Showcase of Medical Discoveries

Wednesday
April 10, 2019

UAMS Winthrop P. Rockefeller
Cancer Institute Rotunda (10th Floor)

4:30 – 6:00 p.m.

FDA U.S. FOOD & DRUG ADMINISTRATION
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Showcasing research conducted at the FDA National Center for Toxicological Research.

UAMS Office of Research

For more information, contact:
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#1

Division of Microbiology at FDA's National Center for Toxicological Research

Steven L. Foley *and Carl E. Cerniglia*
FDA, National Center for Toxicological Research,
Division of Microbiology

The Division of Microbiology's goals are to perform fundamental and applied research to address critical issues in support of the FDA mission. The Division's research projects are based on expertise of division staff and consultation with scientists from other FDA Centers, regulatory agencies, academia, and industry.

The Division staff includes research scientists, research support staff, postdoctoral fellows, undergraduate and graduate students, visiting scientists, and program support specialists. Researchers in the Division have the facilities and equipment to help address the scientific challenges encountered by FDA and other government organizations. The Division of Microbiology scientists are actively engaged in research addressing FDA issues with special emphasis on:

- Host-microbe interaction
- Antimicrobial resistance
- Microbiome assessment
- Microbial contamination
- Foodborne pathogens and virology
- Office of Women's Health, tobacco, and nanotechnology projects

Deputy Director — Division of Microbiology

Steven L. Foley, Ph.D.

Background

Dr. Steven Foley is a Supervisory Research Microbiologist and Deputy Director of the Division of Microbiology at NCTR. Originally from Minnesota, Dr. Foley earned his Bachelor of Science in zoology and his Ph.D. in cellular and molecular biology/infectious diseases from North Dakota State University in Fargo. The focus of his research was on the expression and purification of the increased serum survival (Iss) protein, which was identified as an important virulence factor for Avian Pathogenic *Escherichia coli*, and the development and characterization of monoclonal antibodies towards Iss. He then completed a postdoctoral fellowship with the FDA Center for Veterinary Medicine, where the focus of his research was developing methods for source tracking of *Salmonella* from food-animal species. Following his postdoctoral fellowship, Dr. Foley served as an assistant professor at the University of Central Arkansas (UCA) where he taught courses in biology and microbiology and conducted research related to antimicrobial resistance in *Salmonella* and *E. coli*. During his time at UCA, Dr. Foley also served as a science advisor for the FDA Office of Regulatory Affairs, where he provided technical advice on research needs and methodologies. He continued as a science advisor after accepting a position as an associate research scientist with the National Farm Medicine Center at the Marshfield Clinic Research Foundation (MCRF). At MCRF, Dr. Foley led a research program focused on antimicrobial resistance and virulence of foodborne and zoonotic pathogens. In 2009, Dr. Foley joined NCTR and his research team has been focused in the fields of zoonotic diseases, food safety and tobacco-associated microbiology. In addition, Dr. Foley completed the Leadership Arkansas program and the Leadership in a Democratic Society program through the Federal Executive Institute and is serving as the co-chair of the NCTR Institutional Biosafety Committee and an adjunct professor in the Food Science Department at the University of Arkansas.

Research Interests

Dr. Foley's research program has been multifaceted to address FDA research needs in the areas of antimicrobial resistance and virulence in foodborne pathogens and tobacco-associated microbiology. Plasmids that are present in *Salmonella enterica* and other enteric pathogens often contain multiple genes that can encode for antimicrobial resistance, increased colonization, and/or overall virulence. A long-term goal of Dr. Foley's research is to better understand the role of plasmids in increased virulence among *Salmonella enterica*. In his previous studies, DNA sequencing of plasmids identified factors that are potentially important for increased virulence in *Salmonella*. For example, his team's research has demonstrated the contribution of plasmid-encoded VirB/D4 Type 4 Secretion Systems to increased invasion and survival in model systems. Several other plasmid types also appear to encode potential virulence factors, often along with antimicrobial-resistance genes, thus our interests are to further elucidate their roles in virulence and whether there is a co-selection of increased virulence and resistance. Another related interest is to better understand the factors that impact plasmid-transfer efficiency; preliminary studies have shown that differential exposure to certain antimicrobial agents can impact the efficiency of plasmid transfer. The team's ongoing research builds upon these earlier studies to evaluate a larger diversity of plasmids associated with *Salmonella* to identify their impact on pathogenicity (both looking at the pathogen and host sides of the equation) and refine the understanding of the role of antimicrobial exposure that may influence plasmid transfer among *Salmonella* and other enteric organisms. The second broad area of research in Dr. Foley's laboratory is related to microbiology of smokeless tobacco products. He has been utilizing culture-based, molecular, and bioinformatics approaches to identify and characterize the bacterial and fungal populations in smokeless tobacco products and evaluate their potential to contribute to carcinogen formation.

#2

Microbiome Research in the Division of Microbiology at FDA's National Center for Toxicological Research

Youngbeom Ahn, Huizhong Chen, Bruce Erickson, ***Steven Foley****, Kuppan Gokulan, Mark Hart, Jinshan Jin, Sangeeta Khare, OhGew Kweon, Seong-Jae Kim, Fatemeh Rafii, Doug Wagner, and Carl Cerniglia*

FDA, National Center for Toxicological Research, Division of Microbiology

Research scientists in the Division of Microbiology at the Food and Drug Administration's National Center for Toxicological Research (NCTR) are actively engaged in multiple microbiome-related research projects that impact various areas of public health. The mission of the Division of Microbiology is ***to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science***. To this end, several of these research projects being conducted in the Division involve the development of tools to more efficiently study members of the microbiome. These projects focus on the development of tools to study the microbiome and studies that focus on the impact of FDA-regulated products on the human microbiome. Overall, the research being conducted within the Division of Microbiology should provide advances that will contribute to improved public health for the citizens of the United States.

#3

Division of Bioinformatics and Biostatistics

Joshua Xu
FDA, National Center for Toxicological Research,
Division of Bioinformatics and Biostatistics

The Division of Bioinformatics and Biostatistics (DBB) is one of the six research divisions at National Center for Toxicological Research of the U.S. Food and Drug Administration (NCTR/FDA). The division was established in May 2012. The division consists of four programs critical to the NCTR missions and FDA review process including bioinformatics and biostatistics research and support, scientific computing activities for Center wide needs and functions as a part of NCTR's full-service computer center (e.g., software development, database management, high performance computing etc.,), and the newly established "review-to-research and return (R2R)" program. The division currently has about 50 staff members with some 40% of the level of effort in research, and the remaining 60% in support and service. The division's mission is to apply bioinformatics and biostatistical methods to conduct research and support diverse needs in precision medicine, drug safety, biomarker development, drug repositioning, risk assessment, and more within FDA. The division's research programs are designed and aligned to FDA product center needs, both current and prospective. We strive to ensure NCTR linkages with sister product centers are strengthened, and that the division's capabilities continue to evolve to be more diverse, robust, and capable of meeting future requirements of the FDA. The division's activities can be conveniently grouped into 5 themes: (1) Precision Medicine – assessing emerging technologies and their application for rare diseases and clinical application, (2) Predictive Toxicology – improving drug safety with predictive modeling and biomarker development, (3) Biostatistics and Bioinformatics Methodologies – applying and developing new methods, (4) R2R Program – enhancing the interaction between review and research, and (5) Service and Support – primarily supporting NCTR regulatory science research. These five themes guide/govern both the division's current activities and future directions, although individual projects within each theme might be refined, revised or even replaced to adapt to changing FDA or NCTR priorities.

Computer Scientist — Division of Bioinformatics and Biostatistics

Joshua Xu, Ph.D.

Background

After graduating with a Ph.D. in electrical engineering from Texas A&M University in 1999, Dr. Xu worked as a senior software engineer for a congressionally funded mobile telemedicine program at the Texas Center for Applied Technology, a research and development center of the Texas A&M University System. In this position he designed and developed many vital modules through software development and hardware integration. In 2007, he joined ICF International to work as an onsite contractor for the National Center for Toxicological Research. Dr. Xu's primary responsibilities included:

- data analysis
- method development
- design and development of bioinformatics tools systems to manage and analyze:
- genomics data
- genetics data

In 2012, Dr. Xu joined the newly formed Division of Bioinformatics and Biostatistics to focus on genomics and image analysis. In 2018, he became the Branch Chief for Research-to-Review and Return (R2R) in the Division.

Research Interests

Dr. Xu's experience includes about 16 years developing telemedicine systems, bioinformatics software and systems. He has been working closely with the FDA's Voluntary eXploratory Data Submission program to review and analyze the submissions involving genetic data and personalized medicine. He specializes in: 1)software design and implementation, 2) database design and development, 3) data mining, 4) signal and image processing algorithm development, 5) high performance computing, and 6) analyzing data to associate disease and toxicity with genetic profiles. Dr. Xu's experience includes more than 17 years developing bioinformatics software and systems and conducting bioinformatics research. He has worked closely with the FDA's Voluntary eXploratory Data Submission program to review and analyze the submissions involving pharmacogenomics, genetic data, and personalized medicine. Dr. Xu has in-depth expertise and experience in software design and development, data mining, data integration, next-generation sequencing data analysis, image analysis, high-performance computing, and analyzing data to associate disease and toxicity with genetic profiles. He has led several systems-development projects at NCTR including SNPTrack—an integrated solution for managing, analyzing, and interpreting genetic association study data. His recent endeavor has been with the Sequencing Quality Control (SEQC2) project, a large and international collaborative consortium led by FDA to evaluate the technical reliabilities and scientific applications of the next generation sequencing (NGS) technologies.

Dr. Xu's research interests lie in targeted deep sequencing, genomics, bioimaging data analysis, text mining, and machine learning. He is leading a large working group as part of the SEQC2 consortium to assess the reproducibility and detection sensitivity of onco-panel sequencing, including liquid biopsy. Onco-panel sequencing targets a few small regions of the genome and can detect rare, but clinically relevant, sub-clonal mutations. Accurate diagnosis and subsequent tailoring of therapy depends on thorough characterization of tumor mutational spectra. A cross-lab evaluation of eight pan-cancer comprehensive panels and five circulating-tumor DNA liquid-biopsy assays is currently underway. The working group has over 200 participants from academia, government agencies, and industry (including eight companies providing onco-panels and 29 testing laboratories). The scope and complexity of this comprehensive study is unprecedented and aims to provide recommendation in support for FDA's mission in regulatory oversight of NGS diagnostic tests.

#4

In *silico* Drug Repurposing for Rare Diseases Treatment Development at NCTR

Zhichao Liu[1], *Hong Fang*[1], *Donna L. Mendrick*[1], *Anne Praiser*[2], *William Slikker*[1], *Weida Tong*[1]
[1] *National Center for Toxicological Research and* [2] *Center for Drug Evaluation and Research, US Food and Drug Administration, Jefferson, Arkansas 72079, USA*

There are tremendous unmet needs in drug development for rare diseases. Computational drug repositioning is a promising approach and has been successfully applied to the development of treatments for diseases. However, how to utilize this knowledge and effectively conduct and implement computational drug repositioning approaches for rare disease therapies is still an open question. We aim to:

1. Develop bioinformatics frameworks for *in silico* drug repositioning in rare diseases.
2. Prioritize rare diseases regarding their repositioning opportunities.
3. Translate the novel genetic finding into rare disease treatment development.

In this showcase, we will elaborate on our drug repositioning projects with three examples including (1) Topic modeling of FDA drug labeling for exploring rare disease treatment options; (2) Deciphered miRNA transcription factor feed-forward loops to identify drug repurposing candidates for cystic fibrosis; (3) Potential Reuse of Oncologic Drugs for the Treatment of Rare Diseases. It aims to seek for potential collaboration opportunities and clinical opinions and supports for better position of our ongoing effects.

Visiting Scientist — Division of Bioinformatics and Biostatistics

Zhichao Liu, Ph.D.

Background

Dr. Zhichao Liu's background spans the fields of chemistry, biology, and computer science. In the past eight years, he took part in several cutting-edge projects in both industry and academia. Specifically, he developed several high-efficacy, quality-control strategies of processing analytical techniques and applied them to the tobacco industry. In recent years, Dr. Liu focused on developing the standard pipeline to balance the efficacy and safety in drug repositioning and drug-safety areas. The research aims to provide the standard *in silico* pipeline for drug repositioning and early drug-safety detection by retrieving, integrating, and organizing the information from chemical, biological, and clinical spaces. This helps the industry seek the optimal route to accelerate the drug-development efficacy from an advanced regulatory-sciences perspective.

Research Interests

- Developing innovative approaches for *in silico* drug repositioning for rare diseases
- Developing flexible and integrative risk-prediction systems for drug-safety evaluation
- Developing and applying standard pipeline for genomic data (e.g. microarray/NGS) analysis for drug- safety and -efficacy questions
- Developing strategies and frameworks to facilitate text-mining performance for diverse document types and infrastructures
- Designing and developing databases and visualization systems that allow interactive exploration of complex interior relationships embedded in biological-data profiles

#5

Non-Clinical in vivo Bioimaging at NCTR

S. Liachenko, *X. Zhang, C. Wang, F. Liu, S. Liu, N. Sadovova, A. Tripp, W. Slikker Jr.*

FDA, National Center for Toxicological Research,
Division of Neurotoxicology and Office of Center Director

The NCTR in vivo bioimaging facility is comprised of two MRI (Bruker 4.7 tesla 40 cm bore, and Bruker 7 tesla 30 cm bore) and two PET/CT scanners (Siemens PET Focus 220 + dedicated standalone CT, and Siemens Inveon PET/CT combo). Numerous imaging projects have been and are being executed at NCTR. Some of these include the development of neurotoxicity biomarkers, the assessment of the safety of pediatric anesthesia, gadolinium brain retention, and others. Several of these are featured in the poster.

Director, Bio-Imaging Lab — Division of Neurotoxicology

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

Background

Dr. Serguei Liachenko studied at the Russian State Medical University in Moscow from 1981-1988. He then worked at several institutions in Russian pharmaceutical and biotechnology business. In 1994, he obtained an M.D. and a Ph.D. degree in pharmacology from the Russian Research Center for Bioactive Compounds in Moscow. After relocating to the U.S. in 1995, he completed postdoctoral training at the University of Pittsburgh from 1995-2002. During this time; he received the National Research Service Award from NIH. In 2002, Dr. Liachenko joined the Bio-Imaging Center of Emphasis at Pfizer, Inc. In September 2009, he accepted the position of director of Bio-Imaging at NCTR.

Research Interests

Current research interests include:

- discovering and developing nonclinical imaging biomarkers of drug toxicity and efficacy using magnetic resonance imaging and spectroscopy.
- investigating glutamine cycle involvement in neurotoxicity, neurological disorders, and addictive substances abuse
- elucidating the mechanisms of toxicity using magnetic resonance.
- continuous improvement of imaging/spectroscopic methods in nonclinical applications

Dr. Liachenko's expertise includes:

- biology
- pharmacology
- animal research
- bio-imaging, magnetic resonance, imaging analysis
- algorithms development
- additive (3D printing) and subtractive (3D milling) rapid prototyping.

Dr. Liachenko's research goals include the development of imaging/spectroscopy biomarkers of toxicity to the stage of formal qualification by FDA, development of MRI toolboxes for routine applications in drug safety research, and elucidation of biochemical pathways of drug addiction using minimally invasive imaging technologies.

#6

FDA U.S. FOOD & DRUG ADMINISTRATION
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Evaluation of olfactory pathway neuropathology in a transgenic rat model of Alzheimer's disease

Sumit Sarkar *and Elvis Cuevas*
FDA, National Center for Toxicological Research
Division of Neurotoxicology

Alzheimer's disease (AD) is one of the most debilitating neurological diseases leading to impairments in cognitive, sensory and motor functions. Olfactory dysfunction in humans has been recognized as a potential biomarker for the early detection of AD. Olfaction is a process which initiates from a sensory neuron input to the olfactory bulb (OB), that is decoded in the piriform cortex followed by downstream stimulation of neurons in the hippocampus. An exploration of olfactory pathway alterations at different stages of disease progression, characterized by amyloid plaques (AP) deposition in the rat transgenic (Tg) AD model, holds the potential to describe a spatial-temporal pattern of neurodegeneration, related to early olfactory loss. Therefore, the aim of the present study was to characterize the neuropathology and protein alteration in the OB of AD Tg rats at different stages of disease progression (4-20 months). Histochemical staining with a styrylbenzene derivative, fluoro-styrylbenzene (FSB) was used to detect both AP and neurofibrillary tangles (NT). Fluoro-Jade C (FJC) was used to detect degenerating neurons and axonal degeneration. Western blot was used to detect proteins related with the amyloidogenic pathway including: APP (Amyloid-beta [Aβ] Precursor Protein), oligomeric Aβ, as well as pTAU (mainly component of NT), RAGE (receptor for advanced glycation end products, and LC3 (Light Chain 3). In the ADTg rats, moderate to intense AP deposition started around 6-8 months and progressively increased over time. Initially, non-fibrillar AP was detected at 6 months, that evolved to fibrillary AP after 8 months. Neurodegeneration observed as FJC positive neurons was observed after 8 months and progressed over time. No amyloid pathology or neurodegeneration were observed in control rats. Increased levels of APP and oligomeric Aβ started to be evident after 9 months in the AD Tg rat and increased over-time; compared to non-Tg rat. Levels of pTau were increased at all ages and gradually increased over-time. AD Tg rats showed increased levels of RAGE and LC3B starting at 15 months and increased over-time in AD Tg rat compared to non-Tg rat. Together, these data suggest that olfactory dysfunction worsen with disease progression in AD, which may correlate with early loss in olfaction. Further studies are necessary to fully characterize the amyloid pathology in the complete olfactory system including the entorhinal cortex and the hippocampus.

Research Biologist — Division of Neurotoxicology

Sumit Sarkar, Ph.D.

Background

Dr. Sumit Sarkar received his doctorate in neuropharmacology and neuroscience from Nagpur University in Nagpur, India in 2001 where he acquired a thorough background in neuropharmacology and toxicology. It was there that he performed research that contributed to the mechanistic understanding of neuropeptides, especially opiates, and their interactions with gonadotropin-releasing hormones in the hypothalamus. Dr. Sarkar studied how neuropeptides serve to affect the regulation of gonadotropin hormone release from the pituitary of the teleost, *Clarias batrachus* or walking catfish. He received further training during his postdoctoral tenure in Dr. Ronald Lechan's lab in the Division of Endocrinology and Metabolism at Tufts-New England Medical Center in Boston, Massachusetts in 2000 where he studied "the role of neuropeptides in regulating TRH and CRH in the brain of rodents during fasting and bacterial infection."

His postdoctoral research helped him confirm that phosphorylation of CREB is an essential step in activating thyroid and stress hormones in the brain, especially in the hypothalamus. This specific work earned him the 2002 Abbott Laboratory "Thyroid Research Clinical Fellowship Award for Best Poster Presentation" at 84th Annual Meeting of Endocrine Society. Additionally, he investigated satiety and feeding-inducing hormones and their effects on thyroid and stress hormones in the brain. Dr. Sarkar then joined the Pharmacology and Toxicology Department at Indiana University, School of Medicine to study "emotional stress-induced cardiac regulation and fever to explore common hypothalamic origins and brain stem mechanisms" (2004-2006). Afterwards, he worked as a clinical research fellow at the Simon Cancer Research Center in Indianapolis for one year. In 2008, he joined Boston Children's Hospital, Harvard Medical School as a research scientist to study the "role of Endoplasmic Reticulum stress in inducing obesity and diabetes."

In November 2008, Dr. Sarkar joined the NCTR. Since joining the FDA, Dr. Sarkar has published 30 peer-reviewed publications on which he is the first and corresponding author for eleven and seven, respectively. Dr. Sarkar received an FDA "Special Act Award" for exceptional productivity and special accomplishments as a research scientist and an "Outstanding Service" Group Recognition Award for exemplary work as a member of the NCTR summer student committee. Dr. Sarkar is an adjunct assistant professor in Pharmacology and Toxicology at University of Arkansas for Medical Sciences. He serves as an expert grant reviewer on several scientific committees including the Alzheimer's Association since 2012. Currently, he is a reviewer for *Current Alzheimer Research*, *Alzheimer's* and *Dementia*, *Neurotoxicology, Journal of Neurochemistry, Molecular Neurobiology, Neurotoxicology and Teratology, Toxicology In vitro, Food Chemical Toxicology*, *Journal of Toxicology*, *Journal of Drug* and *Alcohol Research* and a manuscript for Dove Press. In 2018, Dr. Sarkar became a member of the editorial board of *Metabolic Brain Disease* published by Springer.

Research Interests

Over the last eight years, Dr. Sarkar's research work has been focused on the effects of various neurotoxicants in the brain vasculature and other components of the neurovascular unit. The components of the neurovascular units (pericytes, microglia, astrocytes, and neurons, as well as basal lamina) act as an intricate network to maintain the neuronal homeostatic microenvironment. Thus, disruptions to this intricate cell network due to neurotoxicant exposure can lead to neuronal malfunction and symptoms characteristic of central nervous system diseases. Dr. Sarkar's laboratory investigates the role of neurovascular elements, ER stress, and endothelial dysfunction in neurodegenerative disorders with a special emphasis on Parkinson's and Alzheimer's disease using rodent models.

#7

Sex differences in repolarization reserve, a possible mechanism for sex-related differences in drug-induced QT prolongation and Torsades de pointes

*Feng Wei, Chengzhong Cai, Beverly Lyn-Cook, and **Li Pang***
FDA, National Center for Toxicological Research
Division of Biochemical Toxicology and Division of Systems Biology

Sex differences in drug-induced QT prolongation and Torsades de pointes (*TdP*) are well documented. Women are at a higher risk than men for experiencing Torsades, but the underlying molecular mechanisms are still not clear. One possible explanation is that women have longer baseline QT interval and reduced repolarization reserve. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) carry their donor-specific genetic/genomic information and may be a useful model for understanding mechanisms of sex differences in drug-induced *TdP*. As I_{Ks} is an important repolarization reserve current, in this study, we examined the responses of male- and female-derived iPSC-CMs to the I_{Kr} blocker dofetilide in the presence and absence of the I_{Ks} blocker JNJ 303. We found that compared to male-derived iPSC-CMs, female-derived iPSC-CMs showed larger FPDc prolongation and were more proarrhythmic when I_{Kr} and I_{Ks} were both blocked than blocking either of them individually. Interestingly, the expression of I_{Ks} beta subunit KCNE1, but not the alpha subunit KCNQ1, was higher in male-derived cardiomyocytes than in female-derived iPSC-CMs. Similar results were also found in a second batch of male- and female-derived iPSC-CMs that were derived from the same donors and another pair of male and female iPSC-CMs that were derived from different donors. KCNE1 has been reported to slow activation and abolish inactivation of *KCNQ1*. As higher levels of KCNE1 gene expression have been found in male adult heart (both epicardium and endocardium) than that in female adult heart, the increased expression of potassium channel KCNE1 may explain, at least partially, the reduced repolarization reserve and the increased susceptibility of females to drug-induced *TdP*.

Staff Fellow — Division of Biochemical Toxicology

Li Pang, M.D., M.S.

Background

Dr. Li Pang received an M.D. from North China Coal Medical College, China in 1993 with the second highest G.P.A. out of more than 300 students. As a result, she was selected to enter Peking Union Medical College Hospital for further training in endocrinology and started her research career in the cardiovascular field. In 1998, Dr. Pang received a scholarship from the University of Montreal and later joined Montreal Heart Institute to study ion-channel remodeling in heart failure and atrial fibrillation. In 2005, she was recruited as a faculty member for the Department of Pharmacology and Toxicology at the University of Arkansas for Medical Sciences, where she collaborated with other scientists and developed an NIH-funded RO1 project to provide novel long-term therapy for hypertension — serving as a co-principle investigator (PI). In December 2011, Dr. Pang joined FDA as a Commissioner's Fellow and received intensive regulatory science training. Upon graduation, she was converted to an FDA staff fellow and in December 2013. Currently, Dr. Pang is serving as the PI of three NCTR protocols, one of which is supported by the FDA Office of Women's Health and two are supported by the FDA Center for Drug Evaluation and Research (CDER). Dr. Pang has established wide and diverse collaborations with scientists at other FDA Centers, academia, and industry. She received both an individual award and the NCTR "Director's Award" in 2015 as a member of the *In Vitro* Cardiotoxicity Research Group.

Research Interests

Dr. Pang's research interests have included ion-channel remodeling in cardiovascular disease, pharmacogenetics, gene therapy, and biomarker identification. Her current research goal is to characterize comprehensively human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) for drug-induced cardiotoxicity detection using a wide range of techniques, including:

- cellular and molecular biology
- biochemistry
- viral gene transduction
- patch clamp
- microelectrode array
- impedance
- high-resolution imaging.

She is also interested in using the hiPSC-CMs model to investigate genetic and hormonal effects in sex differences of drug-induced QT prolongation and *Torsade de Pointes*. A third ongoing project is to identify potential biomarkers for early diagnosis of radiation-induced heart disease.

#8

Assessment of a 28-day oral exposure to the dietary supplements nattokinase and lumbrokinase individually or in combination with aspirin in Sprague-Dawley rats

*Camacho L[1], **Silva CS**[1], Vanlandingham MM[1], Patton RE[2], Olson GR[2], Felton RP[1], Ferguson SA[1], Law CD[1], Swift S[3], Abdel-Rahman A[3], Fitzpatrick S[3]*

[1]FDA, National Center for Toxicological Research, Division of Biochemical Toxicology, [2]Toxicologic Pathology Associates, [3]FDA, Center for Food Safety and Applied Nutrition

Nattokinase (produced by *Bacillus subtilis* var. *natto*) and lumbrokinase (produced by earthworms) are fibrinolytic enzymes promoted as health supplements for improving cardiovascular and circulation health in humans. The possibility exists that these fibrinolytic enzymes may increase the risk of bleeding, especially when taken together with other clot-modifying compounds. We report the findings of a 28-day study in which male and female Sprague-Dawley rats were dosed daily by oral gavage with nattokinase or lumbrokinase (1000 mg/kg body weight (bw)/day), individually or in combination with aspirin (10 or 100 mg/kg bw/day). No treatment-related changes were observed in body weight, grip strength or motor coordination behavior assessments, bleeding time, or clinical pathology. Both doses of aspirin inhibited the arachidonic acid-induced platelet aggregation. This effect was observed also when aspirin was co-administered with nattokinase or lumbrokinase, except in the low aspirin plus lumbrokinase combination group. The reduced effect of the low aspirin dose in the presence of lumbrokinase was probably due to the hydrolysis of aspirin by lumbrokinase esterases. Statistically significant effects were observed also in males treated with the high aspirin for a few parameters measured by thromboelastography; the magnitude of the effects was more pronounced in the high aspirin plus nattokinase combination group. At the histopathology level, the only treatment-related observations were an increase in the incidence of mucosal erosion and regenerative epithelial cell hyperplasia in the glandular stomach in the high aspirin groups, individually or in combination with lumbrokinase or nattokinase. This work was sponsored under an interagency agreement between the FDA/NCTR and the NIEHS/NTP (FDA IAG # 224-12-0003/NIEHS IAG # AES12013).

#9

Gene expression and DNA methylation alterations in the glycine *N*-methyltransferase gene in diet-induced nonalcoholic steatohepatitis-associated liver carcinogenesis

Barbara Borowa-Mazgaj, *Aline de Conti, Volodymyr Tryndyak, and* ***Igor P. Pogribny***
FDA, National Center for Toxicological Research, Division of Biochemical Toxicology

Non-alcoholic fatty liver disease (NAFLD) is becoming a major etiological risk factor for hepatocellular carcinoma (HCC) in the United States and other Western countries. In the present study, we investigated the role of gene-specific promoter cytosine DNA methylation and gene expression alterations in the development of NAFLD-associated HCC in mice using (i) a diet-induced animal model of non-alcoholic fatty liver disease (DIAMOND), (ii) a Stelic Animal Model (STAM) of NASH-derived HCC, and (iii) a choline- and folate-deficient (CFD) diet (CFD model). We found that the development of NAFLD and its progression to HCC was characterized by down-regulation of the glycine N-methyltransferase (GNMT) and this was mediated by progressive *Gnmt* promoter cytosine DNA hypermethylation. Using a panel of genetically-diverse inbred mice, we observed that GNMT down-regulation was an early event in the pathogenesis of NAFLD and correlated with the extent of the NAFLD-like liver injury. Reduced *GNMT* expression was also found in human HCC tissue and liver cancer cell lines. In in vitro experiments, we demonstrated that one of the consequences of GNMT inhibition was an increase in genome methylation facilitated by an elevated level of S-adenosyl-L-methionine. Overall, our findings suggest that reduced *Gnmt* expression caused by promoter hypermethylation is one of the key molecular events in the development of NAFLD-derived HCC and that assessing *Gnmt* methylation level may be useful for disease stratification.

Research Biologist — Division of Biochemical Toxicology

Igor Pogribny, M.D., Ph.D.

Background

Dr. Igor Pogribny received an M.D. in 1982 and a Ph.D. in biochemistry and oncology in 1986 in the Ukraine and joined NCTR in 1999. He is the recipient of the FDA “Outstanding Service Award” (2006) and FDA “Commissioner’s Special Citation Award” (2011).

Research Interests

Dr. Pogribny’s research interests and experience are centered in three major areas: 1) to elucidate the role and significance of genetic and epigenetic alterations in the development and progression of cancer, 2) to investigate and establish the role of epigenetic alterations as indicators of exposure to genotoxic and non-genotoxic carcinogens — critical for the primary prevention of neoplasia in humans, and 3) to conduct research on nonalcoholic fatty liver disease (NAFLD).

NAFLD is strongly associated with metabolic syndrome and obesity. It includes a range of liver injury from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, and is a major health problem and leading cause of chronic liver disease in the United States and Western countries. Dr. Pogribny’s laboratory aims to better understand the role of individual genetic and genomic — including epigenomic — susceptibility factors in the development of NAFLD. Identification of these susceptibility factors may facilitate the development of diagnostic tools that can be used to identify high-risk individuals to help prevent NAFLD-related liver cancer.

#10

Aptamer-based Proteomics Identifies Potential Predictive Biomarkers of Doxorubicin-induced Cardiotoxicity

Li-Rong Yu[1], *Jaclyn R. Daniels*[1], *Zhijun Cao*[1], *Richard D. Beger*[1], *William Mattes*[1], *Issam Makhoul*[2], *Angela Pennisi*[2], *Jeanne Y. Wei*[2], *Jane P.F. Bai*[3], *Julia T. Lathrop*[4], *Jinong Li*[5], *Valentina K. Todorova*[2]

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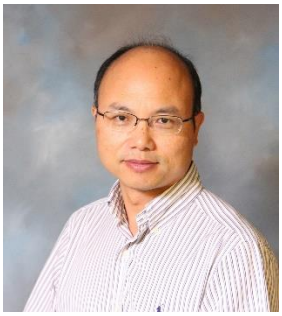
Treatment of cancer patients with doxorubicin (DOX) can cause cumulative dose-dependent cardiotoxicity, yet currently there are no clinically validated biomarkers for the prediction of cardiotoxicity caused by DOX treatment. In this study, 70 breast cancer patients were enrolled under an IRB-approved protocol and treated with a combination of DOX (60 mg/m^2) and cyclophosphamide (600 mg/m^2). Blood samples were collected prior to treatment (T0), and after the first (T1) and second (T2) cycles of DOX treatment. Cardiac function was assessed by a multi-gated acquisition (MUGA) scan before the start of DOX treatment and at completion of four cycles of chemotherapy. The SOMAscan® proteomic platform was used to profile 1,305 proteins in each plasma sample and statistical analyses compared protein levels at the three time points in two groups of patients: the cardiotoxicity group (N=14, those patients with a reduction of >10% in left ventricular ejection fraction after the completion of treatment) and the normal function group (N=56, i.e., normal LVEF after the completion of treatment). Fourteen proteins showed different abundance levels in plasma at T0 in the cardiotoxicity group as compared to the normal group. In addition, six proteins at T1 and one protein at T2 were decreased in the cardiotoxicity group. Kaplan-Meier analysis, log-rank test, and Cox regression analysis indicated that plasma levels of these proteins were associated with increased rate and risk of cardiotoxicity. These putative biomarkers may be useful for the prediction of DOX-induced cardiotoxicity, however further validation is needed using a larger cohort of patients.

Team Leader — Division of Systems Biology



Li-Rong Yu, Ph.D.

Background

Dr. Li-Rong Yu studied biology at the Hangzhou Teachers College in China and graduated with a B.S. degree in 1991. He then attended the Institute of Hydrobiology, Chinese Academy of Sciences and earned an M.S. degree in hydrobiology in 1994. There he studied the origin, evolution, and phylogeny of the *pseudogobiini* fishes in East Asia. From 1994 to 1997, Dr. Yu worked as an engineer at the Institute of Reservoir Fisheries, Chinese Academy of Sciences, and studied the effects of nutrition and physiological factors on fish growth. He then attended the Shanghai Institute of Biochemistry, Chinese Academy of Sciences to study cancer proteomics and received a Ph.D. degree in biochemistry and molecular biology in 2000. In the same year, Dr. Yu conducted postdoctoral research in quantitative proteomics and biological mass spectrometry at the Pacific Northwest National Laboratory. From 2002 to 2007, Dr. Yu worked at the Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., National Cancer Institute in Maryland. He worked as a scientist, senior scientist, and group head with studies focusing on the development of mass spectrometry-based quantitative proteomic technologies and their application to cancer biology. In 2007, Dr. Yu joined NCTR as a team leader and director for proteomics. His current research has focused on the development of proteomic biomarkers and understanding the mechanisms of drug-induced organ toxicity at the proteome level.

Research Interests

Predictive clinical biomarkers of chemotherapy-induced cardiotoxicity: Anthracycline-based chemotherapy is a major treatment tool for cancer patients, among which doxorubicin (Dox) is commonly used. However, a serious adverse side-effect is life-threatening cardiotoxicity Currently, imaging tests are the most practical cardiotoxicity monitoring tools; however, they are costly and can only capture cardiotoxicity once functional impairment occurs. Blood biomarkers, such as cardiac troponin T and are sensitive biomarkers of early cardiac-tissue damage, but their ability to predict cardiotoxicity is limited. Therefore, development of new biomarkers to identify potential cardiotoxicity prior to the occurrence of overt cardiac-tissue damage would help prevent permanent damage and/or identify patients at highest risk for cardiac damage. To achieve this goal, breast-cancer patients are recruited, and blood samples are collected before Dox treatment and at early time points after treatment. Blood samples are analyzed using systems biology approaches to identify early biomarkers to predict Dox-induced cardiotoxicity.

Proteomic biomarkers and mechanisms of drug-induced liver injury (DILI): DILI is one of the most frequent causes of drug-development failure and withdrawal of approved drugs. Overdose of acetaminophen (APAP) is well-known to cause clinical hepatotoxicity with a potential for acute liver failure. By using a rat model treated with different doses of APAP for different periods of time, we found molecular pathways evolved progressively from scattered and less significant perturbations to more focused and significant alterations in a dose- and time-dependent manner. Imbalanced expression of heme oxygenase 1 (HMOX1) and biliverdin reductase A was associated with hepatotoxicity. Furthermore, abundance changes of 31 proteins were uniquely correlated to liver damage, and HMOX1 could be a potential plasma biomarker of liver injury as verified via cross-species and validated between plasma and liver tissues.

Metabolomics & proteomics approaches to address pre-analytical variability in human plasma samples: Research in the healthcare area for the identification and validation of new biomarkers, drug targets, and treatment monitoring approaches often starts with the analysis of existing biobank samples or clinical samples collected. The quality of these collected biobank and clinical samples can be impaired by altering pre-analytical processing steps that will confound the analytical results and decrease the value of research. Differences in sample processing will alter the composition of metabolites and proteins in blood samples and could affect diagnostic test results. To address these issues, blood samples were acquired from healthy volunteer subjects and processed to plasma under different conditions including blood and plasma storage time, temperature, and centrifugation force. Proteomics and metabolomics approaches are employed to discover biomarkers of sample quality related to variations in pre-analytical processing of clinical plasma samples.

#11

Somatic Cancer Driver Mutations as Biomarkers of Cancer Risk

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We have been investigating how to use cancer driver mutations (CDMs) as biomarkers to: 1) identify carcinogens and establish how to predict chronic tumor responses from short-term rodent exposures, 2) understand the roles of prevalent, spontaneous CDMs in tissue-specific carcinogenesis and explore their use as human biomarkers of cancer risk, and 3) address topics related to precision cancer treatment. To address these topics, we use three different assays for the quantification of low-frequency somatic mutations. We've developed 16 different Allele-specific Competitive Blocker PCR (ACB-PCR) assays for human and rodent CDMs. Each assay measures a single base substitution, with a sensitivity of 10^{-5}. We use commercially available Droplet Digital PCR (ddPCR) assays for human CDMs, each of which measures a single base substitution at a time, with sensitivities that vary from 10^{-3} to 10^{-5}. Recently, we developed an error-corrected next-generation sequencing (EC-NGS) method that measures hotspot CDMs in 13 different human or rodent amplicons, measuring >100 hotspot CDMs at a time, with a sensitivity of 10^{-4}. Using these methods, we have made several important observations. Some hotspot CDMs are prevalent in normal human tissues, where they serve as substrates and reporters of chemical carcinogenesis. Hotspot CDMs are prevalent as mutant subpopulations in tumors, so a few CDMs can report on large swaths of human cancer. These undetected mutant tumor subpopulations are important for precision cancer treatment; specifically, they can be therapeutic targets or drive resistance to molecularly-targeted therapy. We have identified differences in the prevalence of mutant subpopulations among different breast cancer subtypes. We developed a lung tumor organoid model that allowed us to detect the outgrowth of erlotinib resistance-causing mutations consistent with those identified previously in clinical studies. We expect our new method EC-NGS method will speed development of CDMs as biomarkers of cancer risk.

#12

Genome-wide Mutation Detection by Interclonal Genetic Variation Analysis

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Genetic toxicology assays estimate mutation frequencies by phenotypically screening for the activation or inactivation of endogenous or exogenous reporter genes. These reporters can only detect mutations in narrow areas of the genome and their use is often restricted to certain in vitro and in vivo models. Here, we show that Interclonal Genetic Variation (ICGV) can directly identify mutations genome-wide by comparing sequencing data of single-cell clones derived from the same source or organism. Upon ethyl methanesulfonate (EMS) exposure, ICGV detected greater levels of mutation in a dose- and time-dependent manner in E. coli. In addition, ICGV was also able to identify a ~20-fold increase in somatic mutations in T-cell clones derived from an N-ethyl-N-nitrosourea (ENU)-treated rat vs. a vehicle-treated rat. These results demonstrate that the genetic differences of single-cell clones can be used for genome-wide mutation detection.

Staff Fellow — Division of Genetic and Molecular Toxicology

Javier Revollo, Ph.D.

Background

Dr. Javier Revollo received a B.S. degree in genetics from the University of Wisconsin-Madison in 2000 and a Ph.D. from Washington University in St. Louis in 2006. He pursued postdoctoral studies at the National Institutes of Health (NIH) between 2007 and 2012. He joined FDA as a commissioner's fellow in 2012. During his career, Dr. Revollo has studied several biomedical phenomena, including parasitology, NAD biosynthesis, mammalian aging, and glucocorticoid signaling. He has received numerous awards, including:

- "Fellows Award for Research Excellence" (NIH, 2010)
- "Rodbell Research Award" (NIH, 2010)
- "Presidential Award" from The Endocrine Society in 2011.

Dr. Revollo was recruited to FDA because of his expertise in mammalian genetics and next generation sequencing (NGS).

Research Interests

The flow cytometry-based Pig-a assay detects cells deficient in Glycosylphosphatidylinositol (or GPI)-anchored surface markers and provides a rapid and cost-effective enumeration of cells that are presumed to contain mutations in the endogenous X-linked Pig-a gene. Dr. Revollo is currently working on the validation of the Pig-a assay by genetically characterizing presumed Pig-a mutants derived from the assay.

Another research area that interests Dr. Revollo is the direct detection of somatic mutations. Somatic mutations are genetic alterations in cells that increase cancer risk. They can occur spontaneously but also result from DNA damage induced by the environment (e.g., sunlight) or genotoxic compounds (e.g, carcinogens). Current genetic toxicology assays can only estimate somatic-mutation rates by assaying the function of certain gene markers (e.g., Pig-a) or transgenes. Dr. Revollo is developing NGS methods capable of directly and efficiently identifying somatic mutations in the whole genome — in any tissue, and in any species, or any established cell culture — without the need for selecting and expanding cells that have mutations in only a few specific reporter genes.

#13

Development of Standard Test Method for Poly(ethylene glycol) Coating Quantitation on Gold Nanostructured Materials Using Reversed Phase High Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC-ELSD)

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Inorganic nanomaterials are promising candidates for biomedical applications including for drug delivery, imaging and radiation therapy, with many products in clinical trials. The critical quality attributes to consider for these nanomaterials include size, shape, composition, purity, stability and the surface properties. Many nanomaterial utilize poly(ethylene glycol) coatings to passivate the surface but very few measure various parameters that are required to assure consistency and reproducibility during product development. Parameters such as quantity, density, molecular weight, stability and homogeneity are important to measure. Slight variations in surface coatings on the nanomaterial may lead to differential recognition by the immune system *in vivo* and will lead to an undesirable/altered biodistribution, efficacy, and safety issues. An adequate characterization of these organic coatings on nanomaterials is important through standardized methods and methodologies. Consensus standards through stakeholder involvement can assist product development, quality control and accelerate review of submission to FDA. Here, we are presenting a robust and reliable test method for quantitative analysis of a common surface coating ligand, Polyethylene Glycol (PEG), using HPLC-ELSD. We prepared a series of bio-compatible spherical gold nanoparticles (AuNPs) and gold nanorods (AuNRs) coated with different molecular weight PEGs (e.g., M_w = ~ 5, kDa, ~10 kDa, ~ 20 kDa) followed by isolation, separation and quantitation of coatings using HPLC-ELSD system. Various techniques were compared for appropriate sample preparation to quantify the surface coatings. These methods are being proposed to ASTM International as work items towards standards development with stakeholder collaboration. This HPLC-ELSD method utilized here is a robust and very sensitive method to quantify poly(ethylene glycol) and its derivatives on a nanocrystal surface.

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Staff Fellow — Office of Scientific Coordination

Goutam Palui, Ph.D.

Background

Dr. Goutam Palui received his bachelor's degree in chemistry and master's degree in organic chemistry from Jadavpur University, Kolkata, India, followed by a Ph.D. in chemistry and bio-chemistry from the department of biological science, Indian Association for the Cultivation of Science, India. He then pursued postdoctoral research work at Florida State University and gained experience in the field of nano-biotechnology to develop multi-functional targeted drug delivery and imaging systems for interfacing the nanomaterials in biology. Dr. Palui dedicated seven years to the study of material science in the academic setting. Using his expertise in organic chemistry he designed inorganic nanomaterials to interface with them in biology.

- Synthesis and characterization of various inorganic nanocrystals (metallic such as gold and silver nanoparticles, magnetic such as iron oxide, and semiconductor QDs); developing various synthetic approaches of making new organic coatings based on different multi-dentate, multifunctional module(s); this includes both small molecule and polymers.
- Using nanoparticle as delivery platform for drug or bioactive molecules such proteins and antibody, sensing, imaging and tracking *in vitro*, as well as *in vivo* experiment.
- Designing a specific peptide molecule as enzyme substrates; probing the interaction between protein/enzyme and specific peptide using QD-bioconjugates.
- Developing FRET-based sensors for biology
- Synthesis of chiral polymers and block copolymers to create materials that mirror advanced material functions but have improved properties over their biological counterparts.

Dr. Palui joined NCTR in January 2018 as a staff fellow in the Office of Scientific Coordination (OSC). His current studies at NCTR are focused on the development of nanomaterials and their toxicity assessment both in vivo and in vitro. He is developing standards that can help regulatory agencies and industry and being actively involved in advanced training for postdocs and undergraduate researchers; engineering the surface of nanomaterials with organic coatings and evaluating the toxicity-related issues of nano-drugs regulated by FDA; and developing bio-compatible, multi-functional new nanomaterials that can be used safely for therapeutic purposes. Dr. Palui has co-authored more than 40 publications, book chapters, and reviews.

Research Interest

Dr. Palui designed and synthesized functional "polymer and block copolymer" systems with the goal of creating new materials that address key issues in society. Macromolecules can autonomously self-assemble into a hierarchy of secondary, tertiary, and even quaternary structures which can be utilized as synthetic proteins. Dr. Palui's research activities aim to judiciously incorporate chirality (a chiral molecule/ion is non-superposable on its mirror image) into synthetic homopolymers and block polymers to create materials that mirror these advanced functions but have improved "anti-biofouling" properties for marine vessels, medical implants, or algae-resistant glass. Another aspect of his research includes the synthesis of precision polymer with improved thermal and mechanical properties.

Dr. Palui improved understanding of the biological (as well as non-biological) systems by interfacing the "inorganic nanocrystals" (such as semiconductor, metallic, and magnetic nanoparticles) coated with various synthetic polymer ligands. Engineering the surface of nanoparticles with different reactive end-functionality to attach biomolecules and finally apply them for biotechnology including live-cell imaging, brain imaging, protein tracking, etc. His primary areas of experience include: 1) the design and synthesis of poly (ethylene glycol)-based new biocompatible polymers to coat the hydrophobic nanoparticles, introducing various bio-conjugation techniques—attaching proteins, peptides, and drugs; and 2) the design, synthesis, and characterization of various peptide and pseudopeptide molecules which self-assemble in aqueous, as well as organic media, to provide nano-structured gel materials.

#14

Standard Test Method Development for Lipid Quantitation in Liposomal Formulations

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Liposomal drug products have been approved for clinical use for over two decades with a gradual trend of increasing submissions to FDA on drug products containing nanomaterial. More than 1/3rd of the drug products containing nanomaterial submitted to FDA are liposomes. There are several advantages of liposomal drug delivery, including prolonged half-life, reduced toxicity and potentially improve 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 as well as regulatory review. Here in, we are presenting a fast, reliable and sensitive test method for lipid quantitation in liposomal formulations, using High Performance Liquid Chromatography (HPLC) with Charged Aerosol Detector (CAD). This method can be used for other non-chromophoric material, such as other lipid compositions, polyethylene glycol to assure quality control and to ascertain variations in lipid component profile for regulatory submissions.

Acknowledgments: This work was supported by an Interagency Agreement (IAG) between the U.S. Food and Drug Administration/NCTR and the National Institute of Environmental Health Sciences/NTP (FDA IAG No. 224-18-0502R/NIEHS IAG No. AES12013).