiated pre-packaged food, the food packaging materials must be FDA-approved for irradiation. The list of approved packaging materials for irradiation contains very few modern materials. To evaluate the food packaging material for

safety, information on the chemical changes that occur after irradiation treatment is required. Additives present in polymeric food contact materials form radiolysis products when exposed to irradiation. In this study, we determined the concentration and identity of some of the radiolysis products of two antioxidants, TNPP and Irganox 1076, when irradiated at doses applicable to food.

Polyethylene resin containing TNPP and Irganox 1076 were irradiated at 0.5 - 20 kGy. The antioxidants and their radiolysis products were extracted from the resin using accelerated solvent extraction. The extracts were analyzed using liquid chromatography with mass spectrometry (LC-MS and LC-MS/MS) detection.

The concentration of the antioxidants decreased as the irradiation dose is increased. For Irganox 1076, a 64% decrease from 312 ppm was observed at 4 kGy. The major radiolysis product was an oxidized form of Irganox 1076 corresponding to the loss of two hydrogens. For TNPP, a 97% decrease from 1595 ppm was observed at 4 kGy. The major radiolysis products were tris(nonylphenyl) phosphate, followed by nonylphenol. Eight other minor compounds were tentatively identified as radiolysis products of TNPP. Most of the radiolysis products have low responses, implying low concentrations.

15. A Simple, Rapid, High-throughput Potency Assay for Seasonal and Pandemic Influenza Vaccines: Using Surface Plasmon Resonance to evaluate concentration and composition of industry products

Authors: Coyle, Elizabeth M., FDA/CBER; Hahn, Megan, FDA/CBER; Chilcote, Katarina, FDA/CBER; Manischewitz, Jody, FDA/CBER; King, Lisa R., FDA/CBER; Golding, Hana, FDA/CBER; Khurana, Surender, FDA/CBER

Plain Language Synopsis: Many problems prevent influenza vaccines from reaching the public. Companies have difficulty measuring the amount of vaccine in a vial. Companies use an experiment called "SRID" to do this, but it is not perfect. This abstract

summarizes a new-and-improved experiment called "SPR-based potency" that is better and more accurate.

Abstract:

Background (350 words): After the 2009 H1N1 pandemic, WHO recommended alternative potency assays to SRID to avoid possible bottlenecks in vaccine production. A Surface Plasmon Resonance (SPR)-based potency assay was developed to quantitate hemagglutinin (HA) in monovalent and multivalent influenza vaccine samples. Unlike SRID, it does not need conventional reference antisera for each new season's components and can be used across multiple seasons.

Purpose: The simple SPR assay uses synthetic glycans with sialic acid (SA) receptors to determine the potency of influenza vaccines. Functionally active forms of HA are quantitated for binding to SA. The SA-HA surface is probed with monoclonal antibodies specific to different subtypes and can quantitate the proportion of different subtypes in the formulated multivalent influenza vaccine. It can quantitate Egg-derived, cell-derived, insect-derived vaccines in many formulations like virus like particles (VLPs) and recombinant HA protein-based vaccines for both multivalent seasonal (A/H1N1, A/H3N2, B) and monovalent pandemic (A/H5N1, A/H7N9) strains.

Methodology: Sialic acids with α -2,6 or α -2,3 specificities were immobilized on an NLC (NeutrAvidin) chip in the ProteOn XPR36 SPR system. Various reference hemagglutinin (HA) antigens were used as reference curves and were used to quantitate the HA content in influenza vaccines. Monoclonal antibodies are used to determine the individual proportions of each subtype in the multivalent vaccine.

Results: The determination of HA content in large panel of vaccine lots was compared using SA-glycan SPR potency assay and SRID. The SPR-based HA content measurement showed good agreement like the SRID assay for seasonal (A/H1N1, A/H3N2, B) and pandemic (A/H5N1, A/H7N9) strains. It is stability-indicating, due to the specificity

of the glycans for functional native HA. The assay is able to adequately subtype a seasonal vaccine product, which could be used in formulation and release of vaccines.

Conclusion: The SA-glycan SPR assay can quantify vaccine HA concentration and compositions with good accuracy, repeatability, linear range, precision and robustness. The SPR potency assay could allow manufacturers an alternate method for HA quantitation and subtyping for vaccine product release and stability testing. In a pandemic outbreak, SPR potency could help rapid lot release of influenza vaccines

16. Rapid point of care (POC) diagnostics: experiments in technology development

Authors: Elespuru, Rosalie, FDA/CDRH; Dan, Michelle, Winston Churchill High School; Kim, Yeonju, Thomas Jefferson High School of Science and Technology; Li, Baoguang, FDA/CFSAN; Tang, Xing, FDA/CDRH

Plain Language Synopsis: Technologies such as the polymerase chain reaction (PCR) could be used for rapid detection of disease-causing bacteria, such as Salmonella, in fields, hospitals, or other environments. The appearance of battery-operated devices may make this possible. Lab experiments found that this DNA-based technology could work using methods that we developed

Abstract:

Background: Targeted DNA detection technologies such as the polymerase chain reaction (PCR) could be used for rapid detection of pathogens, such as Salmonella, Klebsiella, or other microbes (as well as larger species), in field, hospital, or other point of care (POC) environments. Allele-specific PCR can't identify unknown agents, but the technology is capable of yes/no answers concerning the presence of targeted species via DNA analysis. Within this context, few if any practical diagnostic systems suitable for use outside of laboratory facilities have been developed. The appearance of lightweight battery-operated PCR devices could change the paradigm for on-site diagnostics.

Purpose: A commercially available battery-operated PCR instrument, Ahram Biosystems' Palm PCR, was tested to assess its usefulness in a POC, or field-based context. Questions asked included: 1) Does the small cell-phone sized instrument work? 2) How does it compare with a standard laboratory PCR machine? 3) What technological issues need to be worked out for the instrument to work in a non-laboratory environment?

Methods: Results with the battery-operated Palm PCR were compared with a standard lab instrument and standard reagents. Experiments were designed to test the function of the Palm PCR without refrigeration or electricity. The following technological hurdles were addressed: 1) a method for isolation of suitable quality DNA from bacteria using non-toxic reagents, 2) methods for operating the instrument to a successful diagnostic endpoint, 3) sources of reagents that would be stable without refrigeration, 4) measuring the endpoint of a positive result (amplified DNA) with the aid of a cell phone, 5) detection of Salmonella bacteria spiked onto spinach as a model for recovery of a pathogen in a field environment.

Results, Conclusion and Impact: 1. The Palm PCR can work in a non-laboratory environment with ordinarily trained personnel (high school students) with results in <2h. 2. Methods were developed to resolve many technological issues, but others, e.g. related to excitation of dyes for detection still need work. 3. Field-based or POC diagnostics could have wide-spread utility in POC environments, including doctor's offices, airports, hospitals and farms, for identification of any targeted species, from bio-threat microbes to bush meat.

17. Modeling of long-term behavioral defects in mice exposed to ZikV early in life.

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Plain Language Synopsis: Prenatal ZIKV infections can result in CNS damage with devastating consequences, even in patients without microcephaly at birth. We have developed an animal model to test the motor and developmental defects that follow infection that should help understand the long-term consequences of the disease and develop therapeutics for these patients.

Abstract:

Patients that were exposed to Zika virus in early and late gestation can have significant developmental and motor defects regardless of the early diagnosis of microcephaly, clinically referred to as Zika congenital syndrome. To treat these patients, it is critical to develop animal models that replicate the defects induced by ZikV infection on the developing CNS. We developed a mouse model of neonatal Zika virus infection of immunocompetent mice, which models 2nd-3rd trimester of pregnancy infection in humans. The inoculation of 1-day old mice results in acute neurological symptoms, including: ataxia, kinetic tremors, and seizures that are observable until 20 - 30 days post-infection (DPI). After this time, the mice recover and appear outwardly indistinguishable from age-matched, uninfected animals. As expected, given the severity of the early infection, immunohistochemical imaging of the CNS 60 DPI indicated sustained lesions. Surprisingly, assessment of gene expression indicated active inflammatory processes in the CNS 2 and 6 months after the mice recover from infection indicating a persistent pathology. To better understand the consequences of the infection we conducted behavioral tests to examine anxiety, memory, and mobility in adult mice that had been infected neonatally with Zika virus. Despite appearing normal in their cages, Zika infected adults showed significant defects in balance and coordination, as well as hyperactivity and decreased anxiety compared to age-matched uninfected controls. The model suggests that ZikV infection results in life-long behavioral deficits and provides a tool to test potential

therapeutics designed to ameliorate the consequences of Zika congenital syndrome.

18. Whole genome sequences of potentially toxigenic fungi from the walnuts, peanuts, and selected fruits

Authors: Gebru, Solomon, FDA/CFSAN; Gangiredla Jayanthi, FDA/CFSAN; Mammel Mark, FDA/CFSAN; Tournas Vasiliki, FDA/CFSAN; Tartera Carmen, FDA/CFSAN

Plain Language Synopsis: Fungi are the major contaminants of foods and they produce mycotoxins that can potentially cause disease. The purpose of this study was to develop a rapid means of identifying fungal contaminants in foods by generating whole genome sequences to determine whether the identified fungus/fungi pose a health concern for consumers.

Abstract:

Foods of plant origin, such as tree nuts and fruits, are known to foster the growth of toxigenic and pathogenic fungal species. It is important to identify fungal contaminants in fresh fruits and nuts because some fungi can grow and produce mycotoxins on these commodities and can cause infections or allergies.

The purpose of this study is to develop a rapid means of identifying fungal contaminants in foods by generating high -uality whole genome sequences; this will provide reliable data for evaluating whether the identified fungus/fungi pose a health concern for consumers.

All the isolates were tested for the presence of live fungi by direct plating on DG18 agar as described in the FDA Bacteriological Analytical Manual (BAM). The DNA was extracted with the AllPrep Fungal DNA/RNA/ Protein kit following the manufacturer's instructions. Whole genome sequencing was performed using a Nextera XT DNA Library Prep Kit with 2 x150 bp paired-end sequencing on an Illumina NextSeq Sequencer.

Twenty-two fungal isolates from moldy seedless grapes, walnut halves, apples, and

peanuts from the Washington D.C. area were sequenced. The sequences were identified to the species level, using an MLST and custom kmer database, as *Aspergillus*, *Alternaria*, and *Fusarium* species. The raw sequenced data and some of the draft genomes were submitted in NCBI under BioProject PRJNA482816.

There are only a few publicly available fungal genomes sequences from food isolates. High-quality newly generated whole genome sequences can provide a quick and accurate answer to epidemiological questions and can be used as a tool for identification and determination of their pathogenic potential.

19. Evaluation of Nanopore sequencing for fast determination of plasmids and virulence markers

Authors: Narjol Gonzalez-Escalona, Marc A. Allard, Eric W. Brown, and Maria A. Hoffmann

Plain Language Synopsis: We tested the availability of nanopore sequencing for fast determination of virulence markers, antimicrobial genes, and plasmids in STECs

Abstract:

Shiga toxin-producing *Escherichia coli* (STEC) of serotype O26:H11/- is the second most important hemolytic uremic syndrome producing *E. coli* worldwide. Sequencing of STECs in outbreak situations is usually performed with the Illumina MiSeq platform using Nextera XT to create the DNA libraries. Nextera XT is a simple and straightforward library preparation method, and the sequencing results are optimal for determining phylogenetic relationships among bacteria. However, when compared to reference genomes it is evident that some portions of the genome are not included or underrepresented in the final library. Additionally, the shotgun sequencing technology results in draft assemblies usually encompassing > 300 contigs for STECs. Thus, much of the bacterial genome is missing or fragmented. The missing data in the final draft assemblies could be crucial for certain uses: 1) plasmid content, composition, and size determination, 2)

phages carrying shiga toxin genes and other virulence markers, and 3) comprehensive virulence marker determination. To resolve this issue, we evaluated the use of MinION nanopore – an alternative sequencing method that produces complete closed genomes. Using this technology, we sequenced the complete genomes of three STEC O26:H11 strains belonging to two different sequence types (ST21 and 29). The data produced was enough to obtain a complete closed genome, consisting of a single chromosome of 5.7 Mb and two plasmids of 95 and 72 Kb for ST21 and a single plasmid of 105 Kb for the ST29 strain. Using these data, we rapidly characterized the virulome and documented the presence of antimicrobial genes in the three strains. The MinION results were compared to the MiSeq-Nextera XT results for accurate and inclusive determination of the virulome of these three strains and found that in every strain MiSeq-Nextera method failed to identify some virulence genes that were not missed by MinION sequencing. The missed genes were in plasmids and the chromosome, highlighting the downside of the Nextera XT method. Additionally, the results obtained with MinION were comparable to the information obtained using the PacBio data. The correlation between the two long read methods for determining plasmids, virulome, antimicrobial resistance genes, and phage composition in STEC O26 strongly indicates that the MinION sequencing technology is an excellent solution for rapidly determining STEC O26 closed genomes and comprehensive analysis of their genomics markers.

20. A genome-recovery pipeline towards the genomic epidemiology of the foodborne parasite *Cyclospora cayetanensis*

Authors: Gopal R. Gopinath; Mark Mammel; Helen R. Murphy; Chaeyoon Lee; Maria S. Almeria; Mauricio Durigan; Laura Ewing; Ben D. Tall; Françoise Thibaud-Nissen*; Alexandre J. da Silva and Hediye N. Cinar

Plain Language Synopsis: A high-quality reference-genome-assembly of *Cyclospora cayetanensis* has been generated using

bioinformatic analyses of sequence datasets from cyclosporiasis patients stool samples. A genome-recovery workflow has been developed to generate new genome assemblies using this bioinformatic resource. This will strengthen the identification and source-tracking of *C. cayetanensis* during investigations of foodborne illnesses.

Abstract:

The foodborne coccidian parasite *Cyclospora cayetanensis* causes an intestinal illness called cyclosporiasis. Lack of established bioinformatic tools for genome recovery and a reliable reference genome for comparison limit isolate-identification and sub-typing critical for source-tracking during outbreak investigations. To recover *C. cayetanensis*-specific genomic data enmeshed in complex metagenomic reads, an in-house bioinformatic workflow was developed and tested with WGS datasets recovered from four clinical samples. Initially, 90 million reads obtained from a stool sample NF1 were mapped to a publicly available *C. cayetanensis* assembly. About 10% of these reads were recovered and confirmed to be specific to *C. cayetanensis*; these reads were used to build an annotated 44 MB de novo assembly with SPAdes and AUGUSTUS. Subsequently, WGS datasets from samples C5, C8 and C10 were processed using NF1 as the template and submitted to the CycloTrakr project [BioProject: PRJNA357477] at NCBI. A significant level of underlying intra-species SNP differences between these four genomes and 12 public assemblies were observed by kSNP3, k-mer based genomic conservation, and Mash-based distance analyses. Uniformity in the quality of genome assemblies and sufficient coverage are critical for genome comparisons and phylogenetic studies, and could be achieved using a reference genome assembly. Towards this, high-quality reads that originally generated individual NF1 and C8 assemblies were combined to create a 44.4 MB and 738 contig assembly. The *C. cayetanensis* reference CcayRef3 [RefSeq: GCF_002999335.1] was annotated with the

NCBI Eukaryotic Genome Annotation Pipeline. Homologs of about 5000 *Eimeria* spp. and *Toxoplasma gondii* proteins, and many Apicomplexan core proteins from CEGMA were identified in the reference genome. CcayRef3-based genome recovery workflow improved the genome build quality in terms of contig lengths, gene prediction, and annotations in existing and new assemblies. Phylogenetic analysis based on the genomes from different geographical locations and using conserved exons derived from the CcayRef3 suggested *C. cayetanensis* to be a parasitic organism with previously unidentified emerging genomic diversity. The genome recovery workflow coupled with an annotated reference genome CcayRef3 from this study will open a new chapter in the molecular epidemiology of *C. cayetanensis* towards better identification and source-tracking strategies to strengthen investigations during cyclosporiasis outbreaks.

21. Survival Study of *Salmonella enterica* in Inoculated Pistachios

Authors: Julie Haendiges, NSF International/FDA/CFSAN; Jie Zheng, FDA/CFSAN; Elizabeth Reed, FDA/CFSAN; Nathan Anderson, FDA/CFSAN; Susanne Keller, FDA/CFSAN; Quincy Suehr, FDA/CFSAN; Jesse D. Miller, NSF International; and Maria Hoffmann FDA/CFSAN

Plain Language Synopsis: In this study, we investigated the survival of *Salmonella enterica* in pistachios. Over a one-year period, samples of inoculated pistachios were tested as well as metagenomic sequenced to look at the persistence of the strains. Our study shows that *Salmonella* can persist in pistachios over time.

Abstract:

Background: Recently, outbreaks of *Salmonella enterica* in low-moisture foods have been increasing. Pistachios have been associated with multiple outbreaks and product recalls due to contamination with *Salmonella enterica*. Two serovars, Montevideo and Senftenberg, have been

responsible for multiple contamination events since 2008.

Purpose: The objective of the study is to evaluate how long *Salmonella enterica* can survive in pistachios at different relative humidities and to look for differences between serovars.

Methods: Pistachios were inoculated with either the *Salmonella* cocktail containing serovars Montevideo, Senftenberg, Anatum, Oranienberg, and Newport or the individual strain by the soak method. The pistachios dried overnight in a hood at room temperature and then were stored at different humidities (30% or 60% RH) at 25°C over a nine-month period. At different time points, triplicate 10g samples were enumerated by first macerating in a 1:9 dilution of Buffered Peptone Water and then directly plating on mTSAYE to assess the total *Salmonella* counts. DNA was extracted to perform metagenomics and molecular serotyping.

Results: The direct plating showed the cocktail inoculation has a reduction of 2.19 (30% RH) and 3.35 log CFU/g (60% RH) after nine months of storage with a reduction of 1.19 log CFU/g after desiccation. Differences in the log CFU between the samples stored at 30% and 60% RH were observed after six months of storage. The single strain inoculation showed that serovar Anatum has less potential for survival with a 2.42 (30% RH) and 2.92 log CFU/g (60% RH) reduction. Serovar Oranienberg also showed lower potential for survival when compared to the others (Montevideo, Senftenberg, and Newport).

Conclusions: This study clearly shows that different serovars of *Salmonella enterica* are able to survive in pistachios over an extended period of time. These findings indicate a potential risk if the product becomes contaminated during harvest or processing.

22. **Molecular and genomic analyses of *Cronobacter sakazakii* isolated from plant-origin foods using microarray, whole genome sequencing, and zebrafish infection models demonstrate relevance to clinically virulent sequence types and phylogeny**

Authors: Jang, Hyein, FDA/CFSAN; Eshwar, Athmanya, University of Zurich; Gopinath, Gopal, FDA/CFSAN; Gangiredla, Jayanthi, FDA/CFSAN; Patel, Isha, FDA/CFSAN; Beaubrun, Junia, FDA/CFSAN; Chase, Hannah, FDA/CFSAN; Addy Nicole, FDA/CFSAN; Ewing, Laura, FDA/CFSAN; Neg

Plain Language Synopsis: The objective of this project was to develop highly discriminatory molecular methods for the characterization of pathogenic strains of *Cronobacter* species. Accurate identification and characterization of *Cronobacter* support FDA's ability to protect the food supply by distinguishing, identifying, and tracking of microbial pathogens that cause foodborne illness.

Abstract:

There is a growing body of evidence suggesting that eukaryotic plants may be the ancestral host for *Cronobacter* species. *Cronobacter sakazakii* (Csak), the primary pathogen, continues to be isolated from ready-to-eat produce, flours, cereals, nuts, and spices; several surveillance studies have shown that Csak can also contaminate food manufacturing environments, posing a risk to susceptible consumers. To date very little is known about the phylogenomic and virulence traits possessed among plant-associated Csak strains. To understand the phylogeny and virulence of plant-associated Csak strains using molecular and genomic analyses. Ninety-two Csak strains were obtained from various plant-derived foods and food manufacturing environments located in the USA, Middle East, Asia, and Europe. The strains were characterized using PCR, DNA microarray (MA), multi-locus sequence typing (MLST), and whole genome sequencing (WGS) analyses. WGS was conducted using Illumina's MiSeq platform and Nextera XT library kit. To assess virulence, Zebrafish infectivity (ZI) studies were performed on

selected strains. PCR analysis showed that the strains possessed the virulence plasmid, pESA3, and 99% (91/92) were positive for the Cronobacter plasminogen activator gene. Twenty percent (19/92) of the strains possessed the filamentous hemagglutinin gene cluster. Combinatorial MLST and MA analyses showed that these strains clustered according to ST with more than 20 STs among six phylogenetically-related clades including the malonate-positive ST64 and clinically relevant ST1, ST4, ST8, ST12, and ST13 clones. WGS analysis resulted in comparative annotations, clarified genome-wide nucleotide polymorphisms, and elucidated distinct clades representing strain clusters from different sources. ZI studies showed that the plant-associated Csak strains are as virulent as other Cronobacter species. Finding virulent Csak strains of clinically relevant STs, which were associated with plant-based foods, suggests that these foods can serve as potential transmission vehicles and supports widening the scope of continued surveillance of this important foodborne pathogen.

23. Application of Metagenomics to Define Microbiomes and Detect Listeria monocytogenes in Smoked Fish and Ice Cream Facilities

Authors: Kocurek, Brandon, FDA/CFSAN; Jarvis, Karen, FDA/CFSAN; Grim, Christopher, FDA/CFSAN; Morin, Paul, FDA/ORR; Howard, Laura FDA/ORR; Ottesen, Andrea, FDA/CFSAN; Timme, Ruth, FDA/CFSAN; Ramachandran, Padmini, FDA/CFSAN; Leonard, Susan, FDA/CFSAN; Rand, Hugh F

Plain Language Synopsis: Metagenomic sequencing permitted Listeria monocytogenes subtyping with equivalent resolution to WGS of individual isolates given adequate sequencing coverage. We also demonstrated that culture-based environmental sampling enrichment methods may under-report Listeria monocytogenes contamination in food manufacturing facilities.

Abstract:

Introduction: Current environmental sampling practices involve targeted culturing procedures. Metagenomics can provide accurate and unbiased analyses that reveal the entire microbiome including pathogens and commensals.

Purpose: This work investigated the microbiomes of environmental swab culture enrichments from smoked fish and ice cream manufacturing facilities to assess the efficacy of culture-based environmental sampling workflows for *Listeria monocytogenes*.

Methods: 16S rRNA gene sequencing was performed on 48 environmental swab cultures, 24 from each food processing facility. Shotgun metagenomic sequencing was performed on 7 of the 24 swabs from the ice cream facility and 6 of the 24 swabs from the smoked fish facility.

Results: Both facilities harbored similar taxa, e.g. *Enterococcus*, *Pseudomonas*, *Lactococcus*, but distribution and abundance of these taxa varied across sampling sites. 16S rRNA gene sequencing of swabs from the smoked fish and ice cream facilities demonstrated 100% (16/16) and 60% (3/5) congruence to culture-based detection results for the presence of *Listeria monocytogenes*, respectively. Conversely, 38% (3/8) and 52% (10/19) of samples that were negative for *L. monocytogenes* by culture from the smoked fish and ice cream facilities, respectively, yielded appreciable 16S rRNA reads specific to *L. monocytogenes*, with relative abundances ranging from <1% to 14%. However, this may be confounded due to the presence of *L. innocua* in seven ice cream swabs. Comparing whole genome sequencing (WGS) data from isolated colonies of *L. monocytogenes* from each facility revealed that shotgun metagenomic reads resolved to within 0-8 SNPs. *L. monocytogenes* from the ice cream and the smoked fish facilities showed the presence of different *L. monocytogenes* lineages, I and II, respectively.

Significance: This study demonstrated

that current culture-based environmental sampling enrichment methods may under-report *Listeria monocytogenes* contamination in food manufacturing facilities. Additionally, we show that shotgun metagenomic sequencing can be used for pathogen subtyping and forensic disposition with equivalent resolution to isolate WGS given adequate sequencing coverage is achieved.

24. Immune checkpoint receptor LILRB4: a new player in regulating viral infection

Authors: Lee, Ha-Na, FDA/CDER/OBP/DBRR-III/COE in Infectious disease and Inflammation; Manangeeswaran, Mohanraj, FDA/CDER/OBP/DBRR-III/COE in Infectious disease and Inflammation; Ireland, Derek, FDA/CDER/OBP/DBRR-III/COE in Infectious disease and Inflammation; Le

Plain Language Synopsis: Blocking immune checkpoints is emerging as a novel therapeutic approach for treating chronic infectious diseases, however, its risk is also concerned. Our study demonstrates the critical role of immune checkpoint LILRB4 in regulating immune responses against viral infection, and the potential risk of checkpoint inhibitors in treating virus-associated diseases.

Abstract:

Immune cells express checkpoint molecules that regulate their function. Blocking selected checkpoints that downregulate immune responses is revolutionizing cancer therapy and recent studies suggest they could be used to enhance the immune response to chronic infections. However, downregulation of the response is critical for reducing inflammation and maintaining tissue integrity. Leukocyte Immunoglobulin-Like Receptor B4 (LILRB4), is an inhibitory receptor expressed predominantly in antigen-presenting cells that downregulates immune cell activation, with no known role in immune responses against viral infection. Assessment of mRNA expression in the brain of mice infected with Zika virus (ZIKV) showed a significant increase in LILRB4 suggesting that it could play a role in regulating the neurological

damage induced by the virus. In the infected brains LILRB4 was predominantly expressed by infiltrating myeloid cells and microglia. Whereas C57BL/6 mice challenged with ZIKV have transient neurological symptoms and survive the infection, mice lacking LILRB4 (LILRB4 KO) exhibited more severe signs of neurological disease including ataxia, hind limb paralysis and seizures, had higher viral burden in the central nervous system, blood and kidney, and succumbed to disease 18-22 days post infection. The increased expression of LILRB4 was partly dependent on the type I interferon (IFN) expression levels. Further, LILRB4 negatively regulated ERK-dependent activation of microglia and macrophages in response to IFN γ , and within this context, its deficiency resulted in hyperactivation of innate immune system in the brain during ZIKV infection. LILRB4 KO mice also showed reduced activation of NK and T cells which may contribute to the impaired clearance of virus. Taken together, our data suggest that type I IFNs induced in response to virus increase the levels of LILRB4-expressing myeloid cells and play a key role in regulating host immune responses against ZIKV infection, thereby limiting viral replication and virus-induced immunopathology. These findings highlight the critical role of checkpoint inhibitors in regulating the immune response and indicate that checkpoint inhibitors that target myeloid cells might pose a potential risk in the context of virus-associated diseases.

25. Immune suppression following radiation exposure in a mass casualty scenario can be alleviated with nicotine products that increase population of cells in first line of defense against pathogens

Authors: Lehtimaki, Mari, FDA/CDER; McFarland, Hugh, FDA/CDER; Gandhi, Deep, FDA/CDER; Surujdin, Ryan FDA/CDER; Rosenberg, Amy, FDA/CDER

Plain Language Synopsis: Radiation exposure in mass casualty situations exposes victims to infections. We have tested nicotine patch applied the day after the radiation incident in a mouse model as a potential treatment. The

nicotine patch treatment reduces the impact of infections a month after the radiation incident.

Abstract:

Radiation exposure in mass casualty situations, such as nuclear reactor melt downs or detonation of nuclear devices, leads to immune suppression, which can expose individuals to infections despite previous immunity provided by vaccinations. Treating mice 24 hours post-irradiation with subcutaneous nicotine in TiterMax -adjuvant or transdermally as a patch, improves their ability fight off wild type *L. monocytogenes* (LM) challenge 4 weeks after the treatment.

In our mouse model, animals vaccinated with attenuated LM lose protective T memory responses following sub-lethal 6 Gy irradiation. Animals treated with nicotine products 24 hours later were evaluated for burden of LM in spleen after challenge with wild type LM. Immune cell profiles were evaluated from mouse spleens at a time point close to the challenge. We used the FlowSOM algorithm to construct self-organizing maps of differentially stained cell populations and arranged them into minimal spanning trees based on similarity in staining. This allowed us to evaluate changes in populations of interest at a single glance at the time of challenge.

We found that re-vaccination with an attenuated strain of LM 1-2 days post-irradiation protects mice from a lethal wild-type LM challenge. Re-vaccination, used here as a positive control for rescue of the immune response to LM, leads to increased populations of LM -specific memory cells as well as increase in granulocyte populations. This is mimicked by 1/8 nicotine patch treatment and subcutaneous injections containing TiterMax emulsion. FlowSOM algorithm-derived minimal spanning trees show that neutrophils and macrophages are predominantly affected by the treatments. The level of effect differs among treatments: 1/8 nicotine patch increases neutrophils, while re-vaccination affects both neutrophils

and macrophages; TiterMax containing injections increase the proportion of both neutrophils and macrophages among total splenocytes.

Nicotine patch, TiterMax, and re-vaccination all seem to have a long-term effect on granulocyte population percentages as the effect of treatment can be detected 22 days after radiation and treatment administration. The effect of nicotine treatment on the innate immune system likely contributes to protection against wild type LM challenge after total body irradiation and offers a potentially cost-effective treatment to radiation affected populations.

26. Platform extension of a real-time PCR method from SmartCycler to Applied Biosystems 7500 Fast for the detection of prohibited materials in animal feeds

Authors: Liu, Kun, FDA/ORR; Pires, Gabrielle, FDA/ORR; Furseth, Heidi, FDA/ORR; Jinneman, Karen, FDA/ORR.

Plain Language Synopsis: The study was to extend the instrument platform of a method used in the animal feed program. Data generated on the reference platform and the new platform were compared. Results showed slightly improved performance on the new platform and supported the instrument platform extension.

Abstract:

A SYBR Green-based simplex real-time Polymerase Chain Reaction (rt-PCR) method is currently used in FDA's Bovine Spongiform Encephalopathy (BSE) compliance program. Unfortunately, the SmartCycler platform was recently discontinued by its manufacturer. In our study, performance of this rt-PCR method was compared between SmartCycler and Applied Biosystems 7500 Fast (AB7500F) platforms. The purpose was to extend the instrument platform to the AB7500F and to enable FDA laboratories to continue using rt-PCR for identifying prohibited materials in animal feeds. A total of 321 DNA templates from bovine, caprine, and ovine species as well as matrix blank controls were tested on both rt-PCR platforms. The false

positive rates for bovine, ovine, and caprine identification were 2.2%, 3.3%, and 2.8% on the AB7500F platform, respectively; whereas they were 6.7%, 6.7%, and 6.4% on the SmartCycler platform, respectively. The false negative rates for all three species were 0% on the AB7500F; whereas on the SmartCycler, the false negative rate was 1.7%, 0%, and 1.3% for bovine, ovine, and caprine identification, respectively. Both platforms were able to detect DNA of prohibited materials at the limit of detection (LOD), which were 0.0001% (v/v) of DNA extracted from 0.25 grams bovine or ovine meat and bone meals, 0.01% of DNA extracted from 0.25 grams caprine meat meal, and 0.0001 µg or greater of caprine gDNA spiked feed matrices (0.25 grams feed per sample). Results showed that there was no significant difference between the AB7500F and the SmartCycler platforms. The false positive and false negative rates were slightly improved on the AB7500F compared to the SmartCycler. In addition, there was not an observed significant difference between AB7500F software versions v1.4 and v2.3. Our results support instrument platform extension of the BSE simplex rt-PCR to the AB7500F system.

27. Whole-Genome Sequence-based taxonomy of *Citrobacter* species, with particular consideration of *C. werkmanii*

Authors: Lomonaco, Sara, FDA/CFSAN; Lascols, Christine, IHRC/CDC/NCEZID; Crawford, Matthew A, UVA/Department of Medicine; Anderson, Kevin, DHS/S&T; Hodge, David R, DHS/S&T; Pillai, Segaran P, FDA/OC; Morse, Stephen A, IHRC; Khan, Erum, Aga Khan University/Department

Plain Language Synopsis: Fourteen *Citrobacter* species are currently recognized, with several that include emergent antibiotic-resistant strains. Bioinformatics-based methods using Whole-Genome Sequence (WGS) data offer improved bacterial species discrimination. However, in some instances databases may list incorrect taxonomic classifications. We used WGS data to evaluate the taxonomic positions of 249 publicly reported *Citrobacter* strains.

Abstract:

Background: Fourteen *Citrobacter* species are currently recognized, several of which are associated with human infections and increasingly observed as antibiotic resistant. Limited species discrimination is provided by MALDI-TOF, DNA-DNA hybridization, and 16S rRNA sequencing. Recently, however, Multilocus Sequence Analysis (MLSA), *recN* gene sequencing, core genome MLST (cgMLST), and Average Nucleotide Identity (ANI) have improved species discrimination.

Purpose: Accurate species identification is important to support epidemiologic investigations, design/test diagnostic platforms, and devise infection control/treatment strategies. We aimed to assess the accuracy of the taxonomic classification of publicly reported *Citrobacter* genomes.

Methodology: 16S rRNA gene sequences, MLSA, *recN*, cgMLST, and ANI were used to evaluate the taxonomic positions of 249 *Citrobacter* strains by comparison to reference strains representing the 14 *Citrobacter* species.

Results: The 249 *Citrobacter* strains were divided into three groups based on ANI values derived by comparison to 14 *Citrobacter* reference strains. The first group (ANI \geq 97%) included 225 strains for which the high ANI values was deemed to provide a reliable species identification. The second group (ANI between 95-97%) included 14 strains, 10 of which were originally described as *C. werkmanii*. However, these latter strains each had ANI values of 96.1% as compared to the *C. werkmanii* type strain, and thus could be considered at the limit for species identity. cgMLST grouped these 10 strains together but separated them from the *C. werkmanii* cluster, including the reference strain for this species. Equivalent findings were obtained using *recN* and MLSA analyses. Finally, the third ANI-based group (ANI <95%) contained 10 strains, with the highest observed ANI of 93.56%, and the remaining values between 92-93% and ~83%, all below the proposed threshold of 95-96% for taxonomically

defining prokaryotic species.

Conclusions: Further studies will be needed to clarify whether the 10 “*C. werkmanii*” isolates that form a separate group represent a different clade within this species, a subspecies, or a distinct, but closely related species. As each of these organisms only shares a ~96% ANI value with the *C. werkmanii* type strain, a minimum ANI of 97% may be the threshold to positively delineate the species of *Citrobacter* isolates.

28. Evaluation of Apparatus used to Test Liquid through Protective Materials: Comparison of a modified dot-blot apparatus to the ASTM penetration cell

Authors: Schwerin, Matthew, FDA/CDRH; Das, Srilekha, FDA/CDRH; Woods, Terry, FDA/CDRH; Wood, Steven, FDA/CDRH; Lucas, Anne, FDA/CDRH

Plain Language Synopsis: Personal protective equipment (PPE) are in the first line of defense for healthcare workers who come in contact with deadly viruses. There are archaic methods to test PPEs. This study evaluated gowns for their ability to resist clinically relevant test soil in the standard ASTM apparatus and in a modified dot-blot.

Abstract:

Personal protective equipment (PPE), such as gowns used in the latest Ebola outbreak in western Africa, are critical in preventing the spread of deadly diseases. Appropriate test systems and test soils are needed to adequately evaluate PPE. ASTM test method F903 Standard Test Method for Resistance of Materials used in Protective Clothing to Penetration by Liquid has been used for decades to test fabrics’ resistance to liquid penetration. However, this test apparatus requires at least 60 mL of test solutions, is labor intensive, and has problems with leakage around the gaskets. We compared the F903 test apparatus to a modified dot-blot apparatus to evaluate the visual penetration of a blood test soil. A series of commercially available gowns and drapes were tested in each apparatus. Using blood test soil at 2 psi, there was no statistically significant

difference between the two methods except for one gown. By comparing this gown in the ASTM test apparatus with and without a screen, the particular screen selected did not account for the difference between the dot-blot and F903 apparatus; however, it is conceivable that a particular screen/fabric combination could account for this difference. The modified dot-blot apparatus was evaluated using three different test solutions: blood, vomit, and a labeled protein (GaR IgG-HRP) in a blood test soil solution. This testing revealed significant difference in penetration for some of the PPE garments. The modified dot-blot had several large advantages over the ASTM apparatus – over six times less sample volume and no edge or gasket leakage. In addition, nitrocellulose can be easily incorporated into the modified dot-blot apparatus, enabling the trapping of viruses and proteins that penetrate PPE – thus, permitting the use of antibodies to quickly and sensitively detect penetration.

29. Establishment of 232 Salmonella Reference Genomes from PacBio Sequencing Data for SNP Cluster Construction

Authors: Luo, Yan, FDA/CFSAN; Kwan, Yao, FDA/CFSAN; Sanchez, Maria, FDA/CFSAN; Rand, Hugh, FDA/CFSAN; Cao, Guojie, FDA/CFSAN; Miller, Daniela, FDA/CFSAN; Turuvanda, Tim, FDA/CFSAN; Timme, Ruth, FDA/CFSAN; Tallent, Sandra, FDA/CFSAN; Allard, Marc, FDA/CFSAN; Brown,

Plain Language Synopsis: 232 isolates spanning the diversity of *Salmonella* have been whole genome sequenced, using the third generation long reads sequencing technology to enrich the NCBI high quality reference genome database, which can provide high resolution and rapid contamination source identification for the outbreak investigations.

Abstract:

Salmonella is a common foodborne pathogen associated with a wide range of products, including poultry, beef, fresh produce, seafood, and various processed foods, making pathogen surveillance and tracking

both important and challenging. In 2012, the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition (FDA-CFSAN) launched the GenomeTrakr network to collect whole genome sequencing (WGS) data from foodborne pathogens to provide superior resolution for isolate typing, outbreak detection, surveillance, source tracking, and drug resistance monitoring. This network submits WGS data to the short-read archive (SRA) at NCBI for processing through the NCBI's Pathogen Detection analysis pipeline; WGS data is used to relate isolates by phylogenetic inference based on single nucleotide polymorphisms (SNPs). This requires the construction of a reference-based SNP matrix, in which a high-quality draft assembly or a closed genome is required for read mapping. The vast majority of the ~ 200,000 genomes submitted for Salmonella are short-read Illumina data and only provide a draft assembly with many contigs (usually 30-300). Thus, there is a need for high-quality reference genomes spanning the diversity of Salmonella that can be used for validation and detailed genetic studies. Therefore, we have started a project to provide long read sequencing data using Pacific Biosciences (PacBio) Sequel platform (V2.1 chemistry, Sequel SRMT cell 1M v2, 10-hour movie), and report on results for 232 isolates here. The PacBio SRA data was submitted to NCBI and de novo assemblies were generated using the PacBio Hierarchical Genome Assembly Process (HGAP) 4.0. We have sequenced 232 Salmonella isolates, representing 178 identified clusters and 32 unclustered isolates that are greater than 50 SNPs from any other currently available genome at the NCBI Pathogen detection website. We were able to completely close 202 Salmonella genomes and the remaining 30 Salmonella genomes resulted in 2 to 5 contigs with an average coverage of 240X. Adding high-quality reference genomes to the NCBI database will help to identify novel plasmids and genomic islands, shed insight on the evolution of antibiotic resistance and virulence, and supplement the construction of SNP matrices, generating more accurate

phylogenetic analyses and supporting outbreak investigations.

30. Development of an immunocompetent mouse model for Dengue virus disease

Authors: Manangeeswaran, Mohanraj; Chowdhury, Monica; Lee, Ha-Na; Ireland, Derek; Lewkowicz, Aaron; McWilliams, Ian; Engel, Kaliroi; Verthelyi, Daniela. Laboratory of Immunology, DBRR-III, OBP, CDER and Center of Excellence in Infectious disease and Inflammation.

Plain Language Synopsis: Dengue virus (DENV) infects 400 million people worldwide annually. Lack of immunocompetent mouse model hinders the development of DENV-specific therapeutics and vaccines. To address this critical unmet need, we report the development of a new mouse model to study DENV disease.

Abstract:

Dengue virus (DENV) is an arboviral disease that infects over 400 million people worldwide every year. While most 1st infections are low grade, second infections with a heterologous DENV can result in severe disease, encephalitis and death through a hypothesized mechanism known as antibody (Ab)-dependent enhancement (ADE). There are no approved effective therapeutics and no licensed vaccines for dengue virus disease. There are no immunocompetent mouse models available to understand host-pathogen interactions, establish determinants of disease, or explore risk factors for ADE. To address this unmet need, we developed an immunocompetent neonatal mouse model in C57BL/6 mice using DENV serotype 2, New Guinea C strain (DENV2). The DENV2 infected mice fail to thrive, and develop lethargy, ataxia, and tremors around 6- 9 dpi and approximately 50% of the infected mice succumbed to disease by 10-12 dpi. The mice have a viremia followed by high levels of virus in the CNS and eyes starting 3 days post infection (DPI). The infection in brains and eyes is associated with significant increase in mRNA expression for interferon-inducible genes (BST2, STAT1&2,

IRGM1, IFIT2, IRF7, IFI35), RIG-I-Like receptors (DDX58 and IFIH1), chemokines (CXCL10, CCL5, CCL12, CCL2) antigen presentation and activation (H2-K1, CTSS, TAP1, TAPBP, PTPRC) complement (C4A, C1QB, C2, C1QA) and FC receptors (FCGR1, FCGR4, FCGR2B) as well as a corresponding reduction in genes related to neuronal function (PCP2, PAX2). Flow cytometry and Immunohistochemistry studies confirm broad areas of inflammation and cellular infiltration in the CNS and eyes. This model will help product developers and reviewers to test the efficacy of immunomodulatory and anti-DENV therapeutics and to improve our understanding of the determinants of increased disease severity following a second heterologous DENV infection or vaccine.

This work was performed under the auspices of the MCMi program and the Center of Excellence in Infectious Diseases and Inflammation.

31. Meningococcal vesicle vaccines deleted for major outer membrane proteins enhance gonococcal clearance in a murine model

Authors: Matthias, Kathryn A., FDA/CBER; Connelly, Kristie L., USUHS; Begum, Afrin A., USUHS; Jerse, Ann E., USUHS; Macintyre, Andrew N., Duke University School of Medicine; Sempowski, Gregory D., Duke University School of Medicine; Gao, Yamei S., FDA/CBER; Bash,

Plain Language Synopsis: *Neisseria gonorrhoeae*, the causative agent of gonorrhea, has a large impact on global morbidity. Resistance has developed to all antibiotic classes and no vaccine currently exists. Here, we demonstrate that outer membrane vesicles isolated from *N. meningitidis* that are deleted for major variable proteins function as an effective gonococcal vaccine.

Abstract:

Background: With an incidence rate of 106 million infections a year, *Neisseria gonorrhoeae* has a significant effect on global morbidity. Rapid development of gonococcal antibiotic resistance, and reports of treatment

failures with last-line cephalosporins, has caused the Centers for Disease Control and Prevention to label *N. gonorrhoeae* as an urgent threat and has sparked renewed interest in development of a gonococcal vaccine. Methods: In this study, we immunized mice with detoxified outer membrane vesicles (dOMVs) isolated from the closely-related pathogen *Neisseria meningitidis* and examined the effect on gonococcal clearance in a murine vaginal colonization model. dOMV vaccines were derived from (1) wild type (WT) bacteria, (2) an isogenic strain (ABR-) deleted for expression of the major outer membrane proteins PorA, PorB, and RmpM, or (3) an isogenic strain (OCh) deleted for PorA and expressing a varying PorB sequence type relative to the parental strain. ELISAs were used to evaluate anti-dOMV IgG and IgA antibody titers present in sera and vaginal washes. Sera were also used to identify potential gonococcal vaccine antigens using immunoblot and immunoprecipitation experiments. Results: Although vaccination with WT dOMVs significantly enhanced gonococcal clearance relative to adjuvant-only controls, vaccination with ABR- dOMVs resulted in clearance of a higher percentage of mice relative to WT dOMV-vaccinated mice one week post-colonization. Higher levels of clearance in ABR- dOMV-immunized mice correlated with significantly increased IgA titers and enhanced immunogenicity of unique meningococcal protein antigens. Conclusion: Immunization with meningococcal dOMVs deleted for PorA, PorB, and RmpM promotes gonococcal clearance in a murine model. Deletion of the major porins likely enhances immunogenicity of proteins that are less abundant on the meningococcal surface but exhibit a high degree of homology with corresponding gonococcal proteins, suggesting the potential utility of these dOMVs as a broadly cross-protective *Neisseria* vaccine.

32. Validation of BSL-2 model of Ebola virus infection to test preclinical efficacy and safety of therapeutics targeting the Ebola glycoprotein

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Plain Language Synopsis: There is critical need for developing therapeutics for Ebola virus (EBOV) infections. Pre-clinical models can facilitate product selection, development, and regulation, but the need for high-containment of EBOV hinders development. These studies establish a novel mouse model that can be used to test therapeutics that target the viral protein used to target and infect cells using low level containment facilities.

Abstract:

The current Zaire Ebola virus (EBOV) outbreak is already the second largest in recorded history, and with no approved therapeutics or vaccines to treat afflicted patients, developing tools to facilitate therapeutic discovery and testing is urgent. Most candidate therapeutics target the EBOV glycoprotein (GP), which determines the viral tropism. We developed an accessible EBOV GP centric BSL-2 animal model where a single inoculation of rVSVΔG-EBOV-GP pseudovirus in neonatal C57BL/6 mice results in transient viremia, progressive neurological symptoms, and death 10-15 days post infection (DPI). The disease time-course allows for testing of therapeutics for efficacy and safety in the context of infection. Here we seek to validate use of the model in product development by testing an anti-EBOV GP therapeutic (SAB-139) previously shown to be efficacious in nonhuman primate models to counteract BSL-4 Ebola virus infection. We find that SAB-139 improves survival in infected mice at -1 (pre-treatment), 1, and 3 DPI, however, mice treated 5 DPI resemble control antibody treated animals. Mice

treated with SAB-139 at 3 DPI have decreased severity of neurological symptoms and improved weight gain compared to control-treated mice. Additionally, we find that viral titers are reduced in the eye, brain, and spinal cord 3 and 6 days post-treatment (6 and 9 DPI equivalent) in these animals. Together, these data suggest that the neonatal rVSV-EBOV-GP infection system can be used to assess pre-clinical therapeutics.

33. Babesiosis Occurrence among the Elderly in the United States: Overall, by State and County of Residence, as Recorded in Large Medicare Databases During 2006-2017

Authors: Menis, Mikhail, FDA/CBER; Forshee Richard A, FDA/CBER; Whitaker, Barbee I, FDA/CBER; Leiby, David A, FDA/CBER; Jiao, Yixin, Acumen LLC; Xu, Wenjie, Acumen LLC; Hu, Mao, Acumen LLC; McKean, Stephen, Acumen LLC; Warnock, Rob, Acumen LLC; Kelman, Jeffrey A,

Plain Language Synopsis: This 12-year study shows significantly increasing babesiosis occurrence among the U.S. elderly during 2006-2017, with highest rates in babesiosis-endemic counties and states. The study also shows variation in babesiosis occurrence by age, gender, race, and diagnosis months likely related to tick exposure, and suggests babesiosis infection spread to non-endemic states.

Abstract:

Background: Human babesiosis is caused by intraerythrocytic protozoan parasites of the genus *Babesia*. *Babesia* infection can be an asymptomatic or mild-to-severe disease that may be fatal.

Purpose: The study objective was to assess incident babesiosis occurrence among the U.S. elderly Medicare beneficiaries, ages 65 and older, during 2006-2017.

Methodology: Our retrospective claims-based study used large Medicare administrative databases. Babesiosis cases were identified based on the recorded diagnosis codes. The study assessed babesiosis occurrence rates (per 100,000 elderly Medicare beneficiaries)

overall and by year, diagnosis month, demographics, state and county of residence. The Cochran-Armitage test for trend was used to ascertain babesiosis occurrence trends by calendar year and age.

Results: During a 12-year study period, 19,469 elderly Medicare beneficiaries had a babesiosis diagnosis recorded, for an overall national rate of about 6 per 100,000 person-years. The study results showed a significant increase in babesiosis occurrence over time ($p < 0.05$): from 4 per 100,000 in 2006 to 9 per 100,000 in 2017. The highest babesiosis rates by state (per 100,000) were identified in: Massachusetts (62), Rhode Island (61), Connecticut (51), New York (30), New Jersey (19), New Hampshire (12), Maine (11), and Vermont (10). The highest rates by county were identified in: Nantucket, MA (1,089); Dukes, MA (236); Barnstable, MA (213); Dutchess, NY (205); Washington, RI (187); Windham, CT (177); Plymouth, MA (177); and Ulster, NY (151). Seventy-four percent of all cases were diagnosed from May through October. Babesiosis occurrence declined with advancing age and was significantly higher among males vs. females and whites vs. non-whites.

Conclusion: Our study shows significantly increasing babesiosis occurrence among the U.S. elderly during 2006-2017, with highest rates in the babesiosis-endemic counties and states. The study also shows variation in babesiosis occurrence by age, gender, race, and diagnosis months likely related to tick exposure, and a potential spread to states previously considered non-endemic. Overall, our study highlights the importance of large administrative databases in assessing the occurrence of emerging infections in the United States.

34. Evaluation and comparison of Genome-Based Salmonella serotyping methods with Bead-Based Salmonella Molecular Serotyping and traditional methods for Salmonella Isolated from Food and Environmental Samples

Authors: MacMaster, Kayleigh, FDA/ORR;

Madson, Shauna, FDA/ORR; Nucci, Melissa, FDA/ORR; Wagley, Gail, FDA/ORR; Jinneman, Karen, FDA/ORR; Moore, Michelle, FDA/ORR

Plain Language Synopsis: Genome-based Salmonella serotyping tools SeqSero v1, SeqSero v2 and SISTR were compared to bead-based molecular serotyping and traditional serotyping methods in identifying 561 Salmonella isolates. Methods with the best ability to identify to a single serovar were SISTR (98.9%) and combined SeqSero v1 and v2 (92%), compared with traditional methods.

Abstract:

Salmonella serotyping is essential to surveillance and outbreak investigations. There are more than 2,500 Salmonella serovars. The antisera required for traditional serotyping are expensive and those to rare antigens often expire before use. Recent methods have been developed that target the genes encoding the antigens recognized by traditional serotyping following the White-Kauffmann-Le Minor scheme. The goal of this study was to evaluate three genome-based serotyping tools and compare the results to the bead-based Salmonella molecular serotyping (SMS) method and traditional serotyping. In a recent study, the SMS method was evaluated for identifying 568 archival Salmonella isolates from FDA regulatory samples or reference strains. Whole genome sequencing data for many isolates in the SMS study are publicly available in the GenomeTrakr depository at the National Center for Biotechnology Information (NCBI). The sequences were downloaded from NCBI and analyzed by SeqSero v1, SeqSero v2, and Salmonella in silico typing resource (SISTR), and results were compared to SMS and traditional serotyping. A total of 461 (81.2%) of the isolates had sequencing data available. The number of isolates considered correctly identified or as expected were 451 (97.8%) by SMS, 460 (99.8%) by SeqSero v1 and SISTR. The ability of each method to narrow to a single serovar was SISTR (98.9%), SeqSero v1 (69.3%) and SMS (46.2%). Sequences that could not be narrowed by SeqSero v1 were reanalyzed by SeqSero v2, which became

available at the end of 2018. The ability to narrow of combined results of SeqSero v1 and v2 (92.2%), showed improvement using SeqSero2. The results of additional real-time Salmonella isolates will also be presented. Genome-based methods provided improved results over SMS, and were comparable to traditional serotyping, though both SMS and traditional methods were faster. Genome-based serotyping should aid in identifying rough, non-motile, or weakly agglutinating isolates, reducing misidentification.

35. Genetic Analysis of Glucuronate and Galacturonate Utilization in Shigella

Authors: Mukherjee, Amit, FDA/CFSAN; Tartera, Carmen, FDA/CFSAN; Mammel, Mark K, FDA/CFSAN; Gangiredla, Jayanthi, FDA/CFSAN; Lacher, David W, FDA/CFSAN; Patel, Isha R, FDA/CFSAN; Elkins, Chris A, CDC.

Plain Language Synopsis: Metabolic phenotypes have been used to distinguish the four Shigella species. A new phenotype is reported here that shows *S. flexneri* utilized glucuronate and galacturonate but *S. dysenteriae*, *S. boydii*, and *S. sonnei* cannot with a few exceptions. The genetic basis of this phenotypic difference was delineated by genome sequencing.

Abstract:

The traditional method to distinguish the four Shigella species, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* is serotyping, but whole genome sequencing (WGS) is also commonly used. Another approach is the use of metabolic phenotype: for example, *S. dysenteriae* cannot utilize D-mannitol, distinguishing it from the other three species which can. To find more metabolic phenotypes that would differentiate the Shigella species, Biolog phenotypic microarray (PM) was conducted on 149 Shigella isolates to identify phenotypes in carbon source utilization. Of the 190 carbon sources assayed, ten were identified that could potentially differentiate the four species, but here we focus on two hexuronates, glucuronate and galacturonate. The number of isolates that utilized these two

hexuronates are: *S. dysenteriae* (n=2/34), *S. flexneri* (n=45/50), *S. boydii* (n=2/23), and *S. sonnei* (n=0/42). This shows *S. flexneri* can grow on these hexuronates, while less than 10% of *S. dysenteriae* and *S. boydii* and none of the *S. sonnei* isolates can grow on them. Serotype 6 isolates of *S. flexneri* did not grow and they belong to Cluster I along with *S. boydii*. The genes involved in the catabolism of glucuronate and galacturonate are in the *uxuR/uxaR* regulon that has a common transporter (*exuT*) and upon uptake they are isomerized by a common isomerase (*uxaC*). The following steps in both pathways are carried out by a dehydrogenase (*uxuB* and *uxaB*) and a dehydratase (*uxuA* and *uxaA*) converging to form 2-keto-3-deoxygluconate which then enters the Entner-Doudoroff pathway. To investigate the genetic basis, we performed WGS of 72 isolates using the Illumina MiSeq Platform and analyzed the sequences of the above genes of the *uxuR/uxaR* regulon using sequences of *E. coli* K-12 as a reference strain. Different types of mutations from deletions, truncations, and missense mutations were found that accounted for the hexuronate negative phenotype. Missense mutations mostly in *exuT* were identified by genetic complementation. Such different mutations leading to the same phenotype reinforces earlier observation of convergent evolution in Shigella. An outcome of these studies is that these genetic markers can be used to develop molecular assays to differentiate the four Shigella species.

36. Methods to Detect Viable Trypanosoma cruzi in Blended Acai

Authors: Neal-McKinney, Jason, FDA/ORA and Lock, Christopher, FDA/ORA

Plain Language Synopsis: *Trypanosoma cruzi*, the causative agent of Chagas' Disease, can be transmitted by ingestion of contaminated produce. We developed a new method to detect viable *T. cruzi* parasites in blended acai, using density centrifugation, growth in selective media, and detection by qPCR.

Abstract:

Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is usually thought of as a vector-borne disease transmitted by triatomine insects. However, *T. cruzi* can also be transmitted by ingestion of contaminated food and beverages. Several large outbreaks in South America have been attributed to consumption of contaminated produce, such as acai. Triatomine insects infected with *T. cruzi* are known to live among food crops, and contamination of produce during harvest can result in viable parasites being present in food. The only method currently available to detect viable *T. cruzi* in food products requires feeding mice the suspect food item and monitoring for infection. In this study, we sought to develop a method to extract and enrich *T. cruzi* from contaminated acai. Using density centrifugation, we were able to concentrate *T. cruzi* and reduce the amount of acai matrix prior to enrichment. The extracted parasites were propagated in a selective media containing antibiotics for up to three weeks. Quantitative PCR was then utilized to measure the amount of DNA in the enrichment culture and monitor growth of the parasites. We observed an increase in *T. cruzi* DNA after two-three weeks of enrichment. Our results demonstrate that we can grow and detect viable *T. cruzi* in experimentally contaminated acai. We are currently using this method to examine commercial acai products for the presence of *T. cruzi* DNA and viable parasites. In the future, this method will be utilized to determine the ability of *T. cruzi* to survive in different matrices and storage conditions.

37. Vet-LIRN Inter-Laboratory Comparison Exercise to *Campylobacter jejuni* in Dog Feces

Authors: Nemser, Sarah, FDA/CVM; Lindemann, Samantha, FDA/CFSAN; Kmet, Matthew, FDA/CFSAN; Pickens, Shannon, Institute for Food Safety and Health, Illinois Institute of Technology; Ulaszek Jodie, Institute for Food Safety and Health, Illinois Institute of Technology

Plain Language Synopsis: The ability to rapidly detect *Campylobacter jejuni* in dog

fecal samples is important for veterinary diagnostic laboratories. The Veterinary Laboratory Investigation and Response Network funded the development of a rapid detection method. An Inter-Laboratory Comparison Exercise was completed; showing that analysts could successfully identify *Campylobacter jejuni* in dog feces.

Abstract:

The ability to rapidly detect *Campylobacter jejuni* in dog fecal samples is important for veterinary diagnostic laboratories. During 2014 to 2017, the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) funded Texas A&M to develop a rapid Polymerase Chain Reaction (PCR) method to detect *Campylobacter jejuni* in dog feces (Texas method). In 2018, a multi-state outbreak of antimicrobial resistant *Campylobacter* in humans linked infections to contact with puppies from certain pet shops. The Ohio Department of Agriculture used a modified version of the Texas method to test and identify positive dog fecal samples during to the outbreak. Subsequently, Vet-LIRN organized an inter-laboratory comparison exercise (ICE) evaluating the performance of the modified method. The ICE was done in collaboration with the Moffett Proficiency Test Laboratory located at the Institute for Food Safety and Health at the Illinois Institute of Technology (IIT/IFSH) and the FDA Division of Food Processing Science and Technology. In October 2018, twelve blind-coded refrigerated canine fecal samples were shipped to seven laboratories with one analyst per laboratory. All participants detected *Campylobacter jejuni* at inoculation levels of 26,500 and 2,650 CFU/g (Colony Forming Units per gram). Seventy-five percent of analysts reported "suspect" for challenge samples, artificially inoculated at 265 CFU/g. Lower detection rates were expected for the challenge samples. The ICE showed that the modified PCR method provides improved detection of *Campylobacter jejuni*.

38. Non-targeted Analysis of Tattoo Inks using Liquid Chromatography High Resolution Mass Spectrometry (LC/HR-MS) and Nuclear Magnetic Resonance (NMR) Spectroscopy

Authors: Fisher (O'Donnell), Christine M., FDA/CFSAN; Ridge, Clark D., FDA/CFSAN; Donnelly, Sarah E., FDA/CFSAN; Kneapler, Caitlin N., FDA/CFSAN; Fardin Kia, Ali Reza, FDA/CFSAN; Croley, Timothy R., FDA/CFSAN; Knolhoff, Ann M., FDA/CFSAN

Plain Language Synopsis: Non-targeted analysis of non-pigment components in tattoo inks is performed using liquid chromatography high resolution mass spectrometry and nuclear magnetic resonance spectroscopy. This methodology can be used to find and identify compounds that can be used to evaluate the safety of tattoo inks and protect public health.

Abstract:

As tattoos have grown in popularity, so have concerns surrounding their safety. Many safety studies conducted on tattoo inks have focused on pigments, bacterial contamination, and polyaromatic hydrocarbons. However, little is known about the remaining components in the carrier solution, which suspends the pigments, prevents bacterial growth, and aids application. Thus, a non-targeted analysis approach using both liquid chromatography high resolution mass spectrometry (LC/HR-MS) and nuclear magnetic resonance (NMR) spectroscopy was implemented to study these components.

A survey of ten ink colors from the same brand and seven black inks from various brands were analyzed by LC/HR-MS and NMR. For LC/HR-MS analysis, several sample preparation techniques were tested as the presence of a variety of polyethylene glycol (PEG) polymers provided a challenge for this analysis. Ultimately an extraction with 75:25 acetonitrile:water followed by a clean-up procedure using mixed mode cation exchange (MCX) solid phase extraction cartridges provided the best removal of PEGs with minimal loss of other components of interest. A computational PEG filter was

developed to remove remaining PEG signals during data processing. Principal component analysis (PCA) were then used to determine the diversity of the tattoo inks based on their components. This analysis revealed that the tattoo inks differ greatly, even between inks of the same brand and/or color. Some components of interest were putatively identified by accurate mass, retention time (RT), and MS/MS fragmentation matches to an analytical standard. These identifications were then verified by NMR analysis.

The same survey of tattoo inks was also analyzed by NMR. Sample preparation for this analysis consisted of an extraction with deuterated acetonitrile containing maleic acid as an internal standard for quantification. NMR analysis also revealed the vast diversity of the tattoo inks when comparing the major components (i.e. water, isopropyl alcohol, PEG, etc.) found in each ink.

The complementarity of these two analytical techniques for non-targeted analysis will be highlighted. This work provides a methodology for analyzing and identifying components of potential concern in tattoo inks that may affect the safety of tattoos and public health.

39. Data Driven Outbreak Prevention: Reducing Foodborne Illness Risk Factors in Restaurants

Authors: Otto, Jessica, FDA/CFSAN/RFPT; Williams, Laurie, FDA/CFSAN/RFPT

Plain Language Synopsis: The 2013-2014 Restaurant Baseline Data Collection investigated the relationship between food safety management systems (FSMS), certified food protection managers (CFPM), and the occurrence of risk factors and food safety behaviors and practices commonly associated with foodborne illness in restaurants from 2013 to 2014.

Abstract:

The FDA Food Code is a model code and reference document for state, city, county, territorial, and tribal agencies that regulate retail food establishments such as

restaurants, retail food stores, food vendors, and food service operations in institutions such as schools, hospitals, assisted living, nursing homes and child care centers. Food safety practices at these facilities play a critical role in preventing foodborne illness. The Food Code establishes practical, science-based guidance for mitigating risk factors that are known to cause or contribute to foodborne illness outbreaks associated with retail and food service establishments and is an important part of strengthening our nation's food protection system.

FDA is conducting a 10-year study to measure the occurrence of practices and behaviors commonly identified by the Centers for Disease Control and Prevention as contributing factors in foodborne illness outbreaks. The five risk factors identified as contributing to foodborne illness are: improper holding temperatures, inadequate cooking, contaminated equipment, food from unsafe sources, and poor personal hygiene.

Initial data collections began in 2013 for full service and fast food restaurant facilities. The results of the initial data collection for each of the facility types will serve as the baseline measurement from which trends will be analyzed. Two additional data collection periods for each of the facility types are planned at 3-year intervals after the initial data collection for the purposes of analyzing trends.

This poster will focus on the results of the Fast Food and Full-Service Restaurant Facility baseline data collection results. As part of FDA's commitment to reducing foodborne illness at retail, the 2013-2014 Restaurant Baseline Data Collection investigated the relationship between food safety management systems (FSMS), certified food protection managers (CFPM), and the occurrence of risk factors and food safety behaviors and practices commonly associated with foodborne illness in restaurants from 2013 to 2014. The data will be used to establish a baseline measurement upon which to assess trends for control of risk factors in future studies. Data from this study

will provide valuable insights that FDA can use to develop policy, educational resources, and guidance to improve retail food safety practices.

40. Single lab validation study for isolation of norovirus and hepatitis a virus from high fat, dairy products

Authors: Efstathia, Papafragkou, FDA/CFSAN; Diana, Ngo, FDA/CFSAN

Plain Language Synopsis: During food testing for foodborne virus contamination, we are frequently challenged as a particular food item (i.e., berries) cannot be individually tested since it is part of a more complex product (i.e., frosting or ice-cream). For this reason, we developed a two-day method to measure low viral contamination in such high fat, dairy products.

Abstract:

There is an increasing need for development and standardization of methods for detection of foodborne viruses, and especially the most epidemiologically important hepatitis A virus (HAV) and human norovirus (NoV) from foods. Currently there are a handful of multi lab validated methods available. Though foodstuffs most commonly epidemiologically linked to illnesses have been produce (greens, lettuce), berries (frozen and fresh) and shellfish, there is increased occurrence of other food types as well, such as various ready-to-eat foods. Historically, in food virology testing, there is a specific procedure, an optimal sample preparation approach for each individual food group that takes into consideration the composition of the food and the expected inhibitory substances that they may later influence the virus detection (molecular or infectivity assays) and thus need to be controlled/removed. As on an international level, there is not an existing reference method for high fat dairy foods, we started a single lab validation study examining the simultaneous detection of human norovirus and hepatitis a virus. Our experimental design follows the guidelines described in the General Guidelines for the Validation of Qualitative Detection Methods for

Microbial Analytes - Unique Isolation and/or Enrichment Challenges from the Document with Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds. Overall, this study provides a rapid and sensitive method for isolating viral particles from three different foods: raspberry ice cream, strawberry frosting, and frozen yogurt that can be used to quantify viral contamination during an outbreak or sporadic event of illness.

41. Evaluating a High-Throughput Targeted Amplicon Sequencing Approach for Simultaneous Detection and Quantitation of Foodborne Bacteria, Viruses and the Parasite, *Cyclospora Cayetanensis*, from Complex Samples.

Authors: Isha Patel, FDA/CFSAN/OARSA; Mark Mammel, FDA/CFSAN/OARSA; Gopal Gopinath, FDA/CFSAN/OARSA; Cathy Snider, Texas DSHS; Chun Wang, Texas DSHS; Katie Kneupper, Texas DSHS; Mauricio Durigan, FDA/CFSAN/OARSA; Emma Patregnani, UMD/JIFSAN; Hediye Nese Cinar, FDA

Plain Language Synopsis: Use of a targeted approach for detecting a low amount of pathogens provides another efficient and effective tool for FDA to identify foodborne pathogens, such as *C. cayetanensis*. This technique may enhance detection of such foodborne pathogens in samples implicated in outbreaks.

Abstract:

Introduction: Next-generation sequencing (NGS) methods provide resolution, scalability, and sensitivity for high-throughput surveillance. However, there are still significant challenges in detecting sub-populations of unculturable pathogens (specificity) present in samples at low levels (sensitivity) with whole genome (WGS) or metagenome (WMS) sequencing approaches.

Purpose: The objective of this study was to demonstrate the usefulness of a targeted amplicon sequencing method to i) identify microbial content at the species level, ii) quantify intraspecies diversity down to strain

level and iii) target low level contaminants that may be present in complex communities in foods and clinical samples. This approach combines the power of WGS and versatility of WMS with robustness of a targeted approach.

Methods: Primer3 software was used to design primers from alignments of multiple sequences of 10 core genes for each of 266 species that included 135 pathogens. The primers were pooled as 1,786 (all), 615 (pathogens) and 30 (*C. cayetanensis* only) pair panels. Microbial DNA community standards and DNA from clinical samples positive for *Cyclospora cayetanensis* were used. PCR amplicons were sequenced using Illumina MiSeq Platform. Our in-house bioinformatic pipeline was used for identification and quantification of the targeted organisms from the sequence reads datasets. Briefly, sequenced reads were matched by BLAST to a database of the gene sequences used in primer design.

Results: For the ZymoBiotics standards, the abundance of the reads corresponded to the relative amount of each pathogenic species present in the standard. For the *C. cayetanensis* samples, data from 1786 and 30 pairs were in 100% agreement. The larger panel additionally provided bacterial community stratification.

42. Comparison of FDA BAM and Metagenomic Shotgun Sequencing Methodologies in the Microbiological Isolation and Characterization of *E. coli* from Recalled Chapati 'Atta' Flour

Authors: Pfefer, Tina, FDA/CFSAN; Gonzalez-Escalona, Narjol, FDA/CFSAN; Reed, Elizabeth, FDA/CFSAN; Ottesen, Andrea, FDA/CFSAN; Ramachandran, Padmini, FDA/CFSAN; Mammel, Mark, FDA/CFSAN; Lacher, David, FDA/CFSAN; Kase, Julie, FDA/CFSAN

Plain Language Synopsis: Flour from a lot recalled in 2017 due to possible *E. coli* O121 contamination underwent testing using FDA BAM methods, shotgun sequencing, and whole genome sequencing (WGS). The agreement of all three methods demonstrates that metagenomics can accurately detect

STEC and other *E. coli* directly from enrichment in flour.

Abstract:

Introduction: Flour has recently emerged as an important outbreak source for *E. coli* O157:H7 and other STEC. In 2016-2017, wheat flour contaminated by O121:H19, O26:H11 and *E. coli* O121 was linked to three outbreaks resulting in 102 illnesses in the US and Canada.

Purpose: Flour from a lot recalled in 2017 due to possible *E. coli* O121 contamination underwent testing using FDA BAM methods, shotgun sequencing, and whole genome sequencing (WGS).

Methods: Twenty subsamples from the Chapati 'Atta' flour (20lb bag) were placed in Whirl-pak bags and 225 mL mBPWp broth was added. Enrichment and subsequent plating onto three different agars proceeded per FDA BAM Chapter 4a. Enrichment aliquots underwent DNA extraction, and DNA was subjected to parallel screening by PCR and Bio-Plex suspension array screening (Bio-Plex) per BAM protocol and shotgun sequencing using the NextSeq 500 system for a metagenomics approach. Shotgun sequencing data analysis included microbial relative abundance, *E. coli* molecular serotyping and virulence gene characterization. Typical-looking agar colonies from enrichments of interest were screened using the Bio-Plex and, upon a positive result, WGS.

Results: None of the 20 enriched flour subsamples were positive for *E. coli* O121 by any approach. However, we did identify *E. coli* O113 and O45 as well as virulence gene *eae* in enrichment subsamples #14, #20, and #8, respectively, all confirmed via PCR, Bio-Plex, and isolate WGS. Enrichment metagenomics data analysis confirmed *E. coli* virulence gene *eae* (#8), serotype O113:H2 (#14) and serotype O45:H19 (#20), even in the presence of other closely-related *E. coli*.

Significance: The depth of resolution achieved in the analysis of shotgun sequencing data and its agreement with the FDA BAM method

and whole genome sequencing demonstrates the potential of metagenomics as a tool to accurately detect STEC and other *E. coli* directly from enrichment in flour.

43. Human antibody repertoire in serum and amniotic fluid following Zika virus infection during pregnancy

Authors: Ravichandran, Supriya, FDA/CBER; Golding, Hana, FDA/CBER; Khurana, Surender, FDA/CBER

Plain Language Synopsis: Zika Virus (ZIKV) causes neurological complications in newborns following virus infection of pregnant mothers. There is limited understanding of immune responses to ZIKV during pregnancy that hampers serodiagnosis. We found novel epitopes in serum and amniotic fluid from ZIKV-infected pregnant female that can be used as diagnostic or therapeutic targets.

Abstract:

Background: Zika Virus (ZIKV) is an arbovirus presenting strong neurotropism. Moreover, ZIKV causes microcephaly, a devastating outcome in newborns, upon transmission of ZIKV from infected mother to the fetus during pregnancy. The recent ZIKV outbreak has thus sparked global efforts to develop diagnostic and serological tests and vaccines. These efforts can greatly benefit from information on the repertoire of antibody response generated following ZIKV infection during pregnancy.

Purpose: To identify new targets in the ZIKV proteins that are recognized by post-exposure antibodies that do not cross react with other flaviviruses upon ZIKV infection during pregnancy. To use this information for development of better differential serodiagnostic test for ZIKV infection and effective countermeasures.

Methodology: Maternal serum and amniotic fluid (AF) were collected 10 days post symptom-onset from a pregnant female at 3-weeks of gestation and another pregnant female in the third trimester. Sera and AF were used for antibody repertoire analysis

using ZIKV whole genome fragment phage display libraries (ZIKV-GFPDL). Bound phages by IgM, IgG, and IgA were sequenced and mapped to the entire ZIKV genome. Selected peptides were evaluated for development of ZIKV-specific serodiagnostic test.

Results: The IgM antibody titers were 100- and 1000-fold greater than IgG and IgA antibodies in the maternal serum sample suggesting a primary immune response to ZIKV infection. In the amniotic fluid (AF), the IgM and IgA antibody titers were similar to the serum levels, but the IgG antibodies was reduced by 1000-fold. A diverse IgM repertoire encompassed the entire ZIKV structural (C, prM, E) and non-structural (NS1, 2A, 2B, 3, 4A, 4B and 5) proteins. IgG antibodies also recognized Domain III of E protein known for being the least cross-reactive with other flaviviruses. IgA antibodies mainly recognized NS5 protein of ZIKV genome. This global antibody analysis revealed several novel targets in the non-structural proteins (NS1, NS2B, NS3, NS4B, and NS5) that were evaluated for diagnosis of ZIKV infection

Conclusion: Our data demonstrate highly diverse antibody repertoire within structural and non-structural proteins in serum and AF following ZIKV exposure during pregnancy. Newly identified epitopes could be used for early detection of ZIKV infection during pregnancy.

44. Immune persistence measured by human complement serum bactericidal activity in pediatric populations immunized with MenAfriVac®

Authors: Reveille, Alexandra, Center for Biologics Evaluation and Research, FDA, USA; Bhat, Niranjana, Center for Vaccine Innovation and Access, PATH, USA; Tang, Yuxiao, Center for Vaccine Innovation and Access, PATH, USA; Kelly, Corey, Center for Vaccine Innovation

Plain Language Synopsis: In these two studies immunity against *Neisseria meningitidis* is assessed by using human complement (hSBA) to measure bactericidal antibody in sera from

two pediatric persistence studies conducted in Bamako, Mali following a national vaccination campaign.

Abstract:

Neisseria meningitidis causes endemic and epidemic meningitis and sepsis resulting in high morbidity and mortality. Introduction of PsA-TT conjugate meningococcal vaccine, MenAfriVac®, through large-scale vaccination campaigns in Africa led to significant decreases in serogroup A (MenA) meningococcal disease. Antibody persistence following immunization is important for continued control of MenA disease. Here, bactericidal antibody is measured using human complement (hSBA) in sera from two pediatric persistence studies conducted in Bamako, Mali following the national MenAfriVac® campaign.

Pers-007 examines the persistence of MenA antibodies 4-5 years following study PsA-TT-007 in which infants were immunized with one or two doses of PsA-TT (5µg or 10 µg). A representative subset of PsA-TT-007 participants (165 from four original study arms, n=660) and an unimmunized age-matched control group (n=165) were enrolled. Sera were obtained from all participants prior to a MenAfriVac® catch-up campaign conducted in 2017, and from a subset of enrollees (56 from each study group, n=280) 28 days and 180 days following receipt of the catch-up campaign dose.

The NIH MenAfriVac Antibody Persistence Study (MAP) conducted in 2012 enrolled a household-based, age stratified sample of 800 residents of Bamako, Mali who were 1-29 years of age at the time of the 2010 MenAfriVac® campaign. In 2014, MAP re-enrolled subjects (with age- and sex-matched replacements as needed) and unvaccinated children born since 2010 to compare with persistence following vaccination in infancy.

hSBA was detectable in sera collected from children 4-5 years following MenAfriVac® vaccination in infancy, with antibody persistence being greatest in those who received two doses (hSBA titer ≥ 4 in 70%

vs. 42% of subjects, two vs. one dose 10 µg, $p < 0.0001$). All previously vaccinated subjects developed high-titer hSBA responses to a booster dose administered at approximately five years of age, irrespective of their infant dose or regimen. At six months following the catch-up campaign vaccination, 98-100% of previously vaccinated subjects maintained titers ≥ 8 , compared with 64% of age matched controls ($p < 0.0001$). hSBA assays of sera from the 2014 MAP participants who were 1 year to 17 years of age at the time of vaccination ($n = 600$) are being initiated to determine hSBA persistence in children and adolescents 3.5 years following vaccination.

Bactericidal antibody measured by hSBA was detectable in children 4 to 5 years following MenAfriVac® vaccination in infancy. By monitoring hSBA immune responses following MenAfriVac® immunization over time we can address questions regarding antibody decline and immune persistence across pediatric age groups.

45. Leveraging Automation for the Identification of Fungal Isolates from Medical Products

Authors: Rodriguez, Allison, FDA/ORA; Chen, Kai-Shun, FDA/ORA

Plain Language Synopsis: A protocol was developed and evaluated for the rapid identification of fungi. The approach incorporates automation to reduce analyst hands-on time. Fungi have been implicated in multiple infections and outbreaks stemming from the use of medical products. Rapid identification of fungi is critical to FDA's mission to safeguard public health.

Abstract:

Fungi have been implicated in multiple infections and outbreaks stemming from the use of drugs and medical devices. FDA routinely tests medical products for the presence of fungi. When fungal isolates are recovered, their identity is determined by DNA sequencing. The current method entails manual DNA extraction and targets the D1-D2 and ITS regions of the ribosomal DNA (rDNA). The resulting DNA sequences are then searched in public online libraries

to determine the genus or species of the organism. This process is time consuming and labor intensive.

This study aimed to streamline fungal identification by automating the DNA extraction and data analysis steps. The QIAcube®, a robotics platform, was evaluated for DNA extraction. The MicroSEQ® Fungal Identification Kit, which targets the D2 region of the rDNA gene, was evaluated along with the MicroSEQ® proprietary fungal DNA library. Public online libraries were used when the MicroSEQ® library failed to identify the isolate within the preestablished confidence level. The DNA of 98 isolates was extracted either manually (44) or using the QIAcube® (54). One isolate was obtained as purified DNA and required no extraction. All the amplicons generated were analyzed and compared using current reference method versus the MicroSEQ® methods.

The QIAcube® yielded DNA comparable to the reference extraction method. Furthermore, the two sequencing methods agreed in the genus identification of 96 of 98 isolates analyzed, with 70 isolates matching at both the genus and species levels. Two isolates were identified as different by both methods, but closely related at the genus level. One isolate identified by the reference method could not be identified by MicroSEQ®. This could be because MicroSEQ® targets a smaller region of the rDNA gene. Thus, providing less data upon which to base identifications. Use of the ITS target permitted identification of four species which MicroSEQ® could only identify to the genus level.

MicroSEQ® supplemented with public libraries was comparable to the reference method targeting D1-D2. The addition of ITS as a target enhanced the ability to identify isolates. Use of automation can reduce analyst hands-on time and minimize the likelihood of errors.

46. Developing a Novel Anti-Biofilm Technique to Eradicate *Mycobacterium chimaera* Biofilms in Heater Cooler Devices

Authors: Archana, Siddam, FDA/ORR; Shari, Zaslou, FDA/ORR; Yi, Wang, FDA/CDRH; Kenneth, Scott Phillips, FDA/CDRH; Matthew, Silverman, FDA/ORR; Patrick, Regan, FDA/ORR; Jayaleka, Amarasinghe, FDA/ORR

Plain Language Synopsis: *M. chimaera* biofilms has been shown to cause infections in patients that underwent cardio thoracic surgeries through contaminated heater cooler devices, exhibit resistance towards antimicrobial agents and persistently re-occurs after intense decontamination strategies. A novel anti-biofilm technique, Cathodic Voltage-Controlled Electrical Stimulation demonstrates a significant reduction of *M. chimaera* bioburden

Abstract:

Background: *Mycobacterium chimaera* is a slow-growing nont-uberculous mycobacterial (NTM) species that is widespread in the environment. It has been recently identified as the causative agent of the ongoing global outbreak of invasive infections among patients that have undergone cardiothoracic surgeries. Aerosolized *M. chimaera* from contaminated heater cooler devices (HCDs), which are typically used to regulate a patient's body temperature during cardiothoracic surgery, has been identified as the potential source of these infections. The reemergence of *M. chimaera* even after subjecting these HCDs to an intense disinfection protocol suggests that these cells are growing in a biofilm, enclosed in a matrix of extracellular polymeric substance (EPS) on HCD surfaces. This study aims to develop an effective decontamination protocol using Cathodic Voltage-Controlled Electrical Stimulation (CVCES) in combination with antimicrobial agents to successfully eradicate *M. chimaera* biofilms on HCDs.

Materials/methods: *M. chimaera* clinical strains, DSM 44623, 2015-22-08-01 (PA HCU isolate) and 2016-20-02 (ND HCU isolate) were used in this study. Biofilm formation on

stainless steel, titanium and silicone coupons were investigated through 3D-Laser Scanning Confocal Microscope (CLSM) and Scanning Electron Microscope (SEM) to study the development and surface morphology of *M. chimaera* biofilms. An application of CVCES of -1.8V (vs Ag/AgCl) for 1 hour to stainless-steel coupons with one day old *M. chimaera* biofilm on stainless-steel coupons was performed to eradicate the biofilm.

Results: *M. chimaera* forms robust biofilms on various medical device materials. CLSM studies showed that these biofilms attach and develops rapidly on these surfaces within a week of incubation. SEM analysis revealed that these cells adhere to stainless-steel and titanium surfaces within 24 hours of incubation and within two weeks, cells were enclosed in the secretion of an EPS. Preliminary data from CVCES experiments shows significant reduction of colony forming units of *M. chimaera*.

Conclusions: This study provides valuable insights into both early and late stages of *M. chimaera* biofilm formation on various medical device surfaces. Application of CVCES decreases *M. chimaera* bio burden by 96% on stainless-steel surface. It is anticipated that findings from this study will potentially aid in controlling and eradicating *M. chimaera* biofilm formation in HCDs.

47. Applying the Validation of Chemical Methods for FDA Foods Program to Regulatory Sampling Assignments and FDA Laboratory Analysis: FY18 Arsenic in Infant Rice Cereal

Authors: Stutts, Dominique, ORS/NFFL; Aleo, Lori, ORS/NFFL; Kovalenko, Anthony, ORS/NFFL; Daniel, Avinash, ORS/NFFL

Plain Language Synopsis: The Northeast Food and Feed Laboratory (NFFL) brought an FDA method online that can separate the most toxic types of arsenic from the lesser toxic types. In doing so, NFFL was able to test infant rice cereals for arsenic types and determine which samples posed a risk of arsenic toxicity.

Abstract:

Elemental Analysis Manual method 4.11 (EAM 4.11) (Kubachka et. al) is the U.S. Food and Drug Administration's (FDA) method to determine arsenic species in rice and rice products. The method uses high- performance liquid chromatography – inductively coupled plasma mass spectrometry to determine and quantitate four different arsenic species in rice matrices: inorganic arsenic (iAs) as the sum of arsenite (As(III)) and arsenate (As(V)), dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA). Method validation is required for regulatory sample analysis under the "Guidelines for the Validation of Chemical Methods for the FDA Foods Program." EAM 4.11 underwent both a Level 2 single laboratory validation and a Level 3 multi-laboratory validation following this guidance. The multi-laboratory validation took place in 2012 and consisted of six participating laboratories.

In an effort to participate in the upcoming FY18 arsenic in infant rice cereal assignment, the Northeast Food and Feed Laboratory (NFFL) of FDA proposed to validate this method before the start of the sampling assignment. Under the guidance of FDA's elemental analysis steering committee (EASC), NFFL illustrated it was capable of performing EAM 4.11 by following the same requirements of the original multi-laboratory validation. This poster presents the steps NFFL took to properly validate EAM 4.11, and how this method validation was applied to FDA regulatory sample analysis. NFFL successfully validated EAM 4.11 and participated in the FY18 infant rice cereal assignment.

48. Evaluation of Human Intestinal Enteroids as a Model for Enteric Virus Infection

Authors: Wales, Samantha, FDA/CFSAN; Yang, Zihui, FDA/CFSAN; Ngo, Diana, FDA/CFSAN; Kulka, Michael, FDA/CFSAN

Plain Language Synopsis: Human noroviruses are responsible for the majority of viral gastrointestinal illnesses. It is vital to

develop a robust assay for virus infectivity to determine whether a food is contaminated. We highlight the work done at CFSAN to test the recently developed method to cultivate human noroviruses using human intestinal enteroids.

Abstract:

Background: Human noroviruses (HuNoV), as well as other human enteric viruses, have been recalcitrant to growth in culture, prohibiting in-depth research into their replication toward development of virus infectivity-based detection assays. The development of a reliable cell culture model for human noroviruses viruses is vital, therefore, for confirming the presence of infectious virus in a potentially contaminated food source, as well as achieving production and consistent availability of HuNoV stocks for use in research and development of mitigation, food extraction, and detection methodologies.

Purpose: The objective of this study is to determine the potential and limitations of the human intestinal enteroids system for the growth of human norovirus strains and other selected enteric viruses.

Methodology: We adopted the methods developed by Dr. Mary Estes' lab at Baylor College of Medicine, using human intestinal enteroids seeded in a monolayer, for infection with human noroviruses, astrovirus, hepatitis E virus, hepatitis A virus, and sapovirus.

Results: The GII.4/Sydney strain provided by Dr. Estes' lab demonstrated robust, consistent replication in the enteroids. Eighteen other norovirus strains were also tested, but less than half of these exhibited signs of replication. Human astrovirus, but not sapovirus, was also able to replicate in this system. Initial studies indicate that HAV, but not HEV, will grow in the enteroids, though more work needs to be done to confirm these results.

Conclusion: The development of the human intestinal enteroids system for enteric virus infection has greatly progressed the field for norovirus replication, however it is limited in

that few norovirus strains are able to grow. More work needs to be done to determine what factors are limiting the growth of other strains/viruses.

49. Identification of Foodborne Pathogens in Environmental Samples using a New Generation Microarray Assay

Authors: Christine, Yu, FDA/CFSAN; Zhihui, Yang, FDA/CFSAN; Chiun Kang, Hsu, FDA/CFSAN

Plain Language Synopsis: The detection of pathogens in food and the environment is essential to identify potential source of outbreaks. A high-density microarray was used to survey the prevalence of enteric viruses in soil and water. The successful identification of viral contaminants will assist the process of developing and implementing effective responses to outbreaks.

Abstract:

Background: Fresh produce is the number one source of food poisoning. Transmission of pathogens during production, processing, and distribution could be the cause of outbreaks. Soil and water may also play a role in contamination of produce during irrigation and growth. There is a paucity of methods for the rapid and simultaneous detection of multiple pathogens in the environment. A new generation custom DNA microarray designed for broad coverage of microbial pathogens may offer an approach for analytical investigation of environmental samples containing multiple enteric microbes.

Purpose: This study was to assess whether the DNA microarray could identify multiple pathogens in soil and irrigation water.

Methodologies: The Affymetrix microarray was designed with the perfect match/mismatch probe sets for individual genes of common foodborne viruses, as well as their surrogates, certain bacteria, phages, and parasites. Soil and irrigation water were collected from domestic farms. One soil aliquot was spiked with norovirus (NoV) GII.1 strain. Moore swabs were used in capture-

filtration of human pathogens in irrigation water. Hepatitis A virus (HAV) HM175/clone 1 (genotype IB) infected-culture supernatant was used for inoculation and process control in selected water samples. To increase the sensitivity of multi-pathogen detection, cDNA derived from RNA extraction was amplified using a commercial kit. Microarray hybridization was performed following the GeneAtlas Manual.

Results: Preliminary data analysis using the MAS5 and PMA algorithm revealed the presence of multiple pathogens in the environment. The inoculated soil sample tested predominately contained norovirus (NoV GII.1 and GII.12); this was confirmed with next-generation sequencing. In addition to the detection of the HAV control strain in the inoculated water sample, both inoculated and non-inoculated water samples showed the presence of HAV IA and NoV GII.4/GII.7 strains. RT-qPCR confirmed the presence of HAV in these water samples.

Conclusion: We demonstrated the application of microarray toward multi-pathogen identification in an agricultural system. Common foodborne viruses were detected in soil and irrigation water. This technology may be useful in evaluating the potential risk of foodborne pathogens at the farm level. The data collected will provide guidance for farm management practices, surveillance, and outbreak investigation.

Poster Session 4

Topic: Addiction (Day 2, P.M.)

50. Title: Evaluating Nicotine Dependence and Patterns of Use Among Exclusive Users of Tobacco Products Using a Potential Cotinine Level Threshold: Data from Wave 1 of the Population Assessment of Tobacco and Health (PATH) Study

Authors: Das, Babita, FDA/CTP; Miller, Mollie, FDA/CTP; Hammad, Hoda, FDA/CTP; Hull, Lynn, FDA/CTP; Del Valle-Pinero, Arseima, FDA/CTP

Plain Language Synopsis: Product use patterns and dependence were assessed in current adult exclusive tobacco users, who provided serum cotinine levels. Findings revealed most exclusive users below 70 ng/mL serum cotinine show less dependence and lower frequency and amount of tobacco use than those above 70 ng/mL, potentially informing FDA nicotine product standards.

Abstract:

Background: FDA has sought comments on a potential product standard to lower nicotine in combusted cigarettes to a non-addictive level, based on observations that exclusive cigarette users who smoke less than or equal to 5 cigarettes per day, equivalent to 0.3-0.5 mg/g nicotine or 50-70 ng/mL serum cotinine, often appear to be non-dependent. In today's landscape, it is unclear how multiple product types and methods of nicotine delivery affect nicotine exposure and dependence in exclusive users of tobacco products. Purpose: This cross-sectional analysis used Population Assessment of Tobacco and Health (PATH) Study Wave 1 data to assess if current adult exclusive users of tobacco products who fall above and equal to or below 70 ng/mL serum cotinine display differential use patterns and nicotine dependence. Methodology: Univariate linear regression analysis was used to examine the relationship between product use frequency, quantity, and Total Dependence (TD) in participants who provided blood samples in the four largest groups of exclusive tobacco product use: cigarettes, ENDS, cigars, and smokeless tobacco (ST). Outcomes were compared between the user groups in those with less than or equal to 70 ng/mL serum

cotinine (below group, BG), and in those with greater than 70 ng/mL serum cotinine (above group, AG). Outcomes were also compared within each user group between AG and BG participants. Results: All BG user groups reported less TD than their AG counterparts; significant for exclusive cigarette, cigar, and ST users ($p < .0001$ for each comparison). All BG groups reported less frequency of use than their AG counterparts ($p < .0001$ for each comparison). All BG groups reported lower quantity of use than their AG counterparts ($p < .01$ for each comparison). TD and frequency differed between all BG exclusive user groups ($p > .0001$) and between all AG exclusive user groups ($p < .0001$). Conclusion: Differences exist in tobacco dependence, frequency, and quantity of use between users with greater than and less than or equal to 70 ng/mL serum cotinine for most exclusive tobacco product users, and highlight product-dependent differences in use patterns and self-reported dependence.

51. Possible Nicotine-Related Health Effects from Electronic Nicotine Delivery System Use by Adolescents and Young Adults: Reports to the FDA

Authors: Limpert, Jean, FDA/CTP; Rudy, Susan, FDA/CTP; Konkel, Karen FDA/CDER; Murphy, Lilun FDA/CTP

Plain Language Synopsis: FDA/CTP has received a limited number of voluntary reports about nicotine-related health effects from e-cigarette use in adolescents and young adults and encourages reporting to the Safety Reporting Portal. FDA's Youth Tobacco Prevention Plan is taking steps to prevent youth use of all tobacco products.

Abstract:

Background: Data from the 2017 National Youth Tobacco Survey indicate that electronic nicotine delivery systems (ENDS) continue to be the most commonly used tobacco product among youth; approximately two million U.S. middle and high school students used in the last 30 days.¹ The role of ENDS in the initiation of nicotine use by youth is concerning; nicotine can harm the developing

brain and is addictive. Research suggests that ENDS use also increases the risk that youth and young adults will subsequently try combusted cigarettes.² ENDS may be small and discreet and resemble other consumer products (e.g., USB device, pen). ENDS may emit fruity or sweet aromas rather than a tobacco smell or have low odor in general. Additionally ENDS may have low-visibility plumes making them difficult to detect.

FDA's Center for Tobacco Products (CTP) receives voluntary reports from the public about adverse experiences associated with tobacco products. The primary mechanism for submitting reports is through the web-based Safety Reporting Portal (SRP).

Objectives:

1. To describe voluntary reports of adverse health experiences related to nicotine exposure in youth and young adult ENDS users.
2. To inform health care providers about the SRP and describe FDA/CTP's current efforts to prevent youth access to and use of e-cigarettes.

Methods: FDA/CTP searched the SRP and legacy databases (1988-October 6, 2018) for adverse experience reports involving adolescent and young adult (ages 25 years and under) ENDS users. We reviewed a total of 33 reports returned by the search, from which we identified eight reports with apparent ENDS nicotine-related health effects.

Results: As of October 6, 2018, eight reports, all from parents, described nine adolescents (aged 15-19 years) and one young adult (23 years old) with apparent nicotine-related health effects associated with ENDS use. Cases reported between 2015-2018 are summarized in the poster.

Conclusions: FDA/CTP has received a limited number of voluntary reports regarding youth and young adults. Reports received by FDA/CTP include symptoms that may indicate nicotine-related adverse health effects, including addiction, overdose, withdrawal,

as well as serious neurologic issues (e.g., seizures) and mental health issues (e.g., suicide attempts). Due to the limited nature of the information in the reports, it is possible that ingredients or factors unrelated to nicotine may have caused or contributed to the health issues.

ENDS use in adolescents and young adults is increasingly common and presents several challenges. ENDS may be discretely used. ENDS nicotine delivery can exceed that of cigarettes. It is possible that the nicotine salts found in some ENDS (e.g., JUUL) may affect nicotine exposure and/or symptoms; additional research is needed. Nicotine is highly addictive and can lead to acute toxicity if exposure is significant. Youth, in particular, who use ENDS may not recognize their dependence while using ENDS or the symptoms that follow abrupt discontinuation, which may be severe. To complicate matters, nicotine withdrawal symptoms are non-specific and may be easily confused with other common adolescent experiences, such as anger, irritability, depression, anxiety, and decreased concentration.

It is important for parents and healthcare practitioners to understand the difficulty in detecting ENDS use and nicotine addiction in youth. Consider the possibility of tobacco use disorder and nicotine withdrawal when evaluating non-specific complaints in adolescents. Resources exist to assist with tobacco product cessation. FDA/CTP encourages reporting of tobacco-related health and product problems to the Safety Reporting Portal (www.safetyreporting.hhs.gov). Additionally, through the Youth Tobacco Prevention Plan, FDA/CTP is taking steps to prevent youth use of all tobacco products.

52. Examining Cigarette Smoking Abstinence Among U.S. Adult Menthol and Non-flavored Cigarette Smokers, 2013-2017

Authors: Feirman, Shari, FDA/CTP; Johnson, Amanda, FDA/CTP; Cullen, Karen, FDA/CTP; Holder-Hayes, Enver, FDA/CTP; Schroeder, Megan, FDA/CTP; Ambrose, Bridget, FDA/CTP

Plain Language Synopsis: The study objective

was to prospectively examine whether abstinence rates differed between current, established, exclusive menthol cigarette smokers and non-flavored smokers. There was no statistically significant relationship between being a menthol smoker (compared to a non-flavored smoker) and abstinence within each racial/ethnic subgroup.

Abstract:

Some studies have found a positive relationship between menthol cigarette smoking and reduced smoking cessation. To investigate this, we evaluated smoking abstinence among menthol and non-flavored smokers in the Population Assessment Tobacco and Health (PATH) Study. We used data from Waves 1-4 of the PATH Study. We used an extended Cox model to estimate hazard ratios (HRs) of achieving smoking abstinence for ≥ 7 and ≥ 30 days at Wave 2, 3, or 4 for menthol versus non-flavored cigarette smokers. In the stratified multivariable model, there was no statistically significant relationship between being a menthol smoker (compared to a non-flavored smoker) and abstinence within each racial/ethnic subgroup. Although the models did not produce statistically significant results when examining the relationships between menthol flavor status, race/ethnicity, and abstinence, qualitatively, there was a consistent directional effect across multiple models. This suggests that menthol cigarette use could be related to reduced abstinence, and that this relationship may be different by race/ethnicity.

Poster Session 4

Topic: Empowering Consumers, Patients, and Other Stakeholders (Day 2, P.M.)

53. Physician and Consumer Capability to Detect and Report Deceptive Prescription Drug Promotion to the FDA

Authors: Betts, Kevin, FDA/CDER/OMP/OPDP; O'Donoghue, Amie, FDA/CDER/OMP/OPDP; Boudewyns, Vanessa, RTI International; Paquin, Ryan, RTI International; Johnson, Mihaela, RTI International

Plain Language Synopsis: Across two experiments, the present research investigated primary care physician and consumer capability to detect and report deceptive prescription drug promotion to FDA. Findings lend mixed support for this capability among both physicians and consumers.

Abstract:

The U.S. Food and Drug Administration's (FDA) Bad Ad program provides an avenue for healthcare professionals to report false and/or misleading prescription drug promotion. Yet, whether healthcare professionals can detect such promotion, and whether they believe it should be reported, remain open questions. Consumer audiences may also be capable of detecting and reporting such promotion, but even less is known about capability in this population. Across two experiments using mock pharmaceutical websites, the present research investigated these questions among a sample of primary care physicians and consumers. Study 1 considered level of deceptiveness, operationalized by number of deceptive claims and tactics on the website. Study 2 considered type of deceptiveness, operationalized by explicit versus implicit deceptive claims (or no deceptive claims) on the website. Findings reveal that upon initial exposure to deceptive promotion, consumers tend to be deceived whereas physicians tend to be suspicious (i.e., not deceived). For physicians and to a lesser extent for consumers, exposure to deceptive promotion tended to increase perceived deceptiveness, which resulted in a combination of less positive attitudes and reduced behavioral intention than would have been expected had deceptiveness gone unnoticed. When

overtly asked whether the promotional pieces included deceptive elements, both consumer and physician capability to detect and report to FDA increased, with physicians maintaining a lower threshold for reporting. These findings offer important implications for programs that involve healthcare professionals and consumers in the detection and reporting of false and/or misleading prescription drug promotion.

54. Post-market safety outcomes for new therapeutic biologics approved by the FDA between 2002 and 2014.

Authors: Bulatao, Ilynn, FDA/CDER; Pinnow, Ellen, FDA/CDER; Day, Brendan, FDA/CDER; Cherkaoui, Sanae, FDA/CDER; Kalaria, Manish, FDA/CDER; Brajovic, Sonja, FDA/CDER; Dal Pan, Gerald, FDA/CDER

Plain Language Synopsis: Expedited programs reduce the time needed to bring therapeutic products to the market. We examined whether fast-track designation, priority review designation, and accelerated approval using a surrogate endpoint are associated with postmarket safety outcomes (withdrawal from the market due to safety or safety-related label updates) for new therapeutic biologics.

Abstract:

Background: Biologics comprise a large and growing market in the U.S. Patterns of postmarket safety outcomes, and their association with pre-market expedited programs have not been systematically described.

Objectives: We examined the association between three expedited programs developed by FDA to reduce pre-market review or development time (fast track designation, priority review designation, and accelerated approval using a surrogate endpoint) and postmarket safety outcomes for new therapeutic biologics (NTBs) approved between 10/1/2002 and 12/31/2014.

Methods: Post-market safety outcomes, defined as a safety-related withdrawal or a safety-related update to the Boxed Warning

(BW), Contraindications (C), Warnings and Precautions (WP), Adverse Reactions (AR) or Drug Interactions (DI) sections of the label from the NTB's approval through 6/30/2018 were recorded following review of label updates posted on two public websites: Drug Safety-related Labeling Changes database and Drugs@FDA. Kaplan-Meier analyses were conducted to examine time to first safety outcome.

Results: 61 NTBs were approved during the study period. Follow-up was 3.5-15.7 years (median, 8.4 years). 30 (49.2%), 42 (68.9%) and 9 (14.8%) NTBs, respectively, were assigned fast track designation, priority review designation, and accelerated approval using a surrogate endpoint. Two NTBs were withdrawn from the market for safety reasons while 53 (86.8%) had at least one safety-related label update. There were 199 individual label updates addressing 900 distinct safety-related issues. There were 24, 24, 119, 133 and 7 individual label updates, respectively, for the BW, C, WP, AR, and DI sections. 65, 34, 334, 560 and 13 safety issues were added to the BW, C, WP, AR and DI sections, respectively. Kaplan-Meier analysis showed that fast track and priority review designation were not associated with the occurrence of a safety outcome, while NTBs classified as accelerated approval using a surrogate endpoint had earlier occurrence of a safety outcome than those not approved via this pathway ($P=0.02$).

Conclusions: NTBs approved via the accelerated approval based on a surrogate endpoint had earlier occurrence of a safety outcome than those not approved via this pathway.

55. Children's Risk of Nicotine Poisoning from Tobacco Products

Authors: Crosby LM, Niazi M and Persoskie A, FDA/CTP

Plain Language Synopsis: Tobacco products are harmful to children; they may be poisoned if they accidentally ingest tobacco or e-cigarette liquid. There is no information about the amount of liquid nicotine that will

sicken or kill a child. We analyzed available information and calculated that the amount of liquid nicotine in a concentrated solution could be fatal to a child under six years old at as little as 1/10th of a teaspoon.

Abstract:

There are no analyses of the potential acute toxicity to children from a single exposure to different tobacco products, based on relevant nicotine concentrations. Tobacco use in the United States is increasingly diverse, with about 37 million adults and 1.6 million middle and high school students classified as current cigarette smokers, while about 8.6 million U.S. adults and 3 million middle and high school students were current e-cigarette users in 2015. Given the continued popularity of these products and their presence in U.S. homes, an analysis of the amount of nicotine that will produce illness or death is important. Using published literature, we estimated potentially toxic nicotine exposures, based on the content and characteristics of five major tobacco product types. After adjusting for inter-individual variability, some preliminary exposure levels have been estimated. The lowest observable effect level in children aged six months to six years is estimated at 0.04 mg/kg for oral exposure. For a one-year old child weighing 10 kg, the lethal dose of orally ingested nicotine is estimated to be between 1 and 14 mg/kg, depending on strength and not adjusting for inter-individual variability. If an e-cigarette liquid contains 30 mg nicotine/mL, then consuming only 0.3 mL (\leq 0.1 teaspoon) liquid could be fatal. Although all tobacco-containing products have caused poisonings in children, poisoning by e-cigarette liquid likely poses the greatest risk to small children. Disclaimer: This information is not a formal dissemination of information by FDA and does not represent the agency's position or policy.

56. Performance Evaluation of Tissue Containment Bags for Power Morcellation

Authors: Duraiswamy, Nandini, FDA/CDRH/OSEL; Herman, Alexander, FDA/CDRH/OSEL; Nandy, Poulomi, FDA/CDRH/ODE; Price,

Veronica, FDA/CDRH/ODE; Gibeily, George, FDA/CDRH/ODE; Hariharan, Prasanna, FDA/CDRH/OSEL

Plain Language Synopsis: Laparoscopic power morcellator use has come under scrutiny lately since there is a risk for the spread of cancer. To alleviate this, some investigators recommend using the device with a tissue containment bag. We identified preliminary metrics and developed in vitro methods to adequately test the performance of these bags.

Abstract:

Laparoscopic power morcellators are medical devices used to divide tissue into smaller fragments to facilitate removal using minimally invasive techniques that rely on small incisions. In some cases, tissue fragments can be left behind leading to significant complications. A published FDA safety communication highlighted the risk to patients undergoing treatment of uterine fibroids with these devices during a hysterectomy or myomectomy. In some cases, women undergoing treatment for fibroids may have an unsuspected uterine sarcoma. Use of laparoscopic power morcellators in these patients carries the risk of spreading cancer cells within the abdomen and pelvic region, which may worsen survival. To minimize the risk of spreading cancerous cells, some investigators have recommended the use of an insufflated tissue containment bags deployed inside the body to isolate the extirpated tissue and morcellator from surrounding tissue/organs. However, the majority of the commercially available specimen bags are not indicated for this type of use.

Device manufacturers evaluate the performance of these containment devices using standalone tensile, burst, dye penetration, and puncture tests without a standardized metric to compare it against. As part of this study, we estimated the morcellation forces, developed new performance test methods that evaluate the propensity of tissue containment bags

to leak when subjected to all clinically relevant forces imparted during a power morcellation procedure. Currently, we have tested seven different legally marketed tissue containment bag materials in a full puncture, dye, bacteriophage, partial puncture/dye, burst, and tensile test. These experiments estimated different safety factors for each bag depending on the bag material, design, and bench testing methodology. These tests without any standardized metrics are not adequate to evaluate the performance of tissue containment bags during power morcellation.

A future simulated usability study will examine potential bag failure modes and other testing methodologies when used with power morcellators. The results from this study may aid in the development of FDA guidance documents, and new testing standards for pre-clinical testing of tissue containment bags used for power morcellation.

57. Using Online Submissions Module to process industry submissions sent to the Center for Food Safety and Applied Nutrition (CFSAN)

Authors: Girmay, Berhane PhD, PMP/FDA/CFSAN; Yu, Xiaoling/FDA/OIMT; Mary, Ditto PhD/FDA/CFSAN; Swift, Sybil, PhD/FDA/CFSAN

Plain Language Synopsis: The CFSAN Online Submission Module (COSM) is an easy step-by-step process to complete a submission, thereby eliminating the printing and mailing of paper submissions. It provides an opportunity for industry submitters to use fully electronic means and for the agency to achieve fully electronic records management.

Abstract:

The CFSAN Online Submission Module (COSM) is an application that provides a real-time user interface for industry users who send submissions to CFSAN OFAS and ODSP. COSM provides two options: for OFAS submissions, COSM eliminates downloading and filling Adobe pdf forms and creating complex data structures. Submitters will log into COSM and process their submissions

following a traditional question-and-answer form interface for the purposes of collecting and assembling a complete submission package. The submission package contains a structured directory of folders and files specific to OFAS submission types that can be uploaded and send through the FDA Electronic Submission Gateway (ESG). Different submission types have different folder structures. The FDA Food Applications Regulatory Management (FARM) system can process those submissions to initiate a workflow for review and taking agency action.

For submissions sent to ODSP, external users will log into the module, create their submissions and, upon completion, they can directly upload the submission into an FDA extranet database. The FARM system can process submissions from both sources (FDA ESG and extranet database) by loading all documents into Documentum, and the meta-data into the FARM's database. The submission data will be populated into a workflow in the FARM system. COSM empowers stakeholders with an easy step-by-step process to complete a submission, thereby eliminating the printing and mailing of paper submissions, and providing an opportunity for industry submitters to use fully electronic means.

58. Arsenic and Lead Determination in D&C Red No. 6 and 7 Lakes Containing Barium Sulfate Using X-Ray Fluorescence Spectrometry

Authors: Hepp, Nancy, FDA/CFSAN

Plain Language Synopsis: A new x-ray fluorescence method was developed for the determination of arsenic and lead in D&C Red Nos. 6 and 7 lakes containing barium sulfate.

Abstract:

An x-ray fluorescence (XRF) spectrometry method was developed for quickly determining whether barium sulfate-containing color additives certifiable as D&C Red No. 6 lakes and D&C Red No. 7 lakes meet FDA's regulatory specifications for arsenic (As) and lead (Pb). Difficulties in preparing XRF standards and analyzing As and Pb in matrices with the heavy x-ray

absorber barium (Ba) were overcome by first preparing As- and Pb-fortified cellulose, then blending color additive samples with fortified and unfortified cellulose to produce XRF calibration materials. Linear calibration equations were generated for each element by calculating the ratios of As, Pb, and Ba line intensities to the Compton-scattered Rh tube line intensity relative to the concentration of each element. Satisfactory compensation for the dramatic changes in intensity caused by the heavy absorption due to barium sulfate was achieved by measuring varying concentrations of As and Pb at several concentrations of Ba and using correction equations. Test samples were analyzed by the XRF method and by inductively coupled plasma-mass spectrometry (ICP-MS). The latter technique poses some difficulties and is time-consuming. In contrast, the XRF method requires very little sample preparation, is nondestructive, uses calibrations that are stable for long periods of time, and offers acceptable method determination limits (1 mg/kg As, 4 mg/kg Pb), which are less than the specification limits (3 mg/kg As, 20 mg/kg Pb).

59. Offering patients more choices: Technical considerations for medical device manufacturers when designing Gastrostomy tubes (G-tubes)

Authors: Herman, Alexander, FDA/CDRH/OSEL; Herbertson, Luke, FDA/CDRH/OSEL; Antonino, Mark, FDA/CDRH/ODE; Silverstein, Joshua, FDA/CDRH/ODE; Myers, Matthew, FDA/CDRH/OSEL; Guha, Suvajyoti, FDA/CDRH/OSEL

Plain Language Synopsis: Gastrostomy tubes (G-tubes), used to deliver nutrition and fluids, are being redesigned using a standardized connector to prevent misconnections. Patients expressed concern that the change would reduce their feeding rate. We developed a novel test methodology and identified adjustable parameters for addressing issues with reduced feeding rates in G-tube patients.

Abstract:

Gastrostomy tubes (G-tubes), also popularly called feeding tubes, allow for the infusion or withdrawal of fluids into or out of the gastrointestinal tract. They are typically used when a patient is not able to intake food or nutrients orally. Due to the potentially deadly repercussions for G-tube misconnections in a clinical setting, a connector was developed using the new ISO 80369-3 standard. The new connector section allows G-tubes to only connect to other enteral devices, such as feeding sets or syringes. The narrow internal diameter of the new connector has caused some patients to express concern. These patients anticipate that this narrowing may drastically increase feeding times, thus impacting their quality of life. In previous experiments, we tested the G-tubes with the new connectors to assess the extent of this flow-rate reduction. When reduced flow rates were observed, it was not apparent how much the new connector contributed towards the reduced flow-rate, because manufacturers often changed other geometric variables unrelated to the ISO 80369-3 connector (e.g. distal tube diameter, or length of the device) concurrently when designing the new connectors. As a result, the impact of flow rate slowing from the connector design change could not be isolated for commercial G-tubes. Thus, a new study was designed to delineate and investigate different design variables that affect the flow-rate through the G-tubes. As part of this study, six different G-tube surrogate models were developed to systematically assess the geometric parameters. Commercial diets and Newtonian analog fluids with matched viscosities were used for testing. The length of the “transition section” encompassing the ISO 80369-3 connector in the new devices was found to be the primary determinant for reduced flow rates. Additionally, our results show that a shortened (≤ 10 mm) transition section, along with a 10% increase in the distal inner diameter of large bore devices (e.g. 24 Fr), can restore flow rates to levels consistent with legacy G-tube designs pre-dating the connector standardization. These strategies for restoring flow rates to previous

levels may help alleviate concerns raised by patients, and other stakeholders, including health care professionals, caregivers, and device manufacturers.

60. Nutrient Consumption among the U.S. Population Using Food Label Information, National Health and Nutrition Examination Survey (NHANES), 2005-2010

Authors: Juan, WenYen, FDA/CFSAN

Plain Language Synopsis: Food labeling information, nutrient intake, label claims

Abstract:

Objective: Compare nutrient consumption amounts between the U.S. population, who reported using food label information versus those who did not use the food label information. Methods: Cross-sectional analysis of data from NHANES 2005-2010 was used to estimate selected nutrient intakes among adults (≥ 18 years, $n=16,802$). The intakes of calories and 42 nutrients were compared among adult males and females who used food label information (users) and those who did not use the food label information (non-users). Food label information includes the Nutrition Facts label (the NFL), which includes information on calories and eight specific nutrients, the serving size on the NFL, the ingredients list, and claims (such as nutrient content claims, health claims, and structure and function claims). Results: Males and females reported using the NFL information more often than other components (e.g., ingredients, claims, and serving size) on the food label. Calorie intake was significantly lower among female NFL label users compared to non-users, but no significant difference in calorie intake was observed among male label users and non-users. Depending on which component of the food label information was used, the intakes of fiber, B vitamins, vitamins D and C, folate, calcium, and potassium were significantly higher and total fat was significantly lower, among label users compared to non-users of either gender. Among NFL users who reported seeking specific nutrient information (calories, fat, trans fat, saturated fat,

cholesterol, sodium, carbohydrate, fiber, and sugar) on the NFL, the intake of calories was significantly lower and fiber intake was significantly higher for males. For females, the intakes of carbohydrate and sodium were significantly lower. Significance: These findings suggest that consumers who used nutrition information on packaged food labels may have improved their nutrient consumption, and that using food label information may lead to beneficial dietary practices.

61. Significance of anti-heat processed milk antibody on ELISA-based detection in a dark chocolate matrix

Authors: Ann V. Nguyen; Kristina M. Williams; Daniel Lee; Lauren S. Jackson; Binaifer Bedford; Jihyun Kwon; Peter F Scholl; Sefat E Khuda

Plain Language Synopsis: Quantitation of milk in chocolate by ELISA can be optimized using matrix-specific incurred standards and extractant, and antibodies capable of recognizing processed milk proteins, depending on polyphenol content.

Abstract:

Introduction: Undeclared milk is prevalent in chocolate products, so specific analytical methods for use with this difficult matrix are required. The performance of milk ELISAs are compromised in chocolate because of the presence of protein-binding phenolic compounds.

Purpose: Antibody specific for heat-processed milk proteins (HPMP-Ab) was generated as a strategy for improved milk detection in processed foods, including chocolate commodities.

Methods: Milk-incurred cookies (0, 3.54, and 17.7 ppm), and tempered dark chocolate samples (62% cacao: 0, 0.9, 1.8, 2.9, 7.6, 28,134 ppm; 100% cacao: 0, 0.075, 0.25, 0.75, 2.5, 7.5, 25, 75 ppm) were evaluated using HPMP-Ab by ELISA. The cookies and dark chocolate samples were extracted with high salt buffer (HSB) /1% Tween (T)-20 and HSB/1%T-20/5% polyvinylpyrrolidone (PVP)/1% fish gelatin

respectively after removing fat with hexane treatment.

Results: In qualitative immunochemical analysis, modified proteins, including aggregates, were found to react with greater signal intensities when using HPMP-Ab. By Sandwich ELISA, HPMP-Ab demonstrated high affinity towards processed purified major milk proteins and proteins of milk-derived ingredients and reference materials subjected to several thermal processing procedures. Acceptable dynamic ranges were observed as evidenced by HPMP-Ab binding curves using incurred cookies and tempered 62% dark chocolate samples, but binding curves were negatively affected using 100% dark chocolate samples due to an increased baseline absorbance/background. The HPMP-Ab ELISA was highly specific, as no cross reactivity was observed when testing a broad range of food commodities using incurred cookies as calibrators. The HPMP-Ab ELISA detected declared/undeclared milk proteins in commercial chocolate samples containing a variety of milk-derived ingredients when tempered 62% dark chocolate samples were used as calibrators.

62. CDER Research Governance Council – A yardstick for Research Governance

Authors: Madhu Lal-Nag, FDA/OTP on behalf of the RGC

Plain Language Synopsis: The Research Governance Council (RGC) was launched as a response to a call for a research governance structure for oversight of CDER's research portfolio, by Dr. Janet Woodcock. The RGC will serve as an informational hub for CDER research and collaboration to impact the future direction of research at CDER.

Abstract:

The CDER Research Governance Council (RGC) was launched in March 2017 as a response to a call for a formal research governance structure for oversight of CDER's increasingly diverse and complex research portfolio, by Dr. Janet Woodcock. The RGC serves as CDER's executive board for oversight of research programs, policy,

communication and resource allocations with a forward-looking perspective on factors that will affect CDER's research programs and priorities. The Council establishes broad goals for CDER research programs with a strong focus on collaboration and innovation. The RGC then reviews and evaluates CDER's research portfolio using approaches that objectively determine impact based on specific research outcomes metrics. Research encompasses a range of CDER intramural and extramural research efforts, including clinical, non-clinical, laboratory, computational, and social science research.

The RGC has made significant strides since its inception and established four central tasks that include research science investment tracking, research prioritization, research program evaluation and the communication of research achievements both internally and externally.

The RGC organized several working groups to help elaborate the essential components of an enhanced research governance structure, including Budget and Spending, Communications, Evaluation of Research Outcomes, Research and Science Priorities as well as Research Tracking. The groups worked in a coordinated fashion to establish the framework for a coordinated research governance structure.

One notable result from this effort is the ability to track and evaluate CDER Regulatory Research Investments and Outcomes. This helps align CDER's Research with its research goals, objectives and priorities, and sets the stage for enhanced communication within the research community at CDER and enables collaborative relationships with external stakeholders as well.

The RGC will continue to foster a community of engagement and accountability and be an informational hub for CDER research and collaboration to positively affect the future direction of innovative regulatory science research at CDER.

63. Can Social Media Promote Pharmaceutical Product Quality

Authors: li, Xue, FDA; Stiber, Neil, FDA; Gonzalez, Fernando, FDA; Tung, Lisa, FDA; Matusovsky, Vlada, FDA; Parvizi, Shahla, FDA; Shojaie, Sharareh, FDA;

Plain Language Synopsis: Social media has become among the most popular Internet platforms, allowing users to communicate and socialize with others around the world. It provides an additional channel for FDA to hear voices from consumers and patients. Attention has turned to the opportunities social media provides for pharmaceutical product quality information.

Abstract:

Background: Social media has become among the most popular Internet platforms, allowing billions of users to communicate and socialize with others around the world. Many features and functions customized on social media enable people to engage in online conversations, share opinions and information, upload pictures and videos through social networks, professional networks, blogs, forums, and other portals. Consumers and patients can access to these online platforms to share user experiences and ask for advice regarding various products, including medical products.

Purpose: Social media provides an additional channel for FDA to hear voices from consumers and patients. Attention has turned to the opportunities that social media provides for pharmaceutical product quality information. Many opinions, alerts, and outcomes may be shared on social media that are not available through existing regulatory approaches, such as complaints and MedWatch. This research will focus on the impact of social media to ensure quality medicines are available through signal detection and proactive stakeholder engagement.

Methodology: Create a list of key words with the context of the information relevant to the topic, including drug products and adverse events. Use keywords to develop queries with the necessary operators. Build and configure components and graphs to organize data and

visualize key factors. Conduct qualitative and quantitative analysis of the data to gain insights associated with the initiatives to advance decision-making processes.

Results: Data collected from social media will be summarized as “mentions” and “mentioned over time.” Data will be further sorted out to identify consumer demographics, geographical locations, points of interest in the conversations, etc. Consumers’ conversations on social media may yield results to identify trends of product quality and corresponding concerns.

Conclusion: Through the efforts of our office in the research of social media by the current approaches, it demonstrated that social media may complement routine post marketing drug quality reports received by the Agency. Social media provides an opportunity for alternative product quality information that directly expresses the patient and consumer experience. These additional perspectives can advance quality and thereby improve patient and consumer outcomes.

64. Content analyses of tobacco product brand marketing on mobile websites, smartphone apps, and social media

Authors: O’Brien, Erin, FDA/CTP; Navarro, Mario, FDA/CTP; Hoffman, Leah, FDA/CTP; Ganz, Ollie, FDA/CTP

Plain Language Synopsis: This research describes how tobacco companies reach people on smartphones. Generally, ENDS, hookah, and cigar brands marketed on mobile websites and social media, and rarely used age gating or displayed health warnings. Cigarette and smokeless brands marketed on mobile websites and apps, and often used age gating and displayed warnings.

Abstract:

BACKGROUND: Most youth and adults have access to smartphones and use them for hours per day. Little is known about how tobacco marketing has evolved to reach people using these devices. The current research describes how cigarette, smokeless

tobacco, hookah, and Electronic Nicotine Delivery Systems [ENDS] companies reach people using smartphone-optimized (mobile) websites, apps, and social media. **METHODS:** We conducted three content analyses to describe how tobacco companies use branded (1) smartphone-mobile websites, (2) smartphone apps, and (3) social media. We identified leading brands based on criteria such as, sales and advertising spending. We searched (1) for websites using a smartphone browser, (2) for apps on the Google Play and Apple app stores, and (3) for social media pages on the platforms of Instagram, Facebook, Twitter, YouTube, Pinterest, and Tumblr. **RESULTS:** Overall, about half of brands had mobile websites, and only cigarette and smokeless brands with the highest market share had smartphone apps. Websites offered internal social media, games, sweepstakes, and videos. All cigarette and most smokeless websites required age-verified accounts for entry, while few other websites did. Most ENDS websites required accounts for making online purchases. All cigarette and smokeless websites displayed health warnings, but no hookah and few ENDS and cigar websites did. All mobile apps provided time-sensitive, location-based coupons. Most ENDS, hookah, and cigar brands had at least one social media page, while very few cigarette and smokeless brands did. Many pages contained links to branded websites and online stores, and pages’ posts featured images of specific products. Few pages used any age gating, and less than one-quarter had a visible health warning. ENDS pages had the most engaged audience. **CONCLUSIONS:** Tobacco companies use websites, apps, and social media to market their products. Results can inform tobacco regulatory activities and prevention and cessation interventions.

65. Pre-ANDA Metrics: OPQ Perspective

Authors: Prodduturi, Suneela, CDER/OPQ/IO; Lee, Su-Lin, CDER/OPQ/IO; Tian, Geng, CDER/OPQ/IO; Wood, Erin L, CDER/OPQ/IO; Wei, Xiangyin, CDER/OPQ/IO; Hughes, Kathryn, CDER/OPQ/IO; Tyner, Katherine,

CDER/OPQ/IO

Plain Language Synopsis: The Pre-abbreviated new drug applications (Pre-ANDA) Program, established in GDUFA II, aims to clarify regulatory expectations and assist ANDA applicants to develop more complete submissions for complex products. This poster describes an analysis of Pre-ANDAs received in the 15-month post-launch period (since 01-OCT-2017) for various quality metrics.

Abstract:

Background: The Pre-abbreviated new drug applications (Pre-ANDA) Program, established in GDUFA II, aims to clarify regulatory expectations and assist applicants to develop more complete submissions for complex products. It is expected to promote an efficient ANDA review process and reduce the number of review cycles required for approval. This should ultimately expedite the availability of these complex products to the patients.

Purpose: The objective is to analyze the Pre-ANDAs received in the 15-month post-launch period (since 01-OCT-2017) for various quality metrics, to report on Office of Pharmaceutical Quality's (OPQ) efforts, and to share lessons learned by OPQ teams with stakeholders.

Methodology: The Pre-ANDAs received prior to 31-DEC-2018 were analyzed for various metrics including number of Pre-ANDAs granted/denied, dosage forms and intended route of delivery, type of submissions, types of Chemistry, Manufacturing, and Control (CMC) questions received, and timelines.

Results: The Pre-ANDA program has been well-received by industry. OPQ worked in close collaboration with the Office of Generic Drugs (OGD) during the entire Pre-ANDA process including triage, review, and communication with the applicant.

The Pre-ANDA statistics indicated:

- 107 Total PANDA Meeting Requests
- 68 (of 107) Accepted PANDA Meeting Requests
- 53 (of 107) Accepted by OPQ
- 1 Accepted OPQ-led meeting

- 100% on-time OPQ triage

Of the Pre-ANDA meeting requests that were granted in FY2018, the most were for products with complexity based on route of delivery (50%), followed by drug-device combinations (26%), dosage form (16%) and Active ingredient (16%). Analysis of the denied Pre-ANDAs indicated that 54% were denied mainly due to the meeting package being incomplete while 31% were denied as the requests were not related to complex products. Additionally, CMC questions were categorized to understand the challenges or regulatory gaps which in turn should help understand and align internal research and policy.

Conclusions: Implementation of the Pre-ANDA Program has required engagement, collaboration, and communication across multiple OPQ sub-Offices and CDER. Critical steps for FY2020 include shortening the meeting package triage timeline from 30 days to 14 days, and to develop advanced trackers to monitor the effectiveness (reduction in the number of review cycles) of OPQ pre-ANDA assessment efforts as the Pre-ANDAs become ANDAs.

66. Virus Recovery Affected by Contact Surface Physicochemistry of Polymer and Glass

Authors: C Shieh, FDA/CFSAN/Moffett; R Yan, IIT; Y Wang, FDA/CFSAN/Moffett; T Duncan, FDA/CFSAN/Moffett

Plain Language Synopsis: Viral transmission between food contact surfaces can result in contamination of the foods being prepared. In this research both food contact surface chemistry and smoothness were found to critically affect virus removal. The surface cleanability could be compromised by scratches or pinholes occurring during usage or manufacture.

Abstract:

Enteric viruses have been recognized as a major causative agent for foodborne illnesses worldwide. Viruses are transmissible via their adhesion/attachment to contact surfaces of food containers or tools.

This research evaluated the recovery or removal of coliphage MS2 as a virus surrogate from abiotic surfaces affected by (1) surface chemistry and (2) topographic smoothness ($< 1 \mu\text{m RMS}$), roughness, and porousness.

Abiotic surfaces of polypropylene (PP), polyvinyl chloride (PVC), polyethylene (low and high densities, LDPE and HDPE), and glass (borosilicate and soda lime) were characterized by atomic force microscopy (AFM), profilometry, tensiometry, and infrared spectroscopy. Observing the MS2 inactivation rate at 0 and 0.045 log/day respectively in tryptic soy broth (TSB) and PBS, we prepared MS2 in PBS containing 1% TSB as inocula onto 9 surfaces, incubated at 4 °C for 24 h, and quantified recoverable viruses by infectivity.

The virus recovery order from 4 smooth surfaces was PP > HDPE \geq LDPE \geq soda lime glass, with strong hydrophobic PP and PE noted. AFM revealed pinholes (diam. 21 ± 3 nm) on the borosilicate glass. These pinholes (being < 28 nm-diam. of MS2) or increased roughness possibly caused virus-trapping, thus decreasing virus recovery. Significantly ($p \leq 0.007$) more viruses were recovered from smooth compared to rough surfaces. All 9 surface recoveries were distributed into 6 statistical groups, with the highest and lowest being smooth PP ($76 \pm 12\%$) and hole-bearing borosilicate glass ($32 \pm 6\%$), respectively. The recoveries of PVC, PP and PE (including smooth and rough surfaces) were classified into 5 of the 6 groups.

The contact surface cleanability could be compromised by scratches or holes occurring during usage or manufacture. Our results illustrated that not all plastic surfaces release attached viruses with equal efficiency; the same was observed for two glass surfaces investigated.

67. The Role of Flavor in Electronic Nicotine Delivery System (ENDS) Advertising: A Comparison of Two Leading Brands

Authors: Moran, Meghan, JHU; Czaplicki, Lauren, JHU; Lagasse, Lisa, JHU; Cino, Samantha, JHU; Trigger, Sarah, FDA/CTP; Zandberg, Izabella, FDA/CTP; Sawdey, Michael, FDA/CTP; Kennedy, Ryan, JHU

Plain Language Synopsis: Flavors are a key reason many young people use e-cigarettes, but it is unclear how the current market leaders, JUUL and VUSE, use flavors in their advertisements. The objective of this study was to examine and compare the presentation of flavors in advertising for these two brands.

Abstract:

Background: As of 2018, JUUL has rapidly gained the largest share of the ENDS market. Research indicates that exposure to e-cigarette advertising is a risk factor for e-cigarette use, but little is known about JUUL's marketing strategy.

Purpose: This study examines and compares JUUL's advertising tactics to those of VUSE, which previously held the largest share of the ENDS market in the U.S.

Methodology: ENDS ads were obtained for the period between 2015-2017 from two market research agencies, which track a variety of media channels (e.g., magazines, radio, online). Ads were coded for claims, features, and appeals by one primary coder and reviewed by a second coder (Krippendorff's $\alpha=0.86$, 93% agreement).

Results: The study identified 20 unique JUUL and 284 unique VUSE ads. No JUUL ads conveyed flavor explicitly, but 30% ($n=6$) displayed colored pods, 10% ($n=2$) mentioned flavor and 10% ($n=2$) described the nicotine in the product ("nicotine salts"). JUUL ads made claims about technological innovation (30%, $n=6$), compared the product to smoking (75%, $n=15$), and featured young people (30%, $n=6$) and bright colors (90%, $n=18$). These ads were placed primarily in online media, and magazines targeting retailers. Most VUSE ads (89%, $n=252$) explicitly advertised the product's flavor, featuring clear flavor

labels and color coding and were placed in traditional media targeting consumers, such as print, TV, radio, and direct mail. Flavors were reinforced by complementary colors and imagery.

Conclusion: VUSE engaged in traditional advertising regarding the appeal of their flavors, while the few JUUL ads we found focused on technological/design innovation over flavor and advertised more prominently online and in business-to-business magazines. JUUL has increased its market share significantly despite an apparent lack of consumer advertising in the sample of ads examined in this study. The contrasting strategies between JUUL and VUSE, may illustrate how different products are positioned in the market. Additional research on the venues outside of mainstream print and digital media targeted at consumers, where JUUL is advertising, can provide insight into how claims, colors, and imagery are being used to promote JUUL products.

68. Developing an FDA Front-of-Pack “Healthy” Icon for Voluntary Use on the Food Label

Authors: Verrill, Linda, FDA/CFSAN; Wu, Fanfan, FDA/CFSAN

Plain Language Synopsis: FDA is exploring the development of a front-of-pack “healthy” icon for voluntary use on the food label and, following a thorough literature review, is planning to test a variety of “healthy” icon prototypes with consumers using focus groups, surveys, and an experimental study.

Abstract:

Responding to the need to help consumers make healthy food choices and to encourage industry to offer healthy products, the U.S. Food and Drug Administration (FDA) is exploring the development of a front-of-pack (FOP) “healthy” icon for voluntary use on the food label. It is planning to test a variety of “healthy” icon prototypes with consumers. This effort is part of FDA’s Nutrition Innovation Strategy, which aims to find new ways to reduce the burden of chronic disease through improved nutrition. The “healthy” icons are intended to graphically indicate that the

product complies with FDA’s soon-to-be-updated definition of “healthy” for voluntary use as a claim on the food label. An FDA literature review has revealed that FOP icons that provide guidance are preferred and are better understood by a wider segment of the population in countries, where tested, compared to those icons that only depict numerical summaries of product nutrients. FDA has developed icon prototypes to first test in a set of focus groups, after which more stylized icons will be further focus group-tested. The presentation will cover the full research plan, including details on the methods and examples of the prototypes to be tested.

69. CDER OPQ Extramural Stewardship Program

Authors: Vivian Wang, FDA/CDER; Kathryn Hughes, FDA/CDER; Katherine Tyner, FDA/CDER

Plain Language Synopsis: The Office of Pharmaceutical Quality (OPQ) of CDER has successfully collaborated with academia, industry, and other government agencies to leverage external expertise to strengthen pharmaceutical drug quality and encourage the adoption of emerging technologies and innovation.

Abstract:

The extramural stewardship program in the Office of Pharmaceutical Quality (OPQ) of CDER has successfully collaborated with academia, industry, and other government agencies to leverage external expertise to strengthen pharmaceutical drug quality and encourage the adoption of emerging technologies and innovation. Since the program was established in 2017, OPQ has issued roughly \$10 million each year through cooperative agreements and contracts to many organizations. The funded projects span advanced manufacturing to fingerprinting of complex products. This poster will discuss the scope and outcomes of the OPQ Extramural Regulatory Program and how they increase the quality of drug products for the American public.

70. Development of an approved, regulatory method for the detection of Natural Rubber Latex in Cosmetic products

Authors: Keeshan Williams, FDA/ORA; Jennifer Canale, FDA/ORA; Anna Marie Brown, CFSAN/OCAC; Stanislav Vukmanovic, CFSAN/OCAC; Anne Lucas CDRH/OSEL

Plain Language Synopsis: This project is focused on developing a validated method for the detection of Natural Rubber Latex in Cosmetic products that will be used to verify products bearing the “latex free” or “does not contain latex” labeling, in support of the requirements of the Federal Fair Packaging and Labeling Act.

Abstract:

Latex and latex-derived ingredients are used in a variety of cosmetic products and present a potential risk to consumers with latex hypersensitivities or allergy. About 1-6% of the general public is classified as being allergic to natural rubber latex (NRL), and about 6-8% of allergic individuals experiencing anaphylaxis upon latex exposure. Although FDA does have validated methods for detecting NRL proteins on the surface of some products (e.g. medical gloves, condoms, catheters), FDA does not have any validated methods for the detection of NRL in cosmetics. This project aims to address this need through the development of a validated, regulatory method for detecting NRL proteins in a variety of cosmetic products. Orthogonal detection modalities are used to quantitatively detect the presence of NRL in cosmetic products, whereby polyclonal and monoclonal antibodies (ELISA-based), respectively, serve as screening and confirmatory analyses. Extraction protocols currently used for the determination of NRL in medical products (e.g. medical gloves) were adapted and optimized for various cosmetic products, including body paints, eyeliner, palette and cream makeup, eye lash adhesives and hair adhesives. The analysis of products (eyeliner, body paint, hair adhesive and eyelash adhesive) labeled as “latex-containing” and “latex-free” that have been successfully evaluated using both polyclonal

and monoclonal ELISA-based methods will be presented. Efforts are currently underway to evaluate the effects on extraction efficiency and method performance with the incorporation of various detergents into the extraction protocol for samples fortified with NRL (IRM 913 - Ammoniated Latex Antigenic Protein reference antigen). The implementation of this method in the cosmetics industry could have a positive public health outcome affecting the labeling of cosmetics and reducing the incidences of anaphylaxis to cosmetics containing NRL proteins.

71. Let's Eat! A Qualitative Study Exploring Low-Income Caregiver Behaviors in Food Shopping and Preparation

Authors: Yu, Kathleen, FDA/CFSAN; Wu, Fanfan, JIFSAN/UMD; Dennard, Elizabeth, ORISE

Plain Language Synopsis: This study with low-income caregivers investigates how access to supermarkets affects the availability and type of food that caregivers purchase; and their experiences with making healthy food choices for their family at the grocery store and at home. Our results can inform future education and outreach efforts to this population.

Abstract:

Background: Research shows that children from low-income backgrounds have a higher rate of overweight status compared to those who are not. Additionally, low-income parents are more likely to experience barriers to accessing healthy foods for their families.

Objective: The study investigates (1) how access to supermarkets affects the availability and type of food that caregivers purchase; and (2) their experiences related to making healthy food choices for their family at the grocery store and at home.

Methods: This study comprises 12 focus groups of low-income, U.S. adult primary caregivers of children between 3-6 years. Groups are segmented by race/ethnicity and access to supermarket (defined by USDA as

distance to supermarkets) within Texas and the mid-Atlantic region.

Measurable Outcome/Analysis: Preliminary analyses of transcripts were conducted by three independent researchers to identify emergent themes.

Results: Overall, caregivers understood what comprises a healthy meal and stated interest in preparing healthy meals. Three major challenges were mentioned: (1) Budgetary concerns were a major barrier to purchasing healthy foods; (2) Some commented that they often find poor-quality healthy food options where they shop; and (3) Some said they find it difficult to shop for healthy options when their children are with them. Respondents also mentioned strategies to overcome identified barriers, such as using shopping lists and sticking to a specific path through the store. It was interesting to find that most caregivers in the “low access” groups overcame the barrier of distance by having access to a car (car ownership, carpooling) or using grocery delivery services.

Conclusion: This study highlighted that low-income caregivers are motivated to provide healthy meals for their families, but experience barriers to doing so effectively. Thus, it is important to provide greater support to mitigate the identified barriers. Such support includes shuttles for supermarkets with high-quality foods; expanded areas for grocery delivery services; greater opportunities for affordable childcare services.

Acknowledgements: The contractor for this project is Fors Marsh Group. We’d like to acknowledge the contributions of Miriam Eisenberg Colman, PhD; Shane Mannis, PhD; and Claire Constance, MPH.

Poster Guidelines

The 2019 FDA Science Forum:
Transforming Health: Innovation in FDA Science

Wednesday, September 11, 2019 - Thursday, September 12, 2019

FDA White Oak Campus, Great Room

POSTER EXHIBITION GUIDLINES:

1. Posters should reflect the findings, major conclusions, and impact of the work on public health.
2. Posters should be in landscape format and no larger than 6 x 4 feet (72x48).
3. Poster authors should set up their poster on the panels provided at least one hour before the start of their session. Posters must be removed immediately after each session.
4. Please register at the 2019 Science Forum Poster registration table to receive an envelope with pins and poster number.
5. Authors are responsible for bringing, mounting, and removing their posters. The poster exhibition staff will remove and dispose of posters that are not taken down.
6. The Great Room does not have a storage room, and the staff assumes no responsibility for posters left behind.
7. Please note that your poster session and your final poster number are in your acceptance e-mail. (*Example: Session 3 - 54 indicates that your poster exhibition session is 3, and the number of your poster is 54.*)

For detailed information go to:
www.fda.gov/scienceforum

Schedules

Poster exhibitions are scheduled to take place on the dates and time below:

Day 1: September 11, 2019

Poster Session 1

Great Room Section C and Room 1504

Time	Topics
9:50 a.m.–10:50 a.m.	Precision Health Advanced Technology

Poster Session 2

Great Room Section C and Room 1504

Time	Topics
3:40 p.m.– 4:40 p.m.	Advanced Technology Product Accessibility, Integrity, and Security Predictive Tools

Day 2: September 12, 2019

Poster Session 3

Great Room Section C and Room 1504

Time	Topics
9:00 a.m.–10:00 a.m.	Predictive Tools Advancing Digital Health and Artificial Intelligence

Poster Session 4

Great Room Section C and Room 1504

Time	Topics
1:00 p.m.– 2:00 p.m.	Advancing Digital Health and Artificial Intelligence Outbreak! Addiction Impacting Public Health Through Electronic Media: Empowering Consumers, Patients, and Other Stakeholders

Acknowledgements

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SESSION WORKING GROUPS

Session 1: Precision Health

Rhonda Moore, PhD, CDER/OND/ODEIV/DNDP (co-chair)
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Qiang Shi, PhD, NCTR/OR/DSB
Charlie Yongpravat, PhD, CDRH/ODE/DCD/VSD

Session 2: Advanced Technology

Richard Gray, PhD, CDRH/OSEL/DBP (Chair)
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Daren Freedberg, PhD, CBER/OMPT
Zhaohui Ye, CBER/OMPT
LCDR James Coburn, CDRH/OSEL/DBP
Ryan Ortega, OC/OCS/OSPD

Session 3: Product Accessibility, Integrity, and Security

Stephen Perrine, M.S., M.G.I.S, CFSAN/OAO/FDECS (co-chair)
LCDR Leslie Rivera Rosado, Ph.D., CDER/OPQ/OBP/DBRRIV (co-chair)
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Maryna Eichelberger, Ph.D., CBER/OCBQ/DBSQC
Brian Fitzgerald, B.Sc., CDRH/OSEL
Gerald Poley, M.D., CDER/OCD/CTECS

Session 4: Predictive Tools

Donna Mendrick, PhD, NCTR/OCD/ADRA (Chair)
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Session 5: Advancing Digital Health and Artificial Intelligence

Qi Liu, PhD, CDER/OTS/OCP/DCPV (Chair)
Richard Forshee, PhD, CBER/OBE (Panel Moderator)
Berkman Sahiner, PhD, CDRH/OSEL/DIDSR
Errol Strain, PhD, CVM/OR/DAFM
Rhonda Moore, PhD, CDER/OND/ODEIV/DNDP
Weida Tong, PhD, NCTR/OR/DBB
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Christopher Scully, PhD, CDRH/OSEL/DBP
Richard Gray, PhD, CDRH/OSEL/DBP
Jeffrey Archer, MS, ORA/ORS/OFFLO/ARKL

Session 6: Outbreak!

Surender Khurana, PhD, CBER/OVRR/DVP (Co-chair)
Chad P. Nelson, PhD, CFSAN/OCD (Co-chair)
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Emily Braunstein, PhD, CBER/OD/ADR
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Matthew R. Schwerin, BA, CDRH/OSEL/DAM
Daniela Verthelyi, MD, PhD, CDER/OPQ/OBP/DBRR-III

Session 7: Addiction

Katherine Bonson, PhD, CDER/OCD/CSS (Co-chair)
Chad Reissig, PhD, CDER/OCD/CSS (Co-chair)
Syed Ali, PhD, NCTR/OR/DNT
Sarah Arnold, MD, MPH, CDER/OND/ODEII/DAAAP
Chad Burger, MS, CTP/OCE/DEM
Christopher Ellis, PhD, CDER/OTS/OCP/DARS
Naomi Kruhlak, PhD, CDER/OTS/OCP/DARS
Berk Oktem, PhD, CDRH/OSEL/DBCMS
Karla Price, MS, CTP/OS/RS
George Rochester, PhD, CTP/OS/DPHS
Pavel Takmakov, PhD, CDRH/OSEL/DBCMS

Session 8: Impacting Public Health Through Electronic Media: Empowering Consumers, Patients, and Other Stakeholders

Christine Lee, PharmD, PhD, CDER/OCD (Chair)
Steve Bradbard, PhD, CFSAN/DPHIA
Lyle Canida, PharmD, CBER
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Suvajyoti Guha, PhD, CDRH/OSEL
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Map of Building 31, Great Room

