U.S. FOOD & DRUG ADMINISTRATION

Listing of Abstracts

2022 Student Scientific Research Day

August 11, 2022

FDA Annual Student Scientific Research Day

2022 Listing of FDA Student Abstracts

Background

Every year, FDA gives high school, college, and graduate students from different backgrounds and scientific disciplines the opportunity to train with mentors from across FDA on regulatory science research projects. Students are exposed to the broad expanse of regulatory science activities underway across the Agency as well as the range of scientific disciplines they call on. Students also learn first-hand about the Agency's domestic and global impact. After completing their FDA training, students are encouraged to explore careers in public health and STEM.

FDA is committed to recognizing the importance of mentor-led student research in STEM related fields. Annually, FDA holds Scientific Research Day to recognize and highlight the importance of FDA student programs and the direct impact their research projects have on advancing regulatory science at FDA. The FDA Office of Scientific Professional Development (OSPD) works with an Agency-wide planning committee to coordinate the FDA student research recognition activities annually.

In addition to the recognition program, FDA showcases the abstracts submitted by our students for the public on www.FDA.gov.

This book contains the abstracts from the 2022 FDA summer students. Among these participants, OSPD received 1 abstract from the Center for Biologics Evaluation and Research, 43 submissions from the Center for Drug Evaluation and Research (CDER), 32 from the Center for Device Evaluation and Research (CDRH), 11 from the Center for Food Safety and Applied Nutrition (CFSAN), 12 from the National Center for Toxicological Research (NCTR), and 1 from the Office of the Commissioner (OC.)

There were 100 total abstract submissions.

Program Goals

1. Recognize FDA student research and contributions to FDA.
2. Present FDA student research on a public website annually.
3. Support STEM education for students in FDA scientific priority areas.

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2022 Listing of FDA Student Abstract Titles listed by FDA Center/Office

2022 FDA Student Abstracts (listed by FDA Center/Office)

Center for Biologics Evaluation and Research (CBER)

1. **Abstract Title:** *Preparation of reference standard for quantitative RT-PCR assay for VP1-171D mutants quantification in the novel oral poliovirus vaccine type 2*
   Authors: Golikova, Anastasiya, FDA/CBER (Student); Manukyan, Hasmik, FDA/CBER (Mentor); Singh, Olga, FDA/CBER (Researcher); Chumakov, Konstantin, FDA/CBER (Mentor); Laassri, Majid, FDA/CBER (Mentor)
   FDA Strategic Initiative: Public Health Emergency Preparedness and Response
   Abstract:
   - **Synopsis**
     - The Plasmid_171 was amplified in E. coli, characterized by Illumina sequencing and evaluated by the quantitative RT-PCR assay. The sequencing data was analyzed by HIVE's Heptagon profiler to confirm the DNA sequence of the plasmid. The plasmid was found to be appropriate for use as a reference standard for the quantitative RT-PCR assay to quantify VP1-171D mutants in the nOPV2 vaccine.
   - **Purpose**
     - Frequent emergence of mutations is an inherent property of RNA viruses with implications for replication, pathogenesis, adaptation to their hosts as well as safety and efficacy of live viral vaccines. The conventional attenuated Sabin polioviruses used in oral poliovirus vaccine (OPV) can revert rapidly to neurovirulence during vaccine manufacture in cell cultures and in vaccine recipients. The novel OPV type 2 (nOPV2) was developed to be more genetically stable and prevent reversion to virulence. It was approved by the World Health Organization for emergency use to control outbreaks caused by circulating vaccine-derived polioviruses. It was shown that VP1-171D mutants can emerge in batches of nOPV2 and modestly increase neurovirulence in mice. Here we report the preparation of reference standard for a quantitative RT-PCR assay which is used for quantification VP1-171D mutants in nOPV2 batches.
   - **Methods**
     - The plasmid containing genome of nOPV2 with mutation VP1-171D (Plasmid_171) was used to transform One Shot™ TOP10 Chemically Competent E. coli, the transformed bacteria were grown over night at 37°C and were used for the plasmid extraction. The extracted plasmid was visualized by electrophoresis on 1% agarose gel, subjected to Illumina sequencing using MiSeq system, and evaluated by the quantitative RT-PCR assay. The Illumina sequencing data was analyzed by the FDA's High-performance Integrated Virtual Environment (HIVE) – Heptagon profiler to confirm the DNA sequence of the plasmid.

- **Results**
    - The result of sequencing confirmed the DNA sequence of the plasmid and the presence of the concerned A to G mutation at position 3053 which corresponds to amino acid change N171D in VP1 protein. The quantitative RT-PCR assay using the Plasmid_171 as reference resulted in accurate quantification of the mutants.
- **Implications**
    - The obtained plasmid was aliquoted in 1x Tris-EDTA buffer and saved at -20°C for use as a reference standard for the quantitative RT-PCR assay to quantify the VP1-171D mutants in the nOPV2 batches. The quantitative RT-PCR assay was developed by the FDA for quality control of the nOPV2 vaccine.

Center for Drug Evaluation and Research (CDER)

1. **Abstract Title:** *Categorization of Confirmatory Evidence of Effectiveness for Rare Disease Marketing Applications*
   Authors: Bagheri, Brandon FDA/CDER(Student); Nugent, Bridget FDA/CDER (Mentor); Thomas, Audrey FDA/CDER (Mentor); Lee, Kerry Jo FDA/CDER (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **Synopsis**
        - Rare disease drug development can be challenging, often due to limited understanding of the underlying biology of rare diseases and small patient populations. To be approved for marketing, a drug must be safe and effective for its intended use and fulfill FDA's statutory requirement for substantial evidence of effectiveness. For rare diseases, submitting 1 adequate and well-controlled trial with confirmatory evidence has been a feasible path to demonstrate substantial evidence of effectiveness for new drugs. In this project, we review and categorize confirmatory evidence used in rare disease marketing applications to better understand what constitutes strong evidence of effectiveness. This research will be used to inform future drug development for rare diseases.
    - **Purpose**
        - There are over 7,000 rare diseases (defined as impacting < 200,000 people in the US) with 30 million people suffering from them nationwide. Rare disease drug development can be challenging due to limited understanding of the underlying biology of these diseases and small patient populations. To be approved for marketing, a drug must be safe and effective for its intended use. FDA guidance explains how to demonstrate substantial evidence of effectiveness, which typically consists of 2 adequate and well-controlled trials or 1 adequate and well-controlled trial plus confirmatory evidence (CE).

Submitting 1 adequate and well-controlled trial with CE has been a feasible path to demonstrate substantial evidence of effectiveness in many rare disease applications. In this study, we categorized CE submitted in marketing applications for rare diseases from 2021-2022. This analysis will provide a better understanding of what constitutes "strong" CE to inform rare disease product review and future drug development for rare diseases.

- **Methods**
  - We identified New Drug Applications and Biologics License Applications for rare diseases that resulted in an approval or complete response from 2021-2022. The data were filtered to include only applications that submitted 1 adequate and well-controlled trial plus CE to fulfill FDA's statutory requirement for substantial evidence of effectiveness. Review documents were collected from an internal FDA database, CE information was mined by 2-3 independent investigators, and manually QC'ed. Analysis will be conducted with R and Python to summarize categories of CE and identify trends. This project is focused on instances where mechanistic and translational science approaches are submitted as CE. The approvals will be further defined to characterize the scientific approaches used to support rare disease applications.
- **Results**
  - Based on our review of the CE submitted in marketing applications for rare diseases from 2021-2022, we identified 6 distinct categories of evidence: Clinical Evidence from a Related Indication, Mechanistic or Pharmacodynamic Evidence, Evidence from a Relevant Animal Model, Evidence from Other Members of the Same Pharmacological Class, Natural History Evidence, and Evidence from an Additional Clinical Study. Additional categories may be added as more data are analyzed. Our initial analysis suggests that multiple types of CE are commonly submitted to support a single, pivotal adequate and well-controlled trial in rare disease applications. Although the results were not finalized at the time of abstract submission, we aim to further categorize the types of translational research used as CE in rare diseases and analyze patterns of CE across therapeutic areas by regulatory action (approval, complete response.)
- **Implications**
  - Rare diseases drug development faces significant challenges. Currently, rare disease marketing applications are reviewed by many divisions across CDER, since rare diseases occur across numerous therapeutic areas. This research project will provide an evidence base that can be used to describe the types of clinical and non-clinical data that have been considered by review teams across CDER as CE. The results will be shared broadly across CDER to facilitate unified approaches for assessing confirmatory evidence for rare disease applications. The results may contribute to better transparency and consistency when FDA discusses the requirements for CE with industry, thus facilitating rare disease

drug development. Communicating to reviewers and sponsors categories and types of CE used for past applications may help when considering the most appropriate scientific approach for a particular product.

2. **Abstract Title:** *Statistical Considerations for Using Tolerance Interval to Determine Product Specification*
Authors: Chen, Chang, FDA/CDER (Student); Zhao, Xutong, FDA/CDER (Mentor); Tsong, Yi, FDA/CDER (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

- **Synopsis**
  - Tolerance interval method is often used to determine the lower and upper specifications of a drug product. Under the normality assumption, the tolerance interval estimated by data is the confidence interval with a pre-specified confidence level. The width of the confidence interval decreases as the sample size increases. Therefore, the product specification determined by the tolerance interval method may be overly wide when the sample size is small. In this project, we discuss the sample size requirement for setting product specification. We also discuss the suitable coverage for a specific sample size. The statistical issues are illustrated with a simulated example.
- **Purpose**
  - Drug product specification needs to be determined that the probability of below the lower limit and of above the upper limit should be controlled at the same level. Therefore, when they are determined by data, two one-sided tolerance intervals should be used under the pre-specified confidence level of 95%. Given the difference in design, the estimation procedures are different. The objective of the project is to derive the sample size requirement for the estimation for a tolerance interval with a given coverage and confidence level for different study designs. We further examine the consequences when applying the wrong formula for the design and sample size. Typically, when dealing with continuous measurements, the sponsor determines the specification under normality assumption using tolerance intervals estimated by pooling the data collected from different batches. Furthermore, a high coverage interval is often used regardless of the limited sample size. The purpose of the project is to clarify the proper methods to be used according to the study design.
- **Methods**
  - Under normality assumption, the study design and sample size play important roles in determining how the tolerance interval is estimated and what coverage interval is proper and applicable. When the data are collected from multiple batches, the tolerance interval should be determined under the random effect model. When the data are collected from a single batch, the tolerance interval needs to be determined under a

single normal distribution. In this project, we derive the formulas for the two designs for the tolerance interval calculation. Based on the formulas, we derive the sample size determination for a given coverage P. For a given sample size, we derived the appropriate and corresponding coverage. Furthermore, for the objective of examining the difference in the specification limits when the wrong formula is used for a given design, we generate a data set with 30 batches and 10 observations per batch for a total of 300 observations. We use the simulated data set to illustrate the impact when applying the wrong formula for specification determination.

- **Results**
    - The conventional specification determined by sample mean +/- 3 sample standard deviations has been criticized because it is a point estimate of the interval of 99.73% coverage of a normal distribution. Since the estimate is derived from the collected data, it has estimation error. Therefore, a confidence interval of the interval should be used. The tolerance interval method should be used to incorporate the estimation error. When we use the pooled data without separating the between batch and within batch variations, the tolerance interval will have a different result.
- **Implications**
    - The results of this project provide the evidence of properly using the tolerance interval to determine the product specification limits. First, for any given intent coverage P, the sample size needs to be properly determined for different study designs. Second, in the data collection process from a study, the sample size determines the proper coverage P allowed to be used. These rules will serve as the review guidance for product specification evaluation.

3. **Abstract title:** *The Importance of Effective Scientific Leadership and Collaboration*
   Authors: Davies, Zarina, FDA/CDER (Student); Johnson-Williams, Dr. Bernadette, FDA/CDER (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract:
    - **Synopsis**
        - The purpose of this project was to design a framework to be utilized for leadership amongst health professionals. This study aimed to highlight the importance of scientific leadership and collaboration to create a framework for efficient teams and workstreams to use when conducting programs. The researcher interviewed six staff members within the Executive Program and Project Management (EPPM) team of the Office of Clinical Pharmacology within a time frame of three months. The focus of the interview questions was to determine existing best practices and improvements for efficiency. Thematic analysis uncovered the following five core competencies: strong communication, problem-solving, time management, flexibility, and cultural competence. This project suggests that establishing a framework focusing on

skills needed for effective scientific leadership and collaboration can enhance workflow productivity and serve as a strategy for developing successful health programs. This research serves as valuable information that can guide key practices for program development in health organizations.

- **Purpose**
  - The purpose of this project was to design a framework to be utilized for leadership amongst health professionals. Core competencies emphasizing scientific leadership and collaboration amongst health science professionals can enhance interdisciplinary teams. This is vital because scientific leadership benefits public health outcomes. This study aimed to highlight the importance of scientific leadership and collaboration for efficient teams and workstreams. Core competencies were used to create a framework for staff to apply as an effective strategy when conducting programs.
- **Methods**
  - The researcher interviewed six staff members within the Executive Program and Project Management (EPPM) team of the Office of Clinical Pharmacology. Virtual interviews were conducted within a time frame of three months. The focus of the questions was to determine existing best practices and improvements for efficiency. The researcher participated in activities to observe projects and daily staff activities. Responses from interviews were analyzed to identify competencies to generate a framework upon completion.
- **Results**
  - Thematic analysis uncovered the following five core competencies: strong communication, problem-solving, time management, flexibility, and cultural competence. Many participants indicated staff willingness to work together, responsiveness to feedback and constructive criticism, and staff engagement in programs with innovative subject matter as strengths within the office. Participants stated the following areas for improvement: identifying the best method of communication to help minimize misunderstandings amongst stakeholders; encouraging time management amongst customers with competing priorities; finding innovative ways to approach tasks and being considerate to include all perspectives in discussions.
- **Implications**
  - This project suggests that establishing a framework focusing on skills needed for effective scientific leadership and collaboration can enhance workflow and productivity. Utilization of a competency framework based on the aforementioned areas can serve as a strategy for developing successful health programs with positive outcomes. This further supports the literature which states effective leadership and staff collaboration produce optimal teams which thrive on commitment and excellence. This project, however had some limitations. A small sample size was used because the group within the office has a small number of staff. Interviewees although holding different positions, all belonged to the same group within the FDA. Expanding interviews amongst individuals from different offices could be something to consider for future research. Despite these limitations, staff within EPPM could still apply the

framework when working on programs with stakeholders. This research serves as valuable information that can guide key practices for program development in health organizations.

4. **Abstract Title:** *Advancement in PAT for Crystallinity Control Strategy and its Application in Pharmaceutical Manufacturing*
   Authors: Kayalar, Canberk FDA/CDER (Student); Ma, Chaoying FDA/CDER (Mentor)
   FDA Strategic Initiative: Increasing Choice and Competition through Innovation
   Abstract:
   - **Synopsis**
     - The physicochemical properties such as crystallinity and polymorphic form and its stability of active pharmaceutical ingredients (API) can impact pharmaceutical manufacturing process by affecting flowability, tableting, as well as finished product performance such as dissolution and bioavailability. Due to conditions that API endures during manufacturing and/or storage, the API may undergo polymorphic form change and/or conversion of amorphous/crystalline states, which is a common risk for advanced manufacturing, such as Additive (also commonly known as 3D printing, 3DP) and Continuous Manufacturing. The changes in crystallinity can lead to unexpected consequences if not identified and controlled in a timely manner. In some cases, such failures of life saving medication can lead to catastrophic outcomes that threatens patient's health and wellbeing. This investigation explores the current states of crystallinity monitoring and control strategy among approved NDA/ANDA which employ advanced manufacturing processes. Further, evolution of crystallinity monitoring and control strategy for high-risk drugs, along with the progress in formulation technology and manufacturing process is investigated through case studies, where high-risk drug refers to its manufacturing process and/or drug product quality that is sensitive to crystallinity change. The outcome of this data exploration can help us identify gaps between industrial practices and state-of-the-art process analytical technology (PAT) tool capability.
   - **Purpose**
     - The API's physiochemical properties such as crystallinity and polymorphic form can impact pharmaceutical manufacturing process such as flowability tableting, as well as finished product performance such as dissolution and bioavailability. Due to conditions API endures during manufacturing and/or storage, crystallinity of the API may change, which can lead to unexpected consequence if not identified and adequately controlled in a timely manner. Such failures of life saving medication can lead to catastrophic outcomes that threatens patient's health and wellbeing. The purpose of this investigation is to assess current states of crystallinity monitoring and control strategy and its application in pharmaceutical manufacturing.
   - **Methods**
     - Rigorous literature search is crucial to understanding the past and current practices pertaining to crystallinity monitoring and control strategy for high-risk drugs. The current states of crystallinity monitoring, and control strategy are

investigated among approved NDA/ANDA which employs advanced manufacturing process such as Additive (3D Printing), Continuous manufacturing, and conventional manufacturing. Risk assessment for crystallinity change regarding to additive (3D printing) manufacturing process, including selective laser sintering, stereolithography, binder jetting, and fused deposition modeling are performed. Further, high risk drugs (manufacturing process and/or drug product quality that is sensitive to crystallinity change), will be explored, along with the progress in formulation technology and manufacturing process, as well as factors of solid dosage forms (hard capsule, extended-release capsule, and granules). PAT methods for crystallinity monitoring, parameters, equipment, and outcomes are investigated. In addition to approved ANDA and NDA submissions, withdrawn applications for high-risk crystalline drug are also investigated to assess the potential impact of crystallinity change to decision of marketed product withdrawal.

- **Results**
  - To understand the current state of PAT for crystallinity monitoring and control strategy, case studies are performed for approved products using 3D printing and continuous manufacturing. Various analytical techniques, including the classical techniques of pXRD, DSC, FTIR, as well as the more advanced techniques of NIR, Raman, terahertz-pulsed, ssNMR, are examined for their applications in pharmaceutical manufacturing process. Mitigation strategy and controlling strategy for crystallinity monitoring and control of additive manufacturing process are explored and proposed. The advanced analytical techniques such as NIR, and Raman, are becoming more common for advanced manufacturing which frequently deal with crystallinity change during manufacturing and stability. Furthermore, application of crystallinity monitoring and control for a proven high risk crystalline drug are examined for submissions in a span of decades, and recent research publications, to identify the gap between industrial practice and state-of-the-art PAT tool capability. While data simulation to improve XRD sensitivity is reported for academia research, it is not considered an applicable tool for industry and regulatory submission. The approved and withdrawn submissions are investigated to identify pit falls and trends that results in product failure and its relevance with crystallinity change in the product.
- **Implications**
  - This investigation will bring further understanding of potential risk of crystallinity change in advanced manufacturing process. The investigation explores the suitability and analytical limits of PAT for crystallinity control strategy which will facilitate the review of future high risk crystalline drug submissions such as advanced manufacturing. This also have a potential to guide the industry and agency to implement appropriate techniques to monitor and control the crystallinity of API(s) throughout the drug product manufacturing process and its shelf life to enhance and ensure consistent drug product quality and clinical performance. The results will be presented in FDA

Student Science Day, FDA OPQ Summer ORISE Research Presentation, and archived in DPM II Summer ORISE sharepoint folder.

5. **Abstract Title:** *Efficacy Extrapolation for Pediatric Product Development for GERD*
Authors: Kim, Yurim, FDA/CDER (Student); Yi, Sojeong, FDA/CDER (Mentor); Kim, Insook, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
  - Multiple drug products have been approved for treatment of symptomatic Gastroesophageal Reflux Disease (sGERD) and erosive esophagitis (EE) in pediatric patients. The approved products include Proton Pump Inhibitors (PPIs) and H2 Receptor Antagonists (H2RAs), where the mechanism of actions is to inhibit gastric acid secretion and consequently increase the gastric pH. The current guidance for pediatric GERD indicates efficacy extrapolation may be feasible for pediatric efficacy in some cases. As a new class of products, potassium-competitive acid blocker (PCAB) is under development for GERD and EE in adults, we surveyed the product labeling and publicly available FDA reviews for PPIs and H2RAs to review how the pediatric efficacy and dosages were supported for pediatric approval. The efficacy of gastric acid reducers for sGERD and EE in pediatric patients was established by efficacy extrapolation from adults for PPIs and H2RAs. In addition, pediatric studies were conducted for PK and safety as well as efficacy or PD depending on the product. Our study revealed the different levels of efficacy extrapolation was used to support the pediatric indication for PPIs and H2RA over time requiring additional pediatric efficacy and/or PD data in pediatric patients.
- **Purpose**
  - Multiple drug products have been approved for treatment of symptomatic Gastroesophageal Reflux Disease (sGERD) and erosive esophagitis (EE) in pediatric patients. The approved products include Proton Pump Inhibitors (PPIs) and H2 Receptor Antagonists (H2RAs), where the mechanism of actions is to inhibit gastric acid secretion and consequently increase the gastric pH. To facilitate the pediatric development for the new class of gastric acid reducers for sGERD and EE, we surveyed how pediatric efficacy and dosage were supported for approved PPIs and H2RAs by obtaining the availability of safety, efficacy, PK, and PD data. We also evaluated whether the extrapolation of efficacy could further streamline the pediatric program of new drug class for the treatment of sGERD and EE.
- **Methods**
  - We evaluated the approved drug product labeling and publicly available FDA reviews for PPIs (Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, Rabeprazole, and Dexlansoprazole) and H2RAs (Famotidine, Cimetidine, Ranitidine, Nizatidine). Pediatric indications for PPIs and H2RAs include treatment of symptomatic GERD (sGERD), healing of erosive

esophagitis (EE), or maintenance of healing of EE. We gathered information how the labeling of the products for pediatric patients was supported: the availability of pediatric safety, efficacy, PK, and PD data in addition to those in adults and how the extrapolation of adult efficacy supported the product efficacy in pediatric patients. Evaluating presence of the pediatric data compared to those of the adults, the level of extrapolation for each drug could be obtained. Approaches to pediatric dosing selection are presented as followed: Pharmacokinetic (PK) and Efficacy Approach, PK and Pharmacodynamic (PD) Approach, and PK-only Approach.

- **Results**
  - Among six PPIs approved in adults, six PPIs were indicated for EE, and five PPIs were indicated for sGERD in pediatric patients. Four PPIs (Esomeprazole, Lansoprazole, Pantoprazole, and Rabeprazole) were not indicated for the maintenance of healing of EE in pediatrics. Per labeling, the efficacy extrapolation to pediatrics was used for all PPIs. Across PPIs, two levels of extrapolation were shown in major pediatric populations: Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole, and Dexlansoprazole used PK and Efficacy Approach to support pediatric dosing selection; Esomeprazole used PK and PD Approach. In detailed analysis, pediatric patients of age 1 month to less than 1 conducted PK and PD approach in Omeprazole, Esomeprazole, and Pantoprazole, in addition to the major pediatric populations of age 1 to 16 years.
    Among four H2RAs approved in adults, three of the drugs (Famotidine, Nizatidine, and Ranitidine) were approved for the use in pediatrics. Among three approved were indicated for sGERD and EE in pediatric patients and only ranitidine but not Famotidine and Nizatidine is approved for the use of maintenance of healing of EE in pediatrics. Across H2RAs, two levels of extrapolation were shown: Famotidine and Nizatidine used PK and PD Approach; Ranitidine used PK and Efficacy Approach.
- **Implications**
  - Overall, healing of EE and treatment of sGERD in adult and in pediatric populations can be extrapolated by adjusting dosage with adequate PK studies carried out in pediatric programs. Maintenance of healing of EE, however, requires additional safety and efficacy studies in pediatric patients as the data to the necessity of maintenance in pediatrics remain limited.

6. **Abstract Title:** *Immunogenicity Change upon Single Transition for Adalimumab Biosimilars*
   Authors: Martini, Brett, FDA/CDER (Student); Ji, Ping FDA/CDER (Mentor); Doddapaneni, Suresh FDA/CDER (Mentor); Sahajwalla, Chandrahas FDA/CDER (Mentor); He, Lei FDA/CDER (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
   - **Synopsis**
     - Lack of previous experience and complex study design challenge the clinical pharmacology characterization of a proposed interchangeable

biosimilar product. The number and duration of the switches during a switching study potentially affect the development of immune response and impact of PK. R programming software and data extracted from each individual clinical study reports provides analysis for several variables over the course of the study, including anti-drug antibody (ADA) incidence, neutralizing antibody (NAb) incidence, anti-drug antibody titers, and trough concentration. Comparison of the transition and non-transition arms for each study allows understanding the impact of transition and explores the opportunity for the simplification of the biosimilar drug development.

- **Purpose**
  - An interchangeable biosimilar is a biosimilar product that demonstrates the same clinical result with no greater risk in terms of safety or diminished efficacy as the reference product in any given patient, if administered alternating or switching between the use of the biological product and the reference product more than once to an individual than using the reference product without such alternation or switch. The clinical pharmacology characterization of a proposed interchangeable product remains challenging due to the complexity of study design and the lack of previous experience. For example, the switching study requires at least three switches between the proposed interchangeable product and the reference product. The number and duration of these switches may affect the development of immune response and consequently impact on PK. The goal is to investigate the impact of a single transition from reference product to a biosimilar on immunogenicity to inform a switching study and identify opportunities to simplify the regulatory approval process of biologic submissions.
- **Methods**
  - A comprehensive review of specific FDA-approved adalimumab biosimilars clinical study reports was conducted. Data, including BLA #, proprietary name, non-proprietary name, indication, anti-drug antibody (ADA) incidence, neutralizing antibody (NAb) incidence, anti-drug antibody titers, trough concentration, from each report was extracted for further analysis. After gathering and transposing data to Microsoft Excel, R computer programming software was utilized to generate tables and figures. Figures and tables are separated by each respective adalimumab clinical trial arm and transformed into panel plot by study population indication. Timepoint of single transition was highlighted on plots across the course of the clinical trials. Figures and data are prepared for dissemination in future research.
- **Results**
  - The manuscript summarizes a total of six adalimumab biosimilars. Data analysis generated by the clinical comparison for the anti-drug antibody

(ADA) incidence, neutralizing antibody (NAb) incidence, anti-drug antibody titers, and trough concentration for the transition and non-transition arms of each product submissions. ADA and NAb incidence, regardless of the arm or indication, result in a non-clinically significant change upon comparison of values before and after transition. Similarly, the average ADA titers, regardless of the arm or indication, result in a non-clinically significant change upon comparison of values before and after transition. Coefficient of variation percentage for the trough concentrations of each biologic demonstrate an absence of a clinically significant change pre and post transition point. In summary, regardless of safety, efficacy or PK variables, comparison of the switching and non-switching arms across the length of the study results in a non-clinically significant change in respective value after single transition.

- **<u>Implications</u>**
    - Results of the study indicates the opportunity for the simplification of the biosimilar drug development.

7. **Abstract Title:** *Exploration for exclusion of females of reproductive potential as bioequivalence study population in product-specific guidances for generic drug development*
   Authors: Paleracio, Christian Grace, FDA/CDER (Student); Tsui, Cynthia, FDA/CDER (ORISE Fellow); Nguyen, Duyen, FDA/CDER (ORISE Fellow); Li, Karen, FDA/CDER (Mentor); Frost, Mitchell, FDA/CDER (Mentor); Kim, Myong-Jin, FDA/CDER (Mentor); Shon, Jihong, FDA/CDER (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **<u>Synopsis</u>**
        - Product-Specific Guidance's (PSGs) describe the FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific reference listed drugs (RLDs). A safety-related consideration during PSG development is whether to enroll females of reproductive potential in bioequivalence studies with pharmacokinetic endpoints (PK BE studies). Information on RLD labeling such as toxicology data (e.g., genotoxicity, embryofetal toxicity, and fertility) and contraception recommendations are usually reviewed to guide the final determination of whether to include females of reproductive potential in a PK BE study. This project compiles a list of drugs for which the PSGs recommend excluding females of reproductive potential. The products' pharmacology and toxicology profile and exclusion rationale are collected to assess and identify trends and similarities that can serve as a resource to develop a standardized framework to inform future recommendations. Following the analysis of specified drug products excluding females of reproductive potential in PSG recommendations, several observations were made. Out of 61 drugs, 15 are positive for genotoxicity, 27 are reported to

produce serious embryofetal toxicity including fetal deaths in at least one species at exposures below or similar to the drug's maximum recommended human dose, and 29 have significant impact on fertility. There are 11 and 42 drugs with labeling recommendations on the use of contraception methods prior to and after termination of therapy, respectively. The current findings could provide an insight into the factors and levels of information that should be considered for the exclusion of females of reproductive potential as a PK BE study population. A standardized framework for the selection of BE study subjects developed based on this work can help improve consistency and promote efficiency of PSG development and ensure subject safety in PK BE studies for generic drug development.

- **Purpose**
    - Product-Specific Guidances (PSGs) describe the FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific reference listed drugs (RLDs). A major element for PSGs is the selection of the study population for bioequivalence studies with pharmacokinetic endpoints (PK BE studies) from safety perspectives. Exclusion of females of reproductive potential has been recommended for drugs with a teratogenicity potential. The decision for each drug has been generally determined based on its toxicological and toxicokinetic profile. This project aims to develop a standardized framework for an informed decision regarding study population selection by understanding the toxicological, PK, and clinical profiles of drugs for which PSGs recommend the exclusion of females of reproductive potential. Such framework can help improve consistency and promote efficiency of PSG development and ensure subject safety in PK BE studies for generic drug development.
- **Methods**
    - We first searched for PSGs recommending the exclusion of females of reproductive potential or a similar restriction for a study population (e.g., post-menopausal or surgically sterile, exclusion of all female subjects), using the PSG Search Shiny App, a full-text search tool of the FDA's PSG data. The initial search included only PSGs on oral drug products recommending PK BE studies in healthy subjects or the general population. The PSGs identified from the initial search were then filtered to exclude drugs recommending the exclusion of females of reproductive potential based only on indications. The following information of each identified drug after filtering were collected using its approved labeling and new drug application (NDA) submission package: reproductive toxicology data (e.g., genotoxicity, embryofetal toxicity, and fertility), contraception recommendations, PK characteristics (e.g., half-life), and enrollment of healthy subjects during the NDA program. Key components supporting the PSG recommendation for each drug were captured and their common features were sorted.

- **Results**
    - We identified PSGs corresponding to 61 RLDs recommending the exclusion of females of reproductive potential or a similar recommendation (e.g., post-menopausal or surgically sterile, exclusion of all female subjects), after excluding PSGs of drugs administered via non-oral route or contain recommendations of BE studies with clinical endpoint. Out of 61 RLDs, 15 are positive for genotoxicity, 27 are reported to produce serious embryofetal toxicity including fetal deaths in at least one species at exposures below or similar to the drug's maximum recommended human dose, and 29 have significant impact on fertility (2 and 6 drugs are reported to have irreversible or reversible impact, respectively, and 21 drugs lack reversibility data). Many of the identified drugs are for cancer treatments or antiviral agents. For most of the drugs, patients are advised to use effective contraception during their treatment. There are 11 and 42 drugs with labeling recommendations on the use of contraception methods prior to and after termination of therapy, respectively.
- **Implications**
    - The current survey provided an insight into the factors and levels of the information that should be considered for the exclusion of females of reproductive potential in PK BE studies. This collective information could serve as a resource for developing a decision framework on the selection of the PK BE study subject in relation to reproductive toxicity. A standardized decision framework for the selection of the PK BE study population will contribute to promoting the efficiency of PSG development and improving the consistency among PSGs. In addition, the current data collection and analysis performed for the selection of female subjects can be extended into consideration for the exclusion of males of reproductive potential in PSGs in future research.

8. **Abstract Title:** *Population PK Modeling of Monoclonal Antibody (mAbs) Drugs in Young Pediatrics*
   Authors: Wei, Hui, FDA/CDER (Student); Yu, Jingyu (Jerry), FDA/CDER (Co-Mentor); Liu, Jiang, FDA/CDER (Co-Mentor); Zhu, Hao, FDA/CDER (Co-Mentor); Ma, Lian, FDA/CDER (Co-Mentor); Wang, Yun, FDA/CDER (Mentor)
   FDA Strategic Initiative: Public Health Emergency Preparedness and Response
   Abstract:
    - **Synopsis**
        - Although it has been on the market for over a decade, confusion remains regarding the pharmacokinetics (PK) and optimal dosing of monoclonal anti-body (mAb), especially in young pediatric population. The major objective of this analysis was to conduct population PK modeling and simulation to

evaluate prediction accuracy of maturation functions in young pediatric patients. Monoclonal anti-body PK data from clinical studies were used for model development. The model was developed in a stepwise approach. Body weight or body surface area effect on clearance and volume distribution were used as the covariates in the allometric scaling factors approach. An age descriptor that combines gestational age and postnatal age (PAGE) using an asymptotic-exponential model best described mAb clearance in pediatric patients. A database of mAbs approvals in pediatric patients was built. Clearance and volume distribution of mAbs were better described by the function of maturation process. The maturation function model fitted the population better than the traditional linear body weight-based modeling. Maturation function applied to the pediatric group gave the lowest OFV. The approach of the best allometric scaling factors estimated by datasets ended up with a lower OFV compared with the standardized allometric scaling factors modeling plan. The developed model can be used to characterize the population PK of mAb in young pediatrics and quantify the effects of individual covariates on variability in mAb disposition. The application of maturation functions improved the model prediction accuracy in young pediatric patients. The approach of the best allometric scaling factors estimated by datasets improved the model fitting of the standardized allometric scaling factors widely used in the current pediatric modeling. The developed model combined functions of the maturation process and the allometric scaling, which can explore better exposures for various dosing scenarios of monoclonal anti-body in young pediatric population.

- **Purpose**
    - The objectives of this analysis were to 1) collecting monoclonal antibody drug information from the database with approval in pediatric patients; 2) exploring PK parameter changes and maturation processes for pediatric patients; 3) conducting Poppk modeling and simulation to evaluate prediction accuracy of maturation functions in young pediatric patients, and 4) assessing the impact of different modeling strategies to dosing recommendations in pediatric patients.
- **Methods**
    - Monoclonal anti-body (mAb) PK data from clinical studies were collected and used for model development. The model was developed using a stepwise approach: 1) a database with approval in pediatric patients was built by extracting PK information from DocuBridge system, 2) a compartment structural model with allometric scaling factors was fit to the pediatric data, 3) the maturation function was applied to the model on the top of the best allometric scaling factors, and 4) stage three model was applied to the different age groups of patients, infant group, pediatric group

and the entire clinical study population, to find the final model which described the whole population best. Body weight or body surface area effect on clearance and volume distribution were used as the covariates in the allometric scaling factors approach. An age descriptor that combines gestational age and postnatal age (PAGE) using an asymptotic-exponential model best described mAb clearance in pediatric patients. Gestational age was imputed as 40 weeks for full-term infants and adults in the modeling.

- **Results**
    - A database of monoclonal anti-body (mAb) approvals in pediatric patients was built. Clearance and volume distribution of mAbs were better described by the function of maturation process as the interindividual variability of these two parameters decreased. The maturation function model fitted the population better than the traditional linear body weight-based modeling approach as the objective function value (OFV) was significantly decreased, and a tighter fitting was observed from visual predictive check of goodness of fit (GOF) plots. Maturation function applied to the pediatric group gave the lowest OFV across the applications of infants group only and the entire population group, thought the number of subjects enrolled in each age group may affect the results. The approach of the best allometric scaling factors estimated by datasets ended up with a lower OFV compared with the standardized allometric scaling factors modeling plan.
- **Implications**
    - The developed nonlinear mixed-effect modeling (NONMEM) can be used to characterize the population PK of monoclonal anti-body (mAb) in young pediatrics and quantify the effects of individual covariates on variability in mAb disposition. The application of maturation functions to the pediatric population improved the prediction accuracy of modeling in young pediatric patients. The approach of the best allometric scaling factors estimated by datasets improved the model fitting of the standardized allometric scaling factors widely used in the current pediatric modeling. The developed model combined the descriptions of clearance as functions of the maturation process and the allometric scaling, which can be used to explore better exposures for various dosing scenarios of monoclonal anti-body in young pediatric population compared to the traditional linear body weight-based dosing regimens.

9. **Abstract Title:** *Human Abuse Potential Study Results in the Context of Abuse Detected Post marketing*

   Authors: Yohanka Caro, Silvia Calderon, Ling Chen, Saranrat Wittayanukorn, Sara Karami, Sheheryar Muhammad, Modupeola Adereti, Rajdeep Gill, Rose Radin, E. Gregory Hawkins, Chad J. Reissig, Dominic Chiapperino

   Abstract:

- **Synopsis**
    - This research focused on Human Abuse Potential studies for drugs that were determined to have a low potential for abuse, but no evidence of actual abuse post marketing. Similar to previous studies which assessed the relative performance of commonly used measures in the human abuse potential study, I analyzed data from six different HAP studies in order to determine whether other forms of analysis on subjective measures (such as Drug Liking and Take Drug Again) may offer a better understanding of patterns of abuse, once the drug is marketed.

      The overall research objective was to determine if the Human Abuse Potential study was too sensitive and therefore predicting signals of abuse inaccurately for the drugs in the study. The drugs analyzed in this study included the dual orexin receptor antagonists in Schedule-IV, and selective anticonvulsants in schedule-V.

      Overall, HAP studies are highly predictive in demonstrating that a particular drug can produce abuse related effects in people who recreationally use drugs. However, patterns of real-world abuse may be influenced by many factors (e.g., the indication of the drug, drug availability, economic factors, and the number of years a drug is on the market, etc.). These other factors inadvertently influence the likelihood that a drug may be abused. As a result, it may be useful to utilize other mechanisms that may better model these outstanding factors and predict the real-world abuse potential of a drug.
- **Purpose**
    - Human abuse potential (HAP) studies include multiple measures to assess subjective effects that correlate to drug-taking behavior. A positive HAP study informs regulatory decision making on whether or not a drug has abuse potential and, aids in determining an appropriate level of control in the Controlled Substance Act (CSA). It is less clear whether these data would predict the likelihood of abuse or misuse in the broader community setting post marketing. A positive signal in a human abuse potential (HAP) study may not always translate to high levels of actual drug abuse or misuse in the post marketing setting.

      Determination of the relative abuse liability premarket and actual abuse post approval is valuable in order to understand the risks to public health and safety. This study was conducted to determine whether or what type of additional data collection and analyses may better predict abuse patterns in the post marketing setting.

- **Methods**
    - We analyzed data from several HAP studies submitted to the FDA as a basis for placement in schedules IV or V, in which visual analog scales (VAS) were used to measure subjective effects such as drug liking, feeling good, feeling bad, and other drug effects, for the test drugs, positive controls and placebo. To evaluate cases of abuse detected post marketing, drug utilization and epidemiological data of abuse, and related adverse outcomes were collected from 2010-2020.

      Because of the lack of evidence of actual abuse for these controlled substances, we assessed the relationship between the subjective effects within each HAP study by calculating the effect size. This was done by determining the difference between the VAS scores of the test drug (T) and placebo (P), divided by the standard deviation of the difference.

      The effect size was used to determine the magnitude of the difference/or the association between the test drug and placebo. The closer the effect size is to zero, the closer the effect of the test drug resembled placebo.
- **Results**
    - The results of this study indicated that no generalizations can be drawn across drug classes. As a result, the findings for each drug need to be discussed independently.

      For the dual orexin receptor antagonists, suvorexant had no apparent dose-effect relationship for the positive subjective effects nor the Bad Effects. While in the case of daridorexant, increasing doses demonstrated a positive dose-effect relationship for positive subjective effects and Bad Effects.

      In the case of the anticonvulsants, the drugs analyzed demonstrated a positive dose-dependent relationship for positive subjective effects and Bad Effects, but there was no observed difference in the association between Bad Effects and Take Drug Again. For Take Drug Again there is a dose dependent decrease in effect size which corresponds to a dose dependent increase in Bad Effects. As bad effects increase, take drug again decrease. The Bad Effects of the anticonvulsants seem to have an impact on Take Drug Again.
- **Implications**
    - There may be several reasons why the abuse detected post marketing may not be consistent with a positive signal in the HAP studies and the abuse related adverse events (e.g., euphoria) reported in clinical trials. Actual patterns of abuse post marketing may be influenced by many factors

including but not limited to drug indication, availability, economic factors, the number of years a product is on the market, etc.

As new drugs with new mechanisms of action and lower abuse potential are being developed, other study approaches (e.g., behavioral economics, money vs. drug choice) may be worth further investigations to determine if they may provide more predictive data determinative of actual abuse post marketing.

10. **Abstract Title:** *Establishing an Analytical Workflow for Adaptive Immunity Profiling with NGS Technologies*
    Authors: Chen, Alex, FDA (ORISE Fellow)/CDER (Student); Xiao, Wenming, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Immune cell receptor profiling is important to the FDA's mission because it will advance precision medicine and provide orthogonal, functional evidence for the efficacy and safety of cancer immunotherapies and immunomodulatory drugs. High-throughput sequencing of BCR gene regions and downstream analysis of these reads allows us to simulate and reconstruct B cell receptors, repertories, and clonotypes. However, the use of effective analytical tools and computational environment is essential due to the nature of data generated from the next generation sequencing (NGS) technologies. In this study, we established a workflow with MiXCR, a universal software for fast and accurate analysis of raw T- or B- cell receptor repertoire sequencing data on precisionFDA for FDA researchers and their outside collaborators. Our results indicated that MiXCR is comparable to other software tools that utilizes different approaches for analysis of adaptive immunity profiling. Compared to TRUST4, an assembly-based receptor profiling software, MiXCR was able to produce similar results shown by the rank of IgH V region gene segments. Although our current study focused on RNA-seq and Exome-seq data, there is great potential to expand the breadth of MiXCR. This pipeline can be applied to data from whole genome or single cell sequencing, as well as data derived from targeted TCR/IG library amplification like 5'RACE, Amplicon, and Multiplex assays.
    - **Purpose**
        - Immune cell receptor profiling is important to the FDA's mission because it will advance precision medicine and provide orthogonal, functional evidence for the efficacy and safety of cancer immunotherapies and immunomodulatory drugs. High-throughput sequencing of BCR gene regions and downstream analysis of these reads allows us to simulate and

reconstruct B cell receptors, repertories, and clonotypes. However, the use of effective analytical tools and computational environment is essential due to the nature of data from the next-generation sequencing (NGS) technologies. In this study we focused on MiXCR, an adaptive immunity profiling software for fast and accurate analysis of raw T- or B- cell receptor repertoire sequencing data. By understanding the proper utilities MiXCR provides, we can optimize our processing pipelines and allocate resources more efficiently to get results that might aid in further research or review process.

- **Methods**
    - PrecisionFDA is a collaborative high-performance computing platform for next-generation sequencing assay evaluation and regulatory science exploration. Through precisionFDA, we created apps that allow scientists to experiment with all the different functionalities of MiXCR and compared results from other analysis software. The apps are essentially shell scripts that run inside a Linux virtual machine on the cloud. By specifying the appropriate input parameters, users can run multiple executions simultaneously. This highly secured cloud-based structure enables us to share data sets and analytical tools across FDA firewall, promoting research collaborations.
- **Results**
    - We established the MiXCR pipeline on PrecisionFDA for FDA researchers and collaborators. The pipeline returns multiple files based on export specifications, including an overall summary with information on clonotype from all immune repertoires or specific immune repertoires. After processing RNAseq data from 13 B-cell lines on the CDR3 region with MiXCR and TRUST4, an assembly-based receptor profiling software, we compared the top three V region gene segments and ranked them based on clone frequency. MiXCR and TRUST4 produced similar results where many of the top three gene segments were the same but their ranks varied. Out of the 13 samples ran by MiXCR, two (U-266 and BC-3) had the same top three gene segments and the same ranks while two other samples (RAMOS and U-2946) had the same gene segments, but different ranks compared to TRUST4. The rest of the samples often had two of the three same gene segments as TRUST4, but their ranks varied.
- **Implications**
    - Our results indicated that MiXCR is comparable to other software tool that utilizes different approach for analysis of adaptive immunity profiling. With more research and investigation into the customizable features of MiXCR, we can apply the current pipeline we have to single cell sequencing and whole genome sequencing data. Since our current study focused on RNA-

seq and Exome-seq data, there is great potential in creating an analytical pipeline to work on data derived from targeted TCR/IG library amplification like 5'RACE, Amplicon, and Multiplex assays as well.

11. **Abstract Title:** *Analysis of Sorbitol Content in Oral Liquid Formulations of Pediatric Medications*
Authors: Chen, Nikki, FDA/CDER (Student); Ton, Lexi, FDA/CDER (Student); Le, Thomas, FDA/CDER (Student); Cote, Brianna, FDA/CDER (Mentor); Vinueza, Gelareh, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **<u>Synopsis</u>**
    - Published case studies have hypothesized sorbitol's potential effect on decreasing drug absorption and bioavailability. The objective of this study was to examine the prevalence of sorbitol as an excipient in FDA approved, orally administered, liquid formulations labeled for pediatric use to evaluate whether current medications are dosed at efficacious levels due to decreased bioavailability from sorbitol. The oral liquid formulation products with pediatric indications were extrapolated from the interagency agreement between the Institute of Child Health and Human Development (NICHD) and the FDA. The most up-to-date label was pulled from public sources. Data collected included generic/trade name, formulation, submission number, BCS class, total product sorbitol content, discontinuation status, daily dosing for pediatric patients, and maximum daily dose for pediatric patients. Sorbitol amounts were compared after age group dosing was converted into per kilogram dosing through the CDC data table of weight-for-age charts. The established standard for a product with a known sorbitol effect on pediatric patients is lamivudine to which our preliminary data was compared to. For the 53 dosing regimens reviewed thus far, the sorbitol exposures range from 23.98 mg/kg/day to 3807.690877 mg/kg/day of sorbitol with an average of 1121.352 mg/kg/day, mode of around 887 mg/kg/day, and median value of 893.9504 mg/kg/day of sorbitol. Preliminary results suggest that dosing may need to be re-evaluated for pediatric liquid formulation drug products. The study of concomitant sorbitol with lamivudine provided evidence of decreased plasma drug concentration-time curve values as sorbitol amount increased. With the low-dose sorbitol from the lamivudine study (3.2 g) being lower than the current study's highest per kilogram sorbitol amount per day (3.8 g), should the drug react similarly to lamivudine, roughly a 14% decrease in plasma drug concentration would be seen.
- **<u>Purpose</u>**
    - Drug product excipients, such as sorbitol, provide stability or palatability in pediatric formulations but can have unwanted adverse effects. Published case studies have hypothesized that sorbitol's potential effect on decreasing

drug absorption and bioavailability is stemmed from drug solubility and intestinal permeability which are reflected in a drug's biopharmaceutical classification system (BCS) category. Potentially due to sorbitol's osmotic laxative effects, decreased bioavailability is seen for sorbitol-containing drugs because of increased fluid in the gastrointestinal tract and from enhanced gastrointestinal motility which can reduce drug contact time for absorption in the small intestine. The objective of this study was to examine the prevalence of sorbitol as an excipient in FDA approved, orally administered, liquid formulations labeled for pediatric use to evaluate whether current medications are dosed at efficacious levels due to decreased bioavailability from sorbitol.

- **Methods**
    - The oral liquid formulation products with pediatric indications were extrapolated from the interagency agreement between the Institute of Child Health and Human Development (NICHD) and the FDA. The most up-to-date label for each drug product was pulled from Drugs@FDA and Labels@FDA. For labels not available on FDA sites, Google searches and DailyMed were used to locate labels. Data collected included generic/trade name, formulation, submission number, BCS class, total product sorbitol content, discontinuation status, daily dosing for pediatric patients, and the maximum daily dose for pediatric patients. Daily sorbitol intake and maximum daily sorbitol intake were calculated using FDA approved dosing and the product's sorbitol content. Since pediatric dosing regimens often vary by age, the publicly available CDC data table of weight-for-age charts were used to calculate daily and maximum drug dosing per kilogram for the youngest age in each dosing age group at the 50th percentile.
- **Results**
    - The established standard for a product with a known sorbitol effect on pediatric patients is lamivudine for which the FDA approved an increase in the dose for pediatric patients from 8 to 10 mg/kg in September 2017. This was as a result of a sorbitol exposure study with lamivudine solution in healthy adult subjects. This study showed that concomitant use of sorbitol decreased lamivudine exposure in a dose-dependent manner. In this project, 53 dosing regimens were reviewed thus far for which the sorbitol exposures range from 23.98 mg/kg/day to 3807.690877 mg/kg/day with an average of 1121.352 mg/kg/day of sorbitol. The mode of the data set found more values around 887 mg/kg/day of sorbitol and a median value of 893.9504 mg/kg/day of sorbitol. The evaluated dosing regimens are for the 9 drugs analyzed thus far, but data collection is ongoing for the total of 370 drug products.

- **Implications**
    - Preliminary results suggest that dosing may need to be re-evaluated for pediatric liquid formulation drug products. The study of concomitant sorbitol with lamivudine provided evidence of decreased plasma drug concentration-time curve values as sorbitol amount increased. With the low-dose sorbitol from the lamivudine study (3.2 g) being lower than the current study's highest per kilogram sorbitol amount per day (3.8 g), should the drug react similarly to lamivudine, roughly a 14% decrease in plasma drug concentration would be seen. As data collection and evaluation continues, further analysis will be conducted considering the BCS category of each product to see whether decreased absorption is category specific. A limitation of this study is needing to exclude products from the analysis due to unavailable formulation information as well as estimated per kilogram dosing for products not labeled in milligrams per kilogram.

12. **Abstract Title:** *Crystallinity Monitoring and Control Strategy for High-Risk Drug Manufacturing: Current Status Assessment from Approved NDA/ANDA Towards Future Implementation in Advanced Manufacturing*
    Authors: Chen, Yingjie, FDA/CDER (Student); Kurtyka, Bogdan, FDA/CDER; Meng, Zhaoyang, FDA/CDER; Obrzut, Daniel, FDA/CDER; Madurawe, Rapti, FDA/CDER; Wu, Huiquan, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Polymorphism and crystallinity change are prevalent observations in pharmaceutical manufacturing. As different crystalline forms may have dissimilar physical and chemical properties, they impose significant risks on manufacturing processes, product quality, efficacy, and patient safety. Through data collection, statistical analysis, and process modeling efforts, this project aims to conduct a technology survey from recently approved applications to identify common industrial practices of monitoring and controlling crystallinity change in high-risk drug manufacturing. The critical parameters impacting crystallinity are investigated, and rational improvements to monitoring and control strategies are recommended for future considerations.
    - **Purpose**
        - Many drug products are developed with drug substances in crystalline forms. In solid states, these crystals can have different arrangements, resulting in polymorphism and the potential of crystallinity change. As different forms may have dissimilar physical and chemical properties, they can impose risks on manufacturing, product quality, efficacy, and safety. It

has been well recognized that change in crystallinity may impact dissolution, affecting clinical performance of a drug. Therefore, crystallinity is defined as a clinically relevant critical quality attribute (CQA) and needs to be monitored and controlled.

The purposes of this project are to conduct a survey from recently approved applications to identify industrial practices of monitoring and controlling crystallinity change in high-risk drug manufacturing, to conduct data analysis and modeling to identify gaps between current practices and the state-of-the-art in advanced manufacturing, and to suggest rational improvements.

- **Methods**
  - For the technology survey, a keyword search is performed on approved ANDAs and NDAs from 2017-2022 to identify applications that may involve crystallinity change. The results are screened per different risk levels. For each entry, information related to the drug classification, risk mitigation strategy, and monitoring technology are extracted with key technology parameters. Statistical summary and principal component analysis (PCA) are used to visualize the data and analyze key factors impacting the adoption of different strategies. The current practices are then compared with state-of-the-art tools. For representative cases, flowsheet-based process modeling is conducted to reveal the critical process parameters that may lead to crystallinity change and to establish their design space. High-risk unit operations such as granulation and compression are selected to illustrate how variability in the process conditions can be propagated into drug product CQAs, especially crystallinity.
- **Results**
  - Thus far, the study has screened over 4000 approved ANDAs and NDAs with keyword searches and surveyed over 300 cases for risk factors and/or control strategies. It has been observed that Powder X-Ray Diffractogram (PXRD) in pharmaceutical development phase is the most popular strategy used to mitigate the risk of crystallinity change, followed by the characterization of drug substance with PXRD or IR, setting PXRD specifications for drug substance release, and drug product release. Very limited cases use in-line monitoring and in-process controls for crystallinity, and most applications only employ one layer of control.
- **Implications**
  - With the collected dataset, statistical analysis results, and modeling efforts, the study provides detail information of current strategies used in monitoring and controlling crystallinity change in drug product manufacturing, and how they mitigate the associated risks. The project also supports future efforts in identifying technological advancements and addressing gaps in CMC of pharmaceutical solid polymorphism.

13. **Abstract Title:** *High-throughput Bioanalytical Method for Determination of Risperidone and its Active Metabolite in Rabbit Plasma: An application to bioequivalence evaluation of complex drug formulations in rabbits*

Authors: Chintapalli, Abhinav, FDA/CDER (Student); De Palma, Ryan, FDA/CDER (Mentor); Matta, Murali K., FDA/CDER (Mentor); Rouse, Rodney, FDA/CDER (Mentor)

FDA Strategic Initiative: Increasing Choice and Competition through Innovation

Abstract:

- **Synopsis**
  - Generic drugs are an important part of the drug market because they are typically less expensive than the "brand-name" drug, but require the same dosage, safety, stability, strength, effectiveness, and route of administration. The only difference between the brand-name and generic drug lies in its formulation. Demonstrating bioequivalence of complex drug formulations can be difficult since non-active pharmaceutical ingredients vary between formulations. The current study was designed to assess the bioequivalence of a risperidone complex extended-release dosage form. As a part of this evaluation, three long-acting risperidone microsphere formulations were developed using a computational model; one to serve as a reference product, one developed to be bioequivalent, and one developed to not be bioequivalent. To test the bioequivalence of these formulations, a 3-arm cross over study in rabbits was conducted. Risperidone & Paliperidone- (Risperidone Metabolite) were detected using a novel LC-MS/MS method that was validated according to current FDA guidance. Comparability of PK profiles of the 3 formulations was then used to establish bioequivalence.
- **Purpose**
  - The primary purpose of this study is to determine the concentrations of Risperidone and Paliperidone within the rabbit serum for three different drug formulations. These data will be used to develop PK profiles for the three drugs and help us determine bioequivalence, however, this is only the primary endpoint. The method used in this study is the first reportable simultaneous detection method for risperidone and paliperidone in rabbit serum which will be tested. The validity of the computational model-informed drug development method that was used to develop the different formulations will also be tested for its utility and predictive ability.
- **Methods**
  - This study was a 3-arm crossover study. Three cohorts of 6 rabbits were created, and each cohort was administered one of the drugs with blood collected over a 26-day period. Following a 2-week washout period, each cohort received a different formulation with the same sampling points. In the final period, each cohort was given the formulation they had not yet

received, and the same sampling schedule was followed. Blood was centrifuged and serum extracted and stored at -70° C. All samples were analyzed using the LC-MS/MS method. Extraction of analytes from serum involved using 100 µL of acetonitrile containing internal standards. The 100 µL of the internal standard dilution was added to 96-well filter plates and an aliquot of 30 µL of serum was added to each well. Samples were shaken for 5 mins and centrifuged at 4000 rpm for 5 min. The collection plate containing supernatant was placed in autosampler and 2 µL was injected into a mass spectrometer. Researchers were blinded to the identity of each rabbit and formulation of drug that was administered.

- **Results**
  - This is an ongoing study and the results are yet to be determined. We are unable to comment on whether the PK profiles of the different formulations will be comparable, however, the results from the pilot PK study indicate that the LC-MS/MS method used will be an accurate and effective way to calculate the concentrations of analytes simultaneously within rabbit serum.
- **Implications**
  - The bioanalytical method that was used in this study has been validated as a precise and accurate simultaneous detection method for risperidone, and its primary active metabolite- paliperidone. Although this method was developed for rabbit serum, the sample prep and method parameters for human serum would not differ significantly.

14. **Abstract Title**: *Analysis of Human Data in PLLR Converted Labelings*
    Authors: Dao, Ashley, FDA/CDER (student); Ceresa, Carrie, PharmD, MPH, FDA/CDER (Mentor); Kratz, Katherine, MD, FDA/CDER (Mentor); Dinatale, Miriam, DO, FDA/CDER (Mentor); Johnson, Tamara, MD, FDA/CDER (Mentor); Yao, Lynne, MD, FDA/CDER (Mentor); Maynard, Janet, MD, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis:**
      - In 2015, the Pregnancy and Lactation Labeling Rule (PLLR) took effect. The PLLR removed pregnancy letter categories, created new labeling requirements for subsection 8.1, Pregnancy, and subsection 8.2, Lactation, and added subsection 8.3, Females and Males of Reproductive Potential. The objective of this research project is to determine the impact of the PLLR on the inclusion of human data in subsections 8.1 and 8.2 and to identify gaps in human data that may guide research for pregnant and lactating people.
    - **Purpose**
      - In 2014, FDA published the final rule "Content and Format of Labeling for Human Prescription Drug and Biological Products; Requirements for

Pregnancy and Lactation Labeling," known as the Pregnancy and Lactation Labeling Rule (PLLR). The PLLR went into effect on June 30, 2015. The PLLR removed pregnancy letter categories from prescription drug labeling(s) and created new requirements for subsections 8.1 to 8.3. Subsection 8.1, Pregnancy, contains a risk summary about the drug's effect on pregnancy, including labor and/or delivery, and a discussion of data supporting the risk summary; this data was formerly found in the "Pregnancy" and "Labor and Delivery" subsections. Subsection 8.2, now titled Lactation, replaces the former 8.3 subsection, "Nursing Mothers." With the PLLR, subsection 8.3, Females and Males of Reproductive Potential, was introduced. The objective of this research project is to determine the impact of the PLLR on the inclusion of human data in subsections 8.1 and 8.2 and to identify gaps in human data that may guide research for pregnant and lactating people.

- **Methods**
  - PLLR-converted labeling approved between June 30, 2015, and December 31, 2021 was reviewed using the Drugs@FDA or DailyMed websites. Subsections 8.1 and 8.2 in labeling were evaluated for the presence of human data. Human data were evaluated and categorized as quantitative (numerical data) or qualitative (descriptive information only). Quantitative data were further subcategorized to demonstrate whether or not pharmacokinetic (PK) data were present. "PK data" was defined as labeling in subsections 8.1 or 8.2 with information related to drug's absorption, distribution, bioavailability, metabolism, and excretion as a function of time. The analysis for this study included calculating the proportion of PLLR-converted labeling that contained human data by year, and the proportion of labeling that contained qualitative versus quantitative and/or PK data.
- **Results**
  - A total of 1797 PLLR-converted labelings were analyzed. Human data were present in subsection 8.1 in 810 of 1797 labelings (45.0%); and in subsection 8.2 in 675 of 1797 labelings (37.6%). In subsection 8.1, 593 (73.2%) contained qualitative data and 217 (26.8%) contained quantitative data. Among the 217 labelings containing quantitative data, 16 (7.4%) labelings included PK data. Of the 809 labelings with human data in subsection 8.1, 274 state that the data were not sufficient to inform safe use in pregnancy. In subsection 8.2, 483 (71.6%) contained qualitative data, and 192 (28.4%) contained quantitative data. Among the 192 labelings with quantitative data, 80 (41.7%) included PK data. When PLLR went into effect in 2015, 27.5% of labeling included human data in subsection 8.1 and 19.6% included human data in subsection 8.2. By 2021, 47.3% of labeling included human data in subsection 8.1 and 40.4% contained human data in subsection 8.2. Of the 675 labelings with human data in subsection 8.2, 424 state that the data were not sufficient to inform safe use in lactation.
- **Implications**
  - From the start of the PLLR implementation process in 2015 through December 31, 2021, the proportion of labeling containing human data in subsections 8.1 and 8.2 increased. Although PLLR led to an increase in human data included in labeling, less than half of the labelings analyzed overall contained either qualitative or quantitative human data in

subsection 8.1 (45%) or subsection 8.2 (37.6%). Data included in subsections 8.1 and 8.2 were more often qualitative than quantitative. PK data were less frequently found in subsection 8.1 (7.4%) than in subsection 8.2 (41.7%). Human data were more often present in subsection 8.1 than in subsection 8.2. These results indicate a need for increased human data collection for drugs and biological products used during pregnancy and lactation and to update prescription product labeling as more human data become available.

15. **Abstract Title:** *Updates on Risk Mitigation for Nitrosamine Impurities*
Authors: Desai, Saaniya (ORISE Fellow); Shakleya, Diaa (Mentor); Faustino, Patrick (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response
Abstract:

- **Synopsis**
  - Most of us are exposed to nitrosamines in some form through low levels in water and food (such as meat, vegetables, or dairy) as they are organic compounds. However, they can also be found in drugs during the manufacturing process, as nitrosamines form from a chemical reaction that can occur during drug synthesis. Specifically, these nitrosamines include N-Nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA). The acceptable intake level of NDMA is 96 ng/day. Increasing this exposure causes an increased risk of cancer, as they can be metabolized into alkylating agents that can damage DNA which can cause mutations and lead to cancer. There could be multiple potential sources of these impurities in manufactured drugs. While extra filtration and purification steps could eliminate some of these impurities, due to the number of potential sources for impurity formation, there is a need for effective control strategies. Studying and testing compounds that directly inhibit nitrosamine formation is one step forward in decreasing the risk that excessive nitrosamines have on patients who need drugs that are found to be contaminated with nitrosamine impurities. OTR labs are engaged in producing a strategy to mitigate or reduce the formation of nitrosamines in drug products.
- **Purpose**
  - The daily acceptable intake of nitroso-related impurities, carcinogenic impurities found in some drug products, is 96 ng/day. Consuming more than this is a public health issue due to the toxicity of these compounds, as they can be converted to alkylating agents that modify DNA bases and induce mutations. Formation of these impurities requires a nitrogen containing compound susceptible to forming the impurity, a nitrosating agent (such as nitrate or nitrite, which can be found as impurities in excipients), and acidic conditions that favor the nitrosating reaction; however, the source of these impurities is unknown. Impurities formed during synthesis can be removed

via purification, but nitrosamines formed in the drug product cannot be removed in this way. Inhibiting formation during synthesis could lower the level of these impurities. This project relates to the evaluation of inhibitors and antioxidants in order to decrease the formation of nitroso-related compounds in drug products.

- **Methods**
    - Interactions between nitrosating agents from nitrites in excipients and amines in active molecules or in impurities (such as DMF and/or DMA) could cause the nitrosamine content in drug products.
      Nitrosamines can be detected by a highly sensitive detector after separation from a sample using liquid or gas chromatography (LC/GC) and mass spectrometry methods; using a full evaporation static headspace GC method with nitrogen phosphorous detection (FE-SHSGC-NPD) has been proposed to overcome challenges in testing such as limitations by sample preparation steps and expensive equipment.
      A study by Jireš et al. revealed that changing the pH with Na2CO3 could remove the DMA precursor and stop N-nitrosation. The optimal pH for the nitrosation reaction is 2.5-3.4, so an acidic pH would enhance the reaction. Stability testing is also important, as consumers may store drug products in fluctuating conditions over periods of time. Quality control studies use accelerated condition data to determine expiration dates while room temperature data is used for shelf life (Dharani et al., 2022).
- **Results**
    - Information regarding the presence and impact of nitrosating agent precursors in excipients is scarce so far, but there is a case study regarding reactive components in common excipients (Boetzel et al., 2022). The FDA has also approved a review of novel drugs to confirm the commonly used excipients were relevant. A recent stability study by Dharani et al. also showed that there was variation in NDMA content before and after stability conditions were used, indicating that the products might contain the impurity before they were exposed to these stability conditions so the source might be in the drug, excipients, method, or processing. This also indicates that an investigation into the source of the impurity is crucial in stopping its formation. In November of 2021, the FDA also suggested manufacturers use antioxidants or pH modifiers such as Na2CO3 to inhibit nitrosamine impurity formation in a mitigation study, as these would change the pH of the micro-environment.
- **Implications**
    - Decreasing the amount of nitrosamines in drug products is essential for public health, as an intake of more than 96 ng/day over a long period of time is associated with an increased risk of cancer. It was recently found

that elevated levels of this impurity were found in certain drugs used to treat high blood pressure, heartburn, acid reflux, and diabetes. It is important that patients get the treatment they need, and if it is inaccessible due to impurities found in the drugs they need then it is essential for this to be remedied. This data will indicate the risk to consumers and encourage companies to test products that could be at risk for elevated nitrosamine levels before releasing it to the market. Finding methods to reduce the formation of nitrosamines and related impurities in drug products will make it safer to put products out on the shelf. It will also prevent future drugs from potential recall due to excessive levels of impurities. Overall, these initiatives are a vital response to a public health emergency, as impurities in medicine will have an impact on patients.

16. **Abstract Title:** *Pharmacokinetic and Pharmacodynamic Modeling of Opioid and Sedative/Psychotropic Drug Effects on Ventilation and Pupillometry*
Authors: Ette, Mfonabasi , FDA/CDER (Student); Igo, Matthew, FDA/CDER (Student); Han, Xiaomei, FDA/CDER (Mentor); Florian, Jeffry, FDA/CDER (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response
Abstract:

- **<u>Synopsis</u>**
    - The use of opioid drugs is accompanied by the risk of adverse effects such as sedation and effects on ventilation. Use of some sedative psychotropic drugs with opioids have been shown to have additional effects on some pharmacodynamic measures. A clinical study (NCT04310579) was conducted to investigate the effect of sedative psychotropic drugs paroxetine and quetiapine on ventilation and sedation in healthy volunteers. A population pharmacokinetic and pharmacodynamic model was developed to characterize the pharmacokinetics and pharmacodynamics of oxycodone alone as well as describe interactions from concomitant drugs on these measures.
- **<u>Purpose</u>**
    - Opioids can cause adverse effects such as sedation and potentially fatal respiratory depression. Benzodiazepines are known to increase these risks, and product labeling has been updated with a boxed warning regarding increased risk with co-use of these medications. There is concern whether concomitant administration with other sedative psychotropic drugs could also have similar effects. A clinical study (NCT04310579) was conducted to investigate the effect of the sedative psychotropic drugs (midazolam, paroxetine and quetiapine) with oxycodone on ventilation and sedation in healthy volunteers. The primary objective of the study was to determine whether the combination of psychotropic drugs (paroxetine, quetiapine and midazolam) with an opioid (oxycodone) decreases the ventilatory response

to hypercapnia compared to an opioid alone. Secondary objectives included assessment of whether each psychotropic drug affects the pharmacokinetics of oxycodone. Data from the study were used to explore pharmacokinetic and pharmacodynamic relationships when study drugs were administered alone or in combination.

- **Methods**
  - Data Collection: Pharmacokinetic and pharmacodynamic data were obtained from 45 subjects. This included pharmacokinetic data for study drugs and metabolites (oxycodone, oxymorphone, midazolam, paroxetine, quetiapine, and norquetiapine), ventilation assessments (focusing on baseline minute ventilation and minute ventilation at end-tidal partial pressure carbon dioxide of 55 mmHg [VE55]), pupil diameter, and subject-reported sedation based on a 100 mm visual analog score.

    Model Development: A population pharmacokinetic and pharmacodynamic model was developed to characterize the pharmacokinetics and pharmacodynamics of oxycodone, capture the inter and intra-subject variability, and described drug-interactions from concomitant drugs. The structural model was implemented with ordinary differential equations. Indirect response models were applied to the ventilation, pupillometry and sedation data. Models were validated with visual predictive checks to assess predictive performance. Nonlinear mixed effects modeling software (NONMEM) version 7.5 and R software version 4.2.0, nlmixr package were utilized in model development. All graphics were rendered in R.
- **Results**
  - A two-compartment model appropriately described and predicted the pharmacokinetics of both oxycodone and oxymorphone in both parts of the study. At a significance level of 0.05 midazolam had a significant effect on oxymorphone clearance. At a significance level of 0.01, paroxetine had significant effects on the clearance of oxycodone and oxymorphone. The ventilation data was adequately characterized by an indirect response model with concentration-dependent inhibition of production. The addition of midazolam was associated with a greater reduction in VE55 compared to oxycodone alone. The pupillometry data was adequately characterized by an indirect response model with concentration-dependent inhibition of production for oxycodone. Paroxetine and quetiapine exhibited opposing effects of mydriasis and miosis, respectively, on response.
- **Implications**
  - Pharmacokinetic and pharmacodynamic models were developed to describe data from a completed clinical study with opioids and sedative psychotropics drugs. The pharmacokinetics of both oxycodone and oxymorphone were adequately described with compartmental models. The observed effects of all three concomitant drugs suggest that these drugs

alter the pharmacokinetics of oxycodone and oxymorphone. Pharmacodynamic effects from oxycodone and concomitant drugs on ventilation and pupillometry were described with indirect response models, showing independent contribution of the concomitant drugs to different pharmacodynamic measures. Further research is necessary to determine the relationship between ventilation and changes in pupil diameter for opioids administered alone or in combination with sedative psychotropic drugs.

17. **Abstract Title:** *Comparing Data from NASH Biomarker Submissions*
Authors: Ganesh, Prem, FDA/CDER (Student); de la Cruz, Aida, FDA/CDER (Student); Bandukwala, Abbas, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:
    - **Synopsis**
        - Non-Alcoholic Steatohepatitis is expected to be the leading cause of liver transplants in the next decade, yet there is no FDA approved treatment. The current standard for a NASH diagnosis is a liver biopsy, that carries a higher risk of morbidity and mortality. Clinical-trial enrollment is further hindered by a lack of surrogate endpoints used to identify changes in the progression of NASH. Prognostic and diagnostic biomarkers are needed for the success of clinical trials, both to diagnose and enroll patients and to quantify the success of a potential new drug. The purpose of this study is to generate a table for comparing relevant data from NASH submissions to the Biomarker Qualification Program (BQP) with different parameters based on user input. A user interactive table will facilitate biomarker qualification as data comparison of both clinical and analytical data points can be streamlined by reviewers.
    - **Purpose**
        - Non-alcoholic steatohepatitis (NASH) is a chronic and progressive liver disease characterized by liver fibrosis, ballooning, and steatohepatitis. Currently, there is no FDA approved treatment, and drug development is further hindered by a lack of non-invasive biomarkers that could serve to enrich clinical trials or serve as an endpoint marker for drug trials. The Biomarker Qualification Program (BQP) currently has nine NASH enrichment biomarker submissions at varying stages of review. The identification and qualification of these diagnostic and prognostic biomarkers would decrease the number of liver biopsies, and help identify patients more likely to have NASH which will allow for more efficient drug trials. The purpose of this study is to create a user-interactive table displaying both clinical and analytical data submitted by organizations, such

as academic and consortium groups, to facilitate evaluation of submitted biomarkers.

- **Methods**
  - Requesters submitted biomarker qualification regulatory submissions such as Letters of Intent (LOI) and Qualification Plan (QP) which were reviewed. Spreadsheets containing relevant clinical and analytical data were created. Analytical data points included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and enrichment. Clinical values included validation and training cohort demographics and decision point values. Submitted biomarkers included fluid based and imaging-based biomarkers as well as composite biomarkers consisting of a combination of both. The data was organized into tables. After consulting with subject matter experts and discussions with those involved in the biomarker qualification process, functions, queries and other coding was developed to create a user interface that would search the tables and provide a summary of important data from these submissions.
- **Results**
  - Based on the relevant data, a user interface was created allowing the user to select parameters to populate the table from the original data and compare clinical and analytical data for the selected biomarkers or submissions. Allowing the user to set the parameters lets different reviewers use the system as needed and streamlines the process for new data to be added to the database when new biomarkers are submitted to the FDA. Repeat submissions of selected biomarkers such as PRO-C3, MRI-PDFF, and MR imaging can be compared either from submission to submission or from the individual biomarker to the composite which includes it.
- **Implications**
  - The submissions were either prognostic or diagnostic biomarkers seeking to assess the fibrosis stage and other clinical endpoints to identify patients for drug development clinical trials. Since some of the composite biomarkers and the non-composite use the same biomarkers, this table allows FDA reviewers to directly compare the information from each submission. While this project was focused on NASH and its relevant submissions, the same idea can be adapted for other biomarkers submitted to the BQP. This project will allow those involved in the BQP to easily visualize the submissions, resubmissions, and their relevant data, and save time in finding information, and make the process more consistent across the BQP.

18. **Abstract Title:** *Sample Size Determination for Machine Learning Classifiers with an Application to Electronic Phenotyping*
Authors: Fang, Xinying, FDA/CDER (Student); Saha, Satabdi, FDA/CDER (Student); Song, Jaejoon, FDA/CDER (Mentor); Dharmarajan, Sai, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
    - Advances in automated document classification has led to identifying massive numbers of clinical concepts from handwritten clinical notes. These high dimensional clinical concepts can serve as highly informative predictors in building classification algorithms for identifying patients with different clinical conditions, commonly referred to as patient phenotyping. However, from a planning perspective, it is critical to ensure that enough data is available for the phenotyping algorithm to obtain a desired classification performance. This challenge in sample size planning is further exacerbated by the high dimensionality of the covariates and the inherent imbalance of the response class. To enable efficient sample size planning, model-based and learning curve-based approaches have been developed to predict the sample size with a linear classifier or by fitting a learning curve to inverse power law models. Further, to consider imbalanced data and correlated features, we derive formulas for sample size dependent performance metrics that are sensitive to class imbalance (AUC and MCC), and propose a two-step approach where we first perform feature selection using the innovated High Criticism thresholding method, then determine the sample size by optimizing the two performance metrics. Meanwhile, we develop an R package named planningML along with an R Shiny app to facilitate the convenient implementation of the proposed approach. From the application on two imbalanced electronic phenotyping datasets from the MIMIC-III database, we compared the combinations of four feature selection methods and two sample size determination methods with a LASSO estimator, and found out that our method can predict similar sample size as LASSO, but require much less data. Therefore, in high-dimensional classification analysis with imbalanced data and correlated features, our approach can efficiently and accurately predict the sample size needed for machine-learning based classification.
- **Purpose**
    - We propose an approach and develop software to determine the training data size required for building various supervised machine learning classifiers with an application to electronic phenotyping where class imbalance is a concern to varying degrees. We offer practical recommendations for planning the application of ML and a statistical software package which makes our methods easy to implement.

- **Methods**
    - For the situation with class imbalanced data and correlated features, we derive formulas for sample size dependent classification performance metrics that are sensitive to class imbalance such as Area Under the reciever operating characteristics Curve (AUC) and Matthews Correlation Coefficient (MCC), and propose a two-step approach for sample size planning. In our approach, we first perform feature selection using the innovated Higher Criticism thresholding method, then determine the optimal sample size by optimizing the two aforementioned performance metrics. Meanwhile, we develop a freely accessible R package named 'planningML' along with an R Shiny app to facilitate the convenient implementation of the proposed sample size determination approach.
- **Results**
    - We benchmark the performance of our sample size determination methods by comparing the predicted performance under a given optimal sample size to the performance of a LASSO estimator in two imbalanced electronic phenotyping datasets. We show that our method can determine sample size requirement for a linear ML classifier such as LASSO for a desired performance on AUC and MCC.
- **Implications**
    - In high-dimensional (p>>n) classification analysis with imbalanced data and correlated features, our approach can efficiently and accurately predict the sample size needed for machine-learning based classification. Our software enables easy implementation of our approach, facilitating the planning of machine learning classification studies.

19. **Abstract Title:** *Characterizing the Current Dose Finding and Optimization Landscape Across Therapeutic Areas – A Retrospective Analysis of 2018-2020 Novel Drug Approvals*
    Authors: Gong, Christine, FDA/CDER (Student); Liu, Jiang, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - In the drug development of traditional cytotoxic cancer therapies, dose selection follows a toxicity-driven approach based on the maximum tolerated dose (MTD). However, the dose-response profiles of new drug modalities indicate that higher doses do not always provide greater efficacy. This project aims to enhance understanding of dose selection in various therapeutic areas, to investigate limitations in current dosing strategies, and to support future policy development for oncology dose finding and optimization. Preclinical and clinical data were summarized pertaining to the dose selection and dose finding strategies that justified study of the

first-in-human (FIH), proof-of-concept (POC), confirmatory, and approved doses of 160 new molecular entities. Among the oncology entities analyzed, a median of 6.5 doses was evaluated in FIH trials, a median of 1 dose was evaluated in both POC trials and confirmatory trials, and a median of 1 dose was approved. Regarding the MTD paradigm, 91.30% of oncology drug approvals (n=46) proposed MTD identification as a Phase 1 study objective, compared to 7.89% of non-oncology approvals (n=114). Though widely utilized in oncology drug development, the MTD paradigm is no longer appropriate for novel oncology products; alternatively, a pragmatic, holistic, and multidisciplinary approach to dose finding and optimization can be explored. Such approach may involve evaluation of multiple dose levels during later phases of drug development, as well as integration of model-informed approaches to support dose selection.

- **Purpose**
    - Dose selection for traditional cytotoxic cancer therapies follows a toxicity-driven approach based on the maximum tolerated dose (MTD), which presumes that "more is better" for efficacy. However, in recent decades, oncology drug development has shifted focus to molecularly targeted agents and immunotherapies, whose dose-response profiles indicate that "more is not always better." Use of the MTD paradigm for these new drug modalities causes undue toxicity in patients and is associated with product withdrawals. This project aims to enhance understanding of dose selection in various therapeutic areas and to investigate limitations and improvements to current dosing strategies. The totality of evidence used to select the first-in-human (FIH) starting dose, dose escalation range, recommended phase 2 dose, registration trial dose, and approved dose will be summarized. Such analyses will help streamline regulatory recommendations regarding dose finding and optimization in oncology drug development.
- **Methods**
    - Preclinical and clinical data were summarized from public and FDA-internal sources (i.e., Drugs@FDA, EDR, DARRTS, drug approval labels, multi-discipline reviews, clinical study reports) for each of 160 new molecular entities approved between 2018 and 2020. A comprehensive database was established with the following information: preclinical data utilized to select clinical starting doses and dose escalation steps/cap, biomarkers studied to support the proof-of-concept (POC) dose range, benefit-risk analysis employed to individualize approved treatments, and model-informed drug development (MIDD) applied to characterize dose-response relationships. The novel drugs were categorized into 15 therapeutic areas and differentiated as oncology or non-oncology products. In each therapeutic area, the dose ranges and number of doses studied in the FIH, POC, and

confirmatory trials were compared to approved doses. Use of the MTD paradigm and approval of previously unstudied doses were further investigated.

- **Results**
    - Among oncology therapeutic areas, a median of 6.5 dose levels was evaluated in FIH trials, with a median 15-fold difference between the minimum and maximum dose studied in each trial. A median of 1 dose was evaluated in POC trials and confirmatory trials, with a median 9-fold difference between the highest dose studied and the FIH starting dose. A median of 1 dose was approved, with a median 8-fold difference between the highest approved dose and the FIH starting dose. Among non-oncology therapeutic areas, a median of 5 doses with a 24-fold dose range was evaluated in FIH trials. Medians of 3 doses with an 11-fold, 2 doses with a 10-fold, and 1 dose with a 10-fold difference relative to the FIH starting dose were evaluated in POC trials, in confirmatory trials, and upon approval, respectively. Regarding the MTD paradigm, 91.3% of oncology drug approvals (n=46) proposed MTD identification as a Phase 1 study objective, compared to 7.89% of non-oncology approvals (n=114).
- **Implications**
    - The findings estimate the dose ranges and number of doses studied across drug development stages in oncology and non-oncology therapeutic areas. The approximations established from 2018-2020 data can inform current trial design and policy development regarding dose selection for cancer drugs. Furthermore, the MTD paradigm is no longer appropriate for novel oncology products, which are designed to target molecular alterations that drive cancer progression; alternatively, a pragmatic, holistic, and multidisciplinary approach to dose finding and optimization can be explored. To identify optimal dose(s) for administration during clinical trials, multiple dose levels should be evaluated in a sufficient number of targeted patients. To improve efficiency at each stage of drug development, model-informed approaches that integrate the best understanding of dose-exposure-PD-response relationships with up-to-date nonclinical and clinical data should provide quantitative support for dose selection.

20. **Abstract Title:** *Assessing Important Information Gaps Associated with Initial Pediatric Study Plans for New Oncology Drug and Biological Products*
    Authors: Gafford, Marilyn, FDA/CDER (Student); Guinn, Daphne, FDA/CDER (Mentor); Charlab Orbach, Rosane, FDA/CDER (Mentor); Burckart, Gilbert, FDA/CDER (Mentor); Reaman, Gregory, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:

- **Synopsis**
    - The RACE for Children Act requires sponsors to propose an initial Pediatric Study Plan (iPSP) of new molecularly targeted drugs and biologics that are intended for treatment of adult cancers whose target is relevant in pediatric cancer. A landscape analysis was performed to identify trends in agreement and information gaps associated with a sponsor's first iPSP submission for oncologic new molecular entities received in 2021. Comments made during iPSP review that were sent to the sponsor were collected for each evaluated iPSP. Each of the FDA comments were reviewed and nine data flags were developed. For iPSPs with a sponsor proposed plan for a full waiver, the most common clinically relevant information gap was inadequate justification based on molecular target. Any other sponsor proposed plan (deferral, partial waiver, partial waiver and deferral, or study) had information gaps related to clinical study features, clinical pharmacology, and/or missing clinical or nonclinical data. This landscape analysis of iPSPs shows the trends in comments that often occur during initial review and may help to provide sponsors with more comprehensive guidance on developing a more complete iPSP for oncology products.
- **Purpose**
    - Pediatric drug development typically relies on adult drug discovery with evaluation in pediatric populations beginning late in development or after approval for an adult indication. To address this need, the RACE for Children Act requires pediatric evaluation of new molecularly targeted drugs and biologics that are intended for treatment of adult cancers whose target is relevant to pediatric cancer. When a sponsor submits an initial Pediatric Study Plan (iPSP), sponsors may include plans for pediatric studies, plans to request deferral of studies, or plans to request a partial or full waiver of studies. FDA evaluates the justification for the planned request and provides considerations so that an iPSP can be efficiently agreed upon. To understand the most common information gaps associated with iPSPs, a landscape analysis of iPSPs was performed to identify trends in agreement and information gaps associated with first iPSP submission for new molecular entities. Understanding the trends in iPSP submissions can help to inform future guidance for developing iPSPs.
- **Methods**
    - iPSP information was extracted from an internal FDA database. iPSPs had to meet the following criteria: received January 1, 2021, through December 31, 2021, associated with a new molecular entity, the first submission of an iPSP. Summary comments made during PSP review that were sent to the sponsor were collected by each recommended section found in the Appendix of Pediatric Study Plans: Content of and Process for Submitting

Initial Pediatric Study Plans and Amended Initial Pediatric Study Plans Guidance for Industry. Six data flags were created in a pilot examination of five iPSPs then refined upon review of all comments. The nine final data flags are: inadequate justification based on molecular target, inadequate justification based on disease, missing clinical or nonclinical information, clinical study features, clinical pharmacology, agreement with sponsor, formatting, pediatric age waiver, and other. Multiple data flags could be assigned to each comment based on content. Characteristics and trends related to submitted iPSPs, comments, and data flags were analyzed.

- **Results**
    - 65 iPSPs were identified. 352 comments were analyzed. 428 data flags were assigned. Of the 65 iPSPs, 42 planned to request a full waiver; 13 planned to request a partial waiver/deferral; 7 planned to request a deferral; 2 planned a study; and 1 planned to request a partial waiver. For plans to request a full waiver (65%), the most common information gap was inadequate justification based on molecular target. Any other plan (35%) includes a pediatric study and had information gaps related to clinical study features (30.0%), clinical pharmacology (27.4%), or missing clinical/nonclinical data (16.3%). Clinical Study Features could include study design, inclusion/exclusion criteria, or study timeline. Clinical Pharmacology could include dosing, PK/PD, or formulation. For small molecule iPSPs (34), 50% planned to request a full waiver, and the most common information gaps were clinical pharmacology related (22.8%). Of the 31 biologic-associated iPSPs, 81% planned to request a full waiver, and the most common information gap was inadequate justification based on molecular target (20.9%).
- **Implications**
    - The results of this study highlight the most common information gaps in the first submission of an iPSP received in 2021. The most common clinically relevant information gap when sponsors plan to request a full waiver is inadequate justification based on molecular target, such as evaluating new targets and information for non-relevant pediatric targets. When proposing a study, including a planned requests for deferral or partial waiver, the most common information gaps relate to the clinical study features, clinical pharmacology, and/or missing clinical or nonclinical data. These most common topics relate to justification for the study design, recommendations for the study or participants, dosing, PK/PD, drug formulation, BA/BE, or missing data. This landscape analysis shows the trends in comments that occur during initial review and may provide sponsors with more guidance on developing a complete iPSP. Overall, ensuring that pediatric studies are considered early in drug development

allows for timely access to safe and effective novel oncology treatments for pediatric patients.

21. **Abstract Title:** *Bayesian dynamic information borrowing approaches in the rare disease and sequentially enrolled pediatric trial context.*
Authors: Haine, Lillian, FDA/CDER (Student); Sun, Hengrui, FDA/CDER (Mentor); Valappil, Thamban, FDA/CDER (Mentor); Mullick, Charu, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **<u>Synopsis</u>**
    - Bayesian trials are proposed as investigators look to improve trial design efficiency via informative priors, which effectively borrow information from previous studies. Informative priors may be useful in two contexts: 1) the rare disease context - where limited patient population might make it nearly impossible to reach full trial enrollment, and 2) the pediatric context, where trials often enroll sequentially from older to younger age cohorts and borrowing from a previous age cohort could be reasonable and improve trial efficiency. Yet prior specifications directly impact the final trial results and naively borrowing from previous data might produce erroneous results. Therefore, careful consideration must be given as to how to determine an appropriate amount of information to borrow from previous studies. This work compares several approaches to prior specification in a simulation study and two case examples. The approaches are: 1) fully informative, 2) uninformative, 3) Robust Meta-Analytic-Predictive Priors, 4) power prior, 5) Bayesian hierarchical model, 6) calibrated Bayesian hierarchical model, and 7) Multisource Exchangeability Model. In simulations, we see that dynamic borrowing approaches appropriately borrow from the previous study data when the previous and subsequent study data have similar event rates and avoid borrowing when the event rates diverge. In the rare disease case example, most of the dynamic borrowing approaches avoid borrowing from the previous data because the two event rates are very different. For the sequential pediatric trial context all age cohorts have very similar event rates and the borrowing approaches leverage the previous age cohort data. This work illustrates that the approach used to facilitate borrowing from previous data needs to be given careful consideration as this will have a direct impact on the final study results and some borrowing approaches are more suitable to certain contexts than others.
- **<u>Purpose</u>**
    - Bayesian trials are proposed as investigators look to improve trial efficiency via informative priors, which effectively borrow information from previous studies. Informative priors may be useful in two contexts: 1) the rare disease context - where limited patient population might make it difficult to

fully enroll a trial, and 2) the pediatric context, where trials often enroll sequentially from older to younger age cohorts and borrowing from a previous age cohort could improve trial efficiency. Yet informative priors directly impact the final trial results that may lead to regulatory decisions so careful consideration must be given as to how to determine an appropriate amount to borrow from previous studies. Here we investigate several Bayesian dynamic borrowing approaches and their utility via a simulation study and two real trial applications, providing greater insight into the operating characteristics of these dynamic borrowing approaches and their applicability to the rare disease and sequentially enrolled pediatric trial context.

- **Methods**
  - We compare several approaches to prior specification in a simulation study and two case examples. The approaches are: 1) fully informative, 2) uninformative, 3) Robust Meta-Analytic-Predictive Priors, 4) power prior, 5) Bayesian hierarchical model, 6) calibrated Bayesian hierarchical model and 7) Multisource Exchangeability Model. We compare these approaches in two examples that illustrate two contexts where borrowing is especially useful. One, a rare disease context, where we borrow from previous study data to investigate the use of MYOZYME to treat infantile-onset Pompe disease. Two, a sequentially enrolled pediatric trial investigating HARVONI to treat hepatitis C infection. Here, there were 3 age cohorts enrolled sequentially: 1) 12 to <18 years, 2) 6 to <12 years, and 3) 3 to <6 years. We apply a sequential borrowing framework, borrowing from individuals in the older age group closest to the current age group, so for the 6 to <12 years analysis we borrow from the 12 to <18 years data. We apply all approaches and compare the mean posterior response rate and 95% credible intervals.
- **Results**
  - In simulations, we see that dynamic borrowing approaches are more able to avoid borrowing when the previous study and the subsequent study have different event rates when compared to the fully informative approach and have higher power when compared to the uninformative approach. Of the borrowing methods, the calibrated Bayesian hierarchical model appears to be the most effective at minimizing bias, while all other borrowing approaches behave similarly, introducing a minimal amount of bias. In the first case example, or the rare disease context, the previous study data had a much higher event rate than the subsequent study and as such, most of the dynamic borrowing approaches avoid borrowing from the previous data and have similar results to the uninformative approach. For the second case example, or the sequential pediatric trial context, all age cohorts have similar event rates. Thus, the borrowing approaches have results to the fully informative approach and higher precision when compared to the

uninformative approach as they are leveraging the previous age cohort data.

- **Implications**
    - Bayesian trials that effectively borrow information from previous studies via informative priors are particularly useful in the rare disease and the sequentially enrolled pediatric trial contexts, where increasing trial efficiency via decreased necessary sample size is desired. We show that using a dynamic borrowing method has advantages, as naively pooling previous and current study data could introduce substantial bias and even produce erroneous study results. Additionally, the approach used to facilitate borrowing from previous data needs to be given careful consideration as this will have a direct impact on the final study results and some borrowing approaches are more suitable to certain contexts than others.

22. **Abstract Title:** *A Comparison of Statistical Methods for Evaluation of Slope-based Estimated Glomerular Filtration Rate in Chronic Kidney Disease Randomized Controlled Clinical Trials*
Authors: Hakhu, Navneet, FDA/CDER (Student); Koh, William, FDA/CDER (Mentor); Zhou, Dali, FDA/CDER (Mentor); Li, Feng, FDA/CDER (Mentor); Clark, Jennifer, FDA/CDER (Mentor); Chen, Ling-Wan, FDA/CDER (Mentor); Zhang, Jialu, FDA/CDER (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

- **Synopsis**
    - Estimated glomerular filtration rate (eGFR) is a surrogate measure of kidney function based on serum creatinine via a blood test and patient demographic characteristics. Two-year eGFR total slope has been used as a primary endpoint in randomized controlled clinical trials of patients in earlier stages of chronic kidney disease (CKD). Common statistical methods exist to analyze eGFR total slope. In recent years, investigational treatments evaluated in clinical studies have been observed to have short-term acute reversible effects on eGFR that may impact the long-term rate of decline in eGFR (Heerspink et al., 2020). Vonesh et al. (2019) proposed a two-slope model to account for the possibility of this acute effect. Our objective for this project was to compare the different statistical methods for evaluating slope-based eGFR as a primary endpoint in CKD trials. The results of this project can aid sponsors at the planning stage and regulators in their review of proposed designs and new drug application submissions.
- **Purpose**
    - Evaluation of new interventions for patients at risk for or in the earlier stages of chronic kidney disease (CKD) on clinical outcomes (e.g., dialysis, kidney transplant, end-stage kidney disease, or death) may require clinical

trials with long follow-up periods. One approach to accelerate the time to observe a trial endpoint is to use a surrogate outcome. Estimated glomerular filtration rate (eGFR) is one example of a surrogate measure of kidney function based on serum creatinine via a blood test and patient demographic characteristics. Two-year eGFR total slope has been a primary endpoint in randomized clinical trials of patients in earlier stages of CKD. Common statistical methods exist to analyze eGFR total slope. Recently, Vonesh et al. (2019) proposed a two-slope model to account for the possibility of a change point between an initial acute and reversible phase followed by a chronic phase observed in CKD trials (Heerspink et al., 2020). Our objective for this project was to compare statistical methods for evaluating slope-based eGFR as a primary endpoint in CKD trials.

- **Methods**
  - We were interested in estimating the difference in total slopes between treatment and control arms for the full analysis population. We defined total slope as the average first-order trend in the annualized rate of change of eGFR two years post-randomization. We assumed no intercurrent events (dialysis, kidney transplant, or death) have occurred to assess whether the different statistical analysis methods yielded similar estimates. We considered four broad classes of statistical methods: (i) analysis of covariance (ANCOVA); (ii) mixed models repeated measures (MMRM); (iii) linear mixed effects models (LME); and (iv) generalized estimating equations (GEE)—including two-slope versions for LME and GEE using splines to allow for a potential change point between an initial acute and reversible phase followed by a chronic phase. We conducted simulation studies under different null and design alternative hypotheses (with or without a change point) and evaluated statistical operating characteristics (e.g., bias, precision, type I error rate, coverage probability).
- **Results**
  - Our simulation studies showed that when the average rate of change in eGFR was linear over time within each randomized treatment arm, all models considered yield unbiased estimates of the difference in total slopes, with nominal type 1 error rate for all models expect the LME with random intercepts only. When there is a potential acute reversible effect, our simulations showed that estimates from the considered models may be different.
- **Implications**
  - Pre-specified analyses, especially for the primary endpoint, is critical to maintaining the integrity of clinical trials, including CKD trials. Our evaluation of statistical methods to estimate eGFR total slopes is an important step towards understanding the pros and cons of the different

available statistical methods. The results of this project can aid sponsors at the planning stage and regulators in their review of proposed designs and new drug application submissions.

23. **Abstract Title:** *NIR: A PAT Solution for Crystalline Monitoring and Control for High-Risk Product Manufacturing*
Authors: Henson, Samuel FDA/CDER (ORISE Fellow); Mazumder, Sonal FDA/CDER (Mentor)
FDA Strategic Initiative: Innovative and Emerging Technology Initiatives
Abstract:

- **Synopsis**
  - Crystallinity detection is currently performed off-line before and after manufacturing of drug products. With increased PAT method application, particularly in-line NIR measurements, there is an opportunity to increase the knowledge of in-process materials without adding new process analyzers. NIR has been shown to successfully detect the spectral differences between crystalline forms of an API. Data reconstruction, spectral simulation, and various modeling strategies demonstrate the ability of NIR to differentiate between crystalline forms of API. Various preprocessing strategies extract the relevant spectral information, allowing the models to perform as expected. Binary blends including only the crystalline forms highlight the spectral differences between crystalline forms and are successfully analyzed by both classification and prediction models. Multi-component mixtures including excipients represent more realistic pharmaceutical system. A control strategy is presented using classification modeling to indicate change to crystalline form followed by prediction modeling to quantify both the desired and undesired crystalline form. Understanding the mechanism by which crystalline change occurs facilitate corrective actions within the manufacturing process. The practicality of this control strategy allows ease of implementation given the currently employed in-line NIR control strategies for API potency. Increased NIR use in NDAs/ANDAs provides the platform for suggesting this approach as a realistic crystal form detection strategy. Evidence for crystalline detection via NIR allows FDA regulations for crystal form to reflect the current level of science while minimizing the experimental demand on manufacturers. Overall, improving the crystalline detection strategy and its associated regulations create safer products and protect the public health, both of which are key components of the mission of FDA.
- **Purpose**
  - Active pharmaceutical ingredient (API) crystallinity, one of the most important critical quality attributes (CQA) of a drug product, can affect quality, efficacy, or in extreme cases, patient safety. This phenomenon, responsible for the ritonavir catastrophe of the 1990s, is currently detected

in polymorph screening and final product testing. This off-line detection strategy, primarily via X-Ray Diffraction, assesses the crystalline state of the API. Relying on off-line final product testing may not be sufficient for detecting API crystallinity during manufacturing. The industry is moving towards in-line process analyzers via process analytical technology (PAT). Near-infrared spectroscopy (NIR), a common PAT tool, detects CQAs in-line real time. The goal of this project is to demonstrate how in-line NIR data can be used to detect both API potency and crystallinity changes. In-line monitoring and control of API crystallinity ensures consistent quality throughout product lifecycle.

- **Methods**
    - A dataset containing relevant API crystal forms is constructed from literature and drug applications. Spectral reconstruction is followed by spectral simulations, preprocessing, and modeling performed in MATLAB. Spectra for API with known crystalline forms are imported for use in two simulation strategies: binary blends consisting of only the API crystalline forms; and multi-component mixtures incorporating excipients to simulate realistic pharmaceutical formulations. Spectra were preprocessed with mean centering, standard normal variate (SNV), and 2nd derivative SavGol smoothing for each model. Classification modeling was accomplished via clustering and principal component analysis (PCA), while prediction modeling was accomplished with partial least squares (PLS). Loading plots were used to assess the spectral variance captured, and scores plots were used to assess the classifications. Model fit plots with associated metrics are used to demonstrate the prediction model performance.
- **Results**
    - An extensive summary of process monitoring and control strategies from approved NDA/ANDA manufacturing facilities is constructed. Spectral reconstruction with noise simulation provides datasets for model exploration. Preprocessing with SNV shows successful extraction of relevant spectral information. Classification models show promise for separating samples by crystal form in both binary blends of crystal forms and in multi-component blends with excipients present. Prediction models successfully predict the content of each API crystal form in both types of blends, indicating potential in-line application. A control strategy relying on in-line NIR spectral data and a multi-model approach is suggested, using classification modeling to identify potential crystalline changes and prediction models to quantify the extent of crystal form change. Existing monitoring and control strategies will provide in-line NIR spectral data, requiring only the additional models to implement.

- **Implications**
    - The current state of crystallinity detection is limited and leaves the door open for situations like ritonavir. The current approval process relies on off-line monitoring strategies discussed previously. Applying knowledge of crystalline transformations streamlines the monitoring and control strategy to the unit operations which present conditions known to induce crystalline change. Implementation of the suggested control strategy in which crystalline changes are both detected and quantified facilitates early detection and mitigation. As the industry moves toward broader in-line analysis via PAT tools, NIR data will become more widely applied to multiple unit operations, allowing crystallinity assessment at each stage throughout the manufacturing process. Early crystallinity detection prevents potentially harmful or ineffective products from ever entering the market, which keeps patients safe and aligns with the overall mission of FDA.

24. **Abstract Title**: *Knowledge Management of Drug Coating Process for CDRH-led Cardiovascular Device-Drug Combination Products*

    Authors: Hu, Maggie, FDA/CDER (Student); Xiao, Jingbo, FDA/CDER (Mentor); Tang, Yubing, FDA/CDER (Co-Mentor)

    FDA Strategic Initiative: Unleashing the Power of Data

    Abstract:

    - **Synopsis**
        - Collaboration between the CDER and CDRH is vital to streamline the assessment of combination products. The Office of Pharmaceutical Manufacturing Assessment (OPMA) at CDER started performing manufacturing assessments for CDRH-led cardiovascular device-drug combination products in 2020 through the Inter-Center Consult Requests (ICCRs). However, there exists a lack of centralized prior knowledge on the drug coating process and corresponding in-process controls (IPCs) of the approved products in OPMA's current database. A centralized depository for the manufacturing process information on approved products will aid the knowledge management and facilitate the CDER manufacturing assessment. This project seeks to enhance knowledge-aided manufacturing assessment for the CDRH-led cardiovascular device-drug combination products, specifically drug-eluting stents (DES) and drug-coated balloons (DCB). These combination products are used to treat de novo (new) or restenotic (recurring) atherosclerotic lesions in the femoropopliteal artery, coronary artery disease, and improve luminal diameter in patients with diabetes or high cholesterol. Through collation and analysis of manufacturing process information on these approved device-drug combination products, redundant efforts to collect this information are reduced and the consistency of the CDER manufacturing assessment for device-drug combination products can be improved. This project helps to achieve the OPMA's mission to assure that high quality pharmaceuticals are consistently manufactured over the product lifecycle.

- **Purpose**
    - Collaboration between the CDER and CDRH is vital to streamline the assessment of combination products. The Office of Pharmaceutical Manufacturing Assessment (OPMA) at CDER started performing manufacturing assessments for CDRH-led cardiovascular device-drug combination products in 2020 through the Inter-Center Consult Requests (ICCRs). However, there exists a lack of centralized prior knowledge on the drug coating process and corresponding in-process controls (IPCs) of the approved products in OPMA's current database. A centralized depository for the manufacturing process information on approved products will aid the knowledge management and facilitate the CDER manufacturing assessment.
- **Methods**
    - This project seeks to enhance knowledge-aided manufacturing assessment for the CDRH-led cardiovascular device-drug combination products, specifically drug-eluting stents (DES) and drug-coated balloons (DCB). These combination products are used to treat de novo (new) or restenotic (recurring) atherosclerotic lesions in the femoropopliteal artery, coronary artery disease, and improve luminal diameter in patients with diabetes or high cholesterol.
- **Results**
    - Using the FDA's Premarket approval (PMA) database, a list of up-to-date approved DES and DCB device-drug combination products was identified. The manufacturing information regarding the drug coating process was collected and deposited into a pre-designed template to perform analysis between applications for similar device-drug combination products. Through collation and analysis of manufacturing process information on these approved device-drug combination products, redundant efforts to collect this information are reduced and the consistency of the CDER manufacturing assessment for device-drug combination products can be improved.
- **Implications**
    - Results from this study provide more readily accessible data in the OPMA and improve the manufacturing assessment productivity within the CDER. This project helps to achieve the OPMA's mission to assure that high quality pharmaceuticals are consistently manufactured over the product lifecycle.

25. **Abstract Title:** *Bayesian Borrowing Methods for Type 2 Diabetes Pediatric Studies*
    Authors: Ji, Yuanyuan, FDA/CDER (Student); Crackel, Roberto, FDA/CDER (Mentor); Mulugeta, Yeruk, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Pediatric studies in T2DM often face recruitment challenges. It has also been found that the patient level variability is larger than what was assumed in the sample size calculation, which has led to underpowered

studies. To counter these challenges, borrowing from the adult population can be an appropriate methodology. However, this methodology remains undeveloped in T2DM. The purpose of this project is to develop these methods that will allow us to provide guidance to sponsors when they face recruitment challenges and opt to borrow from adult data. The outcomes of this project include the application of Bayesian borrowing methods to 2 completed pediatric studies and simulation studies to evaluate operating characteristics.

- **Purpose**
  - Pediatric studies in Type II Diabetes Mellitus (T2DM) can often be challenging due to recruitment issues. In addition, it has been found that the observed patient level data variability is larger than what was assumed in the sample size calculation, which has led to undersized/underpowered pediatric studies. To counter these challenges, borrowing information from the adult population using Bayesian approaches can be an appropriate methodology in drawing reliable statistical inference in pediatric studies. However, the methodology of said borrowing from adult data remains undeveloped in a T2DM adult/pediatric context. The purpose of this project is to develop these methods that will allow us to provide guidance to sponsors when they face recruitment challenges and opt to borrow from adult data.
- **Methods**
  - Given a pediatric study with a non-significant treatment effect, we constructed a mixture prior distribution by identifying and synthesizing relevant adult data using summary level statistics and then down weighting the information to incorporate uncertainty. The amount of borrowing from the adult studies is measured by the prior effective sample size. The prior distribution is then combined with the observed pediatric data to form the posterior distribution. After data analysis, the amount of borrowing necessary to have a significant treatment effect is determined. In addition, when considering ongoing pediatric studies, it will be desirable to prespecify parameters and explore operating characteristics so that agreement between the FDA and the sponsor can be reached. Therefore, simulations were carried out under various assumptions to help aid in drawing recommendations.
- **Results**
  - An analysis that retrospectively considered a recently reviewed pediatric study whose results are published in the label was considered. Since the original analysis of the adult studies addressed missing data that is no longer acceptable, they were reanalyzed with current techniques and the Bayesian analysis was performed again. The amount of borrowing needed

to attain a significant treatment effect is about 85-90%. Simulation studies evaluating operating characteristics will be summarized. The analysis and results of a second pediatric study are still pending, where methods to match the pediatric population with the relevant adult population will be considered.

- **Implications**
    - As pediatric studies in T2DM are ongoing and may likely be underpowered, the need for identifying a pre-specified analysis that leverages adult data is increasing fast. The results and outcomes of this project will help in aiding and guiding sponsors. So far, we have identified the following areas of need:
      1. The possibility to reanalyze historical adult data
      2. Prespecify prior mixture distributions and parameters
      3. Explore operating characteristics
      4. Submit code for validation
      5. Determine the prior effective sample size needed to borrow from the adult population to ensure adequate power
      6. Prespecify sensitivity analyses

26. **Abstract Title:** *Impact of PBMC Co-culture on ADCC Activity in Breast Cancer Cells*
    Authors: Ju, Anna, DBRRI/OBP/OPQ/CDER/FDA (Student); Endo, Yukinori, DBRRI/OBP/OPQ/CDER/FDA (Mentor); Wu, Wen Jin, DBRRI/OBP/OPQ/CDER/FDA (Co-Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Recently, immunotherapy has grown increasingly renowned for its efficacy in treating cancer with fewer side effects than traditional treatment options. More specifically, antibody immunotherapies target surface antigens on tumor cells, eventually causing cell death. Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism whereby humoral effector cells induce apoptosis on target cells opsonized by a specific antibody via non-phagocytic mechanisms. This interaction can mediate cell death mechanisms, most notably cytotoxic granule release. Newly developed biological products often require the use of bioassays to identify critical quality attributes. Cell-killing-based ADCC bioassays commonly employ human natural killer (NK) cells and/or human peripheral blood mononuclear cells (PBMCs) to establish a mechanism of action. Data from ADCC bioassays are notably inconsistent and are often attributed to product quality, target cell antigen expression, or PBMC donor variability. Our preliminary data revealed that coculture of target cancer cell with PBMC can influence epidermal growth factor receptor (EGFR) expression. EGFR is a well-known antigen expressed in metastatic cancers, and cetuximab is an FDA-approved IgG1-based therapeutic antibody targeting EGFR. While the mechanism of antigen expression alteration has not been elucidated, we aim to determine whether this change in the EGFR expression can influence cetuximab-dependent ADCC activity in target cells.

- **Purpose**
  - Recently, immunotherapy has grown increasingly renowned for its efficacy in treating cancer with fewer side effects than traditional treatment options through immune response mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC). Cell-killing-based ADCC bioassays employ human natural killer (NK) cells or human peripheral blood mononuclear cells (PBMCs) to establish a mechanism of action. Data from ADCC bioassays are notably inconsistent and are often attributed to product quality, target cell antigen expression, or PBMC donor variability. Our preliminary data revealed that coculture of target cancer cell with PBMC can influence epidermal growth factor receptor (EGFR) expression., an antigen expressed in metastatic cancers. Cetuximab is an FDA-approved IgG1-based therapeutic antibody targeting EGFR. While the mechanism of antigen expression alteration has not been elucidated, we aim to determine whether this change in the EGFR expression can influence cetuximab-dependent ADCC activity in target cells.
- **Methods**
  - To first examine changes in protein expression of EGFR in target cancer cells after being co-cultured with human primary PBMCs, the indicated cancer cells were first co-cultured with human primary PBMCs for 24 hours in a 1:4 ratio. We analyzed protein expression in the whole cell lysate through Western blot analysis. Levels of EGFR protein expression in the cancer cells co-cultured with PBMCs were compared to that in monocultured cancer cells. We then performed lactate dehydrogenase (LDH) assays using the Promega LDH Glo-Assay Kit. On the first day, we seeded 1x10^5 cancer cells in 96-well plates to prepare for multiple different cetuximab antibody conditions. The cancer cells were placed in up to 5 different conditions: no additional PBMCs or NK cells, monoculture cancer cells with PBMCs, monoculture cancer cells with NK cells, PBMCs coculture cancer cells with PBMCs, or PBMCs coculture cancer cells with NK cells. After incubating 24 hours, we added cetuximab and NK cells. After incubation for another 24 hours, the media was collected on the third day to be analyzed via LDH assay.
- **Results**
  - The monocultured cancer cells with no additional PBMCs or NK cells added showed the lowest relative light units (RLU) out of all the conditions without regard to the concentration of cetuximab antibody added. The monocultured cancer cells with added PBMCs or NK cells had increased RLU in a dose-dependent manner of cetuximab. he monocultured cancer cells with added NK cells had higher RLU compared to its PBMC counterpart and had its peak LDH concentration at around 1 μg/mL cetuximab. In contrast, the co-cultured cancer cells with added PBMCs or NK cells showed lowered LDH concentrations across all cetuximab concentrations compared to their monocultured counterparts. The monocultured cancer cells with added NK cells showed clearer dose-dependent manner LDH results than those in monocultured cancer cells with added PBMCs.
- **Implications**
  - The clearer results of LDH activity in the monocultured cancer cells with added NK cells indicated a successful establishment of ADCC assay using NK cells rather than PBMCs. ADCC activity relies most heavily on NK cells out of all other

PBMCs. NK cells only make up < 10% of PBMCs, so larger amounts of isolated NK cells would contribute to the clearer, heightened, dose dependent ADCC activity. Then, we found that the co-cultured cancer cells with added PBMC or NK cells had lowered LDH concentrations in comparison to their monoculture counterparts. We previously observed that co-cultured cancer cells with PBMC for 24 hours resulted in decreased EGFR expression in the cancer cells. Our results indicate that decreased EGFR in the cancer cell line co-cultured with PBMC results in lower ADCC activity using cetuximab. Based on our results, PBMC may influence antigen expression in target cells, resulting in producing inconsistent ADCC activities.

27. **Abstract Title:** *Development and Analysis of a Novel DOOR Endpoint for Complicated Intra-abdominal Infection (cIAI) Using Nine Registrational Trials for Antibacterial Drugs*
Authors: Kinamon, Tori, FDA/CDER (Student); Gopinath, Ramya, FDA/CDER (Mentor); Waack, Ursula, FDA/CDER (Mentor); Needles, Mark, FDA/CDER (Mentor); Rubin, Daniel, FDA/CDER (Mentor); Collyar, Deb, PARI; Doernberg, Sarah, UCSF; Evans, Scott, George Washington/ARLG; Hamasaki, Toshi, George Washington/ARLG; Thomas Holland, Duke University/ARLG, Howard-Anderson, Jessica, Emory University School of Medicine/ARLG, Chambers, Henry, UCSF/ARLG; Fowler, Vance, Duke University School of Medicine/ ARLG; Nambiar, Sumathi, Johnson and Johnson/ARLG; Kim, Peter, FDA/CDER; Boucher, Helen, Tufts University School of Medicine/ARLG
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:
    - **<u>Synopsis</u>**
        - Utilizing patient-level data from nine registrational Phase 3 clinical trials for complicated intraabdominal infection (cIAI), we developed and tested a desirability of outcome ranking (DOOR) endpoint for cIAI that may provide an improved understanding of trial participants' overall outcomes.
    - **<u>Purpose</u>**
        - Desirability of outcome ranking (DOOR) uses an ordinal ranking system to evaluate global outcomes in clinical trial participants by incorporating safety and efficacy assessments into a single endpoint. In this study, we developed and applied a DOOR endpoint for cIAI clinical trials.
    - **<u>Methods</u>**
        - We reviewed nine Phase 3 noninferiority trials for cIAI with electronic patient-level data (n=5022 participants) submitted to the FDA between 2005-2021. We (1) applied a DOOR prototype and modified it based on clinically meaningful events that trial participants experienced, resulting in a cIAI-specific DOOR endpoint; and (2) applied the cIAI-specific DOOR endpoint to trial datasets, assigned each participant a DOOR rank, and estimated the probability that a participant in the study treatment arm in each trial would have a more desirable DOOR rank or component outcome than if assigned to the comparator arm.

- **Results**
    - In the first part of our study, we identified common events that patients with cIAI experienced while in the clinical trial environment. We noted that a significant proportion of participants underwent additional surgical procedures related to their baseline infection, infectious complications were uncommon overall but were diverse, and that participants with poorer outcomes had more infectious adverse events, serious adverse events (SAEs), and procedures. These observations informed our development of the cIAI-specific DOOR endpoint. In the second part of our study, we found that the cIAI-specific DOOR distributions between treatment and comparator arms in all trials were similar. DOOR probability estimates for the trials ranged from 47.4% to 50.3% and were not statistically significant. Component analyses provided visual displays of risk-benefit assessments of the study treatment vs. the comparator.
- **Implications**
    - We developed a cIAI-specific DOOR endpoint to better elucidate the events that patients experienced in these trials. The component analysis allowed more nuanced evaluation of the factors that contributed to the composite DOOR probability estimate. Our study was limited by its retrospective approach and trial design heterogeneity. Similar data-driven approaches can be utilized to develop syndrome-specific DOOR endpoints for other infectious disease indications. The DOOR endpoint could be used as an additional tool to help sponsors evaluate their products and detect differences between treatment arms. Developed with the input of physicians and patient representatives, the DOOR endpoint may enable improved definition of what is important to patients, and by doing so, better incorporates the patient voice into clinical trial analysis

28. **Abstract Title:** *A Landscape Analysis of Valganciclovir/Ganciclovir Treatment for Congenital CMV in the US, 2008-2021*
    Authors: Nania, Flannery, FDA/CDER (Student); Krishnan, Anirudh, FDA/CDER (Student); Viswanathan, Prabha, FDA/CDER (Mentor); Komatsu, Takashi, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - The goal of this study is to characterize the current landscape of congenital cytomegalovirus (cCMV) infections and their treatment with antivirals [(v)GCV] in the USA. To conduct the study, claims data were obtained from Sentinel Distributed Database from 2008-2021. Two cohorts were identified by indexing at: G1) diagnosis of a cCMV infection and G2) prescription of (v)GCV. G1 was subdivided into 3 groups that received treatment within 45 days, 180 days, and did not receive treatment within 180 days. All groups were stratified by age, sex, race, and year. Outcomes indicating disease

severity and/or treatment effects such as clinical features, hematological outcomes and hearing loss were assessed. Based on our analysis, hearing loss increased over time for all cohorts, severe hematological events occurred infrequently, and current treatment patterns do not fully align with current guidelines. We hope this research contributes to a better understanding of provider and family decision making and patient outcomes, which can facilitate future clinical trials and efforts to improve the treatment of this disease. We would also like to acknowledge contributions by Adebola Ajao, Amy Bishara, Ann McMahon, Ashish Rai, Danijela Stojanovic, Hengrui Sun, Jency Daniel, and Mayura Shinde to this project and its related components.

- **Purpose**
  - Congenital cytomegalovirus (cCMV) infection is the leading cause for non-hereditary sensorineural hearing loss (HL) in childhood in the United States and is associated with a variety of other sequelae in infants, including neurodevelopmental impairment. Neonates infected with cCMV are symptomatic in 10-15% of cases, and asymptomatic in 85-90% of cases. There are two drugs, ganciclovir (GCV) and its prodrug valganciclovir (vGCV) [(v)GCV], that are recommended by American Academy of Pediatrics (AAP), which are not approved by the FDA for treatment (Tx) of cCMV. AAP recommends 6 mos of (v)GCV treatment to be initiated in the 1st month of life for neonates with moderate-severe symptoms. However, antiviral use is associated with modest improvements in clinical outcomes and adverse hematological events, mainly neutropenia, which affects treatment plans. The goal of this study is to characterize the treatment landscape of cCMV in the US. This includes examining factors influencing the decision to treat, duration of treatment, and the alignment of those trends with current guidelines.
- **Methods**
  - We used claims data from Sentinel Distributed Database (2008-2021) to identify two main cohorts. The first cohort (G1) consists of infants diagnosed with cCMV within the first 45 days of life and was further divided into 3 sub-cohorts: (G1a) infants treated with (v)GCV within 45 days, (G1b) infants treated with (v)GCV within 180 days, and (G1c) infants not treated with (v)GCV within 180 days. The second cohort (G2) was identified by indexing on prescriptions for (v)GCV for Tx of cCMV for infants and children less than 5 yrs of age. All cohorts were assessed for disease severity through the following categories: hearing loss (baseline, 60, 180 and 365 days), clinical features (jaundice, hepatosplenomegaly, microcephaly), and hematological outcomes (neutropenia, G-CSF, pRBC transfusion). The cohorts were divided by age, race, sex, and year. We compared baseline disease severity and periodically assessed outcomes to determine trends in (v)GCV use over time, proportion of children who receive Tx over time and sought to identify factors that influence the decision to start antiviral Tx.
- **Results**
  - Of 1,500 infants with cCMV identified in G1, 15% was G1a, 20% was G1b, and 80% was G1c. The mean age of cCMV diagnosis was 8 days (SD 12). Among G1 infants, 70% had one or more clinical symptom at baseline, 23%

of whom were treated with (v)GCV. The most common symptom at baseline among all groups was jaundice (48-49%), followed by thrombocytopenia (36% of G1), and brain abnormalities (19% of G1). At baseline, 44-47% of 1a-b infants experienced thrombocytopenia, as did 33% of 1c. Brain abnormalities were seen in 32-34% of 1a-b infants and 15% of 1c. HL increased in prevalence in 1a-b from 19-22% to 58-62%, as well as in 1c infants from 6% to 18% from baseline to 365 days. Neutropenia was seen in 21% and 12% of 1b and 1c infants within 180 days of cCMV diagnosis, respectively. In G2, 372 infants with cCMV received (v)GCV. The mean age for G2 was 78 days (SD 138). HL increased in prevalence from 34% to 58% from baseline to 365 days. Major symptoms were similar to those of G1. The most prevalent hematological outcome was neutropenia as seen 60 days (15%) and 180 days (19%) after index.

- **Implications**
    - Our analysis indicated that reports of hearing loss progressed with time and that severe hematological events occurred infrequently across all groups, though the impact of treatment on these factors needs further examination. We also found that treatment patterns may not align with current guidelines, and work is ongoing to further assess the reasoning and implications of this observation. Another focus of our ongoing work is insight into treatment duration. With this ongoing analysis we hope to contribute to a better understanding of provider and family decision-making and patient outcomes, which is necessary for clinical development of antiviral treatments in this population. This information may inform the design of future clinical trials of antiviral drugs for cCMV, as treatment options and guidelines must progress in order to better address this disease and its long-term effects on infants.

29. **Abstract Title:** *Simulation Study of the Evaluation of Bayesian Network for Synthetic Data Generation*
Authors: Yunyi Li, FDA/CDER (Student); Tae Hyun (Ryan) Jung, FDA/CDER (Mentor); Di Zhang, Former FDA/CDER (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation & Unleashing the Power of Data
Abstract:
    - **Synopsis**
        - With machine learning algorithms, synthetic data may precisely capture the complexities of real-world data (RWD) with preservation of their statistical features and lower the hurdle in which RWD are limited, incomplete or cannot be obtained easily. However, there is no consensus and guidance on the statistical methods that are most reliable for generating and validating synthetic data for various data scenarios. In our study, the state-of-art methodologies for generating and validating synthetic data were reviewed. To get a comprehensive guidance for using synthetic data generated by Bayesian Network algorithm, a simulation study with a wide range of conditional structure complexity, sample size ranging from 50 to 15,000, number of variables ranging from 3 to 27, various combinations of variable types were evaluated. Besides commonly used optimization algorithms,

such as PC, Grow-Shrink, hill climbing, the performance of other 6 algorithms for structure learning were tested as well. Furthermore, we used a single arm NDA review to generate synthetic data and evaluated the similarity and the reliability compared to the real data. For model selection, Bayesian Information Criterion scores were calculated, Partially Directed Acyclic Graphs and Directed Acyclic Graphs were plotted. Metrics such as standardized mean difference (SMD), propensity score mean squared error (pMSE), the area under receiver operating characteristic curve (AUROC) and the area under Precision-Recall curve (AUPRC) were calculated for synthetic data validation. Based on simulation studies, validated by parameter estimation and metrics, synthetic data generated by Bayesian Networks with careful model selection could successfully preserve statistical features, especially when sample size is large and interactions between each variable are simple. Our simulation results also imply that when RWD are limited, well-generated synthetic data for RWD with complex data structures can be used as an alternative to traditional external controls.

- **Purpose**
    - In single-arm studies, external control arms, also known as synthetic control arms, can be generated using real-world data (RWD) pulled from historical trials, medical claims, and other sources of patient data when randomization is impractical, infeasible or unethical to perform. In October 2020, the FDA supported the use of a Medidata synthetic control arm® in a Phase III registrational trial in recurrent glioblastoma. This external control arm is created using a large volume of cross-industry historical clinical trial data. Alternatively, through the application of machine learning algorithms, synthetic data can be accurately generated and capture the complexities of RWD including the preservation of important statistical features. This feature will lower the hurdle in which RWD are limited, incomplete or cannot be obtained easily. However, there are neither regulatory science research nor consensus on the statistical methods for generating and validating the synthetic data in various data scenarios and the regulatory utility is unclear.
- **Methods**
    - We used the comprehensive Bayesian Network to generate synthetic patient data. Models with a wide range of conditional structure complexity, including scenarios with sample size ranging from 50 to 15,000, number of variables ranging from 3 to 27, different combinations of variable types (continuous, dichotomous, categorical with 3, 4, 5 levels) were evaluated. Constraint-based (PC, Grow-Shrink, Incremental Association Markov Blanket etc.) and score-based (Hill Climbing, Tabu Search) structure learning algorithms were tested. Bayesian Information Criterion (BIC) scores were calculated to quantify the performance of each algorithm. Partially Directed Acyclic Graphs (PDAGs) and Directed Acyclic Graphs (DAGs) were plotted to visualize the results of structure learning. Parameter estimation standardized mean difference (SMD), propensity score mean squared error (pMSE), the area under receiver operating characteristic curve (AUROC) and the area under Precision-Recall curve (AUPRC) were calculated for synthetic data validation.

- **Results**
    - For the scenarios with a small number of variables, relationship between variables is simple, a correct conditional probability structure and promising synthetic data can be generated based on a small sample size. For complicated interaction structures, even with a large sample size, a correct DAG cannot always be guaranteed. However, based on the DAG with the highest BIC scores, validated by parameter estimation, SMD, pMSE, AUROC and AUPRC, a promising synthetic data set could be generated as well. For sparse variables, unobserved categories or missing values could influence the result of structure learning.
- **Implications**
    - Based on simulation studies, generating synthetic patient data using data-driven Bayesian Network, successfully captured the complexities of the relationship between each variable with preservation of their statistical features not only with the large sample size, medium-sized number of variables and complex data structure but also with the small sample size and simple data structure. It implies that well-generated synthetic data for RWD with complex data structures can be used as an alternative to traditional external controls.

30. **Abstract Title:** *Manufacturing Deficiencies in Liquid Drug Products, Data Extraction and Analytics*
Authors: McChesney, David, FDA/CDER (Student); Aldridge, Allison, FDA/CDER (Mentor); Pai, Vidya, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the power of data
Abstract:
    - **Synopsis**
        - The purpose of this study is to extract and analyze data for manufacturing deficiencies to determine the ten most common deficiencies in liquid drug product applications. These deficiencies are identified in relation to the chemistry manufacturing and controls (CMC) listed in the drug product application. OPMA assesses the manufacturing and controls information in an application through a risk-based review which is captured in an Integrated Quality Assessment (IQA) document. The review determines if a process is appropriately developed and controlled to ensure that quality is built into the manufacturing process. If a deficiency is identified, the assessor will issue an Information Request (IR) to inform the applicant that an area needs to be addressed. IRs were extracted from nearly nine hundred IQA forms from the last 3 years. IRs are then categorized by the development area for which they have been identified within the IQA. Currently the data is being sorted in order to create trends, provide analytics and statistics from these deficiencies. The analysis results will further improve the efficiency and consistency of the regulatory assessment process. The data will be useful for training assessors and assist in harmonizing deficiency language. Through industry communication, it will help improve the first-cycle approval rate by FDA clarifying expectations for liquid unit operations. Thus, increasing public access to quality liquid drug products at a faster rate.

- **Purpose**
    - The purpose of this study is to extract and analyze data for manufacturing deficiencies to determine the ten most common deficiencies in liquid drug product applications. These deficiencies are identified in relation to the chemistry manufacturing and controls (CMC)listed in the drug product application. OPMA assesses the manufacturing and controls information in an application through a risk-based review which is captured in an Integrated Quality Assessment (IQA) document. The review determines if a process is appropriately developed and controlled to ensure that quality is built into the manufacturing process. If a deficiency is identified, the assessor will issue an Information Request (IR) to inform the applicant that an area needs to be addressed.
- **Methods**
    - A tool was created to search FDA document repository to find review (IQA) documents for liquid dosage forms. After retrieval of the IQAs from past 3+ years from New Drug Applications (NDA) and Abbreviated New Drug Applications (ANDA), data extraction of IRs was done using a proprietary 'Extraction Tool.' The tool is a Microsoft Macro that was developed in-house to compile the data into a single file in Excel. IRs are then categorized by the development area for which they have been identified within the IQA. The data will be analyzed in Excel to determine the percentages of each type of IR.
- **Results**
    - The IRs have been extracted from the IQAs and placed in Excel. At this stage, data extraction from 877 IQA documents has yielded 7048 IRs. Currently the data is being sorted in order to create trends, provide analytics and statistics from these deficiencies. The top ten most common deficiencies for liquid drug products will be presented at the conclusion of the fellowship (August 11, 2022).

31. **Abstract Title:** *Review of Model-Informed Drug Development (MIDD) Pilot Program Interactions from 2018 and How Advice was Incorporated into Development Programs*
    Authors: Jay Mehta, FDA/CDER (Student); Jeffry Florian, FDA/CDER (Mentor); Raajan Naik, FDA/CDER (Mentor); Rajanikanth Madabushi, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - The Model Informed Drug Development (MIDD) Pilot Program was initiated in April 2018 to provide an opportunity for drug developers and FDA to discuss application of MIDD approaches to the development and regulatory evaluation of medical products in development and to provide advice on how particular MIDD approaches can be used in a specific drug development program. As the program continues to develop, it is important to review MIDD interactions to gain insight on how advice is being incorporated into drug development and how to improve the program

moving forward. Three MIDD interactions from the first year were chosen to review meetings, meeting packages, clinical pharmacology reviews, and sponsor-submitted documents. A timeline was constructed to examine the role of MIDD in each development program. In doing so, the impact of the MIDD meeting on the development program could be observed as it moved forward. Each story uniquely displayed the utility of the MIDD interactions.

- **Purpose**
  - The objective of the Model-Informed Drug Development (MIDD) Pilot Program is for drug developers and FDA to discuss application of MIDD approaches to the development and regulatory evaluation of medical products in development and provide advice about how particular MIDD approaches can be used in a specific drug development program. Since April 2018, the FDA has received 58 MIDD meeting requests of which 45 have been granted. A survey of industry participants indicated the value of MIDD Pilot Program interactions. However, information on how advice was included following MIDD meetings is separate and only a few case studies of how development programs have been impacted are available. The objective of this study was to examine a subset of MIDD meetings from the first year and compose a history of how development progressed through review of sponsor-submitted and FDA-archived documents.
- **Methods**
  - The study focused on three drug development programs that participated in MIDD meetings from the initial year, spanning different indications and therapeutic areas. Development programs in Phase 2 or earlier and requests from the initial year of the Pilot Program were selected due to more time passing since the MIDD meetings to see how MIDD approaches were incorporated into development. The assessment consisted of reviewing MIDD meeting requests, background packages, and archived meeting minutes. These materials provided information on MIDD approaches the sponsor intended to use and whether the FDA agreed. The second part of the assessment evaluated sponsor submitted documents, meeting minutes/packages from other interactions, and FDA reviews following the MIDD meeting to see how advice had been incorporated. Each case was examined to see what advice was provided at the MIDD meetings, how that advice was incorporated into development, and where the development program was currently.
- **Results**
  - The three cases highlighted how MIDD approaches were planned to be incorporated into development, and whether alignment was reached between sponsors and FDA on the MIDD approaches. MIDD approaches used included population pharmacokinetic, exposure-response, and systems

pharmacology modeling to support proposed developed decisions. Meetings helped support proposed dosing, including dosing regimens different from what may have been previously evaluated, less frequent and more patient friendly dosing to improve adherence, and dosing strategies to mitigate safety concerns. Review of post-meeting interactions showed how MIDD meeting discussions were included into subsequent decisions. Use of MIDD approaches was often documented in protocols for the development program and discussions from MIDD meetings were referenced as supportive information for proposed activities. Agreements from the MIDD meetings were used to support using similar approaches for different indications or populations.

- **Implications**
    - Most information about outcomes from MIDD Pilot Program interactions has been limited to cases where sponsors were seeking post-approval modification of a regimen or from cases discussed publicly. This assessment focused on cases where the drugs were earlier in development and showed the impact of MIDD meetings could be traced forward within development programs. MIDD meetings helped achieve alignment between sponsors and FDA on MIDD approaches to use in a development program and how those approaches could inform and support decisions around dose selection, trial design, and safety mitigation. Outcomes from MIDD Pilot Program interactions will continue to be evaluated as the participating programs advance forward and additional impact stories are expected to be identified. As the MIDD Pilot Program continues to show significant value, MIDD approaches are expected to play an increasingly prominent role in drug development and regulatory decision making.

32. **Abstract Title:** *Development and Validation of an HPLC Method for Detection and Quantification of Pravastatin*
    Authors: Adam Mirza (Student), Jiang Wang (Mentor), Haiou Qu (Mentor)
    FDA Strategic Initiative: Increasing Choice and Competition through Innovation
    Abstract:
    - **Synopsis:**
        - FDA's key mission is to ensure that dugs are safe and effective for patient use. Analytical tests such as Drug Assay and Dissolution are utilized to ensure drug safety and purity. Sensitive analytical instrumentation and techniques such as high-pressure liquid chromatography (HPLC) are used for performing these tests. In this case, an HPLC method was used to analyze pravastatin in dissolution samples.

- **Purpose:**
    - An efficient way to predict the absorption of a drug in the body is through in vitro testing. An vitro dissolution test based on the Biopharmaceutics Classification System (BCS) was utilized for testing pravastatin, a statin drug for treating hyperlipidemia. A high-pressure liquid chromatography (HPLC) method was developed to detect and quantify pravastatin within dissolution samples.
- **Methods:**
    - The Waters Alliance HPLC system equipped with a UV PDA detector, an autosampler, column heater and quaternary pump was utilized. The wavelength was set at 238 nm for UV detection. A Waters Cortecs C18 2.7 µm column (2.1 x 50 mm) in series with a VanguardTM C18 2.7 µm cartridge (2.1 x 5 mm) was used. Pravastatin and resolution reference rosuvastatin were separated by maintaining a temperature of 450 C and an isocratic mobile phase flow rate of 0.80 mL/min. The mobile phase was comprised of 75% MPA-H2O/0.1% Trifluoroacetic acid/25% MPB-Acetonitrile (v/v) for a 4-min chromatographic elution time. The data was acquired and processed using the Waters Empower software.
- **Results:**
    - The method was successfully applied to pravastatin and validated in accordance with USP <1225> Validation of Compendial Procedures. The method is selective and sensitive and may be used for the detection and quantification of pravastatin in dissolution samples.
- **Implications:**
    - An HPLC method was used and validated to successfully detect and quantify pravastatin in dissolution samples. This method helps the FDA's goal to ensure the safety and efficiency of pharmaceutical drugs because pravastatin can be properly tested for correct dosage and purity. Furthermore, with confidence of a correct dosage, the absorption of pravastatin in the body can be more accurately predicted.

33. **Abstract Title:** *Exploration for Exclusion of Females of Reproductive Potential as Bioequivalence Study Population in Product-Specific Guidances for Generic Drug Development*
    Authors: Grace Paleracio, MPH, CDER (ORISE Summer Fellow/PharmD Student); Cynthia Tsui, PharmD, CDER (ORISE Fellow); Duyen Nguyen, PharmD, CDER (ORISE Fellow); Karen Li, PharmD, CDER (Mentor); Mitchell Frost, MD, CDER (Mentor); Myong-Jin Kim, PharmD, CDER (Mentor); and Jihong Shon, MD, PhD, CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:

- **Synopsis**
    - Product-Specific Guidances (PSGs) describe the FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific reference listed drugs (RLDs). A safety-related consideration during PSG development is whether to enroll females of reproductive potential in bioequivalence studies with pharmacokinetic endpoints (PK BE studies). Information on RLD labeling such as toxicology data (e.g., genotoxicity, embryofetal toxicity, and fertility) and contraception recommendations are usually reviewed to guide the final determination of whether to include females of reproductive potential in a PK BE study. This project compiles a list of drugs for which the PSGs recommend excluding females of reproductive potential. The products' pharmacology and toxicology profile and exclusion rationale are collected to assess and identify trends and similarities that can serve as a resource to develop a standardized framework to inform future recommendations. Following the analysis of specified drug products excluding females of reproductive potential in PSG recommendations, several observations were made. Out of 61 drugs, 15 are positive for genotoxicity, 27 are reported to produce serious embryofetal toxicity including fetal deaths in at least one species at exposures below or similar to the drug's maximum recommended human dose, and 29 have significant impact on fertility. There are 11 and 42 drugs with labeling recommendations on the use of contraception methods prior to and after termination of therapy, respectively. The current findings could provide an insight into the factors and levels of information that should be considered for the exclusion of females of reproductive potential as a PK BE study population. A standardized framework for the selection of BE study subjects developed based on this work can help improve consistency and promote efficiency of PSG development and ensure subject safety in PK BE studies for generic drug development.
- **Purpose**
    - Product-Specific Guidances (PSGs) describe the FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific reference listed drugs (RLDs). A major element for PSGs is the selection of the study population for bioequivalence studies with pharmacokinetic endpoints (PK BE studies) from safety perspectives. Exclusion of females of reproductive potential has been recommended for drugs with a teratogenicity potential. The decision for each drug has been generally determined based on its toxicological and toxicokinetic profile. This project aims to develop a standardized framework for an informed decision regarding study population selection by understanding the toxicological, PK, and clinical profiles of drugs for which PSGs recommend the exclusion of females of reproductive potential. Such framework can help

improve consistency and promote efficiency of PSG development and ensure subject safety in PK BE studies for generic drug development.

- **Methods**
  - We first searched for PSGs recommending the exclusion of females of reproductive potential or a similar restriction for a study population (e.g., post-menopausal or surgically sterile, exclusion of all female subjects), using the PSG Search Shiny App, a full-text search tool of the FDA's PSG data. The initial search included only PSGs on oral drug products recommending PK BE studies in healthy subjects or the general population. The PSGs identified from the initial search were then filtered to exclude drugs recommending the exclusion of females of reproductive potential based only on indications. The following information of each identified drug after filtering were collected using its approved labeling and new drug application (NDA) submission package: reproductive toxicology data (e.g., genotoxicity, embryofetal toxicity, and fertility), contraception recommendations, PK characteristics (e.g., half-life), and enrollment of healthy subjects during the NDA program. Key components supporting the PSG recommendation for each drug were captured and their common features were sorted.
- **Results**
  - We identified PSGs corresponding to 61 RLDs recommending the exclusion of females of reproductive potential or a similar recommendation (e.g., post-menopausal or surgically sterile, exclusion of all female subjects), after excluding PSGs of drugs administered via non-oral route or contain recommendations of BE studies with clinical endpoint. Out of 61 RLDs, 15 are positive for genotoxicity, 27 are reported to produce serious embryofetal toxicity including fetal deaths in at least one species at exposures below or similar to the drug's maximum recommended human dose, and 29 have significant impact on fertility (2 and 6 drugs are reported to have irreversible or reversible impact, respectively, and 21 drugs lack reversibility data). Many of the identified drugs are for cancer treatments or antiviral agents. For most of the drugs, patients are advised to use effective contraception during their treatment. There are 11 and 42 drugs with labeling recommendations on the use of contraception methods prior to and after termination of therapy, respectively.
- **Implications**
  - The current survey provided an insight into the factors and levels of the information that should be considered for the exclusion of females of reproductive potential in PK BE studies. This collective information could serve as a resource for developing a decision framework on the selection of the PK BE study subject in relation to reproductive toxicity. A standardized decision framework for the selection of the PK BE study population will

contribute to promoting the efficiency of PSG development and improving the consistency among PSGs. In addition, the current data collection and analysis performed for the selection of female subjects can be extended into consideration for the exclusion of males of reproductive potential in PSGs in future research.

34. **Abstract Title:** *Characterization of Adverse Reactions Included in the U.S. Prescription Information for Topical Corticosteroids*
Authors: Sona Ghorashi, FDA/CDER (Student); Rachel Seong, FDA/CDER (Student); Vicky Chan, FDA/CDER (Mentor); Kelly Harbourt, FDA/CDER (Mentor); Melissa Reyes, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **<u>Synopsis</u>**
    - The U.S. Prescribing Information (USPI) is one of the primary tools to inform prescribers on the safe and effective use of FDA-approved prescription drug products. The topical corticosteroids (TCS) represent one drug class with drug products of varying duration on the market, new formulations and active pharmaceutical ingredients (APIs), use for multiple indications, and a range of potency of effect. We sought to characterize the adverse reactions included in labeling to better understand why class labeling may differ among the various TCS products. We utilized several publicly available online databases to identify representative approved, prescription and over-the-counter (OTC) TCS for characterization. We chose one representative USPI for each API, formulation (e.g., cream, ointment, lotion, spray), and concentration. We obtained the most recent USPIs for each representative USPI from Drugs@FDA or DailyMed and screened each USPI for pre-selected adverse reactions reported in tertiary references. We identified 21 different APIs that are included in FDA-approved prescription and OTC TCS, associated with 221 unique NDAs and abbreviated NDAs (ANDAs) for non-combination TCS. After confirming active marketing status, 81 representative TCS of varying combinations of API, formulation, and concentration were identified and characterized. The USPI is a continually changing document that contains the current, evidence-based information available to ensure safe and effective use. Safety profiles for individual TCS drug products may vary as NDAs and ANDAs represent unique drug products despite similar mechanism of action among the API across the TCS class.
- **<u>Purpose</u>**
    - The U.S. Prescribing Information (USPI) is one of the primary tools to inform prescribers on the safe and effective use of FDA-approved prescription drug products. The topical corticosteroids (TCS) represent one drug class with drug products of varying duration on the market, new formulations and

active pharmaceutical ingredients (APIs), use for multiple indications, and a range of potency of effect. We sought to characterize the adverse reactions included in labeling to better understand why class labeling may differ among the various TCS products.

- **Methods**
  - We utilized several publicly available online databases to identify representative approved, prescription and over-the-counter (OTC) TCS for characterization. We chose one representative USPI for each API, formulation (e.g., cream, ointment, lotion, spray), and concentration. We obtained the most recent USPIs for each representative USPI from Drugs@FDA or DailyMed and screened each USPI for pre-selected adverse reactions reported in tertiary references.
- **Results**
  - We identified 21 different APIs that are included in FDA-approved prescription and OTC TCS, associated with 221 unique NDAs and abbreviated NDAs (ANDAs) for non-combination TCS. After confirming active marketing status, 81 representative TCS of varying combinations of API, formulation, and concentration were identified and characterized.
- **Implications**
  - The USPI is a continually changing document that contains the current, evidence-based information available to ensure safe and effective use. Safety profiles for individual TCS drug products may vary as NDAs and ANDAs represent unique drug products despite similar mechanism of action among the API across the TCS class.

35. **Abstract Title:** *Evaluation of Neurofilaments as Potential Pharmacodynamic Biomarkers in Amyotrophic Lateral Sclerosis*
    Authors: Goldenberg, Antony, FDA/CDER (Student); Bhattaram, Atul, FDA/CDER (Mentor); Sharma, Vishnu, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:

- **Synopsis**
  - The objective of this study was to evaluate the role of neurofilaments in the prognosis of amyotrophic lateral sclerosis (ALS). Specifically, this project examined the association between neurofilaments (both light chain and heavy chain in cerebrospinal fluid as well as plasma) and clinical endpoints. Clinical endpoints included ALS Function Rating Scale-revised, disease progression slope and mortality. These findings may provide valuable insights in evaluating the role of neurofilaments as a potential surrogate endpoint.

- **Purpose**
  - Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive degeneration of upper and lower motor neurons, resulting in muscle wasting and typically leading to death from respiratory failure within 3 to 5 years of onset. As per the FDA Guidance for Industry 2019 on Developing Drugs for Treatment in ALS, the FDA encourages sponsors to incorporate exploratory biomarkers in all phases of drug development. Among various biomarkers studied for ALS, neurofilaments, a marker of axonal injury and neurodegeneration, are considered promising biomarkers of ALS because of their significantly elevated levels in patients with ALS. Both light (NfL) and heavy (pNfH) chain neurofilaments present in cerebrospinal fluid (CSF) and plasma have been studied in the literature as a potential prognostic biomarker for ALS. The objective of this study is to determine the role of neurofilaments in the prognosis of ALS.
- **Methods**
  - A PubMed search was used to collect studies evaluating the neurofilament levels in ALS patients. Relationships between neurofilaments (both NfL and pNfH in CSF and plasma) and clinical endpoints were collected. Clinical endpoints included ALS Function Rating Scale-revised (ALSFRS-R), disease progression (DP) slope and mortality. Additional information such as demography, region, and neurofilament measurement methods were also collected. To evaluate the association between neurofilaments and mortality, hazard ratios with 95% confidence intervals were collected. Correlation coefficients were collected to examine the association between neurofilament levels and ALSFRS-R, as well as between neurofilament and DP slope. Heterogeneity analysis was assessed using the Cochran's Q test and the I2 statistic. A random-effects model was chosen for the analysis considering substantial heterogeneity ($I2 > 50\%$ or $p < 0.1$) in the data. The statistical analysis was conducted using 'metafor' package v3.4.0 in R v4.1.3.
- **Results**
  - Out of the 192 papers that the initial MESH search yielded, 18 research articles were used in the analysis. The findings suggested that plasma NfL and pNfH were correlated with each other. Elevated plasma NfL levels were reported to be stable over 40-60 months after ALS diagnosis. A positive correlation was observed between ALSFRS-R total score and neurofilament (NfL and pNfH) present in plasma and CSF. In terms of disease progression, both NfL and pNfH in plasma and CSF were positively correlated with DP slope. In terms of survival, higher pNfH and NfL levels were associated with a higher risk of death in ALS patients.
- **Implications**
  - Study findings suggested a positive correlation between neurofilament levels and clinical endpoints (ALSFRS-R total score, DP slope, and mortality). The applicability of these findings in randomized controlled trials is being

investigated using existing database at FDA and may provide valuable insights in evaluating neurofilaments role as a potential surrogate endpoint.

36. **Abstract Title:** *A cloud-based workflow enables large scale analysis on data from the next generation sequencing technologies*
Authors: Tian, Jiazi, FDA/CDER (Student); Xiao, Wenming, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
  - With the development of next-generation sequencing (NGS), it enables researchers to address complex genomics questions at an unprecedented level. However, the huge data volume for each single sample brings challenges to make the processing data time-consuming and computationally expensive. Therefore, the purpose of this study is to establish a cloud-based workflow for sequence alignment and post processing of large datasets. We leveraged precisionFDA, a cloud-based platform to setup NGS analysis workflow, using the tar archive file of two reads sequence. First, we used BWA to establish sequencing reads alignment on Genome Reference Consortium Human Build 38 (GRCh38). Then we used SAMtools sort and convert alignment files into Binary Alignment Map (BAM) format. Finally, we used MarkDuplicates from Picard, a set of command line tools to markup duplicate reads. Moreover, batch jobs were set up to process multiple samples simultaneously. We used this workflow and batch job submission mechanism on data set of whole-exome sequence (WES) on 279 samples in a cohort study. We used SAMtools flagstat to generate summary report of quality control (QC) metrics on resulting BAM files. Most samples have total reads between 80 million and 170 million. On average, it took about 2 hours to analyze an individual sample on a 36-CPU core. With the setup of batch job submission, we were able to complete the whole workflow for all 279 samples in about 5 hours. QC metrics indicated that nearly 100% reads in all samples were mapped on the reference genome. Therefore, the cloud-based workflow established in this study worked efficiently and analyzed large sequence datasets simultaneously. This workflow can be used to support other applications such as whole-genome sequence (WGS), chromatin immunoprecipitation sequence (ChIP-seq), etc. Moreover, this workflow can be further modified by changing the underline alignment tool for RNA-Seq data, such as replacing BWA with STAR.
- **Purpose**
  - Next-generation sequencing (NGS) has been evolved rapidly in recent decades. It requires specific assays, generates more flexible outputs and has the ability to fully survey mutations for thousands of genes. With these advantages, it has been applied increasingly and enables researchers to

address complex genomics questions at an unprecedented level. The huge data volume for each single sample brings great opportunities along with challenges on data analysis. Raw data is typically in the range of tens of gigabytes per sample, depending on sequencing depth. For example, the raw data size of whole-genome sequencing (WGS) can even reach 250 GB, which makes the processing data and revealing biological insights time-consuming and computationally expensive. To solve this dilemma, cloud-based bioinformatics tools for NGS data analysis have been widely used. However, it is essential to setup a pipeline with appropriate selection and combination of the tools. Hence, the purpose of this study is to establish a cloud-based workflow for sequence alignment and post processing of large data.

- **Methods**
    - We used precisionFDA, a cloud-based platform to setup NGS analysis workflow, which can perform alignments and duplicates identification. Due to the requirement of the platform, the input of the workflow was the combined file of two reads sequence using tar archive. The default number of CPU cores was set as 36. First, we used BWA to establish sequencing reads alignment on Genome Reference Consortium Human Build 38 (GRCh38). Then we used SAMtools sort and convert alignment files into Binary Alignment Map (BAM) format. Finally, we used MarkDuplicates from Picard, a set of command line tools for manipulating sequencing data and formats to markup duplicate reads. By comparing sequences in the 5 prime positions, collecting duplicate reads and ranking the reads, the MarkDuplicates tool generated a new sorted BAM file and its associated index file, in which duplicates reads were located and tagged. Moreover, batch jobs were set up to process multiple samples simultaneously. At last, we used SAMtools flagstat to generate summary report of quality control (QC) metrics on resulting BAM files.
- **Results**
    - We created a general workflow for performing sequence alignment against the common reference genome with QC metrics and scripts for submitting the launch of this workflow as a batch job. We used this workflow and batch job submission mechanism on data set of whole-exome sequence (WES) on 279 samples in a cohort study. Most samples have total reads between 80 million and 170 million. On average, it took about 2 hours to analyze an individual sample on a 36-CPU core. With the setup of batch job submission, we were able to complete the whole workflow for all 279 samples in about 5 hours. QC metrics indicated that nearly 100% reads in all samples were mapped on the reference genome and the percentage of properly paired reads is between 95% and 100%.

- **Implications**
    - According to the QC metrics of resulting BAM files, the workflow established in this study worked efficiently to perform sequence alignment and markup the read duplicates in the samples from a published cohort study. In addition, this cloud-based workflow can run as batches so that the large sequence data sets can be analyzed simultaneously. As resulting processed alignment files are typically the early steps for further downstream analysis, depending on sequencing application, this workflow can be used to support other applications such as whole-genome sequence (WGS), chromatin immunoprecipitation sequence (ChIP-seq), Assay for Transposase-Accessible Chromatin using sequence (ATAC-seq), etc. Moreover, this workflow can be further modified by changing the underline alignment tool for RNA-Seq data, such as replacing BWA with STAR.

37. **Abstract Title:** *One year stability study of in-house sunscreen formulations*
Authors: Wanasathop, Apipa, FDA/CDER (Student); Yang, Yang, FDA/CDER (Mentor); Ashraf, Muhammad, FDA/CDER (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:
    - **Synopsis**
        - The characteristics of sunscreen formulations such as globule size distribution, viscosity, and pH, can have an effect on the transdermal absorption of chemical UV filters. A series of sunscreen formulations were prepared by varying manufacturing process variables (homogenizing speed, mixing temperature, and homogenizing time) in-house. The objective of this study was to evaluate the stability of in-house sunscreen formulations that were manufactured with these controlled variables and stored at 25°/60% RH for one year. Preliminary results have shown that manufacturing process parameters may influence the globule size distributions and product viscosity. After one year of storage, the formulations were found physically stable. There were no significant changes in the visual appearance (cream color, phase separation), pH, and globule size distribution. Other measurements (rheology, UV filters concentration, and IVPT) will be performed and further evaluated.
    - **Purpose**
        - Sunscreen creams are usually formulated as oil in water emulsions. It is a system where an oil phase is evenly dispersed as globules in a continuous aqueous phase. The active ingredients in sunscreen are UV filters, which protect the skin by absorbing UV radiations at the skin surface. It has been shown that chemical UV filters penetrate the skin and result in systemic exposure. There are several factors that may affect the skin absorption

including formulation characteristics and excipient compositions. A series of in-house sunscreen formulations were prepared by systematically varying the manufacturing process parameters that may influence formulation characteristics that may lead to transdermal absorption of UV filters. The objective of this study was to investigate the one-year physical stability of in-house sunscreen formulations that were manufactured and stored at 25°/60% RH. The physical stability was determined by measuring the globule size distribution, viscosity, and pH. The chemical stability was determined by analyzing the contents of UV filters in various formulations.

- **Methods**
  - A series of in-house sunscreen creams (oil in water emulsion) containing 3% (w/w) avobenzone, 10% (w/w) octocrylene, and 6% (w/w) oxybenzone as chemical UV filters were prepared by utilizing the design-of-experiment (DoE) principal. The effects of manufacturing process variables on the characteristics of sunscreen cream were investigated. These variables were mixing temperature (60 - 80°C), homogenization speed (1500 - 5000 rpm), and homogenization time (15, 30, or 45 minutes). The formulations were prepared and dispensed into polypropylene centrifuge tubes, then stored at 25°C/60% RH. For globule size distribution measurement, images were acquired using an Olympus BX51 polarized light microscopy. Size distribution information was obtained using ImageJ software. For rheological properties of the samples, DHR-3 hybrid rheometer with a 25 mm sandblasted parallel plate geometry were used. InLab Viscous Pro ISM pH probe with SevenExcellence multimeter was used to measure the pH of the semisolid. The concentration of the UV filters in the samples will be analyzed by LC-MS.
- **Results**
  - Preliminary results showed that an increase in homogenizer speed decreased the globule size distribution, and all the manufacturing process parameters (temperature, speed, and time) appeared to have an impact on the viscosity of formulations. After storage for one year no significant changes were found in the visual appearance (cream color, phase separation), pH, and globule size distribution. Other measurements are in progress. An in-vitro skin permeation test (IVPT) will also be performed for stable formulations to evaluate the dermal absorption of the chemical UV filters.
- **Implications**
  - The evaluation of a series of in-house sunscreen creams with well-controlled formulation and manufacturing process variables will help identifying sunscreen product quality attributes that may limit dermal absorption of chemical UV filters. The understanding of how these characteristics may impact dermal absorption of UV filters will assist in the product formulation development. The goal is to minimize systemic absorption while maintaining

effective local concentration of UV filters on the skin. Moreover, understanding the formulation stability will ensure that the formulation is safe and effective throughout its shelf life.

38. **Abstract Title:** *Use of kratom to discontinue stimulants: An online & social media analysis*
Authors: Xiaojing(Romy) Wang, FDA/CDER (Student); Jill Settle; Paula Rausch (Mentor); Alexandria Smith
FDA Strategic Initiative: Unleashing the Power of Data; Empowering Patients and Consumers
Abstract:

- **Synopsis**
  - Kratom, an unregulated substance, is utilized as self-treatment for opioid use disorder. Unapproved treatments for stimulant use disorder (StUD) contribute to substantial worldwide burden of disease. We aimed to understand whether and how individuals use kratom to self-treat StUD. A commercially available social listening platform retrieved public online and social media conversations about use of kratom for StUD published between January 1, 2020, and June 21, 2021. Two researchers reviewed 3,820 initial posts, manually coded 398 relevant posts, and conducted thematic analyses. Polysubstance use and misperceptions that kratom is not a drug were common. Posts identified benefits to using kratom, including effectiveness, ability to function, renewed joy, pain relief, harm reduction, and safety and healthfulness. Risks and negative consequences included addiction, side effects and adverse events, guilt, lack of efficacy, others' judgment, drug interactions, and monetary cost. Withdrawal, tolerance, and dependence were discussed. Some justified kratom as necessary despite negative consequences while others wanted to quit. Kratom is being utilized to self-treat StUD, but motivations and methods vary. We identified benefits and concerns associated with kratom use for StUD, including misperceptions that require correction.
- **Purpose**
  - Kratom, an unregulated substance, is utilized as self-treatment for opioid use disorder. Unapproved treatments for stimulant use disorder (StUD) contribute to substantial worldwide burden of disease. We aimed to understand whether and how individuals use kratom to self-treat StUD.
- **Methods**
  - A commercially available social listening platform retrieved public online and social media conversations about use of kratom for StUD published between January 1, 2020, and June 21, 2021. Two researchers reviewed 3,820 initial posts, manually coded 398 relevant posts, and conducted thematic analyses.

- **Results**
    - Polysubstance use and misperceptions that kratom is not a drug were common. Posts identified benefits to using kratom, including effectiveness, ability to function, renewed joy, pain relief, harm reduction, and safety and healthfulness. Risks and negative consequences included addiction, side effects and adverse events, guilt, lack of efficacy, others' judgment, drug interactions, and monetary cost. Withdrawal, tolerance, and dependence were discussed. Some justified kratom as necessary despite negative consequences while others wanted to quit.
- **Implications**
    - While previous studies have described kratom use for its stimulant-like properties or to withdraw from opioids, the present study evinced self-reported use of kratom to withdraw from stimulants. These findings underscore the importance of online and social media data for both signal detection and for understanding *the complexity of substance use.*

39. **Abstract Title**: *Pregnant and Lactating Persons: Past, Present, and Future*
Authors: Wells, Sarah, FDA/CDER (Student); Baisden, Kristie, FDA/CDER (Mentor); Sahin, Leyla, FDA/CDER (Mentor); Yao, Lynne, FDA/CDER (Advisor/Division Director)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:
    - **Synopsis:**
        - Pregnant and lactating persons represents a substantial segment of the U.S. population who may require medical treatment for various conditions. However, pregnant and lactating persons are underrepresented in drug research, which causes gaps in knowledge on the safety of medication use in this population. To advance comprehensive data collection on the safety of drugs in this population, FDA needs to understand the current landscape of pregnancy and lactation PMR issuance. A prior review utilized FDA databases for NDA/BLA approvals and PMRS to evaluate FDA's pregnancy and lactation PMRs issued from January 2007 to December 2020. This review added pregnancy and lactation PMRs issued since the last review (January 2021 to May 2022) and combined the data from the previous review to identify the most updated trends and potential future opportunities. Trends show an increasing number of pregnancy and lactation PMRs being issued from 2007 to 2021 since the passage of FDAAA in 2007. This is an important step in closing the knowledge and safety gap on medication use in pregnant and lactating persons. Nevertheless, there remains a need for more comprehensive data collection in this population. The results from this review suggest there is an opportunity for FDA to increase the consistency in requiring pregnancy and lactation PMR studies, when needed, with future drug approvals.

- **Purpose:**
  - Pregnant and lactating persons represent a unique and important segment of the population who may require medications to treat chronic or acute conditions. However, historically, pregnant and lactating persons have been excluded from drug development trials and continue to be underrepresented in research. Consequently, at the time of NDA/BLA approval, there are generally no human pregnancy or lactation data available to determine the safety of the drug when used during pregnancy or lactation. Such data, when needed, generally must be collected through postmarketing requirements (PMRs). To advance comprehensive data collection on the safety of drugs in this population, FDA needs to understand the current landscape of pregnancy and lactation PMR issuance. A prior review evaluated FDA's pregnancy and lactation PMRs issued from the introduction of the FDA Amendments Act (FDAAA) in 2007 to December 2020. This review added pregnancy and lactation PMRs issued since the last review (January 2021 to May 2022) and combined the data from the previous review to identify more recent trends and potential future opportunities.
- **Methods:**
  - Pregnancy and Lactation PMRs were identified from the "Postmarketing Requirements and Commitments: Downloadable Database File" from the Office of New Drugs. Each PMR was verified using the approval letter within the Drugs@FDA database. The original NDA/BLA approvals from January 2007 to May 2022 were identified using an internal FDA Drug Research and Analysis Host (DASH) database. We excluded drugs that were only approved for use in men, children, or postmenopausal women. Pregnancy PMRs were grouped based on their study design into pregnancy registry, database studies, single-arm/surveillance/pharmacovigilance, pregnancy sub-study in rare disease safety study, and randomized controlled trial (RCT). The data were analyzed for trends in the number and type of pregnancy and lactation PMRs issued across therapeutic areas, and in relation to milestone regulatory events such as public meetings, workshops, and publication of guidance.
- **Results:**
  - The prior review identified 497 NDA/BLA approvals from 2007 to 2020. This review identified 88 from 2021 to May 2022. Of the total approvals, 552 (94%) were drugs used in females of reproductive potential and 110 (20%) were issued pregnancy or lactation PMRs. Pregnancy PMRs (n=89) were issued commonly for relapsing multiple sclerosis (n=7, 10%), plaque psoriasis (n=6, 8%), and migraine (n=6, 8%); lactation PMRs (n=21) for sleep disorders (insomnia and narcolepsy) (n=6, 29%) and chronic idiopathic constipation (n=4, 19%). New data from this review did not change the findings from the prior review. From 2007 to 2012, pregnancy PMRs averaged 3 per year which increased to 12 in 2014. The largest increase in total PMRs occurred 2017 to 2021. We noted issuance of two pregnancy PMRs for new drug approvals (1 in 2016, 4 in 2017, 4 in 2018, 9 in 2019, 5 in 2020, and 5 in 2021). The following PMRs were also issued: single-arm/surveillance/pharmacovigilance (1 in 2017, 6 in 2018, 5 in 2019, 9 in 2020, and 8 in 2021) and lactation (1 in 2017, 2 in 2018, 6 in 2019, 2 in 2020, and 4 in 2021).

- **Implications:**
    - Because data on the use of drugs for medical conditions in pregnant and lactating persons are generally collected postapproval, PMR studies are an important regulatory mechanism to obtain safety data for this population. Since the passage of FDAAA in 2007, there has been a 13-fold increase in pregnancy PMRs and a 4-fold increase in lactation PMRs issued from 2007 to 2021. There is a need for more comprehensive data collection in pregnant and lactating persons, as articulated in FDA guidances, such as the Postapproval Pregnancy Safety Studies and Clinical Lactation Studies Draft Guidances. These findings suggest that there is an opportunity for FDA to increase the consistency in requiring pregnancy and lactation PMR studies, when needed, with future drug approvals.

40. **Abstract Title:** *Challenges and Strategies to Properly Utilize Historical or Real-World Data in Clinical Trials*
Authors: Wu, Fei, FDA/CDER (Student); Wang, Huan, FDA/CDER (Mentor); Cai, Xiaoyu, FDA/CDER (Mentor); Chen, Yeh-Fong, FDA/CDER (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:
    - **Synopsis**
        - Randomized clinical trials (RCTs) are the gold-standard for new drug evaluation. However, RCTs can be very expensive and time-consuming to conduct, especially for some rare diseases. Therefore, the utilization of historical clinical data and real-world data (RWD) in clinical trials is attracting attentions from both academia and industry. These data, obtained from sources other than RCTs, such as electronic health record systems, literature, or registry, can provide useful information on drug safety and effectiveness. However, many challenges occur when applying historical data or RWD to clinical trials. One major concern is related to the selection of the optimal statistical model to mitigate the bias and increase robustness of the estimands. For example, numerous matching techniques have been considered for trials with external controlled arm (ECA) to utilize historical data or RWD, e.g., one-to-one matching, many-to-one matching, propensity score matching, etc. Besides these matching techniques, other methods such as the inverse probability weighting methods and targeted maximum likelihood methods can also be applied. To promote better understandings of these methods, a comprehensive comparison of the pros and cons of these methods under different scenarios in clinical practice is needed.
    - **Purpose**
        - Single arm studies utilizing historical clinical trial data and Real World Data (RWD) have became attractive in trial planning and/or efficacy estimation of new drugs. Many algorithms have been developed for using RWD in clinical trials, including propensity score matching (PSM), inverse probability weighting (IPW), etc. Each method has its own advantages and disadvantages, and there is no general agreement on the usage of these methods. In this study ,we compared different causal methods via

simulations, examined the limitations and valid use of these methods, and proposed a general guidance for RWD analysis.

- **Methods**
  - We compared the performance of different methods in estimating causal effects via simulation studies under different settings. The methods we used were classified into two categories: 1) matching methods (such as PSM and Mahalanobis distance matching (MDM) and 2) IPW methods. We estimated the average treatment effect (ATE) and the average treatment effect on treated (ATT) under different scenarios. We conducted simulations for all methods with various settings. In particular, we generated data with different outcome generation models and treatment selection models. And we assumed different distribution for the covariates incorporated in these models. We evaluated the performance of all methods in terms of different metrics, including bias, standard deviation, and mean squared error (MSE).
- **Results**
  - For matching algorithms, our results showed that the method of nearest neighbor matching with a caliper and without replacement resulted in relatively small MSE in most cases. For IPW methods, the augmented IPW always outperforms other IPW methods. Whether the matching methods are better than the IPW methods depends on the linearity of the true outcome model and the extreme values of propensity scores. If there is strong linear pattern in the true outcome model and there are no extreme values of propensity scores, IPW methods usually result in small bias and outperform matching methods in term of MSE. With extreme values of propensity scores or nonlinearity, the estimators from IPW methods could be largely biased. For true outcome model with strong nonlinearity, MDM usually has a good performance.
- **Implications**
  - It is important to select appropriate causal models based on the characteristics of the data. We recommend conducting statistical tests on the nonlinearity of the data before the selection of methods. If strong nonlinear pattern presents, MDM and coarsened exact matching may be the preferred methods. We also recommend examining the distribution of propensity score before the selection of methods. IPW methods should not be used if there are a large amount of data with extreme values of propensity scores. In the remaining cases, IPW methods are generally recommended.

41. **Abstract Title:** *Data-driven Risk-informed Medical Gas Facility Selection Model*
    Authors: Yang, Yidan, FDA/CDER (Student); Wan, John, FDA/CDER (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:

    - **Synopsis**
      - In this research, we constructed a data-driven predictive model by using FDA inspectional records, this predictive model provides a science-based approach to identify medical gas (MG) facilities that may potentially have a risky impact on public health. Machine learning algorithm random forest

and gradient boosted tree classifier are applied to train the model and resampling method such as cross-validation is also used to evaluate the model performance (accuracy). The experiment outcome indicates that the predictions are fairly accurate, the decision-tree model has a satisfactory performance. In future, as we continually improving the model, it could become a possible option of identifying other risk-informed non-MG manufacturing facilities.

- **Purpose**
  - This research provides a science-based way to evaluate the perceived risk over medical gas (MG) facilities, we leverage the FDA inspectional records and decision-tree type machine learning model to identify the MG facilities that may potentially have a risky impact on public health. This data-driven model can be continually improved as more data comes in the training process.
- **Methods**
  - The data used to train this predictive model are historical FDA ORADSS inspection records, the most recent inspection outcome is considered as the target (response variable) for each facility. The training data contains 25 features characterize the inspection history, manufacturing status and other FDA inspect-related information. A comprehensive factor analysis indicates that decision-tree type model would have a satisfactory performance in this circumstance. Therefore, machine learning algorithm such as random forest and gradient boosted tree classifier are applied to train the model and test the results. Resampling method such as cross-validation is used to evaluate the model performance (accuracy).
- **Results**
  - This data-driven medical gas (MG) model has satisfactory performance with high predicting accuracy. Note that the target (response variable) is originally ordinal and has four-level (OAI, OAI/VAI, VAI and NAI), we also tested the decision-tree model with new target classified as binary (OAI and Others). The result indicates that the predictions are fairly accurate, but the one with binary target has a relatively high accuracy.
- **Implications**
  - This data-driven predictive model provides a science-based way to evaluate the perceived risk over medical gas facilities, it can be continually improved as the machine learning model digests more data in the training process. In future, it could become a possible option to identify other risk-informed non-MG manufacturing facilities.

42. **Abstract Title:** *A Survey of Regression Analysis Methods Adopted in Renal Impairment Studies*
Authors: Zhang, Tina, FDA/CDER (Student); Nguyen, David, FDA/CDER (Student); Al-Khouja,

Amer, FDA/CDER (Mentor); Yi, Sojeong, FDA/CDER (Mentor); Doddapaneni, Suresh, FDA/CDER (DIIP Deputy Director)

FDA Strategic Initiative: Unleashing the Power of Data

Abstract:

- **Synopsis**
  - Renal impairment (RI) studies are conducted to assess the effect of varying levels of renal impairment on pharmacokinetics (PK). Currently, the FDA guidance on renal impairment studies (published in 2010 and draft guidance revised in 2020) recommends conducting a regression analysis, in which measures of renal function and PK parameters are treated as continuous variables. The guidance states that the regression analysis is preferred to an analysis in which renal function is treated as a categorical variable (i.e., ANCOVA) corresponding to the normal, mild, moderate, and severe renal impairment groups; however, it does not describe any standardized methods for conducting the regression analysis. In this study, renal impairment studies conducted for new drug products approved between the years 2015-2021 were investigated based on study design and data analysis methods used. Preliminary results in products approved in 2021 found that in most RI studies, regression analyses were not conducted or secondary to the ANCOVA analyses where renal function was treated as a categorical variable, if used. Notable variability in conducting and reporting the regression analyses was found between RI study reports in terms of PK parameters. This study may be used to help identify best suited data analysis methods in future renal impairment studies.
- **Purpose**
  - The pharmacokinetics (PK) of certain medications may differ in patients with impaired renal function. Renal impairment (RI) studies are conducted to assess the effect of varying levels of renal impairment on PK. FDA guidance on RI studies (published in 2010, draft guidance revised in 2020) recommends conducting a regression analysis, where renal function measures and PK parameters are continuous variables. This method is usually preferred to an analysis where renal function is a categorical variable, i.e., an analysis of covariance (ANCOVA), because regression analyses may allow more precise modeling for renal function and PK. However, RI measures are usually described categorically (i.e., mild, moderate, severe) in the labeling as this description may be easier to clinically translate and determine dose adjustment. The current guidance does not describe any standardized methods for conducting the regression analysis and translating results into dose recommendations. This study aims to investigate any possible differences between regression analysis methods used in past RI studies.
- **Methods**
  - Dedicated RI study reports were identified from drugs approved between years 2015-2021. For each study, collected information included if the study

used a full or reduced design, how RI was defined, and how PK parameters were compared between varying degrees of renal impairment. If the study conducted an ANCOVA and/or a regression analysis, the results were compared to the drug product labeling.

- **Results**
    - Preliminary results found that out of 323 drugs approved from 2015-2021, 108 had dedicated RI studies conducted during their clinical program. Thirty-nine of these studies only included patients with severe renal impairment compared to those with normal renal function (i.e., reduced design). Although the FDA guidance states a regression approach is preferred to an ANCOVA, in most RI studies conducted in 2021, the primary data analysis method used was ANCOVA. Regression analyses were not conducted or secondary to the ANCOVA if used; subsequently, in most cases, the RI study results described in the drug labels were derived from ANCOVA results. Notable variability in conducting and reporting the regression analyses was found between RI study reports in terms of PK parameters.
- **Implications**
    - This study may be used to help identify best suited data analysis methods in renal impairment studies.

43. **Abstract title:** *Adaptive designs for IVPT data with mixed scaled average bioequivalence*
Authors: Lim, Daeyoung, FDA/CDER (Student); Rantou, Elena, FDA/CDER (Mentor); Kim, Jessica, FDA/CDER (Mentor); Choi, Sungwoo, FDA/CDER (Mentor); Choi, Nam Hee, FDA/CDER (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

- **Synopsis**
    - In vitro permeation tests (IVPT) offer accurate and cost-effective development pathways for locally acting drugs, such as topical dermatological products. For assessment of bioequivalence FDA's draft guidance on Acyclovir cream 5% introduces a new experimental design, namely the parallel, single-dose, multiple-replicate per treatment group design, as IVPT pivotal study design. As this design is materially different from previous crossover studies, we examine the statistical properties of its associated hypothesis testing method, mixed scaled average bioequivalence (MSABE). Meanwhile, some adaptive design features in clinical trials can help researchers make a decision earlier with fewer subjects or boost power, saving resources, while minimizing impact on family-wise error rate. Therefore, we incorporate MSABE in an adaptive design combining the group sequential design and sample size re-estimation. Simulation studies are conducted to study the passing rates of the proposed methods—both within and outside the average bioequivalence limits. Further modifications

to the adaptive designs applied for IVPT BE trials, such as Bonferroni's adjustment and conditional power function, are considered.

- **Purpose**
  - In vitro permeation tests (IVPT) offer accurate and cost-effective development pathways for locally acting drugs, such as topical dermatological products. For assessment of bioequivalence FDA's draft guidance on Acyclovir cream 5% introduces a new experimental design, namely the parallel, single-dose, multiple-replicate per treatment group design, as IVPT pivotal study design. The goal of applying adaptive design to IVPT studies through this project is the practical use of the information obtained from the interim stage (pilot), which implies incorporating pilot and pivotal studies.
- **Methods**
  - The FDA draft guidance on Acyclovir cream 5% includes a hypothesis testing method for the associated experimental design, mixed scaled average bioequivalence (MSABE). Meanwhile, adaptive designs in clinical trials can help researchers make a decision earlier with fewer subjects or boost power, saving resources, while minimizing impact on familywise error rate. Therefore, we incorporate MSABE in an adaptive design combining the group sequential design and sample size re-estimation. Simulation studies are conducted to study the passing rates of the proposed methods — both within and outside the average bioequivalence limits. Further modifications to the adaptive designs applied for IVPT BE trials, such as Bonferroni's adjustment and conditional power function, are considered.
- **Results**
  - Our findings are:
    - Adaptive MSABE (a-MSABE) attains robust passing rates.
    - Bonferroni's adjustment offers stronger FWER guarantees than Pocock's α levels, as expected.
    - Conditional passing rate yields unrealistic sample sizes, as opposed to using the true GMR at 0.95 for passing rate calculation.
- **Implications**
  - Adaptive MSABE can save costs and resources by allowing early stopping, with many trials needing fewer than 10 subjects in total. Additionally, simulation studies suggest that fewer than 10 subjects for the pilot study may be sufficient.

Center for Devices and Radiological Health (CDRH)

1. **Abstract Title:** *Autonomous peripheral nerve tissue classification algorithm for polarization sensitive optical coherence tomography images*
   Authors: Amin, Shivam, FDA/CDRH (Student); Hammer, Daniel X, FDA/CDRH (Mentor);

Saytashev, Ilyas, FDA/CDRH (Mentor);

FDA Strategic Initiative: Increasing Choice and Competition through Innovation

Abstract:

- **Synopsis**
  - In addition to clinical approaches for peripheral nerve stimulation (PNS), newly proposed applications to palliate conditions with PNS require searching the stimulus parameter space to determine safe stimulus levels and optimize therapeutic efficacy. Previously, our group demonstrated novel optical biomarkers to assess the safety of PNS using optical coherence tomography (OCT) angiography and polarization sensitive OCT. Proposed techniques for extracting relevant biomarkers from multimodal OCT volumes were based on subjective classification and manual masking of neural segments. We developed an automated algorithm for tissue classification using multimodal OCT volumes. First, we attempt automatic tissue classification using clustering algorithms that group pixels in multi-parameter space based on their intensity values and polarization signatures. Then, we use existing rat sciatic nerve images as inputs to train a neural network that classifies the clusters in each frame of the OCT volume. We were able to visualize voxels that accurately classified nerve fascicles and blood vessels. Our algorithm allows us to improve upon current methods of segmentation and classification by reducing labor time while increasing our segmentation rate. Our work also opened the door to further research and improvement on our methods.
- **Purpose**
  - Real-time biomarkers can aid in the overall assessment of peripheral nerve health. However, the current technique to extract relevant biomarkers from multimodal OCT volumes is based on subjective classification and manual masking of nerve segments. Our aim was to develop an automated algorithm for tissue classification using the multimodal OCT volumes. Automated segmentation will differentiate tissue components (e.g., nerve fascicles, blood vessels, support tissue) for spatial tracing and quantitative analysis (e.g., branching, overall volume, etc.). Moreover, the high-throughput tool will allow us to improve the processing efficiency in a streamlined pipeline, providing high quality data for the assessment of nerve damage in stimulated animals compared to the control animal cohort.
- **Methods**
  - We use PS-OCT data obtained from a previous study designed to assess peripheral nerve damage during overstimulation in a rat sciatic nerve model. For each voxel of the imaged volume, we assign a property according to each modality derived from our PS-OCT processing output. First, we attempt automated tissue classification using k-means and

DBSCAN methods, clustering algorithms that group pixels in multidimensional space. Each of the resultant clusters are assigned to a certain tissue type based upon likeness of their optical properties. Then, we use existing rat sciatic nerve images as inputs to train a neural network (NN) that will classify clusters on every frame of the OCT volume. After classifying the pixels on each frame of the volumetric image, we compare the results from our NN to manually segmented images. The goal is that every pixel labeled by manually segmented images is also labeled as the same tissue by our trained NN.

- **Results**
  - Visualization of the cross-sectional view (B-Scan) shows us that pixels tend to cluster in groups when plotting against intensity, retardation (birefringence), and optical axis orientation. Each group of pixels represents a different class of imaged objects: nerve holder, nerve fascicles, saran wrap, and nerve vasculature. We extracted nerve fascicles and blood vessel classified voxels and visually compared volumetric reconstructions between different classification methods.
- **Implications**
  - Peripheral nerve stimulation (PNS) is used clinically to treat depression and epilepsy, as well as to aid stroke recovery. New neurostimulation applications have been demonstrated recently to relieve chronic pain and immunomodulation. Many applications for relieving conditions with peripheral nerve stimulation (PNS) require a search of the stimulus parameter space to determine safe stimulation levels and optimize therapeutic effectiveness, a process which is time-consuming and expensive using animal studies and end-point histology. Our algorithm allows us to extract relevant biomarkers from volumetric OCT images with an improved method of segmentation and classification by reducing manual labor time while increasing the rate at which we segment. Our work also opened the door to further research and improvement on our methods.

2. **Abstract Title:** *Development of a Recirculating Microfluidic System to Study Interactions Between Opioid Excipients and Blood*
   Authors: Aspel, Zoe FDA/CDRH (Student); Marchena, Isabel FDA/CDRH (Student); Kim, Dongjune, FDA/CDER (Post-doc); Natu, Rucha, FDA/CDRH (Mentor); Malinauskas, Richard (Mentor); Guha, Suvajyoti (Mentor); and Herbertson, Luke (Mentor)
   FDA Strategic Initiative: Preparedness for combatting the Opioid epidemic
   Abstract:

- **Synopsis**
    - To combat the ongoing opioid epidemic, some opioids have been reformulated to include high molecular weight (HMW) excipients such as polyethylene oxide (PEO), which make the pills more difficult to crush into powders for snorting. The purpose of this study is to introduce multiple doses of PEO into the bloodstream over an extended period of time in an *in vitro* setting and determine how PEO impacts blood cell damage. The *in vitro* model was comprised of a proof-of-concept recirculating flow circuit, which included a microfluidic device with a stenotic region to impose higher shear stresses on the passing blood cells. Blood samples were collected at intervals throughout the test, and plasma free hemoglobin concentration was measured to quantify red blood cell damage (hemolysis). Results from the preliminary study show that levels of hemolysis increased in blood with PEO, particularly with prolonged exposure to elevated fluid shear stresses. By applying constant flow and increasing the exposure time to high shear rates, we were able to simulate conditions of long-term opioid abuse and repeated intravenous drug use in an *in vitro* setting. This type of microfluidic system allows us to study blood interactions with PEO or other high molecular weight excipients in a dynamic physiological environment.
- **Purpose**
    - To combat the ongoing opioid epidemic, some opioids have been reformulated to include high molecular weight (HMW) excipients such as polyethylene oxide (PEO), which make the pills more difficult to crush into powders for snorting. While this was intended to deter abusers, it has resulted in additional complications for those who intravenously inject opioids containing HMW PEO. Thrombotic microangiopathy (TMA) is one reported adverse event that can be catastrophic, resulting in microscopic blood clots in the drug user's blood vessels. If these clots begin to obstruct blood vessels, fluid shear stress levels increase in the bloodstream which can damage red blood cells (RBCs) and activate platelets. The purpose of this study is to simulate this adverse event in an *in vitro* setting to examine how the introduction of multiple doses of PEO into the bloodstream over an extended period of time impacts blood cell damage.
- **Methods**
    - An *in vitro* model was developed to assess blood-PEO interactions. The system was comprised of a proof-of-concept recirculating flow circuit, which included a microfluidic device with a stenotic region to impose higher fluid shear stresses on the passing blood cells. PEO solution was prepared by dissolving powdered PEO in phosphate buffered saline. This solution was then added to acid citrate dextrose solution A anticoagulated porcine or human blood and gently mixed for 5 minutes before filling the circuit. For

each test, a constant flow rate was maintained through the microfluidic device using a valve, pressure controller, flow sensor, and closed-loop control monitoring equipment. Blood samples were collected at intervals throughout the test, and plasma free hemoglobin concentration was measured to quantify RBC damage (hemolysis).

- **Results**
  - Results from preliminary study show that levels of hemolysis increased with the addition of PEO and prolonged exposure to shear stress. By increasing the channel length of the microfluidic stenotic region from 3 mm to 6 mm, the plasma free hemoglobin increased significantly from 77 ± 22 mg/dL to 117 ± 4 mg/dL. Similarly, when we increased the blood flow rate in the 3 mm channel to elevate the fluid shear rate from 50,000 s^{-1} to 100,000 s^{-1}, the plasma free hemoglobin levels increased from 77 ± 22 mg/dL to 174 ± 12 mg/dL. These initial results were obtained with blood passing through the test channels only once without recirculating and the total blood exposure time was less than 10 seconds. Further study is needed under more physiological conditions with exposure times on the order of minutes to hours with the recirculating loop and shear rates of approximately 1000 s^{-1}. Similar trends are expected in ongoing experiments, with the extended exposure times leading to more severe hemolysis when PEO is added to the system.
- **Implications**
  - While different test systems have been developed to determine the mechanisms of TMA formation caused by PEO and other high molecular weight molecules in abuse deterrent formulations, the recirculating flow model presented here is more relevant to actual physiological scenarios. The constant flow and increased exposure time to high shear rates represent conditions of long-term opioid abuse and repeated intravenous drug use in an *in vitro* setting. This type of microfluidic system allows us to study blood interactions with PEO or other high molecular weight excipients in a dynamic environment relative to a normal control without PEO. In the future, this model could be used to conduct other safety evaluations, such as dosage testing or studying blood responses to other substances commonly used in conjunction with intravenously injected opioids.

3. **Abstract Title:** *An immunocytochemical analysis of the distribution of sodium channels in microglia and ganglion cells in the primate retina.*
   Authors: Cho, Samuel, FDA/CDRH (Student); Cohen, Ethan, FDA/CDRH (Mentor)
   FDA Strategic Initiative: Increasing Choice and Competition through Innovation
   Abstract:

- **Synopsis**
    - For people with damaged retinas, retinal prostheses aim to stimulate the ganglion cells. Around the fovea, the nerve fiber layer from the peripheral retina poses a barrier to effective stimulation, in addition to the presence of microglia. We characterize the sodium channel subtypes on ganglion cells and immune cells in the fovea.
- **Purpose**
    - For people with damaged retinas, retinal prostheses aim to electrically or optically stimulate the ganglion cells to form action potentials which produce a visual percept of light in the brain termed a "phosphene". If the phosphenes can be generated in a spatially discrete manner across the visual field of a blind person, they may be able to perceive objects or what is termed "form vision." However, there is a problem for sponsors. Around the fovea, a nerve fiber layer of overlying ganglion cell axons from the peripheral retina poses a barrier to stimulating the local central foveal retinal ganglion cells with surface disc electrodes. In addition, the electric fields from stimulus electrodes must pass through immune cells termed microglia. Thus, to effectively stimulate the local retina we need to understand the distribution of voltage-gated channels in the axons, microglia and underlying ganglion cells. Voltage-gated sodium channels can have differing properties and are critical to the generation of an action potential in foveal retinal ganglion cells. The object of this study is to characterize the sodium channel subtypes on ganglion cells and immune cells in the fovea.
- **Methods**
    - Primate eyes were obtained at the point of euthanasia by an approved animal protocol. Eyes were fixed by immersion in formalin. We cut frozen sections 12microns of the fovea and prepared them for immunocytochemistry. We examined the distribution of sodium channels using antibodies against 3 common sodium channel types, NaV 1.1, 1.2, and 1.6. It is thought the sodium channel inner segment "hot spots" that determine ganglion firing thresholds are localized on the proximal initial segment of the ganglion cell axon. To localize these channels to ganglion cells and microglia, we used antibodies against RBPMS and Iba1 respectively. Antibody localization was performed using fluorescent secondary antibodies. We are currently examining the distribution of staining of the fovea using confocal microscopy Z-stacks.
- **Results**
    - We are currently in the process of optimizing the immunocytochemistry of the sodium channel immunofluorescence, and will examine the spatial distribution of the 3 sodium channel types throughout the fovea. This will be plotted as a series of zones of differing sodium channel immunoreactivity using image analysis programs in a simple 3D cross sectional model. This will

allow sponsors to optimize electrode design for effective stimulation of the local retinal ganglion cells

- **Implications**
    - Understanding the distribution of voltage-gated sodium channel types in the fovea is critical for generating effective form vision in the blind patient. A foveal model of ganglion cell activation would help prosthetic designers make stimulating electrodes that more effectively stimulate the local ganglion cells.

4. **Abstract Title:** *Tools for Image Quality Evaluation for Mixed Reality Devices in Medicine*
   Authors: Collins, Brendan, FDA/CDRH (Student); Beams, Ryan, FDA/CDRH (Mentor); Lago, Miguel, FDA/CDRH (Mentor)
   FDA Strategic Initiative: Increasing Choice and Competition through Innovation
   Abstract:

    - **Synopsis**
        - There has been recent increase in efforts to use virtual reality (VR) and augmented reality (AR) devices in medicine. Medical Extended Reality devices are arguably still in early stages of development and there is a lack of existing standards for their evaluation. This project aims to create tools to develop standard test patterns that will be used to assess HMD quality. Using WebXR, an emerging standard in the web AR/VR field, a tool was developed that allows for the creation, editing, and sharing of patterns aimed towards display quality assessment on various head mounted displays (HMDs). Through the tool, users have the freedom to generate, manipulate, and share scenes consisting of shapes, pre-built structures, and patterns common in display quality assessment.
    - **Purpose**
        - There has been a general increase in efforts to use virtual reality (VR) and augmented reality (AR) devices in medical practices. Some of the medical applications for these devices include telemedicine, rehabilitation, surgery, and medical image display. Medical Extended Reality devices are arguably still in their early stages and there are not many existing standards for their evaluation. One cause of this is the differences in hardware and software across head mounted displays (HMDs). HMDs come with various internet capabilities, controllers, and means of connectivity. This can lead to testing methods that work on one HMD requiring fundamental changes to be successfully run on another. The goal of this project is to create tools to develop standard image quality test patterns that will be used to measure HMD quality. Having standard test patterns will allow for improved evaluation of HMDs before premarket submission to FDA, ensuring an efficient and unbiased regulatory analysis.
    - **Methods**
        - Tools were created that can be used on many HMDs. One way to produce software for HMDs despite technological differences is through web development. An emerging standard in the web AR/VR field is WebXR. WebXR works through HTML, CSS, and JavaScript to bring immersive AR/VR

to phones, desktop computers, and standalone HMDs via any compatible web browser. WebXR currently has support for most of the prominent AR/VR devices on the market and is continuously adding support for more HMDs as they are released. Test patterns were created using WebXR, and functionality for various HMDs was added. An emphasis was placed on portability so that all tools behaved the same across various HMD platforms.

- **Results**
    - Using WebXR, a tool was designed and implemented that allows for the creation, editing, and sharing of patterns aimed at display quality assessment. Through the tool, users have the freedom to generate and manipulate scenes consisting of two and three-dimensional shapes to create quality assessment test patterns. Along with shapes, users have access to pre-built structures that are common in display quality assessment. Another core piece of functionality is that created patterns can be shared. Saved patterns are stored using JavaScript Object Notation files, which can then later be uploaded and parsed into a scene in the WebXR environment. Shared patterns and their entities can be locally edited and saved without affecting the original versions. The tool also comes with some built-in patterns that can be edited, saved, and shared.
- **Implications**
    - This tool can aid in the establishment of standard and common tests for manufacturers that are implementing applications with AR/VR headsets as part of a medical device. The ability for the patterns to be edited and shared ensures that these tests can be widely and publicly accessed, meeting a critical goal of the project. The use of this tool for standards purposes will help to streamline the regulatory review process for these devices. As the AR/VR medical field continues to develop, the ability for new image quality assessment tools to be easily designed and implemented will become increasingly important. Ultimately, these tools will allow regulatory scientists to incorporate new technological developments by easily creating and sharing new tests as needed.

5. **Abstract Title:** *Development of a High-Throughput 3DP Engineered Cardiac Tissue Platform*
   Authors: Tromondae K. Feaster, FDA/CDRH (Mentor); Pieper Holeman, FDA/CDRH (Student); Jourdan K. Ewoldt, FDA/CDRH; Maura Casciola, FDA/CDRH; Akshay Narkar, FDA/CDRH; Marshall Ma, Thomas Bifano, Christopher S. Chen, John P. Fisher, Ksenia Blinova FDA/CDRH (Mentor)
   FDA Strategic Initiative: Increasing Choice and Competition through Innovation
   Abstract:
    - **Synopsis**
        - We develop a robust 3D printed platform to generate human 3D microphysiological systems (i.e., engineered cardiac tissues). Engineered cardiac tissues are shown to maintain viability and respond to acute cardiac contractility modulation stimulation signals, providing an high-throughput in vitro engineered cardiac tissue tool to evaluate safety or effectiveness of cardiac electrophysiology medical device.

- **Purpose**
    - The lack of automated robust methods for generating 3D engineered cardiac tissues (ECTs) from human-induced pluripotent stem cell–derived cardiac myocytes (hiPSC-CMs) currently limit the utility and application of this novel technology for medical device safety and effectiveness assessment. Here, we develop a high-throughput 3D printed (3DP) platform to generate 3DP 3D ECTs and enable evaluation of standard cardiac contractility modulation (CCM) signal parameters in vitro.
- **Methods**
    - 3DP ECTs were manufactured utilizing bioink extrusion printing to seed hiPSC-CMs and cardiac fibroblasts encapsulated in a Matrigel-fibrin hydrogel on microfabricated tissue gauges. Morphology, viability, and contractility were evaluated to quantify the functionality of 3DP ECTs compared to traditional manual 3D ECT plating.
- **Results**
    - 3DP ECT displayed robust baseline contractile, electrophysiological (AP), and calcium handling properties. Moreover, acute CCM pulses were applied using a commercial pulse generator to evaluate the utility of 3DP ECTs to assess CCM response in vitro.
- **Implications**
    - Here, we develop a robust high throughput 3DP engineered cardiac tissue platform to investigate tissue contractility. Specifically, to elucidate the acute effects of cardiac contractility modulation stimulation on 3D human engineered cardiac tissue function. These data provide an automated in vitro human-based 3D platform to generate ECTs and quantify cardiac function to evaluate safety and effectiveness of future cardiac electrophysiology medical devices.

6. **Abstract Title:** *Enhancing the simulation software tools for Computer-Aided Triage and Notification (CADt) Devices*
   Authors: Zheng, Jixin, FDA/CDRH/OSEL/DIDSR (Student); Thompson, Yee Lam Elim, FDA/CDRH/OSEL/DIDSR (Mentor); Samuelson, Frank, FDA/CDRH/OSEL/DIDSR (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract:
    - **Synopsis**
        - Computer-Aided Triage and Notification (CADt) devices use artificial intelligence (AI) to prioritize radiological medical images and speed up reviews of cases with time-sensitive conditions such as stroke. Although several publications have shown clinical evidence on the benefits of CADt devices in stroke centers, questions remain on a quantitative assessment to evaluate the clinical effectiveness of CADt devices in different radiology departments. In our

previous work, we adapted queueing theory along with Monte-Carlo simulation to predict the effect of wait-time-savings for patients due to a CADt device as a function of clinical factors such as patient image arrival rate, disease prevalence, radiologist reading rate, and number of radiologists on site. In one of the recent CADt submissions, the effectiveness of the subject device was questioned because the sponsor has multiple cleared CADt devices that can potentially rearrange the same reading list. Therefore, in this work, we consider scenarios where multiple CADt devices are involved to rearrange the same queue of images with different disease conditions. To study such a complex workflow with multiple subgroups of patients, the total number of patients increases, lengthening the processing time of the simulation software. Therefore, in this project, our goal is to optimize the simulation software. Optimization can be achieved by streamlining parameter intake and handling, redesigning job submissions and implementing multi-threading. With the optimized software, we can investigate the impact of wait-time-savings for patients due to a CADt device when used with other CADt devices in different simulated clinical settings and understand the potential clinical effectiveness of CADt devices in such a complex workflow.

- **Purpose**
    - Computer-Aided Triage and Notification (CADt) devices use artificial intelligence to prioritize radiological medical images and speed up reviews of cases with time-sensitive conditions such as stroke. Although several publications have shown clinical evidence on the benefits of CADt devices in stroke centers, questions remain on a quantitative assessment to evaluate the clinical effectiveness of CADt devices. Our team's previous work adapted queueing theory along with Monte-Carlo simulation to predict the effect of wait-time-savings for patients due to a CADt device as a function of clinical factors such as patient image arrival rate, radiologist reading rate, etc. The next step is to expand our model by considering scenarios where multiple CADt devices are involved to rearrange the same queue of images with different disease conditions. This increases the required number of patients, lengthening the processing time of the simulation tool. Therefore, in this project, our first goal is to optimize the simulation software.
- **Methods**
    - Our current simulation software makes use of distributed computing. To study the time-saving impact under different clinical parameters, current job submission sets up each job, defined by a set of parameters, to run 200 trials each of which has around 2000 patients. The process can be sped up via a more well-balanced allocation of computing tasks between individual units in the HPC cluster by redefining a job to process one individual trial. The mathematical framework is also updated to account for additional CADt devices and multiple disease types. Current software also required a full ROC curve to characterize

the CADt device, whereas a typical CADt submission only reports an operating threshold. We thus modify the software to only need a single-point sensitivity and specificity as user input parameters. With the optimized algorithm and submission pipeline, we can investigate the impact of wait-time-saving of multiple CADt devices acting simultaneously in different simulated clinical settings and understand their potential clinical effectiveness in a complex radiologist workflow.

- **Results**
    - With only one CADt device involved, its time-saving ability depends largely on the clinical settings. Consistent with our clinical intuition, our model suggests that CADt devices with a typical AI diagnostic performance (95% sensitivity and 89% specificity) are most effective in a busy, short-staffed clinic. It is found that the impact on the amount of time-savings can be affected by the number of radiologists present in the workflow, patient arrival rate, radiologist rates, and the operating threshold of the device. Assuming no correlation exists among disease conditions, including more CADt devices is expected to decrease the time-saving benefits because true-positive and false-positive cases flagged by all devices are moved up. Our optimized simulation software enables us to confirm our expectation with this complex workflow where multiple disease conditions, modalities, anatomies, and CADt devices are involved in the same reading queue and to quantitatively understand the nuanced effects of bringing multiple CADt devices into a real-world radiology department.
- **Implications**
    - The optimization of our CADt simulation software is essential in expanding our analysis to more complex workflows. As more CADt devices are cleared and become available in the market, we need to understand the potential clinical effectiveness when multiple devices are used on the same reading list with different disease conditions. For example, a reading queue may include brain images with both vessel occlusion and brain hemorrhage. If two CADt devices are involved (one for occlusion and one for hemorrhage), how would the benefits and/or risks of each device be impacted? Our main project goal is to answer that question under various clinical settings (such as patient arrival rate, radiologist reading rate, etc.). However, as we expand the simulation software, more patients are included, increasing the computational time of the software. Our efforts in optimizing the simulation software should successfully reduce both our simulation software's instances at the HPC cluster, which enables us to continue investigating the safety and effectiveness of CADt devices given a complex workflow.

7. **Abstract Title:** *Developing interactive data visualizations to balance workloads*
Authors: Bai, TJ, FDA/CDRH (Student), Thompson, Elim, FDA/CDRH (Mentor), Lago, Miguel, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
    - To facilitate and automate administrative tasks for regulatory science programs, we develop several interactive dashboards that gather, clean, analyze, and visualize information within the division. The pipeline uses Microsoft 365 tools such as PowerBI, PowerAutomate, and SharePoint. Data cleaning and analyses are performed with Python and DAX scripting. We also communicate with our division management so that the dashboards meet their administrative needs. One example dashboard is the Consult Summary Report that summarizes the consults our division did in real time. A management team member can quickly filter the data by consultant, by year, by submission type, etc. to answer his/her administrative questions effectively. Other reports are also set up to keep track of scientific publications, mentor/mentee relationships, and traveling requests. By expediting managerial tasks and cutting down on redundant and duplicated workstreams, our interactive dashboards effectively reduce the repetitive administrative workload of our division.
- **Purpose**
    - In this project, we develop various interactive reports to support, automate, and facilitate administrative tasks within the DIDSR/OSEL/CDRH/FDA. This includes enhancing existing visual reports (e.g., consult summaries and personnel reports), building new visual reports (e.g., travel/conference and publication tracking, etc.), and integrating these reports where relevant. This project examines various modes of data analysis to generate intuitive and insightful conclusions from internal and external data. Not only do these dashboards help our management team visualize the work within the division using to up-to-date information, but individual employees can also review the work they did and/or conferences they attended during employee performance evaluation. Within these interactive reports, the need for redundant, repetitive administrative tasks are reduced, and people's time can be reallocated to focus on supporting the missions of our division.
- **Methods**
    - Reports are built within PowerBI, where data is imported, cleaned, and analyzed with Python and DAX Scripting. Internal data within our division are collected using Microsoft 365 tools such as PowerAutomate and SharePoint. Publicly available data outside our division are also scraped

from relevant websites. Throughout the project, we communicate with our management team to collect feedback and determine their exact administrative needs/desires from which corresponding figures and features are implemented.

- **Results**
    - A few interactive dashboards are created with various figures to effectively communicate the administrative data within our division. For example, in the Consult Summary Report, consult documents are filtered and displayed by their workflow state, data of request, and other key indicators. Correspondingly, consultants are filtered by their open, completed, and total consults. Users can efficiently slice between the two to obtain a better and more detailed understanding of any one consult or consultant. Our reports are designed to be intuitive and interactive, thus best communicating essential information to users in a short amount of time.
- **Implications**
    - The interactive reports expedite various administrative tasks within the division and cut down on redundant or duplicated workstreams. They provide a simple and condensed way for our management team to track things within the division and identify top performers and employees who are available for additional consults. For employees, these reports provide a direct channel for them to review their work and/or request their needs. Additionally, these reports can be useful for people outside the division to effectively visualize the work we did without going through any technical, gritty details. With these interactive reports, division members can re-allocate their time to focus on supporting the missions of our division.

8. **Abstract Title:** *Comparing and Contrasting Two Rounds of Rotary Bend Fatigue Experiments for Improved Preclinical Testing*
   Authors: Katelyn Blackwood (ORISE Student Intern), Jason Weaver (mentor), Mathew Di Prima (mentor), Shiril Sivan (mentor)
   FDA Strategic Initiative: Increasing Choice and Competition through Innovation
   Abstract:
    - **Synopsis**
        - Fatigue characterization is an important component of pre-clinical testing to ensure that medical devices will be safe and effective. The experiments conducted herein compared fatigue performance of nitinol with two different processing methods out to 1 billion cycles. These tests are important because they can help characterize device durability and eventually reduce unanticipated fractures with the Fatigue to Fracture methodology. Fatigue life was tested using rotary bend wire fatigue systems in an environment closely resembling the human body using a phosphate

buffer saline (PBS) water bath which helps ensure accuracy of the data. A range of alternating strains was tested in order to produce e-N curves comparing alternating strain versus the number of cycles until fracture or runout. In many previous experiments the nitinol test specimens underwent the fatigue tests up to only 10 million cycles and so these experiments should provide a better understanding of long-term nitinol durability. Conducting these tests is important because it may lead to an improved understanding of why fractures occur sooner than 1 billion cycles. An initial comparison of the two nitinol fatigue life curves was made and additional analyses will be conducted in the future. Analyzing fracture surfaces can highlight important features of these fractures and help explain why and how they occur. Comparing the data from both rounds of experiments may lead the way to new innovations in nitinol medical devices.

- **Purpose**
    - The purpose of this research is to better understand nitinol fatigue and to prevent nitinol cardiovascular devices from fracturing due to fatigue loading. Fatigue is the process of initiation and propagation of cracks due to cyclic loading applied to a material. Once these cracks form, they grow over time and can finally rupture. Once the experiment is completed the fatigue life can be analyzed by plotting alternating strain versus the number of cycles to fracture. Two rounds of trials were done on nitinol with different material processing and the data will be compared to identify differences in fatigue behavior and the applicability of the Fatigue to Fracture methodology. Fatigue tests were conducted to 1 billion cycles or until fracture which is important because most of the prior studies have been conducted to only 10 million cycles and the 100 million to 1 billion cycle region remains unexplored.
- **Methods**
    - The Rotary Bend Fatigue experiments were conducted using ASTM E2948 Standard Test Method for Conducting Rotating Bending Fatigue Tests of Solid Round Fine Wire and ASTM F3211 Standard Guide for Fatigue-to-Fracture (FtF) Methodology for Cardiovascular Medical Devices. Straight nitinol wires having 0.48 mm in diameter were cut to the desired length and inserted into the chuck of the front motor for the guided tests. Each round of experiments used specimens made from the same lot of nitinol wire, but with slightly different processing. Throughout the experiments different sized mandrels were used to create different strains. The mandrel diameters for both rounds of experiments that were used ranged from 18.3 mm to 178.3 mm. Each wire specimen was submerged in a tank filled with phosphate buffered saline (PBS) solution heated to 37 ^{o} C. Tests were conducted at 24,000 RPM and continued until 1 billion cycles or fracture.

The fracture surfaces were imaged using a scanning electron microscope (SEM) to understand the characteristics of the fracture surface.

- **Results**
    - Fatigue life was characterized into an e-N graph that represents the alternating strain versus the number of cycles to fracture. The results following the end of the first round of experiments (group 1) show that the nitinol wire specimens were able to withstand at least 10 million cycles and fractured between 100 million and 1 billion cycles at strain values near 0.5% and with mostly runouts between 0.27% and 0.40%. The results obtained from the second round of experiments (group 2) concluded very similar results showing evidence of failure past 10 million cycles with fractures occurring between 100 million and 1 billion cycles with strain values near 0.6% and with mostly runouts between 0.28% and 0.40%. By observing the fracture surfaces of the group 1 specimens, the SEM uncovered that the fractures tended to initiate at the location of nonmetallic inclusions.
- **Implications**
    - The results show that the Nitinol wires can survive 1 billion cycles without fracture depending on the imposed alternating strain. When comparing the two rounds of data and their e-N curves group 1 shows a slow initial decay in strain from 1,000 cycles to 10,000 cycles then maintaining a smooth curve as it reached 1 billion cycles. However, the e-N curve for group 2 shows a steeper slope up to approximately 10,000 cycles and then slightly more scatter between 10 million and 100 million cycles. These findings are important to the eventual implementation of the Fatigue to Fracture methodology because the data can be used to evaluate the ability of ASTM F3211 to determine if a device manufacturing change would impact high cycle fatigue life by using low cycle fatigue data. A better understanding of the Fatigue to Fracture methodology could lead to increased applications of nitinol as a material in medical devices as a greater understanding of factors impacting durability is gained.

9. **Abstract Title:** *Optimizing CDRH's Use of Voluntary Consensus Standards: Mapping Committee Jurisdictions for Medical Device Standards*
   Authors: Canavan, Maura, FDA/CDRH (Student); Ajmera, Deesha, FDA/CDRH (Student); Gupta, Purva, FDA/CDRH (Student); Zeng, Jianchao, FDA/CDRH (Mentor); Sullivan, Stacey, FDA/CDRH (Mentor); Woods, Terry, FDA/CDRH (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **Synopsis**
        - The Center for Devices & Radiological Health (CDRH) Standards & Conformity Assessment Program (S-CAP) works to advance the use of

regulatory-ready standards for medical devices throughout their lifecycle to ensure patients have access to safe, effective and high-quality medical devices. As required by OMB Circular A-119: Federal Participation in the Development and Use of Voluntary Consensus Standards and in Conformity Assessment Activities, and under the guidance of S-CAP, over 350 CDRH staff participate in hundreds of standards committees from dozens of Standards Developing Organizations (SDOs) to develop or revise standards relevant to medical devices. Each SDO maintains their standards and the associated committees which have jurisdiction over each standard. However, there is no central repository that contains jurisdiction information for standards from all SDOs. The purpose of this project is to create a repository containing jurisdiction information for each standard relevant to medical devices. The standard jurisdiction information can be leveraged to link information in internal CDRH standards databases about each standard. This information includes the specialty task group and liaison representatives connected to the standard, which CDRH members are on the committee, the ballot history, and what other standards are in the same committee or working group. Having all this linked information will enable S-CAP, CDRH standards liaison representatives, managers, and CDRH staff to easily find the information they need about a particular standard. This new jurisdiction map will fill the knowledge gap between relevant medical device standards, the SDOs who maintain them, and the FDA staff who serve and contribute to their development.

- **Purpose**
    - Center for Devices & Radiological Health's (CDRH) Standards & Conformity Assessment Program (S-CAP) works to advance the use of regulatory-ready standards for medical devices throughout their lifecycle to ensure patients have access to safe and effective medical devices. Over 350 CDRH staff participate in dozens of Standards Developing Organizations (SDOs) to develop standards. Each SDO maintains their standards and associated committees with jurisdiction over each standard. However, there is no central repository of jurisdiction information for standards from all SDOs. Our purpose is to create an internal repository of jurisdiction information for each medical device-relevant standard that will be linked to information in internal databases about each standard. Having this linked information will enable S-CAP, standards representatives, and CDRH staff to easily find information they need about a particular standard. The jurisdiction map will fill the knowledge gap between device standards, SDOs who maintain them, and the FDA staff who use them and contribute to their development.
- **Methods**
    - We identified SDOs listed in the FDA Recognized Consensus Standards public database. We searched each SDO's portfolio of standards and work

items relevant to medical devices and collected information about each standard (published or under development), regardless of recognition status.

- **Results**
    - We recorded the standard designation, version, title, edition, publication date, committee, subcommittee, working group, and maintenance team along with any amendments or corrigenda.
- **Implications**
    - The jurisdiction information we collected will be integrated with existing FDA standards databases to enable S-CAP to track the entire lifecycle of a standard. The updated system will allow CDRH staff to identify connections between liaison representatives, standards, ballot history, and SDO committee structure. This information will streamline the process to search for standards, people (for example subject matter experts and liaison representatives), and organizations. It will enable reviewers to better connect with the resources needed to better understand and leverage standards used in the medical device review process. In addition, this will help S-CAP in their mission to develop and advance regulatory-ready standards. It will also aid in the identification of potential new areas for standards development, make it easier to target collections of related standards for improvements that will make them more useful to industry and review staff, and help get safe and effective medical devices to patients.

10. **Abstract Title:** *Developing multiparameter database of laser-induced photothermal damaging effects and thresholds of nonstandard tissues*
    Authors: Du, Arthur, FDA/CDRH (Student); Ilev, Ilko, FDA/CDRH (Mentor); Shrivastava, Devashish FDA (Co-Author)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Currently, the use of laser radiation in diagnostics and therapeutics devices involving nonstandard tissues (other than ocular and skin tissue) does not have widely recognized consensus standards for laser radiation safety evaluation. This project aims to contribute towards addressing this standard regulatory science and public health need by developing of a comprehensive multiparameter database that will contain the most serious and common safety concerns relating to laser-induced photothermal tissue damaging effects in the near-infrared range on nonstandard tissue such as cortical brain, cardiovascular, kidney, and mucosal tissue. The completed database will include summarized comparison results from clinical and non-clinical studies and will establish evaluation techniques to assess tissue

damaging effects, damage thresholds, nonstandard tissue optical characteristics, and specific nonstandard tissue with significant regulatory and public health safety concerns. The database provided through this research project will also contribute to the development and implementation of novel test methods and tools for standard evaluation of the maximum permissible emission (MPE) thresholds for nonstandard tissues.

- **Purpose**
    - Laser radiation is extensively used in many areas of biomedical science, technology, and everyday life. However, when new technologies and devices are developed, we face significant safety concerns of high-power laser-induced photothermal and other tissue damaging effects. Furthermore, a major challenge is the lack of standard tools for laser safety evaluation of nonstandard tissues other than ocular and skin tissue. This project aims to perform a comprehensive comparison analysis of the device database and published clinical studies on photothermal tissue damaging caused by lasers with specific spectral, temporal, spatial, and dosimetry parameters. The analysis will define a multiparameter database of tissue damaging thresholds predominantly in the near-infrared spectral range. The comprehensive database will serve to determine the prioritized nonstandard tissues with most commonly reported safety concerns. The database will allow for the development of novel tools for standard evaluation of the MPE (maximum permissible exposure) thresholds for nonstandard tissues.
- **Methods**
    - To accomplish the primary project objective for development of a multiparameter database of laser-induced photothermal damaging effects and thresholds of nonstandard tissues, the research methodology is based on conducting comprehensive review and comparison analysis of two major sources of relevant data: recently published clinical studies and device data on laser photothermal damaging effects. The review data will be systematically summarized with a focus on laser-induced damaging effects and thresholds for nonstandard tissues, photothermal tissue damaging mechanisms in the near-infrared (700-1100 nm) range, key laser radiation and tissue optical characteristics. The research approach will also include data review on laser safety evaluation based on maximum permissible exposure (MPE) thresholds for various nonstandard tissues such as cortical brain, kidney, mucosal, and cardiovascular tissues. The completed multiparameter database will include comparison data on these critical laser radiation and tissue characteristics, and MPE evaluation thresholds summarized for specific tissues.

- **Results**
    - The ultimate delivery goal of this project is focused on the development of a laser safety evaluation database associated with laser-induced photothermal damaging effects and thresholds of nonstandard tissues. The resulting database will include comprehensive summarized information and comparison data on the following critical characteristics and tissue photodamaging effects and thresholds: (1) laser radiation with specific spectral, temporal, spatial, and dosimetry characteristics; (2) nonstandard tissue optical characteristics; (3) evaluation techniques used for assessing tissue damaging effects and safety thresholds; (4) established tissue damaging thresholds; and (5) specific nonstandard tissues with significant regulatory and public health safety concerns. The completed database will include summarized relevant review results on clinical and non-clinical studies as well as key results from the medical device database.
- **Implications**
    - This project aims to contribute towards addressing an unmet standard regulatory science and public health need and a major current research gap of lacking comprehensive standard database for laser and optical radiation safety evaluation based on MPE threshold values for various types of nonstandard tissues, since the currently available standard database is limited to ocular and skin tissue only. The multiparameter database developed under this project can be employed for assessing the prioritized nonstandard type of tissues with serious and most reported safety concerns. The database will also contribute to the regulatory science and public health through the development and implementation of test methods and tools for standard evaluation of the MPE thresholds for nonstandard tissues.

11. **Abstract Title:** *Developing a Simple Rocker-bead System to Assess the Impact of Blood Factors on Red Blood Cell Fragility When Testing Medical Devices*
    Authors: Farazdaghi, Arman, FDA/CDRH (Student); Meki, Moustafa, FDA/CDRH (Mentor); Kim, Dongjune, FDA/CDER (Mentor); Natu, Rucha, FDA/CDRH (Mentor); Herbertson, Luke, FDA/CDRH (Mentor); Malinauskas, Richard, FDA/CDRH (Mentor)
    FDA Strategic Initiative: Increasing Choice and Competition through Innovation
    Abstract:
    - **Synopsis**
        - Evaluating the potential for blood damage to occur as a result of interactions with medical devices requires a clear understanding of the different mechanical and chemical parameters that affect red blood cells and, ideally, standardization and control of those variables. We developed a simple and inexpensive testing system, where small vials of blood

containing stainless steel beads (1/8 inch in diameter) are gently rocked to induce red blood cell damage (hemolysis). This will allow for examination of variables that affect hemolysis. Anticoagulated blood from donor animals was gently rocked lengthwise at a 17 degree incline for 1 hour at 18 rpm and 23C. Various bead number (0, 5, 8), vial size (5, 8 mL), and blood volume (3, 5, 8 mL) were investigated. As a positive control, varying concentrations (0, 5, 20, 40 μg/mL) of polyethylene oxide (PEO) were mixed in the blood. After rocking, the blood plasma of the samples was separated and analyzed for hemoglobin levels. The rocking beads were confirmed to be the source of the hemolysis, using a beadless control for comparison. In addition, the bead number was shown to have a positive relationship with hemolysis. Vial length and air volume were also found to be important parameters, consistently showing greater hemolysis as these parameters were increased. Test sensitivity was found to be conditional on the volume of plasma present, being the greatest for the smallest volume of blood (3 mL). The system had high reproducibility as the coefficient of variation for hemolysis was less than 3% when testing 6 samples with 8 beads in each vial. Hemolysis levels increased sequentially with rising concentrations of PEO, demonstrating sensitivity of the test method. This rocker-bead test system will be employed in further study of factors impacting hemolysis (e.g., species, anticoagulant, pH) as such findings may influence the reproducibility and interpretation of the results and standardized testing of medical devices.

- **Purpose**
    - Prior to patient use, blood-contacting medical devices must first be bench tested with animal or human blood to demonstrate biocompatibility. Hemolysis is a useful indicator of hemotoxicity as red blood cells (RBCs) may be damaged when interacting with medical devices and release hemoglobin into the plasma, which can cause adverse events in patients. While general guidelines and testing standards for evaluating device hemolysis are available (e.g., ASTM F1841– Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps), researchers may not account for many of the blood factors that impact RBC fragility and the in vitro test results (e.g., species, hematocrit, anticoagulant, plasma protein, pH). The goal of this study is to develop a simple and affordable test system, based on rocking vials containing blood and stainless-steel beads (SSBs), to investigate the impact of different testing parameters and blood conditions on the results of mechanically-induced hemolysis.
- **Methods**
    - Donor animal blood was anticoagulated with Acid Citrate Dextrose solution A, adjusted to a hematocrit of 35 +/- 1% by adding buffered saline or

removing plasma, filtered at 40 μm, and typically tested within 48 hrs of blood draw. To examine mechanical criterion impacting the sensitivity of the test system, 3, 5, or 8 mL of blood were added to 5 or 8 mL capacity vials holding 1/8" diameter SSBs (0, 5, or 8 beads in each vial). Vials were gently rocked lengthwise for 1 hr on a platform rocker at 18 rpm, 17 deg pitch at 23C. Up to 6 vials under a single test condition were rocked to assess reproducibility of the test. Polyethylene oxide (PEO) powder (7 MDa) dissolved in saline was gently added to blood over a range of concentrations (5, 20, 40 μg/mL) as a positive control, as it induces hemolysis in fluid flow models. At the end of the rocking test, blood samples were centrifuged and analyzed for plasma-free hemoglobin (pfHb) concentration using a spectrophotometer to quantify hemolysis.

- **Results**
    - Rocked SSBs were the source of hemolysis, as baseline pfHb values were similar whether the blood was rocked without beads or held static during testing. Hemolysis increased with vial length and bead number; using 8 SSBs increased the hemolysis by 40-70% compared to using 5 SSBs. Importantly, hemolysis was greater when 3 mL of blood was rocked compared to 5 or 8 mL, even when normalizing results based on blood volumes. This indicates that test sensitivity is dependent on the plasma volume that free hemoglobin dilutes into and possibly the air to blood ratio, even though the vials were gently rocked and no air-blood mixing was observed. The system had high reproducibility as the coefficient of variation for hemolysis was less than 3% when testing 6 samples with 8 SSBs in each vial. Hemolysis levels with the rocker-bead test (RBT) increased sequentially with rising concentrations of PEO, being 2-3X greater in samples with high PEO concentrations compared to the no-PEO baseline condition.
- **Implications**
    - Our preliminary results indicate that we can define a RBT that is sensitive, reproducible, inexpensive, and requires little blood. Testing 3 mL of blood with 8 SSBs in 5 or 8 mL vials (in triplicate) produces a sufficient hemolysis signal (>50 mg/dL pfHb) to reproducibly evaluate how various blood factors impact hemolysis. Future experiments will probe other blood variables (e.g., species, hematocrit, anticoagulant, plasma protein concentration, pH) that can affect in vitro hemolysis testing done by medical companies. To verify the results obtained with the RBT regarding different blood parameters, a flow system that more appropriately simulates actual blood flow in medical devices will be studied. Our research findings may influence how to interpret hemolysis study results on medical devices (e.g., extrapolating values obtained using animal blood to human blood) and

may lead to the revision of widely used standards to ensure better consistency in results and improved device safety.

12. **Abstract Title:** *Measurement of Manufacturing Consistency and Environmental Exposure of 3D Printed Phantoms for Adaptive Optics Ophthalmic Imaging System*
Authors: Fitzgerald, Declan, FDA/CDRH (Student); Rosenthal, Ian, UMD (Student); Agrawal, Anant, FDA/CDRH (Mentor); Liu, Zhuolin, FDA/CDRH (Mentor); Hammer, Daniel, FDA/CDRH (Mentor); Sochol, Ryan, UMD (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

- **Synopsis**
    - Direct Laser Writing (DLW), an additive manufacturing method that uses two-photon polymerization, was used to fabricate retinal phantoms for adaptive optics (AO) ophthalmic imaging system evaluations. While the phantom designs have been continuously revised over the last five years, the overarching goals of providing a stable, repeatable calibration standard when imaged with different AO systems, and of being manufactured rapidly while maintaining high structural integrity has remained the same. Methods to reliably evaluate these characteristics are necessary to qualify the phantom as a regulatory science tool. To ensure that the DLW manufacturing technique can fabricate consistent structures print-to-print, an evaluation process was developed using scanning electron microscopy and AO scanning laser ophthalmoscopy imaging evaluated with ImageJ software. Center-to-center spacing of randomly chosen adjacent features was measured, and the mean and standard deviation values for feature separation were calculated for each microarray and compared across prints. A systematic discrepancy of features measuring 100 nm less than designed was observed but remained consistent across each microarray on multiple prints. To ensure that the phantoms have the mechanical properties necessary to withstand prolonged use without deformation, a variety of exposure tests were developed to emulate environmental during routine use. We confirmed that no significant physical deformation or reflectivity change occurs during all environmental exposures. By demonstrating manufacturing consistency and environmental resilience, we confirmed the microarray phantom may be validated as a regulatory science tool. We expect the retinal phantom regulatory tool will aid in AO ophthalmic imaging device performance assessment for efficient FDA regulatory approval and consistent use in multisite clinical investigations.
- **Purpose**
    - We have previously used Direct Laser Writing (DLW), a 3D printing technique, to fabricate microarray phantoms that simulate the reflective properties of photoreceptor cells in the human eye when observed with

adaptive optics (AO) ophthalmic imaging systems. These phantoms are designed and manufactured with two main goals: (1) To provide a stable, repeatable calibration standard when imaged with adaptive optics systems and (2) To be manufactured rapidly while maintaining high structural integrity. A method for evaluating production consistency and resilience to deformation is necessary for qualification as a regulatory science tool. Our aim is to confirm that the DLW manufacturing provides consistent print-to-print quality by measuring the spacing of individual features, such as microwells or pillars, across multiple prints. Moreover, subjecting the phantom to a variety of environmental stressors allows observation of catastrophic physical deformation or reflectivity changes that can affect phantom appearance and lead to inconsistent imaging device evaluation over time.

- **Methods**
    - Phantoms were printed with a Nanoscribe Photonic Professional GT2 3D printer using two-photon polymerization. We measured consistency between prints with scanning electron microscopy (SEM) images analyzed with ImageJ. Features were identified and traced, overlaying geometries across the SEM image. Once traced, the center was calculated. We measured center-to-center spacing of randomly chosen adjacent features to calculate the mean and standard deviation for each microarray across multiple prints. To ensure consistency over a lifetime of several years, the phantoms were subject to environmental stressors. The exposure tests were selected to emulate routine handling in laboratory or clinical conditions including: 1. Flowing isopropyl alcohol across the phantom surface for 30 seconds, 2. Blowing compressed air across the phantom surface, 3. Submerging in microscope objective immersion oil for 10 minutes, 4. Compressing with a low-lint wipe, and 5. Submerging in water and dish soap with magnetic stirring for 10 minutes. The immersion oil, wipe, and soap-water were applied serially.
- **Results**
    - The most notable result from fabrication was that features consistently measured 100 nm smaller than designed. For example, across multiple prints, wells designed to have a 4.5 µm center-to-center spacing were measured to be 4.4 µm. We speculate that this systematic discrepancy arises from shrinkage during post-processing, which will be confirmed with further experimentation. After IPA and compressed air exposure, we observed no structural changes in the phantom with SEM and no reflectance changes with adaptive optics scanning laser ophthalmoscope (AOSLO). With oil, wipe, and soap-water exposures, we noticed slight deformation along the edge of some microarrays with the print peeled

upwards under SEM, but no difference in reflectance was observed with AOSLO.

- **Implications**
    - The assessment of 3D printed phantom consistency and environmental robustness is essential before the microarray phantom can be used as a regulatory science tool in AO ophthalmic imaging system evaluations. Once qualified as a regulatory tool, we expect the microarray phantom will gain acceptance for use in standardized performance assessment of AO devices in vision science laboratories and ophthalmology clinics. The phantom will be especially important in achieving consistent device performance across multiple devices in multisite clinical investigations. Finally, the microarray phantom will provide more efficient FDA regulatory evaluation of AO system performance, enhancing clinical translation and patient access of this important device technology.

13. **Abstract Title:** *A Survey of Standards Utilization in Current 510(k) Submissions From 2021 and 2022*

    Authors: Gupta, Purva, FDA/CDRH (Student); Ajmera, Deesha, FDA/CDRH (Student); Canavan, Maura, FDA/CDRH (Student); Zeng, Jianchao, FDA/CDRH (Mentor); Woods, Terry, FDA/CDRH (Mentor); Sullivan, Stacey, FDA/CDRH (Mentor)

    FDA Strategic Initiative: Unleashing the Power of Data

    Abstract:

    - **Synopsis**
        - From diagnosis to treatment and rehabilitation, safe, effective medical devices are an integral part of a patient's medical journey. It is important that FDA effectively review devices to bring them to market as quickly as possible to meet patient needs. OMB Circular A-119 requires federal agencies to utilize voluntary consensus standards whenever possible. The 2018 FDA guidance document, "Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices", provides clarity on how standards should be used and cited, allowing sponsors to leverage the correct documentation and appropriate test results more effectively. S-CAP will use the results of this project to help sponsors and review staff avoid common mistakes in standards' use and streamline the pre-market submission and review process. We will survey recent 510(k) submissions to assess use of voluntary consensus standards. We will use the aggregated standards utilization data to identify changes in standards' use after publication of the Appropriate Use guidance, ASCA (Accreditation Scheme for Conformity Assessment) pilot program, and revised form 3514 (Premarket Review Submission Cover Sheet). Our aim is to understand the number and type of standards that are cited in new submissions and how this metric has changed since a previous survey in 2015. Results will be

compared to 2015 data and analyzed to pinpoint areas of inconsistency, including gaps in utilization and analyzing root causes. This project will help provide increased insight into how standards are applied in submissions across medical specialty groups. S-CAP will use the results to improve consistency in standards' use and review in regulatory submissions. The project will inform future improvements to increase the value of standards in regulatory processes and make it easier for them to be used in submissions. Above all, this project aids in the goal to ensure patients have timely and continued access to safe, effective medical devices.

- **Purpose**
    - The Center for Devices and Radiological Health (CDRH) Standards and Conformity Assessment Program (S-CAP) encourages medical device sponsors to use FDA-recognized voluntary consensus standards in their product submissions to ensure device safety and performance and streamline the review process. The goal of this project is to survey recent 510(k) submissions to assess the use of voluntary consensus standards. We will compare the results to a similar survey conducted in 2015. Our aim is to understand the difference between the number and type of standards that are cited in new submissions and how this metric has changed since the publication of the 2018 Appropriate Use guidance and the initiation of the ASCA (Accreditation Scheme for Conformity Assessment) pilot program. We plan to evaluate these changes and utilize the data to identify actions that S-CAP can take to simplify and improve the utilization of standards in regulatory submissions.
- **Methods**
    - A random sample of recent 510(k) submissions from 2021-2022 will be surveyed and analyzed. These data will be de-identified and aggregated for analysis and presentation and then sorted and examined by medical specialty.

      The following standards utilization data will be collected and analyzed:
        - 510(k) submission type (Traditional/Special/Abbreviated),
        - Device class (Class I/II/III),
        - Standard use (Declaration of Conformity (DOC) or General Use),
        - Number of cited standards within medical specialties,
        - Frequency of use of individual standards across medical specialties,
        - Types of test lab (in-house vs. third-party) and accreditation information,
        - Additional Information requests related to standards,
        - Other identified trends.

- **Results**
    - We will curate the standards utilization data collected from 510(k) submissions and compare them to the results of the 2015 utilization survey. We will analyze changes in utilization, both in submission and review, especially with regard to appropriate use of Declarations of Conformity (DOC). We will also highlight factors that may have contributed to improved consistency in standards use, including appropriate and expanded use of DOCs and summary test reports. In addition, we will analyze root causes of gaps in utilization and propose potential solutions.
- **Implications**
    - This project will help provide increased insights into how standards are applied in submissions across medical specialty groups. S-CAP will use the results of this analysis to make informed decisions to improve consistency in standards use in regulatory submissions. Our aim is to understand which standards and supplemental documentation the sponsor included in the 510(k) and the impact that has on device review. In the bigger picture, the metrics collected will summarize sponsors' use of standards, changes since the 2015 survey, and the impact of the 2018 Appropriate Use guidance document and the ASCA pilot program. The project will inform future improvements to increase the value of standards in the regulatory processes and make it easier for them to be used in submissions. Above all, it aids in the goal to ensure patients have timely and continued access to safe and effective medical devices.

14. **Abstract Title:** *Testbed Development for Evaluating the 5G connectivity enablers for Medical Extended Reality (MXR)*

    Authors: Majumder, Haider, FDA/CDRH (Student); Cheng-Yu, Cheng, FDA/CDRH (Student); Matthew, Johnson, FDA/CDRH (Student); Mohamad, Omar Al-Kalaa, FDA/CDRH (Mentor); Yongkang, Liu, FDA/CDRH (Mentor); Ryan, Beams, FDA/CDRH (Mentor); Wei-Chung, Cheng, FDA/CDRH (Mentor)

    FDA Strategic Initiative: Evaluation of 5G connectivity enablers for Medical Extended Reality (MXR)

    Abstract:

    - **Synopsis**
        - Extended reality (XR) is a virtual immersive extension of the real world. When XR is implemented in healthcare applications, it is known as medical extended reality (MXR). Considering the impact of the COVID-19 pandemic, the significance of telemedicine and telesurgery is clearly highlighted in present times. Virtual data communication with the aid of artificial intelligence (AI) can bring revolutionary changes in the healthcare sector to transform conventional medical and surgical practices, which require direct interaction, into alternative, intelligent, contemporary, and efficient approaches. In this work, we aim to build a testbed that facilitates MXR

applications incorporating 5G network connectivity. Moreover, we focused on integration of hardware/software in order to ensure successful implementation of MXR use cases over 5G networks. A framework is also implemented to monitor and evaluate data traffic to identify the real-time network information inherent to the application environment in order to meet the QoS requirements. The development activities can be used as input to a service level agreement between the MXR system operator and 5G network provider and inform the FDA regulatory decision when reviewing 5G-enabled MXR submissions.

- **Purpose**
  - Extended reality (XR) is a virtual immersive extension of the real world. When XR is implemented in healthcare applications, it is known as medical extended reality (MXR). Considering the impact of the COVID-19 pandemic, the significance of telemedicine and telesurgery is clearly highlighted in present times. Virtual data communication with the aid of artificial intelligence (AI) can bring revolutionary changes in the healthcare sector to transform conventional medical and surgical practices, which require direct interaction, into alternative, intelligent, contemporary, and efficient approaches. In this work, we aim to build a testbed that facilitates MXR applications incorporating 5G network connectivity. Moreover, we focused on integration of hardware/software in order to ensure successful implementation of MXR use cases over 5G networks. A framework is also implemented to monitor and evaluate data traffic to identify the real-time network information inherent to the application environment.
- **Methods**
  - MXR encompasses the use of AR and VR in the healthcare sector and it relies on seamless wireless connectivity to facilitate untethered interaction among MXR users, devices, and external application data. To identify the gaps in standardizing network connectivity for MXR applications and evaluating network traffic to meet the QoE requirements, we review the progress presented in research papers by academia and industry. We then highlight promising XR use cases and its compatibility in healthcare applications. The network architectures suitable for MXR use cases over 5G networks have been analyzed. Then, a testbed has been developed to demonstrate the connectivity of MXR applications over 5G networks. Furthermore, we deployed a multi-access edge computing system in the testbed to facilitate data transmission in MXR applications to meet the network QoS such as low latency requirements. We implemented a radio network traffic monitoring tool to evaluate the data traffic in real-time with a vision to investigate 5G network connectivity scenarios.
- **Results**
  - By the end of this proposed project, three main goals will be delivered. The first goal is to build a testbed that integrates MXR use cases with 5G network connectivity. Secondly, we will develop computation models for 5G-enabled MXR system to collect traffic and to determine the QoS requirements for the 5G-enabled MXR system. Finally, we will develop a test method to evaluate the connectivity aspects of 5G-enabled devices. To achieve the first goal of this project, a testbed of integration of MXR use

cases with 5G network connectivity has been built. We have developed an application to serve as a MXR use case for Hololens which can transmit data traffic through the 5G network. In addition, we've built an edge server using EdgeX Foundry software that resides in the edge of our 5G network. The edge server can help to process the data and to provide services for connected user equipment (UE). Moreover, MobileInsight software was used in the system to monitor the data traffic that was transmitting within the 5G network.

- **Implications**
    - This project will fill a gap in the understanding and evaluation of 5G-enabled MXR systems. By building up a testbed, we could deploy various scenarios of MXR over 5G network and using the developed computational models to predict the optimal deployment scenarios of MXR over 5G networks. In addition, this project will not only support scaling the use of MXR applications but also facilitating their development. The identified network traffic KPIs will help us understand the potential impact of 5G network configuration on the various MXR applications. This knowledge can inform the development of evaluation methods for MXR and other 5G-enabled healthcare applications. Moreover, the developed 5G-enabled MXR test method can be used as input to a service level agreement between the MXR system operator and 5G network provider. In other words, the proposed work will help speed up the merging of the telecommunication and medical device industries to integrate 5G connectivity in MXR systems through computational modeling and experimental test methods.

15. **Abstract Title:** *Implementing an analytical computer model developed for laser-induced heat transfer and photothermal damaging effects of nonstandard tissue*
    Authors: Jaikeran, Ryan, FDA/CDRH (Student); Ilev, Ilko K., FDA/CDRH (Mentor); Tan, Xin, FDA/CDRH (Mentor); Shrivastava, Devashish, FDA/CDRH (Mentor)
    FDA Strategic Initiative: Increasing Choice and Competition through Innovation
    Abstract:
    - **Synopsis**
        - The primary objective of this analytical computer simulation project is focused on applying a heat transfer model for evaluating and predicting near-infrared laser-induced thermal effects in nonstandard tissue and the resulting photothermal tissue damaging adverse events with significant and frequently reported safety concerns. These photothermal related safety concerns have been indicated in recently developed and emerging laser therapeutics and diagnostics devices and techniques such as near-infrared brain optical spectroscopy and neural stimulation, photobiomodulation therapeutics, laser lithotripsy and mucosal tissue therapeutics. In majority of these cases, the laser-induced photothermal damaging effects are associated with nonstandard types of tissue, other than ocular and skin tissue, which are not evaluated by standardly available

database and tools. This project aims to contribute towards addressing some of these unmet standard regulatory science and public health needs by implementing of an analytical model based on maximum permissible exposure (MPE) thresholds for laser-induced damaging of nonstandard tissue. The project study will have a broader scientific impact by providing nonstandard MPE database for predicting photothermal tissue damaging effects depending on critical laser radiation parameters and tissue characteristics.

- **Purpose**
    - FDA/CDRH reviews safety and efficacy of medical devices involving non-ionizing laser radiation with multivariable safety characteristics. However, the Agency and scientific community do not presently have tools and standard methods to analyze safety and performance of newly developed and emerging devices. Further challenges are associated with the lack of standard database for laser safety evaluation using maximum permissible exposure (MPE) for nonstandard tissues, as the current database is limited to ocular and skin tissue only. As a first stage of the safety and efficacy evaluation prior to animal and clinical studies, various bench, phantom and analytical computer models are developed. Analytical modeling approaches are extensively employed due to their significant benefits in multiparameter simulation and prediction of laser-tissue interactions including laser-induced heat transfer and photothermal damaging effects. This project aims to utilize analytical computer models to simulate and predict laser photothermal damaging effects and MPE thresholds for nonstandard tissue.
- **Methods**
    - The project objective for implementing analytical computer modeling for evaluating laser-induced heat transfer and photothermal tissue damaging effects will be accomplished by employing the COMSOL multiphysics software and its specific heat transfer module. As analytical test methodology, the COMSOL heat transfer module is developed for simulation and analyzing of thermal effects in biological tissue including laser-induced heat transfer and photothermal tissue damaging effects. The computer simulation approach employed in this project is based on implementing the COMSOL's Bioheat feature following Pennes' approximation using sets of characteristics associated with specific tissue models. As initial proof-of-concept analytical study, we will be implementing the computer simulation approach using predefined characteristics for standardly investigated ocular and skin tissues. Furthermore, the analytical method will be applied to simulate and predict laser photothermal damaging effects and MPE thresholds of nonstandard tissue such as cortical brain, kidney and mucosal tissue.

- **Results**
    - Employing the COMSOL Multiphysics heat transfer computer simulation approach in the case of laser-induced heat transfer and photothermal tissue damaging effects, the resulting major project deliverables will include tabulated data as well as 2-dimentional and 3-dimentional photothermally elevated temperature spatial distributions on the surface and inside the tissue of consideration. The simulated temperature distributions depend on both the laser radiation dosimetric parameters and specific tissue characteristics related to either standard (skin and ocular) or nonstandard (cortical brain, kidney and mucosal) types of tissues. The simulation results will also include plots of time-dependent temperature distributions caused by laser-induced photothermal tissue damaging effects. The temperature distribution results will be used for evaluation and prediction of photothermal tissue damaging effects.
- **Implications**
    - The main results of this computer simulation project can be applied towards addressing a major current regulatory science and public health need of lacking consensus test tools and methodologies to analyze and evaluate safety and performance of recently developed and emerging laser based medical therapeutics and diagnostics devices. Using these analytical models and the data collected from the analytical simulations, the laser-tissue interactions and the resulting potential photothermal tissue damaging effects with significant safety concerns can be mitigated. The project results will also contribute towards laser radiation safety evaluation related to a number of nonstandard tissues unresolved in current laser safety standards.

16. **Abstract Title:** *Pipette tip decontamination and assessment following inoculation with nucleic acid material from Aeromonas hydrophila with multiple modalities.*
    Authors: Kastor, William, FDA/CDRH (Student); Hyuk-Lee, Sang, FDA/CDRH (Student); Glover, Thomas , FDA/CDRH (Student); Fu, Xiao, FDA/CRDH (Student); Donohue, Marc, Johns Hopkins University CHEMBE (Collaborator); Keidar, Michael, George Washington University DMAE/DBE (Collaborator); Soni, Vikas, George Washington University DMAE/DBE (Collaborator); Wood, Steve, FDA/CDRH (Mentor); Karunasena, Enusha, FDA/CDRH (Mentor)
    FDA Strategic Initiative: Public Health Emergency Preparedness and Response
    Abstract:
    - **Synopsis**
        - Investigated three selected methods of cleaning: a common laboratory detergent, ozone gas, and cold atmospheric plasma for their efficacy in cleaning nucleic acids off of exposed pipette tips.
    - **Purpose**

- With the surge of the SARS-CoV-2 pandemic, limitation on production and distribution of single use PPE and medical plastics (used in pipette tips) became a critical issue. Due to these international/national events, there is a need for alternative methods of cleaning these items for reuse. The objective of this investigation was to investigate three selected methods of cleaning: a common laboratory detergent (Alconox), ozone gas, and cold atmospheric plasma (CAP) for this purposes.

- **Methods**
  - In this study nucleic acids extracted from *Aeromonas hydrophila* (ATCC-23211) were utilized to inoculate Biotix® UTip Filtered pack 10µL Pipette Tips. Exposed tips were then subjected to one of the three cleaning methods, and then analyzed for residual nucleic acid material using quantitative real-time PCR (qPCR) and amplification of two housekeeping genes (*16s rRNA*[1] and *rpoB*[2]). Efficacy of cleaning methods was determined by turnover ratio and log reduction in detectable genomic material of the contaminated pipette tips via qPCR.
- **Results**
  - Results from this investigation indicated that Alconox displayed the highest log reduction and turnover ratio (5.943 and 95.89%, respectively). However, physical degradation of the tip box and substantial residue left on the tips were observed. Conversely, ozone exposure displayed the second highest log reduction and turnover ratio (3.349 and 20.63%) with CAP displaying the lowest (2.628 and 14.28%). Damage to the tips from either of these methods was observed to be negligible.
- **Implications**
  - Following these results, further optimization of both ozone and CAP may be warranted to increase their respective efficacies in this application.

17. **Abstract Title:** *Preliminary Studies for Body-Fluid Equivalent Simulated-Use Extraction Vehicle and Solvent Identifier*
    Authors: Lewis, Germanie, FDA/CDRH (Student); Oktem, Berk, FDA/CDRH (Mentor);
    FDA Strategic Initiative: Increasing Choice and Competition through Innovation
    Abstract:
    - **Synopsis**
      - Currently there are no standard methodologies or guidance on the chemical characterization of body fluids and tissues. The purpose of this project is to provide innovative testing strategies that are relevant across a range of analytical instruments and conditions, that can efficiently detect, identify, and quantify chemicals of varying polarities and concentrations which will be used as a regulatory science tool that provides guidance on tissue specific simulated conditions. Relevant surrogates pose a challenge for instrument detection and are currently being explored.

- **Purpose**
    - Medical device availability, reliability and efficacy are intrinsic to medical field for diagnosis, treatment, and preventative care. Medical devices are classified in three categories by their potential risk factors. Class I devices are innocuous devices, such as bandages and thermometers. Class II and III devices pose higher risk and include devices that are used to sustain and support life, such as pacemakers and hip prosthesis. These increased risks can be partly attributed to the contact duration they have with the bodily fluids and tissues. When implanted in the body, chemical constituents from these devices may be drawn out then interact with the body, which results in a cause of toxicological concern. Currently, there are no standard methodologies or guidance for analytically expedient extraction then chemical characterization of bodily fluids and tissues. The purpose of this project is to develop an extraction standard relevant across a range of analytical instruments, that can efficiently detect, identify, and quantify chemicals of various polarities and concentrations.
- **Methods**
    - Extracts were prepared from relevant device materials (e.g. polymers..etc) to measure the power of the extraction vehicle . Similar to the perspective indicated in FDA Biocompatibility Guidance of 2020, polar, semi-polar and non-polar extraction vehicles are used. In addition, simplified versions of body fluids are created(E.g. albumin solution that simulates blood proteins). Extracts are analyzed predominantly by LC/UV/MS methods using two systems, an Agilent 6540B UHD Accurate Mass Q-TOF LC/MS connected to a Dionex Ultimate 3000 UHPLC and a Thermo Scientific Q Exactive HF connected to a Thermo Scientific Vanquish UHPLC. LC/UV/MS separations were performed with the following operating conditions. The column used was an Agilent Zorbax Eclipse Plus C18, 1.8-µm, 2.1 x 100 mm. Mobile phase A was composed of 10 mM Ammonium Acetate/0.05% Glacial Acetic Acid and mobile phase B was composed of Acetonitrile/0.1% Formic Acid. Injection volume was 10 µL, column temperature was 40°C and UV range was 245-331 nm. The solvent gradient flow 0.8 mL/min starting at 10% B to 100% B over 40 minutes.
- **Results**
    - Several pure compounds and mixtures are used to verify methods to perform the analysis by LC/UV/MS methods to include Caffeine, Reserpine, Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate) (Irganox 1010), 1,3,5-Trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene (Irganox 1330), Irgacure 2959, Butylated Hydroxytoluene (BHT), Phthalic Acid Bis(2-Ethyl-Hexyl Ester) (DEHP), Stearic acid and Benzoic acid. The compounds were identified using LC/UV/MS at varying concentrations. The smallest concentration ((µg)/mL) at which each compound was identified were as

followed: 10 (caffeine, BHT, DEHP), 0.1 (reserpine, Irganox 1010, Irganox 1330, Irganox 2959, Benzoic Acid), and not detected (stearic acid).

- **Implications**
    - The efforts here are ongoing to build the foundations of an analytically expedient, blood simulant as a regulatory science tool.

18. **Abstract Title:** *A software framework for AI-based radiologic image analysis*
Authors: Malik, Briana, FDA/CDRH (Student); Gossmann, Alexej, FDA/CDRH (Mentor); Cha, Kenny H., FDA/CDRH (Mentor); Sahiner, Berkman, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
    - We are developing a Python software package which provides a framework for the analysis and research of various AI/ML-based clinical tasks using medical imaging datasets. Our package includes methods for preprocessing, visualization, and feature extraction using modern deep learning methods alongside conventional radiomics approaches. Our feature extraction approaches include deep convolutional neural networks (DCNNs) and variational autoencoders (VAEs). We demonstrate the diverse functionality of our package by combining it with classifiers to perform a number of analysis tasks on a chest computed tomography dataset, a chest radiograph dataset, and a digital breast tomosynthesis dataset.
- **Purpose**
    - Segmentation, classification, and analysis of medical images is often hindered by the time consuming and computationally intensive groundwork (preprocessing, exploratory analyses, feature extraction, etc.) that must be performed prior to further analysis and research. To alleviate this, we are developing a Python software package for the analysis of images in Digital Imaging in Communications and Medicine (DICOM) format, consisting of data preprocessing and visualization methods, as well as deep learning and radiomics approaches to extract image features. Our package will provide a framework for downstream analysis and research of clinical tasks using Convolutional Neural Networks (CNNs) and Variational Autoencoders (VAEs). We demonstrate the functionality of our software by preprocessing, visualizing, and analyzing a chest computed tomography (CT) dataset, a chest radiograph (CXR) dataset, and a digital breast tomosynthesis (DBT) dataset.
- **Methods**
    - The Non-Small Cell Lung Cancer (NSCLC) dataset [1] is a chest CT DICOM dataset containing scans from 422 patients. The SIIM-FISABIO-RSNA COVID-19 Detection Dataset [2] contains 6,334 CXR DICOM scans. The BCS-DBT dataset [3] contains 22,032 DBT volumes from 5,060 patients. Our package first loads and displays

the DICOM image files for interactive viewing, then performs several preprocessing and normalization steps, and extracts imaging features using different approaches. We use deep convolutional neural networks (DCNNs), pretrained on natural images from the ImageNet dataset, to extract features from the medical images. Alternatively, we finetune the pretrained DCNNs by training only certain layers of the model on the medical images, while keeping the remaining layers fixed at their original ImageNet pretrained weights. We extract various radiomics features -- voxel intensity distribution, 2D/3D size and shape of the region of interest, and weight statistics, mesh volume analysis and neighborhood pixel analysis. Additionally, we train variational autoencoder (VAE) models as another deep learning feature extraction approach.

- **Results**
    - We investigate feature extraction on the whole image as well as from ROI contours or bounding boxes. Subsequently, we compare the predictive ability of these diversely extracted features with respect to classifying mortality for the chest CT data, when the different types of features are used as input to classification models -- logistic regression and XGBoost. This is aided by data augmentation techniques (like rotation, flip, gaussian noise, etc.) for DCNN and VAEs. For CXR dataset, we implement multiclass classification for pneumonia detection and ROI appearance (normal, abnormal, etc.) based on the extracted features. For the BSC-DBT dataset we use features extracted from ROIs to classify them into normal, benign, actionable, and cancer categories as specified by the dataset. To compare the classification performance, we use area under the receiver operating characteristic Curve (AUC), which provides an aggregate measure of performance across all possible classification thresholds.
- **Implications**
    - PyRadiomics features resulted in the greatest AUC for the NSCLC chest CT data, outperforming both DL feature extractors, albeit only for XGBoost. On the other hand, for the SIIM-RSNA Chest X-Ray dataset DCNN features resulted in the greatest AUC. Results for the BSC-DBT dataset are still pending.
      We demonstrate these diverse preprocessing, image visualization, feature extraction, and analysis processes to illustrate the use and adaptability of our Python package. We are expanding our package functionality to both Keras and PyTorch for ease-of-implementation. While it is still a work in progress, our software package has the potential to aid the regulatory science research efforts at the FDA by providing a ready-to-use solution to work with and extract valuable information from medical image datasets.

[1] Aerts et al. “Tumour phenotype by noninvasive imaging using a quantitative radiomics approach.” Nature communications 5.1 (2014): 1-9.
[2] https://www.kaggle.com/c/siim-covid19-detection
[3] Buda et al. “Detection of masses and architectural distortions in digital breast tomosynthesis: a publicly available dataset of 5,060 patients and a

deep learning model." arXiv preprint arXiv:2011.07995

19. **Abstract Title:** *ORCA Data Collection: Orthopedic Surgical Meshes, Hip Implants, Additive Manufacturing*
Authors: Marine, Marissa, FDA/CDRH (Student); Mikayilova, Sabrina, FDA/CDRH (Student); Younis, Salma, FDA/CDRH (Student); Sumter, Kyla, FDA/CDRH (Student), Peck, Jonathon, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:
    - **Synopsis**
        - Review of premarket notification (510(k)) submissions for orthopedic implants often involves the comparison of design characteristics and performance testing results between the newly proposed device and previously cleared "predicate" devices. To streamline the review of future 510(k)s, design, performance testing, and manufacturing process information from previously cleared 510(k) submissions were entered into the Orthopedic Rapid Comparative Analysis (ORCA) database for orthopedic surgical meshes, total hip replacements, and additively manufactured orthopedic implants. This allows lead reviewers to efficiently compare new devices to a collection of previously cleared devices for more consistent and confident decision making.
    - **Purpose**
        - Review of premarket notification (510(k)) submissions for orthopedic implants often involves the comparison of design characteristics and performance testing results between the newly proposed device and previously cleared "predicate" devices. In addition, for additively manufactured devices, information on the manufacturing process is often compared to previously cleared devices. To streamline the review of future 510(k)s for orthopedic surgical meshes, total hip replacements, and additively manufactured orthopedic implants, design information, performance testing data, and manufacturing process information were entered into the Orthopedic Rapid Comparative Analysis (ORCA) database.
    - **Methods**
        - Data was extracted from previously reviewed 510(k) submissions, including device materials, features, and dimensional ranges, performance testing methods and results, and manufacturing process information. Once the data was aggregated and filtered to correct invalid data points, analysis allowed for ranges of design, performance testing, and manufacturing process parameters to be presented.
    - **Results**
        - For mesh devices with orthopedic indications, results were collected for performance testing including tensile, tear resistance, suture pull-out or suture retention, puncture, flexibility/handling, ball burst strength testing, and animal

testing. For dual mobility hip devices, results were collected for mechanical testing of non-standardized pull-out and lever-out of the femoral head from dual mobility head. For additively manufactured orthopedic devices, results were collected for residual powder removal including particle extraction and characterization methods.

- **Implications**
    - The ORCA database allows for collection and analysis of design, performance testing, and manufacturing parameters for a wide variety of devices into a comprehensive resource for FDA reviewers. For mesh devices containing orthopedic indications, the collected information on design and performance testing will support future regulatory decisions. Additionally, total hip replacements represent a high-volume review area for the Office of Orthopedic Devices, and thus collection of design and performance testing data for future regulatory decision making is highly useful. For additively manufactured orthopedic devices, the consideration for residual powder removal was raised in the 2017 Guidance "Technical Considerations for Additively Manufactured Medical Devices". To date there is no standardized method to assess residual powder, however, collection of this information from previously cleared 510(k) submissions could lead to recommendations for standardized methods in the future.

20. **Abstract Title:** *Voxel Rendering of Medical Volume Images on an Augmented Reality Head Mounted Display*
    Authors: Johnson, Matthew, FDA/CDRH (Student); Beams, Ryan, FDA/CDRH (Mentor); Zhao, Chumin, FDA/CDRH (Mentor)
    FDA Strategic Initiative: Unleashing the Power of Data
    Abstract:
    - **Synopsis**
        - Augmented reality head mounted displays open new opportunities for intuitive visualizations of medical volume data for surgical planning. We demonstrate an application capable of rendering medical volume data on an AR HMD using a voxel-based rendering algorithm and preexisting engine. The application also allows the user to manipulate the volume data with hand gestures, including translating, rotating and slicing. This application is an essential first step in facilitating the evaluation of surgical planning tasks using AR HMDs.
    - **Purpose**
        - Augmented reality head mounted displays (AR HMDs) have the potential for widespread usage in preoperative planning by rendering and displaying 3D volumetric medical images. Therefore, developing characterization methods for AR HMDs' image quality for medical volume data is critical for regulatory evaluations. To address this regulatory challenge, we developed a 3D rendering

application for displaying medical volume data on an AR HMD that will be used for visual and quantitative testing of image quality and task-based performance.

- **Methods**
    - We developed a 3D rendering application using the Unreal Engine 4.27 for an AR HMD (Microsoft HoloLens 2) that can load and render medical volume images (in MHD, NIFTI, and DICOM formats) as 3D models which can be translated, rotated, and sliced using hand movement. First, the 3D volume is voxelized, with the voxel resolution specified by the user. These voxels use the medical volume image as a texture for their faces, giving the impression when viewed that the voxels comprise an approximation of the original volume image. The whole volume is translucent, with lower intensities in the original image rendered as transparent, allowing the user to see specific regions of interest. For greater detail in the rendered model, the voxel resolution can be dynamically adjusted via the keyboard while the application runs.
- **Results**
    - Our application demonstrates a means of rendering and observing medical volume data in an AR environment and allows for the observation of anatomical features such as the basal ganglia. This is a step forward in performing task-based assessments on AR devices, such as external ventricular drainage (EVD) catheter placement in a physical cranium phantom for preoperative planning purposes, and as such could enable future research in the area of volumetric medical imagery in an augmented reality environment.
- **Implications**
    - By deploying a medical volume image to a consumer-grade AR HMD, which is being used in surgical applications, this application provides a stepping stone for allowing researchers to characterize AR HMDs and their performance in medical contexts, which enables medical device manufacturers and regulators to more easily evaluate these devices.

21. **Abstract Title:** *Decontamination of Additively Manufactured (3D printed) Mask Materials*
    Authors: Robinson, Danielle FDA/CDRH (Student), Lucas, Anne FDA/CDRH(Mentor), Schwerin, Matthew FDA/CDRH (collaborator), Guha, Suvajyoti FDA/CDRH (collaborator), Dawson, Joseph FDA/CDRH (collaborator)
    FDA Strategic Initiative: Public Health Emergency Preparedness and Response
    Abstract:
    - **Synopsis**
        - The overall project goal is to evaluate decontamination methods for any negative effects on PPE performance. This specific portion of the project will focus on decontamination of Additively Manufactured (3D printed) coupons to see if the printed devices/coupons retain basic functionality. During the Ebola outbreak, decontamination of gloves prior to their removal was recommended

by CDC as an extra precaution to prevent the spread of this disease. Identifying which glove/decontamination systems were or were not worth perusing in the event of an emergency in which gloves were not readily available or extra decontamination measures were necessary. Reuse of medical gloves is not recommended, cleared or authorized by FDA.

- **Purpose**
    - During Covid-19 pandemic, there was a severe shortage of all types of PPE needed by health care workers, scientist, and the general public. The overarching project analyzes and evaluates the effect of some decontamination methods on the ability of PPE to retain performance. This specific portion of the project focuses on Additively Manufactured (3D printed) face masks and/or coupons to see if the printed devices/coupons retain basic functionality. As an alternative to conventional masks, 3D printed masks were created by anyone with a 3D printer, but the question of how to decontaminate these masks without damaging effectiveness arose. A related part of the project, as the same decontamination methods are used, is evaluating barrier performance of the gloves following decontamination. The FDA is interested in finding a way to decontaminate the gloves prior to removal without compromising the barrier performance to minimize transmission of infectious organisms before disposal.
- **Methods**
    - For both the gloves and the AM coupons, three decontamination methods were used. (1) the devices were soaked in a 10% bleach solution for one minute, washed off under running tap water for one minute, and soaked in distilled water for at least thirty seconds, excess water was removed by an absorbent paper (2) devices were soaked in a quaternary ammonium solution for five minutes, washed off under running water for one minutes and thirty seconds, and soaked in distilled water for at least thirty seconds, excess water was removed by an absorbent paper and (3) the devices were treated by rubbing 70% ethanol alcohol based hand sanitizer (ABS) on the entire device, followed by wiping off any excess solution. All coupons were then placed on racks and left to air dry overnight. For both AM coupons and glove portions of the study, five different devices per decontamination method were used; for the glove section of the project, a separate set of gloves was used as the blank control, of the AM coupon the device itself prior to any decontamination served as the blank control.
- **Results**
    - The coupons have yet to produce enough data to come to any solid conclusions, one could possibly expect some change in the weight and/or visual appearance of the coupons. The glove study demonstrates that Vinyl medical gloves are poor candidates for decontamination as they had a high number of failures across all decontamination methods. In general, the surgical gloves performed

better than the exam gloves with very few failures detected. This may be due to higher thickness of surgical gloves (0.1 mm ASTM D3577) over that of exam gloves (0.08 mm ASTM D3578).

- **Implications**
    - Identification optimal decontamination method with medical device material, in this case AM mask and medical gloves, is critical in retaining performance of the material. Individuals who make and/or use AM masks, whether in a medical setting or not, will now have some basic information on methods to best decontaminate their masks and what methods to avoid. Because medical gloves are much more delicate and used in a much higher risk environment, reuse of gloves for any purpose following decontamination is not cleared or authorized or recommended by FDA. However, finding a way to decontaminate the gloves prior to removal without compromising the barrier performance to minimize transmission of infectious organisms before disposal is of interest. By establishing what decontamination method does not affect barrier performance post decontamination, the likelihood of health care workers inadvertently contaminating themselves or local environment is reduced.

22. **Abstract Title:** *Modified Procedures for AATCC-100-1993 to Measure and Characterize Viable Bacteria from Wound Dressings*
Authors: Sang Hyuk, Lee, FDA/CDRH, Johns Hopkins University CHEMBE (Student); Kastor, William, FDA/CDRH (Student); Glover, Thomas, FDA/CDRH (Student); Donohue, Marc, Johns Hopkins University CHEMBE (Mentor); Karunasena, Enusha, FDA/CDRH (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response
Abstract:
    - **Synopsis**
        - Wound dressings are immediate responses to cuts and burns that can result in severe complications if not treated correctly. US Food and Drug Administration receive many submissions of antimicrobial wound dressings. However, previous evaluation methods are inefficient and outdated. This study proposes an updated method to evaluate and characterize antimicrobial wound dressings.
    - **Purpose**
        - Chronic wounds are reoccurring healthcare problems in the United States and cost up to $50 billion annually. Improper wound care results in complications such as wound debridement, surgical amputation, and increased morbidity/ mortality due to opportunistic infections. To eliminate wound infections, many antimicrobial dressings are developed and submitted to FDA for evaluation.
    - **Methods**
        - This study proposes an assay to measure time dependent efficacy of wound dressing, using quantitative real time polymerase chain reaction (qRT-PCR). The test organisms included opportunistic pathogens: *Pseudomonas aeruginosa*

(ATCC 15692) and *Staphylococcus aureus* (ATCC 43300). To mimic a wound dressing environment, samples of commercially available wound dressings with silver ion (positive treatment) and dressings without silver ion (negative treatment) were assessed under sterile conditions. All samples were examined by the original protocol, the extended AATCC-100 method, and qRT-PCR. The expressions of specific housekeeping genes were measured (*proC* for *P. aeruginosa* and *16s rRNA* for *S. aureus*). Relative quantification (the Livak method) was used to calculate expression fold changes of virulence related target genes. Target genes of *P. aeruginosa* were *fleN*, *flgG*, *lasI* and *hlgB, lukF* for *S. aureus*. Based on these analyses, log reductions of experimental conditions were compared to identify antimicrobial properties from wound dressing samples

- **Results**
    - These results showed antimicrobial properties of wound dressing samples diminished as incubation days are increased when examined by both methods. Additionally, data from qRT-PCR generally produced lower standard deviation than that of culture methods, therefore demonstrating better precision. Different virulence related gene expressions were observed among the samples, particularly with genes related to motility and quorum sensing for *P. aeruginosa* and genes related to gamma toxin and P-V leucocidin for *S. aureus.* Therefore, prolonged exposure to both silver ion present and absent wound dressings resulted in altered bacterial behavior.
- **Implications**
    - Complementary parallel analysis of samples using these proposed methods better characterize antimicrobial properties of wound dressing efficacy.

23. **Abstract Title:** *Development of a hierarchy of microorganisms known for antimicrobial resistance*
Authors: Scott, Alysia, FDA/CDRH (Student); Linden, Sara, FDA/CDRH (Mentor); Weeks Jon, FDA/CDRH (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response
Abstract:
    - **Synopsis**
        - There is a growing need for effective antimicrobial sterilants to replace ethylene oxide, especially in medical device sterilization. Yet to date there is no detailed resource of suitable microorganisms to be used in testing novel sterilants by manufacturers. This project aims to identify the most appropriate types and strains of microorganisms that are suitable for resistance testing using peracetic acid solutions in order to develop an example of protocol development as a regulatory standard. A literature review was first conducted to select appropriate microorganisms known for their antimicrobial resistance. These microorganisms include several types and strains with the purpose of in-depth

testing to create a hierarchy of antimicrobial resistance. The selected microorganisms will be subjected to minimum inhibitory concentration assays and several exposure conditions to examine the resulting microbial growth. Comparison of microorganism reduction and concentration will be used to categorize the hierarchy of antimicrobial resistance pertaining to the microorganism types and strains. The results of this project will ultimately provide manufacturers looking for regulatory guidance on microorganism selection when testing their novel sterilants, thus reducing ethylene oxide consumption while improving medical device sterilization.

- **Purpose**
  - The desire to move away from ethylene oxide as the primary mode of terminal sterilization has necessitated the development of regulatory science tools to aid in this process. Ethylene oxide has been used due to its effectiveness for sterilization of various types of microorganisms without damaging the devices. In the case of chemical and ethylene oxide sterilant methods, as generally defined by the 2015 FDA reprocessing guide and ISO 14937, a hierarchy of resistance of microorganisms has been used for testing, though never clearly defined by specific organisms or strains. As medical device sterilization modalities move away from ethylene oxide coupled with the lack of regulatory guidelines, there is a high demand for the thoroughly developed hierarchy of resistance of microorganisms to be used for testing including those which are typical of manufacturing contaminants or known pathogenic microorganisms.
- **Methods**
  - Extensive literature reviews were conducted to identify specific strains of microorganisms to be used for testing. For the use of hierarchy testing, we selected microorganisms belonging to several microbial types: gram-positive bacteria, gram-negative bacteria, fungi, enveloped viruses, non-enveloped viruses, mycobacteria, and spores. The criteria for selection included known resistance to other antimicrobial chemicals and sterilants. Additionally, clinical relevance of the selected strains was reviewed. Following strain selection, a minimum inhibitory concentration (MIC) assay will be used to determine the lowest concentration of antimicrobial (paracetic acid) resulting in microbial growth. Additionally, we will test several exposure conditions on microbial sensitivity. The microbial resistance will be characterized by comparing MIC and log reductions achieved within the varied exposure conditions. These comparison results will be used to develop the hierarchy of resistance.
- **Results**
  - The background literature review resulted in the construction of a list of medically relevant microorganisms, as well as standardized methodology for conducting antimicrobial assays. We expect that antimicrobial assays with

these organisms will provide us with a hierarchy of resistance that we can supply to industry for sterilization verification.

- **Implications**
    - This research characterizes a hierarchy of resistance for microorganisms that can be used to assess sterilant effectiveness, as well as provide detailed protocol for selecting an alternative microorganism to be used in testing. By including several microorganism types and strains known for their resistance to antimicrobials, this collection will be a useful reference for novel sterilant validation of effectiveness. Manufacturers and developers looking to ensure effectiveness of a sterilant will be able to use this hierarchy as guidance, microorganism comparison, and a resource for curated strain selection.

24. **Abstract Title:** *Leak Verification for Additively Manufactured Personal Protective Equipment*
Authors: Seay, Nathanael, FDA/CDRH (Student); Porter, Daniel, FDA/CDRH (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response
Abstract:

- **Synopsis**
    - Global supply chains were disrupted by the novel coronavirus disease (COVID-19) pandemic. This disruption impacted the supply of personal protective equipment (PPE) which was critical to slowing the spread of the virus. Necessity made individuals and institutions turn to additive manufacturing (AM) to produce PPE as a stopgap measure. However, to the authors best knowledge, there exists no standardized method to determine the soundness of these devices at the point of care. The objective of the current study is to develop an accessible method for verifying the leak integrity of additively manufactured devices. The current effort is divided into four subcategories. The first being to devise a method for determining cumulative flaw size. The next stage is to relate flaw size to PPE performance detriment. Specifically, to relate flaw size to AM mask leakage. Mask performance is evaluated in a custom aerosol chamber containing aerosolized NaCl particles. The third stage is to relate steps one and two and derive a direct relationship between flaw size indicator and performance detriment. The fourth stage of the present effort is to devise and demonstrate the method to extract the flaw indicator at the point of care.
- **Purpose**
    - Global supply chains were disrupted by the novel coronavirus disease (COVID-19) pandemic. This disruption impacted the supply of personal protective equipment (PPE) which was critical to slowing the spread of the virus. Necessity made individuals and institutions turn to additive manufacturing (AM) to produce PPE as a stopgap measure. However, to the authors best knowledge, there exists no standardized method to determine the soundness of these devices at the point of care. The objective of the current study is to develop an

accessible method for verifying the leak integrity of additively manufactured devices.

- **Methods**
  - The current effort is divided into four subcategories. The first being to devise a method for determining cumulative flaw size. The next stage is to relate flaw size to PPE performance detriment. Specifically, to relate flaw size to AM mask leakage. Mask performance is evaluated in a custom aerosol chamber containing aerosolized NaCl particles. The third stage is to relate steps one and two and derive a direct relationship between flaw size indicator and performance detriment. The fourth stage of the present effort is to devise and demonstrate the method to extract the flaw indicator at the point of care.
- **Implications**
  - Even beyond the possibility of the next pandemic disrupting supply chains again, it seems as though individuals and institutions will continue to innovate and create new means of developing personal protective equipment. The methods developed in the current effort can be used to ensure the soundness of such devices and the safety of those using them.

25. **Abstract Title:** *Developing a Large Diameter In Vitro Flow Loop System for Dynamic Thrombogenicity Assessment of Blood-contacting Medical Devices*
Authors: Srinivasan, Keerthana, FDA/CDRH/OSEL (Student); Serna III, Carlos, FDA/CDRH/OSEL; Diokhane, Charlotte, FDA/CDRH/OSEL (Student); Patel, Mehulkumar, FDA/CDRH/OSEL (Mentor); Jamiolkowski, Megan, FDA/CDRH/OSEL (Mentor), Malinauskas, Richard, FDA/CDRH/OSEL; Lu, Qijin, FDA/CDRH/OSEL (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:
    - **Synopsis**
      - Based on a small-diameter flow loop thrombogenicity test system previously developed previously in our lab, this study aims to develop a large-diameter variant to accommodate thrombogenicity testing of larger medical devices. To ensure that cardiopulmonary bypass roller pumps used in larger diameter test systems perform equally and consistently in driving the blood through separate flow loops, we investigated three pump occlusion setting methods: two conventional methods found in the literature (the gravity drop method and the dynamic pressure method) and a modified dynamic pressure occlusion method we developed. After setting the occlusion pressure, anticoagulated bovine blood was recirculated at 500 ± 30 mL/min at room temperature for 1 hour through PVC tubing loops (9.5 mm ID) containing a thrombogenic material (latex). Preliminary results suggest that the modified dynamic occlusion method could help to improve the consistency and reliability among different roller pumps compared to the conventional gravity drop and dynamic pressure

methods. This improved occlusion setting method will be used in an interlaboratory study to establish standardized test methods for in vitro dynamic thrombogenicity testing of various blood-contacting medical devices.

- **Purpose**
  - Preclinical thrombogenicity evaluations are very important to ensure the safety of blood-contacting medical devices such as catheters and stents. Currently, acute in vivo animal studies, such as the non-anticoagulated venous implant assay (NAVI), are commonly used for device thrombogenicity testing. However, due to inherent animal model limitations and procedure-related confounding factors, these resource-demanding in vivo animal tests can often produce inconsistent or inaccurate results. To mitigate some of these challenges and to provide a more robust thrombogenicity assessment, in vitro blood test flow loops are being developed. Previously, we had investigated the effects of key parameters such as blood species, temperature, and sample diameter on thrombogenicity testing and successfully developed a small diameter (6.4 mm inner diameter [ID] Polyvinyl Chloride [PVC] tubing) flow loop system capable of accommodating samples up to 3.2 mm in outer diameter (OD). In this study, we aim to develop a large diameter flow loop system for thrombogenicity testing of larger medical devices.
- **Methods**
  - Clinical cardiopulmonary bypass roller pumps that can accommodate tubing up to 12.7 mm ID were used to create four parallel large diameter flow loops. To ensure these pumps perform consistently in driving the blood through the separate flow loops, we investigated three pump occlusion setting methods: two conventional methods found in the literature (the gravity drop method and the dynamic pressure method) and a modified dynamic pressure occlusion method we developed. The modified method identified the minimum pump roller occlusion necessary to fill the loops at 20 RPM and to produce a constant flow rate of 500 ± 30 mL/min for each pump. After setting the occlusion pressure, anticoagulated bovine blood was recirculated at 500 ± 30 mL/min at room temperature for 1 hour through PVC tubing loops (9.5 mm ID) containing a thrombogenic material (latex). Thrombus surface coverage and platelet count reduction were measured at the end of each test. In addition, circulated blood from all loops was filtered and visually inspected for non-adherent blood clots.
- **Results**
  - We found that occlusion pressure settings for the dual-roller pumps were influenced by inherent variations between each pump (e.g., roller differences), temperature, and tubing stiffness. Our initial results suggest the commonly used gravity drop and the dynamic pressure methods for setting occlusion were insufficient for our larger diameter flow loop system as substantial differences in thrombus deposition area and platelet count reduction among the four

pumps were observed when these occlusion methods were used. In contrast, similar levels of thrombus formation were observed across all pumps when using the proposed modified dynamic pressure occlusion method. These initial results suggest that the modified dynamic occlusion method could help to improve the consistency and reliability between different roller pumps compared to the conventional gravity drop and dynamic pressure methods.

- **Implications**
    - Since dynamic in vitro thrombogenicity testing relies on side-by-side comparisons of test and control devices, it is critical to ensure all pumps used in the parallel testing of flow loops produce reproducible thrombosis levels when subjected to the same test conditions. The improved thrombosis consistency resulting from using our modified occlusion setting method can help standardize in vitro flow loop systems to assess thrombogenic potentials of blood-contacting medical devices. This modified occlusion setting method will be used in future thrombogenicity studies, such as an upcoming interlaboratory study that we organized through collaboration with the ASTM standards biocompatibility subcommittee. The interlaboratory study will assess test samples with various materials, geometries, and surface roughness patterns to determine the sensitivity and reliability of the test system in differentiating devices with varying thrombogenic potentials.

26. **Abstract Title:** *Quantification of Variability in Gait Metrics Derived from Phone-based Sensors*
Authors: Patwardhan, Shriniwas, FDA/CDRH (Student); Kontson, Kimberly, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
    - Mobile phones come with several sensors, like accelerometers and gyroscopes, that are used by applications to derive gait events (such as toe-off, heel strike) when the user is holding the phone in their hand or has mounted the phone on some part of their body. However, the accuracy and reliability of the sensors in the phones are not well characterized, leading to questions about the impact of these inaccuracies on the gait events derived from the sensor data. This study will quantify the variability in gait events derived from phone-based sensors compared to the same metrics derived from a reference system that captures foot contacts through sensors directly under the feet of participants. Three-axis accelerometry and gyroscopy will be collected from the phones placed on participant's body as they walk along a reference walkway system. Quantifying the accuracy of and variability in phone-based sensors will enable the development of more robust algorithms for deriving gait metrics based on such sensor data. It will also help create guidelines for applications that use such sensor data for gait quantification that truly reflects the variability in the underlying sensor measurements.

- **Purpose**
  - Advanced mobile phones now come with several on-board sensors like accelerometers, gyroscopes, etc. These sensors are used by applications to derive gait events such as toe-off and heel strike when the user is holding the phone in their hand or has mounted the phone on some part of their body. These gait events can then be used to derive spatiotemporal gait metrics such as gait speed, stride length, cadence, etc. that are commonly used to inform mobility status or progression of a clinical condition that impacts mobility. However, the accuracy and reliability of the sensors in the phones are not well characterized, leading to questions about the impact of these inaccuracies on the gait metrics derived from the sensor data. This study aims to quantify the accuracy and variability in phone-based accelerometers and gyroscopes in order to better inform users and App developers of the variability in gait metrics derived by such phone applications.
- **Methods**
  - This study will quantify the accuracy and variability in toe-off and heel-strike gait events derived from phone-based sensors (iPhone X, iPhone 12 Mini, Samsung S10e) compared to the same events derived from a synchronized reference system (Protokinetics Zeno walkway) that captures foot contacts through sensors directly under the feet of participants. A total of 20 able-bodied participants will be recruited. Three-axis accelerometry and gyroscopy will be collected from each phone as participants perform standard gait tasks at a self-selected pace and a hurried pace. The position and orientation of the smart phone on the participant's body will be systematically varied. These data will be passed through several algorithms described in the literature to derive the toe-off and heel-strike gait events and compared to a reference system as a function of body position, orientation, and gait task. Signal accuracy will be assessed through the use of calibrated inertial measurement units as accelerometry and gyroscope data from these units will be compared to each smartphone.
- **Results**
  - Data collection is set to commence in Summer 2022. To date, all phones for the study have been acquired or purchased. Apps to extract the 3-axis accelerometry and gyroscope data from each smartphone are being researched. Synchronization of the walkway system and calibrated inertial measurement unit is achieved through a 3.3V TTL-pulse wave. Data from the smartphone will then be aligned to the inertial measurement unit by attaching the unit to the smartphone and using a "shake" event to align acceleration signals. Preliminary mock data collected from healthy individuals demonstrate protocol feasibility.

- **Implications**
    - Quantifying the accuracy of and variability in phone-based sensors will enable the development of more robust algorithms for deriving gait metrics based on such sensor data. It will also help create guidelines for applications that use such sensor data for gait quantification that truly reflects the variability in the underlying sensor measurements.

27. **Abstract Title:** *Phototoxicity Testing of Emerging Fluorescence Imaging Products: Reactive Oxygen Species Generation and Photocytotoxicity*
Authors: Vig, Shruti, UMD-CP (Student); Gaitan, Brandon, UMD-CP (Student); Frankle, Lucas, UMD-CP (Student); Oskoui, Ellen, FDA/CDRH (Student); Adwan, Miriam, UMD-CP (Student); Elespuru, Rosalie, FDA/CDRH (Mentor); Chen, Yu, UMass Amherst (Mentor); Pfefer, Joshua, FDA/CDRH (Mentor); Chiao-Huang, Huang, UMD-CP (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:
- **Synopsis**
    - Recent advances in optical imaging have the potential to improve patient outcomes. One such promising technology known as contrast-enhanced fluorescence imaging is being increasingly accepted by clinicians due to being real-time, inexpensive, and minimally invasive. A critical challenge that has not been rigorously evaluated is the potential phototoxicity of the fluorophores used for optical imaging procedures. In this study, we have used a commercial cell-free assay to quantify singlet oxygen production rate and a modified 3T3 Neutral Red Uptake (3T3 NRU) assessment strategy to evaluate photocytotoxicity. Results provided strong evidence of the effectiveness of these methods and will help provide a foundation for standardizing photosafety testing of clinical fluorescence imaging products.
- **Purpose**
    - Contrast-enhanced fluorescence imaging is a powerful imaging modality that can aid in surgical visualization of anatomical structures in patients. One of the barriers to entry in translating fluorescent imaging products is the potential for phototoxicity, as agents can produce reactive molecular species (RMS) like singlet oxygen (SO) that cause cell or tissue damage. No optical safety standards address this issue and regulatory photosafety assessment focuses on phototoxicity during solar exposure, which has historically been the primary concern. However, illumination by imaging systems in the visible to near-infrared could cause photochemical damage to internal tissues and organs. There are currently no well-established approaches to screen fluorescence agents for potential phototoxic effects. This research aims to facilitate the advancement of clinical fluorescence imaging by establishing standardized and least burdensome test methods for phototoxicity screening.
- **Methods**
    - We have studied two testing approaches based on commercially available assays: a cell-free chemical assay to quantify SO generation and a modified in-vitro 3T3 Neutral Red Uptake (3T3 NRU) assay. Four imaging agents – ICG, Proflavine, Methylene Blue (MB), and IRDye800 – and three strong RMS-

generating fluorophores – IRDye700, BPD and Rose Bengal (RB)– were evaluated. For the chemical assay, SO production rate was calculated and compared data from the literature. For photocytotoxicity assessment, a 3T3 NRU assay was performed to evaluate the dose-response curve for each agent. Additionally, Mean Photo Effect (MPE) and the Photo Irritation Factor (PIF) values were calculated using the Phototox Version 2.0 software (OECD) for each agent. For both test methods, measurements were performed at agent concentrations and illumination parameters – excitation wavelengths and radiant exposures [RE(J/cm2)] – that were within and above the range of clinical levels.

- **Results**
  - Results indicate that SO production was highly dependent on the radiant exposure and agent concentration. SO production rate for ICG and IRDye800 were more than two orders of magnitude below that of RB. MB and IRDye700 showed high levels of SO production. Proflavine exhibited a spectral overlap with the cell-free chemical assay, yet our tests indicated minimal SO production and appeared robust due to the use of a background subtraction approach. Our preliminary results with the modified 3T3 NRU assay exhibited expected predictions of photocytotoxicity, including for known phototoxic agents, BPD and RB. However, Proflavine exhibited photocytotoxicity using the 3T3-NRU assay despite lack of SO generation with the cell-free assay. This may be due to the production of other RMS – such as superoxide and hydroxyl radicals which could not be detected with the SO specific cell-free chemical assay. Overall, our results for the two methods appeared consistent and in agreement with the literature.
- **Implications**
  - Using these methods to estimate RMS production and related photocytotoxicity should provide practical insights into the photochemical safety of contrast-enhanced fluorescence imaging products. The methods implemented deviate from standard approaches in that they focus on generating results for clinically relevant light and agent doses. While the study focused on quantifying SO production, additional assays will be needed to detect other known RMS produced by fluorophores to establish a comprehensive test methodology. Additionally, evaluating overall photocytotoxicity produced due to collective RMS generation using the in-vitro 3T3 NRU assay may help determine a safe concentration and illumination dose range for clinical imaging. Results from this study provide critical scientific information and help establish consensus standards and regulatory guidelines for innovative diagnostic technologies that are safe for clinical use and hence benefit public health.

28. **Abstract Title:** *Semi-Automated Cone Counting Method for Adaptive Optical Coherence Tomography Volumes*
    Authors: Wolcott, Oliver, FDA/CDRH (Student); Raghavendra, Achyut, FDA/CDRH (Mentor); Hammer, Daniel X, FDA/CDRH (Mentor); Liu, Zhuolin, FDA/CDRH (Mentor)
    FDA Strategic Initiative: Increasing Choice and Competition through Innovation
    Abstract:

- **Synopsis**
    - Adaptive Optics (AO) has enabled the resolution of retinal detail at cellular level in the living human eye by correcting the ocular aberration when combined with other imaging modalities, such as OCT and SLO. It had been suggested that the degeneration of the PR cells can be an early sign of age-related macular degeneration, retinitis pigmentosa and other outer retinal diseases. The counting of these cell through AO-OCT images has completely relayed on manual counting process which is labor intensive and time consuming. The goal of this project is to develop a software program that performs semi-automated cone cell identification in AO-OCT volumes, while maintaining the flexibility to provide manual corrections. By using the unique optical signatures of the cone IS/OS and COST reflections, a semi-automated algorithm was able to initially identified an average of 52% of the total cones counted. After manual correction, the measured cone density is consistent with in vivo measurements from previous AO-SLO [4] and histological studies [5] at similar retinal eccentricities. The results of this study will help establish more sensitive AO-OCT based disease biomarkers and facilitate future implementation of fully automated ML cone counting algorithms.

- **Purpose**
    - Adaptive optics (AO), when combined with other imaging modalities, such OCT and SLO, has enabled the resolution of cellular-level details in the living human eye. It has been suggested that degeneration of photoreceptors (PR) can be early sign of outer retinal diseases, and therefore the cell density can serve as sensitive biomarker for retinal health. In AO-OCT volumes, individual cone photoreceptors appear as a paired reflection at the depth of the IS/OS and COST. Previous publications have described automated cone identification on AO-SLO images aided by machine learning (ML) algorithms [1], however PR density in AO-OCT volumes still comes through the time-consuming manual counting process. The goal of this project is to develop a software program that performs semi-automated cone cell identification in AO-OCT volumes, while maintaining the flexibility to provide manual corrections. The applications of cone density results include: 1. Further development of AO-OCT based cone biomarkers, and 2. Establishment of training data for fully automated ML cone identification algorithms.
- **Methods**
    - AO-OCT volumes were acquired with the FDA multimodal AO [2] and FDA FDML AO systems [3]. AO-OCT data from 3 subjects were collected at various retinal locations (2.5° to 7° to the fovea) and processed using the methods described elsewhere [2, 3]. A software program was developed in MATLAB to semi-automatically identify cones and allow manual correction of the cone locations.

The cones' unique optical signatures and reflections are used as criteria for cone identification. For the semi-automated software cone en face image was generated using the maximum intensity projection across the COST layer. The cone en face image was then thresholded manually by selecting the lowest acceptable pixel brightness to remove background noise. A manual correction was then applied to the semi-automated cone counting results to correct the mis-identified cones using the same criteria. The cone density was finally calculated by taking the ratio between the total number of the cells and total imaged areas, and the results were compared to other in vivo measurements [4] and histological results [5].

- **Results**
  - A semi-automated algorithm was successfully developed with an improved counting efficiency (2-5 minutes/volume). The software initially identifies an average of 52% of the total cones counted. The markings align well with the center of the cells and cone identification does not depend on image brightness. After manual correction, cone density was measured ranging from 18,110 cells/mm2 at 2.5° to 6,944 cells/mm2 at 7° retinal eccentricity, which are consistent with in vivo measurements from previous AO-SLO [4] and histological studies [5] at similar retinal eccentricities.
- **Implications**
  - We have developed a semi-automated software algorithm for cone identification in AO-OCT volumes. Our software allows accurate detection of the cone locations in a timely manner by integrating a manual cell correction method. Future software development is required to further improved the accuracy of the semi-automated cone counting algorithm. The results of this study will help establish more sensitive AO-OCT based disease biomarkers and facilitate future implementation of fully automated ML cone counting algorithms.
- **Citations**

1.) Cunefare D, Cooper RF, Higgins B, Katz DF, Dubra A, Carroll J, Farsiu S. "Automatic detection of cone photoreceptors in split detector adaptive optics scanning light ophthalmoscope images." *Biomed. Opt. express* 7 (5), 2036-2050, 2016.

2.) Liu Z, Tam J, Saeedi O, Hammer DX. "Trans-retinal cellular imaging with multimodal adaptive optics." *Biomedical optics express* 9 (9), 4246-4262, 2018.

3.) Liu Z; Zhang F, K Zucca, Hammer D X. "Multifunctional and multimodal Fourier domain mode-locked laser-based adaptive optics system for ultrahigh speed retinal cellular imaging." *Ophthalmic Technologies XXXII* 11941, 7-13, 2022.

4.) Song, Hongxin, et al. "Variation of Cone Photoreceptor Packing Density with Retinal Eccentricity and Age." *Investigative Ophthalmology & Visual Science*,

Association for Research in Vision and Ophthalmology, Inc., 21 Sept. 2011, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3183974/.
5.) Curcio CA, Sloan KR, Kalina RE, and Hendrickson AE. "Human Photoreceptor Topography." *The Journal of Comparative Neurology*, 292: 497-523, 1990.

29. **Abstract Title:** *Deep Learning Models for Texture Analysis and Evaluation of Bone Health*
Authors: Yan, Ran, FDA/CDRH (Student); Cao, Qian, FDA/CDRH (Mentor); Marupudi, Harsha, FDA/CDRH; Samala, Ravi, FDA/CDRH; Petrick, Nicholas, FDA/CDRH
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
  - Evaluation of bone strength is important for the diagnosis and treatment of osteoporosis. With the recent improvement in spatial resolution of CT systems, it is possible to visualize bone microstructure and extract texture features. It is hypothesized that bone texture could be used to improve the assessment of bone strength compared to using bone mineral density (BMD) alone. This project aims to develop deep learning and radiomics models for estimating trabecular bone strength from high-resolution CT. Blur and correlated noise properties derived from clinical CT systems were used to generate simulated CT images of lumbar vertebrae. A 3D residual network (ResNet) with bottleneck architecture was used to estimate finite element analysis (FEA)-derived bone stiffness and von Mises stress. The root mean square error (RMSE) of the 3D ResNet was 2.57 N/micron for stiffness and 0.77 N/cm2 for mean von Mises stress. The performance of the 3D ResNet was better than both using BMD alone and the radiomics model. Additionally, adding orientation information to the models improved the accuracy of model predictions. We demonstrated that mechanical properties of lumbar vertebral trabecular bone could be inferred from high resolution CT images. Texture features can be derived from both feature-engineering (radiomics) and deep learning approaches. For our dataset and simulation conditions, the deep learning model demonstrated better performance in predicting bone strength. Though bone fracture risk depends on many other factors, including the patient's daily physical activity, this work demonstrates the feasibility of estimating mechanical fragility from imaging features. Identifying effective texture-based imaging features could yield novel endpoints for osteoporosis drug development as well as better monitoring of treatment response.
- **Purpose**
  - Evaluation of bone strength is important for the diagnosis and treatment of osteoporosis. It is currently assessed with bone mineral density (BMD) using dual energy absorptiometry or quantitative CT. With the recent improvement in spatial resolution of CT systems, it is possible to visualize bone microstructure and derive texture features. It is hypothesized that bone texture could be used

to improve the assessment of bone strength. Finite element analysis (FEA) has been widely used for trabecular bone strength analysis for micro-CT images. However, the same analysis could not be reliably performed on clinical CT images due to lower image quality. The purpose of this project is to develop deep learning models and features to estimate FEA-derived mechanical properties from simulated clinical CT images. We also want to compare the performance of deep learning models with that of radiomics models. This work may contribute to novel endpoints for osteoporosis drug development as well as better monitoring of treatment response.

- **Methods**
  - The micro-CT images of 70 lumbar vertebrae were partitioned into training (40), validation (15) and testing (15) datasets. Ten regions of interest (ROIs) from each bone were segmented using Otsu's method. The elastic modulus and the stress distribution for each ROI were computed with FEA. Blur and correlated noise properties derived from clinical CT systems were used to generate simulated CT images for each ROI. A 3D residual network (ResNet) with bottleneck architecture was used to predict bone strength. Training and testing were performed in PyTorch. From the penultimate layer of the trained ResNet model, 512 features were extracted. A total of 92 radiomics features (first order, GLCM, GLRLM, GLSZM, etc) were calculated using PyRadiomics. Additionally, the gradient structure tensor (GST) was used to determine the principal direction for the bone ROIs. A random forest regressor was used to estimate stiffness and stress from the different sets of features. The performance for each model was evaluated with R2 and root mean square error (RMSE).
- **Results**
  - From FEA of the bone ROIs, the mean(std) stiffness is 15.37(6.17) (N/micron), and the mean(std) Von Mises stress averaged across the ROIs is 5.32(1.56) (N/cm2). The performance of the 3D ResNet was 2.57 N/micron (R2 = 0.89) for stiffness and 0.77 N/cm2 (R2 = 0.82) for the average Von Mises stress. The performance of the ResNet model was better than the radiomics model, both models are better than using BMD alone (Radiomics: R2 = 0.69, RMSE = 3.70 N/micron; BMD: R2 = 0.32, RMSE = 5.34 N/micron). The performance the radiomics model was enhanced by adding spatial orientation information (R2 =0.69, RMSE =3.63 N/micron). The performance of using deep features alone was slightly better than the combined model using all three features (R2 =0.88, RMSE =2.65 N/micron).
- **Implications**
  - We demonstrate that texture features could be used to improve the evaluation of bone health compared to using BMD alone. Texture features can be extracted using both feature-engineering (radiomics) and deep learning approaches. Furthermore, these two approaches could be combined to generate a model

with better performance. Due to the anisotropic structure of bone, incorporation of directional information is important for estimating bone stiffness. Though bone fracture risk depends on many other factors, including the patient's daily physical activity, this work demonstrates the feasibility of estimating mechanical fragility from diagnostic imaging.

30. **Abstract Title:** *The Importance of Objective Methods for the Evaluation of Deep Learning Image Processing Devices*
Authors: Younis, Seif, FDA/CDRH (Student); Samuelson, Frank, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
  - The FDA plays an integral role in setting the standard for the evaluation of medical devices used in numerous diagnostic contexts. As a result, it is imperative that the FDA is as informed when evaluating the efficacy of such diagnostic devices. Several manufacturers have emphasized their devices' ability to produce high-quality x-ray images using deep learning neural networks even when their scanners generate images with some level of noise or distortion. But even the best autoencoder models have their limitations, which is why adversarial training was introduced, which have yielded higher-quality output images. We will demonstrate that it is possible to create a "denoising model" utilizing deep learning that outputs realistic looking medical scans from even heavily distorted input images. Such a model may generate believable yet inaccurate x-ray images. Therefore, it is imperative that the FDA require sponsors to utilize objective image evaluation techniques.
- **Purpose**
  - Many device manufacturers have developed deep learning neural networks to remove noise/distortion from their x-ray scans and improve their visual clarity. Some prominent ML frameworks for denoising medical images include autoencoders, which learn to extract key features from a noisy image to output an image with greatly reduced noise. More recently, manufacturers have begun including adversarial discriminator networks in the training of their denoising implementations. These adversarial networks may cause the image denoiser to construct plausible output images that could "blend in" with the training dataset, but not be representative of the particular patient. This presents a problem when applied to very noisy images, as this approach may be functionally equivalent to outright fabrication. In our research, we sought to demonstrate that claims of generating high-quality medical images from noisy scans using adversarial network training ought to be treated with additional skepticism.

- **Methods**
    - We wanted to create our own deep learning model that would simulate removing noise from chest radiographs and return a high-quality recreation no matter how noisy a given input image was. To do this, we chose Python as our primary development language. This enabled us to utilize TensorFlow, a popular machine learning library. TensorFlow greatly simplifies the process of developing numerous types of neural networks, including convolutional neural networks, autoencoders, and generative adversarial networks (GANs). We decided to start by developing an autoencoder model that didn't utilize a GAN. We acquired 12,000 chest radiograph images made publicly available by NIH, ten thousand of which were used to train the model. The other 2000 were used to test its denoising capabilities. We then utilized and compared the effectiveness of several loss functions, including structural similarity and perceptual loss, in refining our model's parameters to develop more detailed denoised reconstructions.
- **Results**
    - After testing numerous autoencoder models with various loss functions, we obtained an autoencoder network which used structural similarity loss that could recreate chest x-rays with low noise and reasonable detail, even at relatively high distortion levels. Still, even this model could not fully recreate the level of detail present in the original image. In the future, we hope to further refine our autoencoder model by increasing the number of iterations and training images used. We also intend to start integrating adversarial network training into our denoising models, which may yield better results compared to simply using an autoencoder by itself. The autoencoder would act as the generator in a GAN model, while a discriminator would distinguish between original and reconstructed images. The task of the autoencoder would be to get better at recreating x-ray images to bypass the discriminator's detection.
- **Implications**
    - Research surrounding the use of GAN training in denoising medical images has touted their ability to create more detailed reconstructions of noisy images compared to autoencoders with MSE loss. While using GANs may produce higher-quality output images, the fundamental flaw with using this method in diagnostic settings is that the focus is less on removing noise from an input image and more on developing a realistic recreation of what the x-ray "should" look like. Such a model may barely reference the original image, to the point that it practically creates these reconstructions out of nothing. Such images may appear to be high quality but are not useful for diagnosing patients. Therefore, it is imperative that the FDA require sponsors to utilize objective image evaluation techniques that demonstrate anatomies shown in medical images are accurate representations of the patients' anatomies.

31. **Abstract Title:** *Establishing an open-access database of wearables gait data to facilitate the discovery of digital biomarkers for Parkinson's Disease*
Authors: Watkinson, Sophia, FDA/CDRH (Student); Kontson, Kimberly, FDA/CDRH (Mentor); Caiola, Michael, FDA/CDRH (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
    - Gait impairment is one of the most well-known and observable symptoms of Parkinson's disease. In recent years, there has been a push to assess gait impairment outside of clinical settings using wearable sensors that deliver quantifiable metrics. The drive to quantify gate impairment using wearables creates a need for a database with gait data collected using a standardized protocol that can be used to validate wearable-derived gait metrics. In the first year of this study, preliminary gait data will be collected from 20 Parkinson's disease patients who will be instrumented with inertial measurement unit sensors and in-sole pressure sensors. Participants will perform a series of tasks such as full-stride gait, gait initiation and termination, tandem gate, timed-up and go test, and balance tests to collect gait data that will be published in an open-access database. The curation of this open-access wearables database will (1) serve as a tool to develop innovative wearable biomarkers, (2) provide an independent data set with which novel wearables-derived biomarkers could be validated, and (3) provide correlational information about clinical evaluations and wearable-derived gait metrics.
- **Purpose**
    - Recently, there has been a push to assess gait impairment in Parkinson's disease patients quantifiably using wearable sensors such as inertial measurement units (IMUs) and insole pressure sensors. There are several studies and databases that provide wearables data for this patient population. However, these databases fail to provide wearables data associated with a reference system, focus mainly on wrist-worn sensors (leaving out other innovative systems such as insole pressure sensors and foot-worn IMUs), and collect continuous movement data, making it difficult to parse out functional movements. These limitations make it difficult to validate and interpret previous and future wearable-derived gait metrics. The purpose of this study is to create an open-access database of gait data from patients with Parkinson's disease through the establishment of a standardized protocol for data collection.
- **Methods**
    - The first year of this study will collect preliminary gait data from 20 Parkinson's disease patients using Xsens IMUs, Moticon in-sole pressure sensors, and a 16-foot Protokinetics Zeno electronic walkway. Wearable sensor placement will be

standardized across all participants, with IMU sensors placed on the xiphoid process of the sternum, left/right wrist halfway between the lateral and medial condyles, left/right lateral thigh midway between the hip and the knee, left/right medial shank midway between the knee and ankle, left/right medial shank just above the malleolus, and left/right dorsum of feet and with Moticon in-sole pressure sensors placed directly in the shoes. Participants will be video-recorded as they perform a series of tasks including full-stride gait, gait initiation and termination, tandem gait, timed-up-and-go (TUG) test, and balance along the Zeno walkway. These sensors will produce data such as acceleration, angular velocity, initial and final contact events, mean stride, stance, swing, and step time that will be included in the database.

- **Results**
  - To date, the three main systems used in the study (IMUs, insole pressure sensors, walkway) have been synchronized using both custom-designed and built-in interfaces. Feasibility of the experimental protocol and system synchronization have been demonstrated through mock data collection from individuals with no neurological disease. Analysis scripts have been written to automate the processing and formatting for all data on the open-access database. The community platform precisionFDA will serve as the initial host of data collected from this research project. This platform allows internal and external stakeholders to download the data within the created 'PD Gait Database' space. Data collection in the patient population is set to begin in Summer 2022.
- **Implications**
  - The curation of this open-access wearables database will (1) serve as a tool to develop innovative wearable biomarkers, (2) provide an independent data set with which novel wearables-derived biomarkers could be validated, and (3) provide correlational information about clinical evaluations and wearable-derived gait metrics. This information can lead to greater confidence in the use of wearable-derived gait data to monitor gait impairment of Parkinson's disease patients outside of a clinical setting and sets the foundation for development of wearable-derived biomarkers for the assessment of Parkinson's disease stage.

32. **Abstract Title:** *Improving usability of a high-throughput in vitro protocol for pulsed electric field cardiac ablation parameters optimization*

Authors: Polina Sobol[a], Maura Casciola[a], Tromondae K. Feaster[a], Anand Ram Vadlamani[a,b], Ksenia Blinova[a]

[a] – Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, Silver Spring, Maryland, 20993, USA

[b] – Department of Biomedical Engineering & Mechanics, Virginia Polytechnic Inst & State University, Blacksburg, VA 24061, USA

FDA Strategic Initiative: Increasing Choice and Competition through Innovation

- **Keywords**
  - Irreversible electroporation (IRE), cardiac ablation, atrial fibrillation (AF), high-throughput human *in vitro* protocol, microplate reader (MPR)
- **Synopsis**
  - Irreversible electroporation (IRE) has gained attention for cardiac catheter ablation in patients with atrial fibrillation (AF) when pharmaceutical treatments fail. Today, catheter ablation is performed clinically through radiofrequency ablation or cryoablation which deliver high heat or extreme cold, respectively, to scar faulty cardiac tissue responsible for AF. However, thermal approaches risk damage to surrounding tissues. Pulsed electric fields (PEF) kill cells by non-thermal mechanisms inducing IRE in the targeted area, which has the potential to improve ablation treatment safety and clinical outcomes. We are developing a preclinical high-throughput protocol to optimize PEF parameter selection for device development as well as regulatory decision making. The scope of this study is to simplify the existing protocol to facilitate preclinical evaluation of PEF cardiac treatments by increasing its accessibility and affordability.
- **Purpose**
  - Atrial fibrillation (AF) results in more than 454,000 hospitalizations each year. One of the treatments for AF is to block the irregular electrical signals by scarring the tissue through the process of cardiac ablation. Pulsed electric fields (PEF) can cause local irreversible electroporation (IRE) of cell membranes, inducing cell death while minimizing thermal damage thus reducing possible off target injuries. However, optimal parameters of PEF cardiac ablation are not yet established mostly due to the lack of standardized preclinical methods to assess PEF effects on tissues. Our Laboratory is developing a novel *in vitro* protocol to correlate PEF parameter selection to treatment outcomes. This protocol uses a confocal microscope to provide high-resolution images of IRE areas exposed to PEF treatment. Here, we investigate microplate reader (MPR) imaging as a simplified alternative to confocal microscopy, reducing the costs and the skillset required for protocol implementation.
- **Methods**
  - We use human esophageal smooth muscle cells (HESMC) to test the feasibility of MPR imaging as an alternative to confocal imaging to assess the effect of different PEF parameters on IRE area. HESMC are grown in monolayer format on a 96-well nanofiber plate at 55-75K cells/well. PEF is delivered by a custom pulsed generator through two needle electrodes. The PEF parameters are: 100 symmetrical biphasic pulses, with phase voltage (V_p) 300, 400, and 600 V, phase duration (t_p) 1, 5, 10 µs, delivered at 10, 100, 1000 Hz pulse repetition frequency (PRF). 4 hours after PEF treatment, the IRE areas will be quantified using propidium iodide (PI) detected by confocal microscopy (4x objective) and MPR imaging. We will optimize MPR scanning parameters by varying the number

fluorescence intensity (FI) measures in a well to minimize MPR scanning time without sacrificing IRE area measurement resolution.

- **Results**
  - For various combinations of PEF parameters, we will quantify the HESMC response using images obtained with confocal microscopy and MPR imaging. To optimize MPR scanning parameters, we will test 1, 12, 21, 32, 52, 97, and 208 equidistant FI locations per well (corresponding to inter-measure distances of 0, 1.5, 1.16, 0.93, 0.71, 0.54, and 0.37 mm), record scanning time and FI, measure MPR imaging-based IRE area and compare it with the IRE area obtained by confocal microscope imaging. Our preliminary results show that when 100 pulses at V_p = 600 V, t_p = 5 µs, and PRF = 10 Hz were applied, the optimal correlation between IRE area measured with confocal microscopy and MPR (6.01 mm^2 and 6.05 mm^2 respectively) was observed with MPR setting at 52 locations per well with a scanning time of 2.8 hours per plate. In our preliminary results, the FI trends obtained with MPR demonstrated expected dose response curves consistent with the trends observed with confocal microscopy, i.e., FI increased with increasing V_p, and t_p, and decreased with increasing PRF.
- **Implications**
  - The development of a high-throughput preclinical protocol for PEF parameters assessment for cardiac ablation based on human cell will reduce animal burden in the development of PEF devices and will support the regulatory process. The aim of the proposed study is to improve the PEF protocol usability by simplifying the protocol and reducing the required equipment cost.
- **Disclaimer**
  - *The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services. This abstract reflects the views of the author and should not be construed to represent FDA's views or policies.*

Center for Food Safety and Applied Nutrition (CFSAN)

1. **Abstract Title:** *Concentration of C. cayetanensis oocysts in potting mix samples by flotation in saturated sucrose*
   Authors: Arida, Joseph, FDA/CFSAN (Student); Almeria, Sonia, FDA/CFSAN (Mentor)
   FDA Strategic Initiative: Public Health Emergency Preparedness and Response
   Abstract:
   - **Synopsis**
     - Contact of produce with soil that has been contaminated with *Cyclospora cayetanensis* may represent an important vehicle in the transmission of this organism. Previous studies in soil showed that a flotation concentration method followed by DNA extraction using commercial kits produced the best sensitivity

for *C. cayetanensis* detection compared to using commercial DNA extraction alone. In order to replicate these conditions, we hope to grow plants in growth chambers using potting mix. However, potting mix is made up of many less dense components including peat moss, wood chips, and perlite which also float in a sucrose concentration. The lack of density causes the flotation of an additional large layer in the supernatant. Five grams of potting mix were seeded with known numbers of *C. cayetanensis* oocysts [20 (n=9), 50 (n=10)]. Unseeded soil samples were included as negative controls. Samples were processed by flotation concentration in a sucrose solution. The supernatant containing the top of the solution (20 ml) was then collected, diluted in water and centrifuged. DNA was extracted from the pellet using a commercially available ZymoBIOMICS DNA Miniprep Kit. qPCR was performed using Qiagen QuantiFast Multiplex PCR Kit with a cut off value for (CT value) *C. cayetanensis* of >38.0. All negative control samples were negative. No inhibition was observed in any of the processed samples. The flotation procedure showed fractional detection of 20 oocysts in 5 g of potting mix. The rates of positivity for 5 g samples seeded with 50 and 20 oocysts were 100%, (n=10) and 33%, (n=9) respectively. The capability of detecting *C. cayetanensis* oocysts in potting mix will facilitate outbreak investigation and increase knowledge on the epidemiology of this important parasite. Adapting the soil protocol to potting mix is critical as potting mix components cause challenges and we may use potting mix instead of soil when growing plants in a lab environment.

- **Purpose**
    - Contact of produce with soil that has been contaminated with *Cyclospora cayetanensis* may represent an important vehicle in the transmission of this organism. There is a scarcity of detection methods for this parasite in soil. Previous studies in soil showed that a flotation concentration method, using high density sucrose solutions, followed by DNA extraction using commercial kits produced the best sensitivity for *C. cayetanensis* detection compared to using commercial DNA extraction alone. To analyze *C. cayetanensis* detection simulating field conditions, we hope to grow herbs in growth chambers using potting mix. However, potting mix is made up of many less dense components than normal soil, including peat moss, wood chips, and perlite which float even in aqueous solutions. These components produce an additional large layer in the supernatant which requires removal before the sucrose solution flotation step. The purpose of the study was to adapt the soil protocol to potting mix which will be used to grow herbs in a lab environment.
- **Methods**
    - Five grams of potting mix samples were seeded with known numbers of *C. cayetanensis oocysts* [20 (n=9), 50 (n=10)]. Unseeded soil samples were included as negative controls. Samples were processed by flotation concentration in a sucrose solution. First the samples were washed in water and

after centrifugation the top was eliminated to avoid perlite and wood fragments. The sediment of the samples was then processed by flotation in sucrose solution. The supernatant containing the top of the solution (20 ml) was then diluted in water and centrifuged allowing oocysts to settle. DNA was extracted from the pellet using two commercially available kits: MP Bio FastDNA Spin Kit for Soil and ZymoBIOMICS DNA Miniprep Kit. The DNA extraction methods included bead-beating to break the oocyst wall according to the FDA BAM Chapter 19b method (BAM 19b). qPCR was performed targeting *C. cayetanensis 18SrRNA* gene as well as an internal amplification control (IAC). In an experiment a new mitochondrial target (MIt1C) was used in the qPCR. The cut off value for (CT value) *C. cayetanensis* was >38.0.

- **Results**
  - All negative control samples were negative. The IAC showed no inhibition in any processed samples. There was no statistically significant difference in level detection between FastDNA Spin Kit for Soil as described in the FDA BAM Chapter 19b method and the ZymoBIOMICS DNA Miniprep Kit. The ZymoBIOMICS DNA Miniprep Kit was chosen as it required less time to complete. Better detection was observed in 5 g versus 10 g of samples. Detection of 50 oocysts in 5 g and 10 g of potting mix was 100%, (n=10), and 37.5% (n=8) respectively. Additionally, there was not statistically significant different in level of detection of sieved potting mix, 100% (n=5) compared to potting mix that was not sieved, 100% (n=5) in 5 g samples. As sieving adds an additional step and there was no difference in level of detection, we recommended against it. The rates of positivity for 5 g samples seeded with 50 and 20 oocysts were 100%, (n=10) and 33%, (n=9) respectively. Therefore, the modified flotation procedure in potting mix was able to detect *as* few as 20 *C. cayetanensis* oocysts in 5 g of potting mix.
- **Implications**
  - The flotation technique provides detection of as few as 20 oocysts in 5 g of potting mix. Adapting the soil protocol to potting mix is critical as we will use potting mix to grow produce in a lab environment. The capability of detecting *C. cayetanensis* oocysts in potting mix will facilitate studies of *C. cayetanensis* detection in grow chambers mimicking environmental field conditions in the laboratory, which would increase knowledge on the epidemiology of this important parasite. We plan on seeding potting mix of produce and monitor how detection is affected by time and environmental factors such as temperature, humidity and/or photope.

2. **Abstract Title:** *Investigating the Variation in State Regulatory Requirements for Temporary Food Establishments*
   Authors: Bunghiuz, Diana, FDA/CFSAN (Student); Liggans, Girvin, FDA/CFSAN (Mentor); Williams, Laurie, FDA/CFSAN (Mentor)

FDA Strategic Initiative: Unleashing the Power of Data

Abstract: In the United States, Temporary Food Establishments (TFEs) are longstanding attractions for celebrating food and beverage consumption and remain popular wherever Americans gather. Artesian and exotic foods can be found alongside fairground classics such as corn dogs and funnel cakes. The U.S. Food and Drug Administration's model 2017 Food Code defines a Temporary Food Establishment (TFEs) as a food establishment that within the calendar year operates for a period no more than 14 consecutive days in length in conjunction with any single event or celebration. While the intent of the Food Code is to provide a model set of provisions states can adopt to regulate food production at retail, variation in states adoption of the TFE provisions has yet to be explored. Inconsistent adoption of the Food Code may have major public health implications. The purpose of this legal mapping study is to 1) develop a coding scheme for collecting and analyzing legal data to compare TFE provisions across jurisdictions and 2) investigate the variation in state regulatory requirements for TFEs among the 10 most populous states (California, Texas, Florida, New York, Pennsylvania, Illinois, Ohio, Georgia, North Carolina, Michigan) and the FDA Food Code. Results from this study will provide the U.S. Food and Drug Administration a more comprehensive understanding of Food Code adoption amongst states, along with the prevalence of state specific TFE regulations and their potential impact on retail food safety. Results will also provide a knowledge base and methodology for future analysis of TFE regulations across all 50 states and Washington, DC.

- **Synopsis**
  - This legal mapping study aims to look at the yet to be explored variation in states' adoption of the TFE provisions of the FDA Food Code. Inconsistent adoption of the Food Code may have major public health implications.
- **Purpose**
  - The purpose of this legal mapping study is to 1) develop a coding scheme for collecting and analyzing legal data to compare TFE provisions across jurisdictions and 2) investigate the variation in state regulatory requirements for TFEs among the 10 most populous states (California, Texas, Florida, New York, Pennsylvania, Illinois, Ohio, Georgia, North Carolina, Michigan) and the FDA Food Code
- **Methods**
  - Identified the retail food safety statutes and regulations in each jurisdiction of the 5 most populous states then generated coding categories to characterize legal attributes pertaining to temporary food establishments. In-depth analysis of specific attributes within TFE topics was then conducted on each of the 10 most populous states (California, Texas, Florida, New York, Pennsylvania, Illinois, Ohio, Georgia, North Carolina, and Michigan) state food service regulations and compared with the 2017 FDA model Food Code.

- **Results**
    - The results of the analysis show variations in specific attributes of the 10 most populous states' regulations for TFEs as well as to the FDA Food Code.
- **Implications**
    - Results from this study will provide the U.S. Food and Drug Administration a more comprehensive understanding of Food Code adoption amongst states, along with the prevalence of state specific TFE regulations and their potential impact on retail food safety. Results will also provide a knowledge base and methodology for future analysis of TFE regulations across all 50 states and Washington, DC.

3. **Abstract Title:** *Determining Vitamin B12 Concentration in Infant Formula using UPLC-UV*
   Authors: Escavage, Jordan, FDA/CFSAN (Student); Wolle, Mesay, FDA/CFSAN (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract:
    - **Synopsis**
        - Vitamin B12 is vital to humans due to its role in red blood cell formation and nervous system function. Therefore, it is important that there are accurate methods in use to determine the vitamin B12 concentrations in nutritional products like infant formula. The concentration of vitamin B12 in infant formula can be verified using ultra-performance liquid chromatography (UPLC) coupled with ultraviolet detection (UV). To achieve this, the formula must first be reconstituted, then the vitamin B12 in the sample must be converted to the more stable cyanocobalamin form. Cyanocobalamin can be selected from the formula matrix using an immunoaffinity column and injected onto a UPLC column with UV detection at 361 nm to determine the concentration of vitamin B12 in the sample.
    - **Purpose**
        - This study presents the establishment and verification of AOAC Method 2014.02 for the quantification of vitamin B12 in infant formula. Vitamin B12 is important in the formation of red blood cells and function of the nervous system in humans, where deficiency can lead to anemia or neurological damage. Vitamin B12 is used to describe all cobalamins, with cyanocobalamin being the most stable and most popular form used to fortify nutritional products. In order to accurately quantify vitamin B12, conversion of all cobalamin forms to cyanocobalamin is important to avoid degradation of the less stable cobalamins during analysis.
    - **Methods**
        - Vitamin B12 was determined following AOAC Method 2014.02 in infant formula samples. A measured portion of sample is mixed with 1% NaCN and 0.4 M NaAc (pH 4.0). The mixture is heated at 100^{o}C for 30 minutes to convert all

cobalamins in the formula to cyanocobalamin. The supernatant is passed through a vitamin B12 immunoaffinity column for cleanup. The analyte is then eluted from the column with methanol, reconstituted in a small volume (0.3 mL) of water and analyzed using ultra-performance liquid chromatography coupled with UV detection. Separation was under gradient elution using a C18 column, with mobile phases consisting of water and acetonitrile with trifluoroacetic acid (0.025%). Cyanocobalamin was monitored at 361 nm. Retail products and standard reference materials will be used for verification of method performance. Quantification of Vitamin B12 will be compared to a liquid chromatograph (LC)-inductively coupled plasma (ICP)-mass spectrometry (MS) method currently under development by FDA.

- **Results**
    - Standard solutions of cyanocobalamin were successfully analyzed using the method described. To assess the performance of the method, a small sampling of infant formulas will be tested. The samples will also be analyzed by an LC-ICP-MS method being developed at the FDA for comparison of method performance.
- **Implications**
    - Determining the concentration of vitamin B12 in infant formula using UPLC-UV can be used to compare the established AOAC method with an LC-ICP-MS method currently under development at the FDA.

4. **Abstract Title:** *Using FDA Datasets to Assess Levels of Toxic Elements in Foods*
   Authors: Elinor Laffargue OFS/DPPB/PPB (JIFSAN Student Intern), Eileen Abt OFS/DPPB/PPB (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **Synopsis:**
        - Toxic elements, specifically arsenic, cadmium, and lead, are taken up by agricultural crops and livestock that we consume and can accumulate in the body and cause health effects. Infants and young children are especially vulnerable to these exposures. FDA data sources, including the Laboratory Flexible Funding Model (LFFM), the Toxic Element Program (TEP) and surveys provide valuable information for understanding the distribution of toxic elements in foods, and where mitigation measures can be targeted, including through development of guidance documents, to reduce exposures.
    - **Purpose:**
        - Toxic elements, specifically arsenic, cadmium, and lead, are found in soil and water from natural and anthropogenic sources. These toxic elements are taken up by agricultural crops and livestock that we consume and can accumulate in the body and cause health effects. Infants and young children are especially

vulnerable due to high consumption of foods relative to their body weight, a less varied diet than adults, and their developing nervous systems. Given the importance of reducing toxic elements in foods, we evaluated various FDA datasets to understand levels of toxic elements in foods and where mitigation efforts might be most appropriate to reduce occurrence and exposures.

- **Methods:**
    - Arsenic, cadmium, and lead occurrence data from 2020 and 2021 collected by state laboratories through the Laboratory Flexible Funding Model (LFFM) cooperative agreement were compiled and evaluated. Evaluation of the data consists of using simple statistics, and graphing software, including Excel and JMP to understand the distribution of toxic elements and variability based on the toxic element properties and food type. Data from these analyses were then used to support a more detailed exploration of cadmium data gathered from FDA surveys (conducted in 2013 and 2021) and 2012-2022 data from the Toxic Element Program (TEP), a targeted monitoring program that monitors levels of certain toxic elements in foods.
- **Results:**
    - Analysis of data on lead in apple and grape juice indicates that, in general, lead levels are below the recently proposed draft action levels of 10 ppb and 20 ppb, respectively. An analysis of toxic elements in root vegetables demonstrates that lead and cadmium concentrations for this category are higher than that of arsenic, with cadmium being highest in potatoes and lowest in sweet potatoes, while lead is highest in sweet potatoes. Further exploration of the LFFM dataset together with FDA survey and TEP data will advance our understanding of toxic elements in foods and help target mitigation measures for cadmium.
- **Implications:**
    - The LFFM data, together with FDA survey and TEP data, provide valuable information for understanding the distribution of toxic elements in foods, and where mitigation measures can be targeted, including through development of guidance documents, to reduce exposures.

5. **Abstract Title:** *An Evaluation of Response Letter Trends for New Dietary Ingredient Notifications*
   Authors: Cruz-Casanova, Paola, FDA/CFSAN (Student); Crone, Teresa, FDA/CFSAN (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **<u>Synopsis</u>**
        - According to the Dietary Supplement Health and Education Act (DSHEA) of 1994, manufacturers are required to submit a new dietary ingredient notification (NDIN) to the FDA at least seventy-five days before marketing some new dietary ingredients. Since the passing of DSHEA, NDINs have been reviewed by what is presently known as the Office of Dietary Supplements Programs (ODSP) in CFSAN/FDA. In 2019, the NDIN submission process began operating via the CFSAN On-line Submission Module with the workflow being tracked in

Appian. To help resolve the issue of information being stored in multiple locations and to allow for more efficient searching of past notifications, we developed a Microsoft Access database which combines information from both the old (1994-2019) and new (2019-present) systems. Data captured included: the name of the new dietary ingredient, a brief summary of the ingredient, type of response letter, supplement description, reviewer comments, and other information gathered from the submission. We also documented when a pre-NDIN meeting occurred prior to a submission. We compared the number of acknowledgements without objections response letters (AKL) for notifications with and without a pre-NDIN meeting. We used the data to determine the effectiveness of pre-NDIN meetings in achieving an AKL final response letter.

- **Purpose**
  - The Dietary Supplement Health and Education Act of 1994 (DSHEA) requires manufacturers to submit a new dietary ingredient notification (NDIN) to the FDA at least 75 days before marketing some new dietary ingredients. Since 1994, NDINs have been reviewed by what is presently known as the Office of Dietary Supplements Programs (ODSP) in CFSAN/FDA. The NDIN submission and tracking process was renovated in 2019. To resolve the issue of needing to search old (1994-2019) and current (2019-present) systems, we developed a new database with information on all the NDINs. For this study, we evaluated trends in the NDIN submission process by tracking types of response letters from various fiscal years. Stakeholders who want to submit an NDIN may schedule a meeting with FDA officials to discuss questions prior to submission. To evaluate if there is a higher likelihood of an acknowledgement without objection response letter after a Pre-NDIN meeting, we determined the response letter type with respect to having or not having a Pre-NDIN meeting.
- **Methods**
  - The Microsoft Access application was used to create a new database. Information such as the ingredient name, NDIN number, type of response letter, and data from the submission was gathered from the old (1994-2019) and new (2019-present) systems. We determined the number of each type of response letter from 1994 to mid-year 2022 and then calculated the percentage of each type of response letter for fiscal years 2013 to mid-year 2022. Pre-NDIN meeting impact was evaluated by calculating the number of each type of response letter for submissions with and those without a pre-NDIN meeting.
- **Results**
  - A total of 1,164 NDINs received a response letter from the FDA from 1994 to present. The overall trend from the last ten years indicated a steady increase in the percentage of AKLs sent for NDINs, with the last two years showing the highest percentages. The average of all the NDINs submitted that received an AKL as the final response letter was approximately thirty-percent overall. Work is on-going to track the Pre-NDIN meetings that led to subsequent submission and a response letter in order to evaluate the effectiveness of pre-NDIN meetings in leading to an AKL final response letter.
- **Implications**
  - The knowledge gained from the results of our study will contribute to advancing further initiatives in making the process of NDIN submissions more effective and consistent. The information retrieved on the Pre-NDIN meeting submission final

outcomes may be beneficial for both ODSP members and external stakeholders to understand how to better communicate during these meetings in order for the notifier to attain a successful result.

6. **Abstract Title:** *Reduction of Toxic Elements: Spices Data Analysis*
Authors: Latimore, Yazmine, FDA/CFSAN (Student); Johnson, Rhoma, FDA/CFSAN (Mentor); Lunzer, Jesse, FDA/CFSAN (Mentor)
FDA Strategic Initiative: Public Health Emergency Preparedness and Response; Unleashing the Power of Data
Abstract:

- **Synopsis**
  - Toxic elements get into our food through air, water, and soil. Toxic elements in foods and beverages present potential health concerns and keeping the supply of foods and beverages safe by monitoring, testing, and evaluating preventive control measures for toxic elements is a priority for the US Food and Drug Administration (FDA). The principal toxic elements that are the focus for this project are arsenic (As), cadmium (Cd), and lead (Pb). This work takes a multi-focused approach by considering existing research and analyzing internal data for a more comprehensive understanding of the principal toxic elements in ten spice categories.
- **Purpose**
  - Toxic elements get into our food through air, water, and soil. Toxic elements in foods and beverages present potential health concerns and keeping the supply of foods and beverages safe by monitoring, testing, and evaluating preventive control measures for toxic elements is a priority for the US Food and Drug Administration (FDA). The principal toxic elements that are the focus for this project are arsenic (As), cadmium (Cd), and lead (Pb). This work takes a multi-focused approach by considering existing research on reduction methods and analyzing internal data for a more comprehensive understanding of the principal toxic elements in ten spice categories.
- **Methods**
  - We examined toxic elements from two perspectives. The first was to perform a scientific literature review of the relevant research performed in the last five years. The second objective was to understand the trends of toxic element levels in spices from data sets composed of monitoring and testing of both import and domestic samples over the years 2016 to 2022. These data come from the FDA's Toxic Elements in Foods Program (TEP). The spices evaluated were cardamon, chili powder, cinnamon, cumin, curry, ginger, oregano, pepper, paprika, and turmeric. A sample size of approximately 1,200 was examined. The mean, median, standard deviation, and 80th, 85th, 90th, and 95th percentile values for each contaminant in each spice were calculated. In addition, the mean and standard deviation values were compared by year.

- **Results**
    - For the literature review, we identified several methods for reducing toxic elements in food. Examples include chelating methods like ethylenediaminetetraacetate (EDTA) to reduce Pb and Cd in yellowfish and tuna, processing aides like bentonite clay to decrease As in grape syrup, diatomaceous earth (DE) to decrease Pb in maple syrup, and cooking methods like boiling pasta to reduce As, Pb, and Cd levels. Our analysis of the TEP data show that the ten spices categories varied in their type and quantity of toxic elements. For example, of the ten spices, pepper had some of the lowest sample means of arsenic, cadmium and lead over the years. Conversely, cinnamon had the highest cadmium and lead sample means of the ten spices. Oregano had the highest arsenic sample means but limited sampling data over these years. These results are not statistically representative of potential toxic element levels found in the marketplace. These results represent our internal data from sources sampled for various purposes. The mean concentrations of these samples are not a health concern.
- **Implications**
    - The literature review serves an important and current survey of methods for reducing toxic elements. The review can be used to propose future research ideas and areas. The data evaluation provides readily accessible baseline information to potentially support future activities and decisions.

7. **Abstract Title:** *Fate of Listeria monocytogenes in Ready to Eat leafy green salad during refrigerated and frozen storage*
   Authors: Meng, Laura, FDA/ORS (Student); Kwon, Hee Jin, FDA/ORS (Student); Weinstein, Leah, FDA/ORS (Mentor); Chen, Yi, FDA/ORS (Mentor)
   FDA Strategic Initiative: Public Health Emergency Preparedness and Response
   Abstract:
    - **Synopsis**
        - In December 2021, a multistate listeriosis outbreak was recognized and linked to ready-to-eat packaged leafy green salads produced by company A, resulting in 16 hospitalizations and 3 deaths. *L. monocytogenes* causes the foodborne illness listeriosis, which is particularly dangerous for the elderly population, those that are immunocompromised, and pregnant women. The ability of *L. monocytogenes* to grow and survive under refrigerator and freezer temperatures makes contamination a large issue in ready-to-eat products. The purpose of our experiment was to investigate how the storage affected the levels of *L. monocytogenes* in leafy greens. To do so, we artificially inoculated a low level of *L. monocytogenes* and monitored bacterial survival and growth for up to 60 days for samples stored in both the refrigerator and the freezer. The samples were periodically enumerated throughout the duration of the 60 days,

resulting in data that indicates that *L. monocytogenes* can survive during frozen storage and proliferate during the refrigerated storage of packaged leafy green salads, regardless of the presence of aerobic microflora.

- **Purpose**
  - Listeriosis is a foodborne disease that is caused by *Listeria monocytogenes*, a bacterium that has the ability to grow and/or survive under severe conditions. The most susceptible populations include elderly people, immunocompromised populations and pregnant women. The ability to grow and persist at refrigerated and freezer temperatures makes *L. monocytogenes* contamination in ready-to-eat products a significant threat to public health. In December 2021, a multistate listeriosis outbreak was recognized and linked to ready-to-eat packaged leafy green salads produced by company A, resulting in 16 hospitalizations and 3 deaths. Enumeration revealed very low levels of *L. monocytogenes* in three reserve outbreak samples that were stored in refrigerated or frozen conditions past the expiration dates. To investigate how the storage affected the levels of *L. monocytogenes* in leafy greens, the leafy greens were artificially inoculated with a low level of *L. monocytogenes* and observed for bacterial survival and growth for up to 60 days.
- **Methods**
  - One *L. monocytogenes* strain isolated from a recalled packaged salad sample from company A was used to artificially inoculate (73.3 CFU/g) 1500 g of fresh leafy green salads. The inoculated salads were evenly distributed into 20 sample bags and stored at 4℃ and -30℃ for up to 60 days. The samples stored at 4℃ were sampled at 7, 14, 21, 28, 42 and 60 days. The frozen samples were sampled at 7, 14, 28, 42 and 60 days. At each sampling point, 25g × 3 replicates were sampled for enumeration. Each sample portion (25g) was placed into a sterile stomacher bag with 225mL of Buffered *Listeria* Enrichment Broth (BLEB) and blended using a stomacher for 30 seconds. The samples with BLEB were directly enumerated using *Listeria* selective agar plates and most probable number (MPN) following the FDA BAM. Uninoculated salads were sampled at each time point to quantify the aerobic bacteria using the 3M™ Petrifilm™ Aerobic Count Plate (ACP).
- **Results**
  - The fresh leafy green salads were inoculated with 1.87 log CFU/g before being subjected to 60 days of storage at 4℃ and -30℃. During the storage at 4℃, the *L. monocytogenes* level decreased slightly from Day 0 to Day 14 before increasing steadily after day 21 and finally reached 4.94 log CFU/g. Increasing levels of spoilage and decomposition in the lettuce samples was observed throughout the 60 days. The level of aerobic bacteria in non-inoculated salads from 7.23 to 8.70 log CFU/g during the 60 days of storage at 4 indicating that *L. monocytogenes* was able to proliferate in the presence of competitive

microflora at 4℃. The level of *L. monocytogenes* remained unchanged during the storage at -30℃.

- **Implications**
    - These results indicate that *L. monocytogenes* can survive during frozen storage and proliferate during the refrigerated storage of packaged leafy green salads regardless of the presence of aerobic microflora such as lactic acid bacteria. Therefore, levels of *L. monocytogenes* in the actual outbreak samples could be very low. This study also demonstrated that *L. monocytogenes* levels can stay unchanged in frozen temperatures in lettuce over a long period of time, which could allow for more flexibility in analyzing samples.

8. **Abstract Title:** *Collation of CFSAN Foodborne Pathogen WGS Metadata from Disparate Research Projects*
   Authors: Scott,Tyree, FDA/CFSAN (Student); Rand, Hugh, FDA/CFSAN (Mentor); Pettengill, Jamie, FDA/CFSAN (Mentor)
   FDA Strategic Initiative: Unleashing the Power of Data
   Abstract:
    - **Synopsis**
        - FDA's Center for Food Safety and Applied Nutrition (CFSAN) researchers have conducted numerous projects that have resulted in a large collection of foodborne pathogen isolates with their associated metadata. Metadata has been stored in disparate formats and locations which reduces the utility of the data. The goal was to create a database integrating the metadata in one central place. This allows for more effective use of this data. A program was written to process the disparate metadata files. The files were organized, and columns standardized so that they were consistent across the various tables and could be merged without errors; this also required managing the different hierarchical levels of each table so they would be labeled correctly once read in. The program was written in Python using various Python packages such as Pandas, Numpy, SQLite, and SQLAlchemy. The resulting code creates a database that can be searched for a specific ID number and all related information will print out. The current program processes 1693 rows of data from four tables into a single uniform table. There are 376 rows of data from Virginia, 156 rows of data from Delaware, 260 rows from North Carolina and 901 rows of data from various other states collected through contract researchers. This represents data collected over eight years. Integrating all this data expedites queries for metadata on isolates linked to outbreaks and helps prioritize outbreak response efforts. The results will be used to improve the FDA's ability to harness data collected over the years on food-borne pathogens from the environment in situations when those pathogens match those found in outbreaks or facility investigations. Finding matches to previously collected isolates provides much needed context in outbreaks and can provide clues to root-cause analysis in

facility investigations. Rapid access to our historical data speeds up FDA's ability to respond to outbreaks and compliance findings.

- **Purpose**
  - Foodborne pathogens are biological agents that can cause a foodborne illness event; these biological agents include viruses, bacteria, and parasites. The CDC estimates that 48 million Americans become sick due to foodborne pathogens each year. The FDA's Center for Food Safety and Applied Nutrition (CFSAN) researchers have conducted various projects over the years that have resulted in a large collection of foodborne pathogen isolates with their associated metadata. Due to the many different projects and the many years of research, metadata have been stored in disparate formats and locations which reduces the utility of the data. My goal for this summer was to create a database so that the metadata could be stored in one central place. This will allow for more effective use of this data for public health safety.
- **Methods**
  - A program was written to process excel files containing the isolate metadata. To ensure the data would be read into the program correctly the excel files had to be organized. I renamed the columns so that they were consistent across the various tables and could be merged without errors; this also required managing the different hierarchical levels of each sheet so they would be labeled correctly once read in. The program was written in Python using various Python packages such as Pandas, Numpy, SQLite, and SQLAlchemy. The resulting code creates a database that can be searched using a specific ID number and all related information will print out. During the 10-week project, a daily progress report provided a summary of activities and results.
- **Results**
  - The current program processes 1693 rows of data from four tables into a single uniform table. There are 376 rows of data from Virginia, 156 rows of data from Delaware, 260 rows from North Carolina and 901 rows of data from the various other states collected by IEH. This represents data collected over eight years. Integrating all this data expedites queries for metadata on isolates linked to outbreaks and helps prioritize outbreak response efforts.
- **Implications**
  - The results will be used to improve the FDA's ability to harness data collected over the years on food-borne pathogens from the environment in situations when those pathogens match those found in outbreaks or facility investigations. Finding matches to previously collected isolates provides much needed context in outbreaks and can provide clues to root-cause analysis in facility investigations. Rapid access to our historical data speeds up FDA's ability to respond quickly and enhances FDA's public health mission.

9. **Abstract Title:** *Determination of mycotoxins in red pepper using LC-MS – a matrix extension study*

Authors: Tran, Ivy, FDA/CFSAN/JIFSAN/ORS (Student); Zhang, Kai, FDA/CFSAN/JIFSAN/ORS (Mentor)

FDA Strategic Initiative: Public Health Emergency Preparedness and Response

Abstract:

- **Synopsis**
  - Red pepper is prone to mold contaminations and the co-occurrence of mycotoxins has been previously reported. To monitor mycotoxins in red pepper and derived food products, there is a need for analytical methods that can reliably determine FDA regulated mycotoxins in these commodities. In this study, the existing FDA compendial method for multi-mycotoxin analysis by LC-MS was evaluated for matrix extension to red pepper. The extended method will assist FDA in gathering data on the occurrence of mycotoxins in these products.
- **Purpose**
  - Red pepper is prone to mold contaminations and the co-occurrence of mycotoxins has been previously reported. To monitor mycotoxins in red pepper and derived food products, there is a need for analytical methods that can reliably determine FDA regulated mycotoxins in these commodities. In this study, the existing FDA compendial method for multi-mycotoxin analysis by LC-MS was evaluated for matrix extension to red pepper. The extended method will assist FDA in gathering data on the occurrence of mycotoxins in these products.
- **Methods**
  - Following the FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program (3rd Ed.), we performed a matrix-extension study using red pepper as the target matrix. Samples were cryogenically homogenized, followed by the addition of 13C-labeled internal standards, extraction, filtration, and LC-MS analysis. Method performance in terms accuracy and precision was evaluated and the influence of sample homogeneity and matrix interferences assessed. Data were compared to the original collaborative method validation study.
- **Results**
  - Sample homogeneity will be characterized using a laser diffraction particle size analyzer, using the AOAC recommended threshold, Dv90<850 µm. Recoveries in a red pepper matrix will be evaluated for aflatoxins and ochratoxin fortified at 10 ng/g; deoxynivalenol, fumonisins, T-2 toxin, HT-2 toxin, and zearalenone at 100 ng/g. Without any major modifications of the compendial method, we expect that method performance in red pepper will be consistent with the original validation study.

- **Implications**
    - The diversity of mycotoxin contaminated food products is overwhelming. It is impossible to exhaustively develop and validate methods for individual matrices. This study serves as an example that shows the matrix extension of a multi-laboratory validated method is a practical and time-efficient approach to achieve mycotoxin analysis in new matrices not included in the original scope of the method validation study.

10. **Abstract Title:** *State Food Freedom Laws and Hunger Free Campus Statutes: Implications for the Future of Retail Food Safety*
Authors: Lowe, Joy, FDA/CFSAN (Student); Love, Cheryl, FDA/ORA (Student); Liggans, Girvin, FDA/CFSAN (Mentor); Williams, Laurie, FDA/CFSAN (Mentor)
FDA Strategic Initiative: Unleashing the Power of Data
Abstract:
    - **Synopsis**
        - A growing number of states across the United States are enacting new laws and policies designed to promote food sovereignty and address disparities in food insecurity. This includes state food freedom laws, hunger free campus statutes, and right to food amendments. While food freedom laws and right to food amendments seek to grant individuals the right to either prepare, consume, or vend homemade food items without any regulatory interference, hunger free campus statutes seek to reduce food insecurity amongst students on college campuses.
    - **Purpose**
        - This study investigates the prevalence and variation in these laws across states to identify commonalities, differences, gaps, and public health rationales.
    - **Methods**
        - Both published and unpublished literature sources will be extracted from various electronic databases. Each article will be thoroughly reviewed in order to identify key themes, gaps, and concepts. Thereafter, each theme will be synthesized by using compare and contrast methods. Emergent themes that are identified during this qualitative research process will be included in the structure of this literature review.
    - **Results**
        - Results of this study will provide the U.S. Food and Drug Administration a more comprehensive understanding of these newly passed laws, their potential impact on retail food safety, and implications for existing and future retail food safety regulations.
    - **Implications**
        - This study will analyze the potential food safety of Food Freedom Laws and Hunger Free Campus Statutes.

11. **Abstract Title:** *Collation of CFSAN Foodborne Pathogen WGS Metadata from Disparate Research Projects*

Authors: Tyree Scott FDA/CFSAN (Student); Hugh Rand FDA/CFSAN (Mentor); Rebecca Bell FDA/CFSAN (Mentor); James Pettengill FDA/CFSAN (Mentor)

FDA Strategic Initiative: Unleashing the Power of Data

Abstract:

- **Synopsis**
  - FDA's Center for Food Safety and Applied Nutrition (CFSAN) researchers have conducted numerous projects that have resulted in a large collection of foodborne pathogen isolates with their associated metadata. Metadata has been stored in disparate formats and locations which reduces the utility of the data. The goal was to create a database integrating the metadata in one central place. This allows for more effective use of this data. A program was written to process the disparate metadata files. The files were organized, and columns standardized so that they were consistent across the various tables and could be merged without errors; this also required managing the different hierarchical levels of each table so they would be labeled correctly once read in. The program was written in Python using various Python packages such as Pandas, Numpy, SQLite, and SQLAlchemy. The resulting code creates a database that can be searched for a specific ID number and all related information will print out. The current program processes 1693 rows of data from four tables into a single uniform table. There are 376 rows of data from Virginia, 156 rows of data from Delaware, 260 rows from North Carolina and 901 rows of data from various other states collected through contract researchers. This represents data collected over eight years. Integrating all this data expedites queries for metadata on isolates linked to outbreaks and helps prioritize outbreak response efforts. The results will be used to improve the FDA's ability to harness data collected over the years on food-borne pathogens from the environment in situations when those pathogens match those found in outbreaks or facility investigations. Finding matches to previously collected isolates provides much needed context in outbreaks and can provide clues to root-cause analysis in facility investigations. Rapid access to our historical data speeds up FDA's ability to respond to outbreaks and compliance findings.
- **Purpose**
  - Foodborne pathogens are biological agents that can cause a foodborne illness event; these biological agents include viruses, bacteria, and parasites. The CDC estimates that 48 million Americans become sick due to foodborne pathogens each year. The FDA's Center for Food Safety and Applied Nutrition (CFSAN) researchers have conducted various projects over the years that have resulted in a large collection of foodborne pathogen isolates with their associated metadata. Due to the many different projects and the many years of research, metadata have been stored in disparate formats and locations which reduces

the utility of the data. My goal for this summer was to create a database so that the metadata could be stored in one central place. This will allow for more effective use of this data for public health safety.

- **Methods**
  - A program was written to process excel files containing the isolate metadata. To ensure the data would be read into the program correctly the excel files had to be organized. I renamed the columns so that they were consistent across the various tables and could be merged without errors; this also required managing the different hierarchical levels of each sheet so they would be labeled correctly once read in. The program was written in Python using various Python packages such as Pandas, Numpy, SQLite, and SQLAlchemy. The resulting code creates a database that can be searched using a specific ID number and all related information will print out. During the 10-week project, a daily progress report provided a summary of activities and results.
- **Results**
  - The current program processes 1693 rows of data from four tables into a single uniform table. There are 376 rows of data from Virginia, 156 rows of data from Delaware, 260 rows from North Carolina and 901 rows of data from the various other states collected by IEH. This represents data collected over eight years. Integrating all this data expedites queries for metadata on isolates linked to outbreaks and helps prioritize outbreak response efforts.
- **Implications**
  - The results will be used to improve the FDA's ability to harness data collected over the years on food-borne pathogens from the environment in situations when those pathogens match those found in outbreaks or facility investigations. Finding matches to previously collected isolates provides much needed context in outbreaks and can provide clues to root-cause analysis in facility investigations. Rapid access to our historical data speeds up FDA's ability to respond quickly and enhances FDA's public health mission.

National Center for Toxicological Research (NCTR)

1. **Abstract Title:** *Exploring CYP450 Enzymes Changes During Pregnancy for PBPK Modeling Use*
   Authors: Brackens, Chloe, FDA/NCTR (Student); Fairman, Kiara FDA/NCTR (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract:
   - **Synopsis**
     - Due to physiological changes during pregnancy, drug pharmacokinetics (PK), including absorption, distribution, metabolism and excretion, is altered. Among these, are changes in enzyme activity and abundance. Drugs such as phenytoin and piperaquine have available PK data that could be used to estimate any physiological changes caused by pregnancy. Therefore, data was gathered from

literature about pregnancy, PK data and various drugs and enzymes. Phenytoin and piperaquine were used to analyze changes in CYP2C9 and CYP2C8 activity, respectively. A percent increase for the Cmax and AUC of piperaquine was calculated. More research should be done to identify other changes in various drugs metabolized by different enzymes for pregnancy. Using drug PK data to predict and show changes in physiology during pregnancy can be useful to the medical and regulatory fields.

- **Purpose**
  - Physiologically based pharmacokinetic (PBPK) modeling can be used to estimate the absorption, distribution, metabolism and excretion (ADME) of drugs. PBPK modeling is useful when little to no data is available about the pharmacokinetics (PK) of a drug, such as during pregnancy. Physiological changes in pregnancy affect ADME due to enzyme activity and abundance changes. However, the margin of change is uncertain and can be difficult to accurately estimate. It is assumed that CYP2C9 metabolizes 10-20% of the most commonly used drugs, such as phenytoin. CYP2C8 metabolizes 5% of currently used drugs, such as piperaquine. Both piperaquine and phenytoin are metabolized by enzymes with known pregnancy ontogeny equations in addition to CYP2C8 and CYP2C9. We aim to use available PK data and CYP activity to estimate changes of enzymes, such as CYP2C9, during pregnancy by comparing pregnancy and non-pregnancy PK data. After analyzing the data, we will propose an equation that demonstrates the enzyme change which will be utilized in other PBPK models to confirm the equation accuracy.
- **Methods**
  - All data was gathered from previously published literature including published equations for CYP2D6, CYP1A2, and CYP3A4. A table consisting of drugs used during pregnancy and metabolizing enzymes was created. Based on this table, CYP2B6, CYP2C8, CYP2C9, and CYP3A5 were researched. A PubMed search was performed for “CYP2C9” and “pregnancy”, resulting in 44 articles. Phenytoin was identified as the most frequently reported probe for CYP2C9 and researched by combining key terms “phenytoin”, “pregnancy”, and “pharmacokinetics.” “Phenytoin” and “PBPK” were also searched. Piperaquine was identified due to the presence of pregnancy and non-pregnancy data. Key terms, “piperaquine”, “pregnancy”, and “pharmacokinetics” as well as “Piperaquine” and “PBPK” were searched. Any available PK data and PK models, especially regarding pregnancy, was collected. Pregnancy and non-pregnancy PK was compared.
- **Results**
  - The PubMed search for “phenytoin”, “pregnancy”, and “pharmacokinetics” with the human data filter yielded 108 results. “Phenytoin” and (“PBPK” or “Physiologically Based Pharmacokinetic Model”) gave 27 results. Overall,

literature suggests that CYP2C9 activity increases over the first, second and third trimesters. The PubMed search for “piperaquine”, “pregnancy”, and “pharmacokinetics” with the human data filter yielded 24 results. “Piperaquine” and (“PBPK” or “Physiologically Based Pharmacokinetic Model”) gave 3 results. Overall, literature suggests that CYP2C8 activity decrease during the first, second, and third trimesters. After our initial review of piperaquine, the AUC and Cmax in pregnant women was approximately 12.37% and 81.37% more than that of non-pregnant women, respectively. We are in the process of comparing additional pregnancy and non-pregnancy PK data for both phenytoin and piperaquine to better quantify changes in enzyme activity through ontogeny equations PBPK modeling.

- **Implications**
    - Using PBPK modeling to estimate enzyme changes and drug pharmacokinetics during pregnancy is very beneficial to the regulatory and medical field. Regulatory reviewers of drug applications can use this information to inform decisions on new and existing drugs that are metabolized by CYP enzymes during pregnancy. Specifically, CYP450 data can be used not only when dosing medications, but also when accessing potential adverse effects from a drug without having to amass large amounts of clinical data on drugs and thus reducing the risk of harm to the fetus or the pregnant persons. Furthermore, this research shines a light on the need to elucidate maternal pharmacokinetics through increased clinical trial participation and novel alternative methods to ensure the health and safety of the pregnant population.

44. **Abstract Title:** *A Comprehensive Investigation of TabNet on Drug-Induced Liver Injury Prediction*
Authors: Anahita Eshaghi, Weida Tong, Zhichao Liu
FDA Strategic Initiative: Increasing Choice and Competition through Innovation and Unleashing the Power of Data
Abstract:

- **Synopsis**
    - Tabular data is one of the most popular formats to record experimental results and collect signals from various analytical technologies in toxicology. Artificial intelligence (AI) has shown great promise in image, video, and text data formats but gained limited success on tabular data. The proposed TabNet is a powerful and interpretable canonical deep learning architecture, which was able to outperform some leading machine learning algorithms such as XGBoost on various tabular datasets. However, it is still an open question whether the initial success of TabNet could be leveraged into different toxicity predictions critical to drug safety evaluation and risk assessment. In this study, we developed TabNet-based drug-induced liver injury (DILI) prediction with chemical structure-based data sets, including DILIst. We comprehensively investigated the prediction power and interpretability of the developed TabNet-based toxicity prediction models. We found that the developed TabNet-based toxicity

prediction models yielded respective (Matthews Correlation Coefficient) MCC, accuracy, AUC, sensitivity, and specificity values of 0.34, 0.69, 0.68, 0.82, 0.50, outperforming the published models with conventional machine learning algorithms (i.e., LR, SVM, KNN, random forest, XGBoost). Furthermore, developed TabNet-based DILI prediction model generated comparative performance with the DeepDILI, which is the current best published DILI prediction mode. Moreover, the proposed TabNet-based DILI prediction model used compound-wise feature selection, enabling the personalized prediction of the individual compound by fusing different feature contributions, enhancing the model explainability, and facilitating real-world applications. In summary, TabNet could be a promising tabular deep learning architecture for toxicity prediction with enhanced model explainability in the preclinical setting.

- **Purpose**
    - This research focuses on using TabNet, a deep learning (DL) program, with tabular data to advance toxicity prediction for drug-induced-liver injury (DILI). TabNet was published by Google and has outperformed a multitude of well-established machine learning algorithms for tabular data. A limitation posed by DL is that tabular data is the data type most used in the real-world and plays a significant role in the biomedical field, yet it remains largely unexplored. By incorporating a tabular DL architecture, this prediction model holds great promise for toxicity prediction. The goal is to leverage the toxicity prediction power of TabNet to evaluate drug safety and risk assessment to reduce adverse drug reactions, a major concern for novel drugs. Specifically predicting DILI, a major cause of drug failure and acute liver failure. This will not only play a large role in advancing DL algorithms for tabular data, but also promote drug safety.
- **Methods**
    - TabNet, a novel deep learning algorithm, was proposed by Google AI for modeling tabular data. There are several advantages of TabNet for predictive model development. First, the TabNet accepts the raw tabular data without any preprocessing required. Second, TabNet enables better learning and interpretability by using sequential attention at each decision step. Third, the feature selection in TabNet is instance-wise, allowing the enhanced model interpretability for individual samples. Here, we employed the utility of TabNet in toxicity prediction. Specifically, we will utilize the drug-induced liver injury as a showcase to comprehensively assess the performance of TabNet and further compared to the state-of-the-art machine learning/deep learning algorithms.
- **Results**
    - We found that the developed TabNet-based DILI prediction model yielded respective (Matthews Correlation Coefficient) MCC, accuracy, AUC, sensitivity, and specificity values of 0.34, 0.69, 0.68, 0.82, 0.50, outperforming the published models with conventional machine learning algorithms (i.e., LR, SVM,

KNN, random forest, XGBoost). Furthermore, developed TabNet-based DILI prediction model generated comparative performance with the DeepDILI, which is the current best published DILI prediction mode. Moreover, the proposed TabNet-based DILI prediction model used compound-wise feature selection, enabling the personalized prediction of the individual compound by fusing different feature contributions, enhancing the model explainability, and facilitating real-world applications.

- **Implications**
    - This research has strong implications for not only tabular deep learning architecture, but also for toxicity prediction, ensuring enhanced model explainability in the preclinical setting. Deep learning has become useful due to its capacity to be able to orchestrate a variety of tasks for large datasets, yet deep learning architecture for tabular data is greatly underexplored. This research will advance being able to analyze and program experimental results with tabular data, one of the most popular data formats. Additionally, this research can have strong positive impacts on drug safety and public health. In drug discovery, the safety of a drug is a major challenge. By creating a model that will improve toxicity prediction, the cost and time of drug evaluations will be reduced while improving drug safety. Specifically applying TabNet's prediction model to drug-induced liver injury (DILI), which continues to be a significantly challenging in regulatory and clinical arenas, a major cause of drug failure will be reduced, and patient safety will be improved.

2. **Abstract Title:** *Pharmacokinetics of Zileuton Nanocrystal Drug Formulation using rat model*
Authors: Lee, Angela, FDA/NCTR (Student); Wickramaratne, Bhagya, FDA/NCTR (Mentor); Khare, Sangeeta, FDA/NCTR (Mentor); Gokulan, Kuppan, FDA/NCTR (Mentor)
FDA Strategic Initiative: Empowering Patients and Consumers
Abstract:
    - **Synopsis**
        - The bioavailability of an oral drug depends on physiochemical properties, dissolution rate, solubility, and permeability in the gastrointestinal tract (GIT). Drug solubility data shows that 40% of approved and 70% of pipeline drugs are poorly soluble (BCS class II and IV) in aqueous solution, which poses a challenge in achieving optimal bioavailability and therapeutic efficacy during drug development. Nanocrystal drug (ND) formulation overcomes the poor solubility of drugs and improves the absorption and bioavailability. Zileuton (ZIL), a BCS class II drug, is used to treat asthma. ZIL inhibits 5-lipoxygenase in the leukotriene production pathway, thereby reducing asthmatic inflammation of the airways, and was chosen as a candidate ND. To evaluate drug solubility and permeability of the ND, 10-week-old male and female Sprague Dawley rats were dosed with gelatin capsules containing one of the following: i) active pharmaceutical ingredient (API), ii) physical mixture (PM), or iii) ND ZIL (600 nm size), or iv) an excipient (Kollidon VA64). The study also consisted of placebo and untreated control groups. The rats were housed in a metabolic cage for the first 24 hours where blood, and urine samples were collected. We evaluated plasma

ZIL concentration at 1hr, 2hr, 4hr, 6hr and 24hr post-ND administration and compared the results with plasma from API and PM treated rats. In addition, we collected fecal and urine samples, performed liquid-liquid extraction, and then used HPLC-MS to determine the concentration of ZIL in each of these samples. In plasma, the level of ZIL was higher in female rats treated with ND, API and PM than males. Our earlier study shows that intestinal phase-1 and phase-II metabolic enzymes are highly upregulated in males as compared to female animals and it can be correlated for the low level of ZIL in males. This study assesses the cytotoxic and immunotoxic effects of nano-formulated drug at the level of GIT.

- **Purpose**
  - While the nanocrystal platform offers several advantages over conventional drugs, due to its complexity, a wide translational gap exists between this platform and its clinical trials. There are several areas requiring further investigation to understand the interaction of nanocrystals in the biological system and its safety assessment. The potential risk factors predicted to be associated with nanocrystals are surface chemistry (charge), biophysical changes due to nano-formulation of API, its interaction with GIT, immunotoxicity, interaction with biological organelles, and biodegradation of nanocrystal drugs/excipients/ingredients. In addition, nanoparticles <150 nm can be internalized by many cell types via opsonization. However, particles >150 nm can be internalized only by macrophages that can be cytotoxic and immunotoxic. Therefore, it is important to investigate cytotoxic and immunotoxic effects of nanocrystal drugs. Science-based evidence is required to understand the following questions: 1) Do nanocrystal drugs affect the permeability of the intestinal epithelial layer? 2) What would be the impact of nanocrystals on intestinal commensal bacteria? 3) Would an increase in concentration of nanocrystal have any impact on commensal bacterial colonization or activation of intestinal immune cells? Here, our focus is to evaluate the drug solubility and permeability of ND in comparison with the non-nanosized parent drug/PM using animal models. Effects of Kollidon will be assessed in a separate study.
- **Methods**
  - To evaluate drug solubility and permeability of the ND, Sprague Dawley rats (10 weeks old male and female) were dosed were dosed with gelatin capsules, via oral gavage, containing one of the following: i) active pharmaceutical ingredient (API), ii) physical mixture (PM), or iii) ND ZIL (600 nm size), or iv) an excipient (Kollidon VA64). Two control groups were used: i) untreated rats and ii) rats treated with empty capsule. The rats were quarantined in a metabolic cage for the first 24 hours, at which time we collected blood plasma, fecal, and urine samples from each group; after the 24 hours, we housed the rats in separate gender and experimental groups, and continued drug treatment for 15 days. On day 15 animals were sacrificed and samples (plasma, fecal, and colon) were collected for various biochemical, immunological, and PK/PD endpoint analysis. We performed liquid-liquid extraction on the samples and used HPLC-MS to determine the concentration of ZIL in each sample. We evaluated plasma concentration of ZIL at various time points after drug treatment (1hr, 2hr, 4hr, 6hr and 24hr) and compared the results with urine and fecal sample results.

- **Results**
    - We analyzed the blood plasma and the urine samples. In plasma, the level of ZIL was higher in female rats treated with ND, API and PM than male rats. Male API rats had the highest plasma concentration 1 hour after treatment, while female API rats had the highest concentration 2 hours after treatment. Both male PM and ND rats had the highest plasma concentration 2 hours after treatment, while both female PM and ND rats had the highest concentration 1 hour after treatment. There was no statistically significant difference between urine concentrations of API, ND, PM, male, or female rats.
- **Implications**
    - The proposed study will generate experimental data to better understand the effects of nanocrystal-drug, API, and parent drug on the intestinal permeability, cytotoxicity, adherence capacity, immune activation, and commensal bacterial community structure in the GIT. The result will provide a proof of principle if these changes may contribute for the adverse effects of nanocrystal-drug use. The study is consistent with the FDA's research priorities to improve safety assessments on drugs, protect public health, and address the 2018 Strategic Priority to "strengthen FDA's scientific workforce and its tools for efficient risk management" by enhancing and "modernizing our regulatory toolbox."

3. **Abstract Title:** *Evaluation of Pericytes in a Double Transgenic (APPPS1) Rat Model of Alzheimer's Disease*

   Authors: Eswaran, Surabhee, FDA/NCTR (Student); Sarkar, Sumit, FDA/NCTR (Mentor); Raymick, James, FDA/NCTR (Lab Technician)

   FDA Strategic Initiative: Public Health Emergency Preparedness and Response

   Abstract:

   - **Synopsis**
       - Alzheimer's Disease (AD) is a neurodegenerative disease in which AD patients experience cognitive and eventually physical decline. AD has been associated with the loss of pericytes, in addition to the formation of amyloid-plaque and neurofibrillary tangles. Pericytes are found in the neurovascular unit, along with endothelial cells, microglia, and astrocytes. These cells are situated within the blood-brain barrier and reinforce its structure, remove toxicity from the brain, and manage blood flow. In this study, the presence of pericytes was evaluated in non-transgenic (NTG) rat and transgenic or AD rat. These rats were injected with Fluoro-Ruby-dextran (FR), a retrograde tracer, in both lateral brain ventricles to observe pericytes. With this method, it was determined there was a significantly larger presence of pericytes in the cortex of the non-transgenic rats than the AD rats. This was evidenced by the non-transgenic rats having area fractions of pericytes that were 0.00318, for male rats, and 0.00711, for female rats, greater than the AD rats. Effect of AD was significant in the hippocampus of the female rat than their male counterparts. Fluoro-Gold (FG), another retrograde tracer, was used to label endothelial cells in the brain. The results have demonstrated that there was a greater area fraction of these endothelial cells in the non-

transgenic rats, in comparison to the AD rats. Lectin, a marker for blood vessels, showed that the microvessels in AD rats were ischemic and shorter in length compared to wild type rats. Lectin also showed vascular inflammation in AD rat brains, due to its ability to label activated microglia. In contrast, the non-transgenic rat brains had healthier microvasculature. These results reinforce the foundational concept of pericyte degeneration during AD, with rats as the model.

- **Purpose**
  - Alzheimer's Disease (AD) is a neurodegenerative disease in which AD patient experience cognitive and eventually physical decline. AD is related to the presence of amyloid-plaques and neurofibrillary tangles, in addition to the loss of pericytes. These developments are linked to the neurovascular unit (NVU). The NVU is composed of pericytes, endothelial cells, microglia, and astrocytes. These cells are found in the blood-brain barrier and together, reinforce its structure and regulate blood flow and brain toxicity. This study is concerned with the NVU, more specifically pericytes, in non-transgenic (NTG) rats and transgenic rats, which have the APPPS1 mutation and therefore exhibit AD-like degeneration with plaques in the brain. This allows for the evaluation of the presence of pericytes in AD and NTG rats. Furthermore, the purpose of this study is to facilitate the reinforcement of foundational concepts, such as pericyte loss and vascular degeneration in AD, and to investigate association between the cells in the NVU during AD.
- **Methods**
  - In this study, the rat AD model used was transgenic, with the APPPS1 mutation. This allowed for the development of parenchymal and vascular plaque in the rat brain. To visualize pericytes in the brain, the rats were injected with Fluoro-Ruby-dextran, a retrograde tracer, in both lateral brain ventricles. After the brains were mounted, the pericytes were evaluated using a fluorescence microscope and NIS Elements BR Analysis. The rats were injected (I.P.) with Fluoro-Gold to view endothelial cells. They were perfused, cryoprotected, and eventually mounted. Microvessel and vascular inflammation were visualized using lectin. The brain sections were incubated in tomato lectin conjugated with DyLight 488 and also mounted. The inflammation and endothelial cells were evaluated using the fluorescence microscope and ImageJ. IBA1 (to view microglia) and GFAP (to view astrocytes) immunolabeling were used to identify associations between cells in the NVU. Free floating sections were incubated in either GFAP or IBA1 antibody overnight, mounted, and observed.
- **Results**
  - The results demonstrated that there was a significantly larger presence of pericytes in the cortex of NTG rats than AD rats. This was evidenced by NTG rats having area fractions of pericytes that were 0.00318, for male rats, and 0.00711, for female rats, greater than AD rats. Effect of AD was significant in the hippocampus of the female rats than their male counterparts. Fluoro-Gold showed that there was a greater area fraction of endothelial cells in NTG rats than AD rats. Lectin analysis indicated that in the cortex, the female NTG rats had 5.35% greater area of vasculature than the AD rats. The male NTG rats had 4.58% greater area than the male AD rats in the cortex. The hippocampus of male and female NTG rats had greater percent areas of vasculature than AD

rats. Lectin also showed that AD rats experienced ischemia, shorter microvessels, and vascular inflammation, in contrast to healthy microvasculature of NTG brains. Regarding the NVU, this study visually demonstrated that activated microglia and astrocytes, pericyte loss, and plaques can be found together in AD brains.

- **Implications**
    - The results of this study reinforce the foundational concept of pericyte degeneration during AD, with rats as the model. It also demonstrates that endothelial cell dystrophy, increased inflammation, and activated microglia are also largely associated with AD. Furthermore, this study visually presented the following relationships: (1) pericyte and endothelial cell degeneration can be found together in AD, and (2) activated microglia and astrocytes are found in the AD brain, more specifically near plaques. These results can be potentially used to further investigate the neuropathological aspect of AD. It could also be beneficial in the maintenance of a strong blood-brain barrier in AD patients and the development of AD treatment, in that pericytes, along with other cells in the NVU, could be targeted to prevent AD progression.

4. **Abstract Title:** *Acute, reproductive, developmental, and genetic toxicities of N-nitrosodiethylamine evaluated in Caenorhabditis elegans*
   Authors: Huynh, Tai, FDA/NCTR (Student); Yan, Yian, FDA/NCTR (Co-Mentor); Pan, Bohu, FDA/NCTR (Co-Mentor); Chen, Tao, FDA/NCTR (Mentor)
   FDA Strategic Initiative: Public Health Emergency Preparedness and Response
   Abstract:
    - **Synopsis**
        - In this study, we proposed using Caenorhabditis elegans as an alternative model to investigate the toxicological effects of N-nitrosodiethylamine (NDEA). NDEA is one of several N-nitrosamines that were detected in FDA-regulated drugs resulting in multiple recalls recently. These contaminations present a public health risk due to their carcinogenicity and mutagenicity. Since C. elegans is a model organism that shares many common structural and genetic qualities with mammals and humans, the results from this study can be inferenced on the possible effects of NDEA on public health. We have conducted experiments to test worm activity using a WMicroTracker (acute toxicity), reproductive (fecundity, brood size, hatchability), developmental (growth rate, body length and lifespan) and genetic (mutation frequencies and types) toxicities using different concentrations of NDEA. Due to the high possibility of detecting other nitrosamines in FDA-regulated drugs, we wanted to compare the results from this study to those from rodents to determine whether C. elegans mode can replace or complement the reproductive, developmental and genetic toxicity studies for this class of chemical in rodents.
    - **Purpose**
        - N-nitrosamines are well known impurities in medicines while a large number of them are mutagenic and carcinogenic. Recently, several N-nitrosamines have

been detected in drugs, leading to renewed interest in understanding how these chemicals exert their biological effects on cellular systems. The toxicological effects of N-nitrosamines have been mainly studied in mammalian cells. Due to the difficulty of the in vitro system for metabolic activation of N-nitrosamines, more studies using in vivo systems are required. In recent years, Caenorhabditis elegans has been becoming a promising in vivo system because it is a relatively cheap and quick way to assess possible toxicities of food and drug products. These small nematodes (~1mm in length for adults) provide advantages due to their simple see-through organismal structure, fast growth and short reproduction period. Because of many common structural and genetic qualities with mammals, C. elegans have become an alternative animal mode for toxicity testing.

- **Methods**
  - In this study, acute toxicity, reproductive and developmental toxicity, and genotoxicity of N-nitrosodiethylamine (NDEA), a typical nitrosamine impurity found in several FDA-regulated drugs have been investigated using C. elegans. NDEA is a well-known mutagenic impurity and a potential human carcinogen that is generated during drug processing. C. elegans were treated with different concentrations of NDEA at L4 development stages for measurement of the compounds' acute toxicity, reproductive toxicity, developmental toxicity, and genotoxicity. Acute toxicity has been evaluated with a WMicroTracker to determine toxic effects of the different concentrations of this compound on the worms' movement and to set a baseline for treatments. The reproductive toxicity will be examined by assessing NDEA's effects on fecundity, brood size, and hatchability. The developmental toxicity will be carried out to investigate NDEA's effects on worms' growth rate, body size and lifespan. The genotoxicity will be evaluated using whole genome sequencing to verify whether NDEA can induce mutations.
- **Results**
  - The results from this study will be compared with those from other in vivo and in vitro toxicity studies to determine whether C. elegans can be a good alternative testing methodology for assessing the developmental, reproductive, and genetic toxicity of N-nitrosamines.
- **Implications**
  - This study may be able to further elucidate the toxicological effects of N-nitrosamines and help support regulations and guidelines to address the public concerns over this type of contaminations in drugs.

5. **Abstract Title:** *Identification of Clinical Signatures for Drug-Induced Liver Injury from Case Reports in Literature*
   Authors: Habib, Mishkat, FDA/NCTR (Student); Zhao, Jingwen, FDA/NCTR; Liao, Tsung-Jen, FDA/NCTR; Hayashi, Paul, FDA/CDER; Minjun Chen FDA/NCTR (Mentor)

FDA Strategic Initiative: Unleashing the Power of Data
Abstract:

- **Synopsis**
  - The purpose of this project is to create a database which can be used to identify and specify DILI clinical signatures for individual FDA approved drugs. To do this, first we collected thousands of DILI cases from literature and recorded patient demographics, curated data from the time when the medication was taken such as symptoms, biopsy description, liver function test results, outcome and also determined the R factor value to deduce the type of injury. We also used either an expert's opinion or a scoring instrument to confirm the effects of a drug on a patient. Data from 600 case reports confirmed that DILI cases are most common in Europe and America and that most patients recovered. This data can be used by clinicians and regulators when DILI issues are encountered.
- **Purpose**
  - Drug Induced Liver Injury (DILI) is an injury of the liver caused by prescribed, over the counter, or herbal medicines. These injuries are the main reasons why many drug candidates failed during clinical trials and are the most common cause of acute liver failure in the US and Europe. In this project, we aim to create a database which will be used to identify and specify DILI clinical signatures for individual FDA approved drugs which includes but not limited to symptoms, onset, 50% decrease date, clinical outcomes, and histological findings.
- **Methods**
  - We firstly collected thousands of DILI case reports from literature. To create the database, we curated data from these case reports about the DILI clinical development course from the time point when the medication was taken. We recorded the patient demographics which include age, race and gender. We also recorded the drug name, start date, end date, onset date and corresponding liver function tests, peak date and corresponding liver function tests, 50% decrease date and corresponding decrease criteria, follow-up date and corresponding outcome, date of biopsy and corresponding biopsy description and symptom date with all the observed clinical symptoms. We retrieved the serum chemistry tests at DILI onset and peak, such as alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBL), and will determine DILI biochemical injury types based on the serum chemistry test at DILI onset by calculating the R factor value using the equation $R=(ALT/ULN)/(ALP/ULN)$. If $R>5$ the injury is hepatocellular, if $R<2$ it is cholestatic, and if $R=2-5$ it is mixed. After creating this database, we conducted statistical analysis to identify potential patterns of the DILI signatures associated with individual drugs or drug classes and also search for correlation between DILI outcome and host factors.
- **Results**
  - We have collected 600 DILI cases which are from different countries around the world. 41% are from North America, 32.97% are from Europe, and 6.7% are from Asia. 51% of the patients were female and 49% of the patients were male. The ages of patients varied from 1 to 90 with the average age being 47. The patients belonged to different races such as White (69%), Hispanic (4.6%), Black

(16%), and Asian (6.9%). The outcome showed that 65% of patients recovered from DILI, 13.8% died, 1.6% died due to Acute Liver Failure, 0.37% went into remission after Acute Liver Failure, and 3.14% underwent a liver transplant, 2.2% faced chronicity, and 1.48% died after liver transplant. 14% of the patients developed a rash, 21% developed fever, 10.6% developed pruritus, and 38% developed jaundice and some of the patients had unknown symptoms. 13.3% of all the patients were rechallenged with the same drug which caused injury to their liver to confirm DILI. Only 11.29% of the cases were analyzed through a scoring instrument and the rest were analyzed through an expert's opinion. The scoring instruments included RUCAM/CIOMS, Naranjo, and CDS among others.

- **Implications**
    - This information would ultimately be used as a reference for clinicians and regulators' decision-making when DILI issues are encountered.

6. **Abstract Title:** *Modeling Alzheimer's Disease's Vascular Pathology Using Human Induced Pluripotent Stem Cells*
Authors: Pidugu, Alekhya, FDA/NCTR (Student); Rosas-Hernandez, Hector, FDA/NCTR (Mentor); Matazel, Katelin, FDA/NCTR (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

    - **Synopsis**
        - Alzheimer's Disease (AD) is a progressive neurodegenerative disease that is the primary cause of dementia. AD pathology is characterized by accumulation of amyloid beta(Aβ) aggregates and build-up of tau protein, contributing to neuronal death. In addition, the function of the blood-brain barrier (BBB) is disrupted by the accumulation of these toxic aggregates. Pre-clinical models used to study AD include transgenic mice and rats that overexpress human genes associated with AD pathology. However, they fail to recapitulate the full spectrum of human AD pathology including neuronal loss and BBB dysfunction. To overcome limitations in modeling AD pathology, novel technologies that recapitulate human-specific characteristics have been developed, including the use of human induced pluripotent stem cells (hiPSCs). hiPSCs are derived from somatic cells and have been reprogrammed into a pluripotent state, enabling derivation of any cell type. In this study, hiPSC-derived brain microvascular endothelial cells (iBMVECs) will be used to characterize some of the pathological features observed in AD brain vasculature. hiPSCs from a healthy individual and an AD patient were cultured in cell-specific media with the chemical cues necessary to induce differentiation into iBMVECs.After differentiation, proteins related to cellular identity and blood-brain barrier function were analyzed by western blot and immunocytochemistry (ICC). Compared to iBMVECs derived from a healthy subject, AD-derived cells presented differential expression of markers of neurovascular function, including decreased expression of the tight junction protein ZO-1 and the membrane-bound transporter LRP-1, which is implicated in Aβ clearance. These results suggest that iBMVECs differentiated from hiPSCs from AD patients can be used to model key features related with the neurovascular pathology observed in AD and may be a powerful tool for efficacy testing of FDA-regulated products for the potential treatment of AD.

- **Purpose**
  - Alzheimer's Disease (AD) is progressive neurodegenerative disease that is the primary cause of dementia. Alzheimer's is believed to be caused by the loss of neuronal connections and the build of amyloid beta and tau proteins in the brain. The function of the blood-brain barrier (BBB), which regulates the entry of molecules into the brain with specific endothelial cells and tight junctions, is disrupted by the accumulation of amyloid proteins. The most common research models used to study Alzheimer's are transgenic mice that overexpress human genes associated with AD. They have been widely used in preclinical testing for potential treatments. However, they fail to recapitulate the full spectrum of human AD pathology including neuronal loss and BBB dysfunction.

    Human induced Pluripotent Stem Cells (hiPSCs) are cells that have been reprogrammed into a pluripotent state that enables derivation of an unlimited source of type of human cell. These cells allow for the derivation of disease-relevant target cells for human disease modeling which can provide information about the cellular and molecular mechanisms involved. In this study, induced brain microvascular endothelial cells (iBMVECs) will be used in order to model and study the human BBB in healthy versus AD patients.
- **Methods**
  - hiPSCs derived from a cognitive healthy individual and from an AD patient with the APOE4 allele were obtained. hiPSCs were plated in Matrigel-coated 6-well plates and maintained in mTeSR™1 medium to avoid spontaneous differentiation. hiPSCs were cultured for 2-3 days until a density of 2-3X105 cells/well was reached and then differentiated in unconditioned medium. Culture medium was replaced daily for 6 days and replaced with serum-free endothelial cell medium.

    Expression of tight junction proteins (claudin-5, occludin and LRP-1) and membrane-bound transporters for which Aβ is a substrate (LRP-1, RAGE and P-gp) were analyzed by immunocytochemistry. After differentiation was achieved, cell were fixed. Then they were Incubated with antibodies overnight, followed by incubation with fluorescent-dye conjugated secondary antibodies. Images were be acquired using a fluorescence confocal microscope. For western blot analysis, 15 µg of protein extract from iBMVECs was loaded into native tris-glycine gels and run for 1 hour at 200 V. Proteins were then transferred to polyvinylidene difluoride (PVDF) membranes, blocked, and then incubated with primary antibodies. After primary antibody incubation, membranes were incubated with secondary antibodies. Images were acquired with Odyssey CLx imaging. Proteins of interest were quantified with Image Studio Ver 5.0 and normalized by β- actin.
- **Results**
  - The hiPSCs were differentiated into iBMVECs as confirmed by the expression of the endothelial marker CD31. The iBMVECs expressed proteins related to blood brain barrier (BBB) functions as shown by immunocytochemistry (ICC) and western blots. These included the membrane-bound transporter proteins P-glycoprotein (P-gp), Low-Density Lipoprotein Receptor-related Protein-1 (LRP-1), and receptor for the advanced glycation end products (RAGE), as well as the

tight junction proteins: zonula occludens-1 (ZO-1), Occludin and Claudin-5. The analysis of the western blot results suggested that compared to the control cells, AD iBMVECs express lower levels of ZO-1 but higher levels of occludin. These results could be due to a feedback mechanism in the AD cells in order to compensate for the lack ZO-1 within the cell membrane of the iBMVECs. The results also suggested that the localization of Claudin-5, Occludin, and ZO-1 is limited to the cell-to-cell contact in control iBMVECs, suggesting functional tight junctions. In AD-derived iBMVECs the tight junction proteins Claudin-5 and ZO-1 are localized at the cell-to-cell junctions, but Occludin is localized at the cell-to-cell junctions and cytoplasm, suggesting dysfunctional tight junctions. Compared to control cell, AD iBMVECs express lower levels of LRP-1, which is involved in Amyloid Beta (Aβ) clearance.

- **Implications**
    - In the future, this data could be corroborated with function assays such as permeability and functional transporter assays to determine whether BBB function is disrupted due to the difference in distribution of tight junctions and transporter proteins in AD cells. Successful implementation of the AD hiPSCs cell model will allow researchers at the FDA and other research agencies to study potential treatments, drugs, and medical devices being developed for AD treatment. The results from this study can lead to other studies using human-specific technologies such as the organs-on-a chip. The chips could be seeded with patient derived hiPSCs in order to model the BBB of healthy versus AD patients. The organ-on-a-chip models also show promise in their pre-clinical capabilities. The implementation of the AD model will allow researcher to analyze the effects of specific drugs, treatments, and immunotherapies designed for AD treatment. These types of studies include but are not limited to permeability assays, analysis of transporters of specific drugs across the BB, and mechanisms of clearance of Aβ and tau plaques. Additionally, the hiPSC model can be further modified to developed studies for other CNS- related and neurodegenerative disorders such as Parkinson's disease.

7. **Abstract Title**: *Investigation into sex and strain specific variability of high fat diet on gastrointestinal tract and microbiome*
   Authors: Ponder, Jacob, FDA/NCTR (Student); Gokulan, Kuppan, (Mentor); Karn, Kumari, (Fellow); Khare, Sangeeta, (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract:
    - **Synopsis**
        - NAFLD is an increasingly prevalent disease that shows interindividual variability in severity, however, the cause of its variability is poorly characterized. A previous study was conducted using 25 strains of Collaborative Cross (CC) mice to determine strain specific differences of a high fat and high sucrose (HF/HS) diet. Recently, host-microbiome interaction, especially intestinal barrier effects and the gut-liver axis, has gained interest. This study focuses on the effect of the HF/HS diet on this interaction. Factors investigated in this study were intestinal epithelial cell integrity and microbial population. The goal of this study is to investigate strain and sex specific differences between two strains used in said previous study (CC042, CC011) that showed highly distinguished responses to

the diet. Tissues from male and female mice who were fed HF/HS diets or normal diets (20 weeks to 40 weeks) were used in this study*. The mRNA expression of 84 genes known to be associated with intestinal epithelial cell integrity were analyzed from mouse ileum using qPCR. Microbial gut population (fecal) was investigated by 16s rRNA sequencing. There was no difference in the mRNA expression in CC042 male animals (20 weeks). CC042 HF/HS females (20 weeks) show downregulation of *Gap junction protein beta 1* (*Gjb1*) (p=0.03; FR -4.59). CC011 male animals showed differential regulation of 3 genes: *Desmoglein-4 (Dsg4)* (p=0.01; FR 3.12), *Claudin-8 (Cldn8)* (p=0.02; FR -16.49), and *Gap junction protein alpha 1 (Gja1)* (p=0.0001; FR -3.35). CC011 HF/HS females (5 of 6) were moribund before week 20; endpoint analysis for this strain, as well as comparison of endpoints with CC042 is currently ongoing. There are currently no non-invasive methods of diagnosis for NAFLD, and this study will potentially aid in determining non-invasive methods for the predisposition to NAFLD.

**This study was a collaboration with Dr. Igor Pogribny, Division of Biochemical Toxicology, National Center for Toxicological Research, US FDA.*

- **Purpose**
  - Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasingly prevalent disease that shows interindividual variability in severity, however the cause of this variability is poorly characterized. NAFLD includes a spectrum of histological changes that begin with fat buildup in the liver (steatosis), which may progress to chronic inflammation (steatohepatitis or NASH), fibrosis, and cirrhosis. Only a subgroup of patients diagnosed with NAFLD progress to NASH and cirrhosis. One of the factors associated with the progression of NAFLD is a high fat and high sucrose (HF/HS) consumption, which is characteristic of the western diet. Recently, host-microbiome interaction, specifically intestinal barrier effects and its role in the gut-liver axis has gained interest. To find the effects on this interaction caused by NAFLD, a previous study was conducted using 25 strains of Cross Collaborative (CC) mice and a HF/HS diet to approximate NAFLD. CC mice are a population of mice designed to approximate the genetic variability of the human population. This study is a continuation of said previous study, focusing on 2 strains (CC042 and CC011) that showed highly distinguished responses to the diet.
- **Methods**
  - Male and Female mice (CC042 & CC011) were fed either HF/HS or normal diets for either 20 weeks or 40 weeks. Banked tissues from these mice were used in this study*. To determine intestinal epithelial cell integrity, mRNA was isolated from the ileum using TRIzol followed by a DNase treatment and cDNA conversion. The mRNA expression of 84 genes known to be associated with intestinal epithelial cell integrity was quantified using qPCR. The raw qPCR data was then processed using GeneGlobe analysis. *Beta-Glucuronidase (Gusb)* was used as a house keeping gene for normalization, and a fold change (FC) magnitude of > 2.00 and a p-value of < 0.05 were used as a threshold to determine significance. The microbial gut population was investigated by 16s rRNA sequencing on DNA isolated from fecal and ileal samples. A more general and expedited analysis of the microbial gut population was performed using

qPCR with primers for predominant phyla and representative genera and species of bacteria.

**This study was a collaboration with Dr. Igor Pogribny, Division of Biochemical Toxicology, National Center for Toxicological Research, US FDA.*

- **Results**
  - The intestinal epithelial cell integrity array included genes involved in the formation of tight junction, adherent junction, gap junction, focal adhesion, desmosomes and hemidesmosomes; a close interaction of these genes maintain the homeostasis at the intestinal mucosa. There was no difference in the mRNA expression in CC042 male animals (20 weeks). In contrast, CC042 HF/HS females (20 weeks) showed differential regulation of several genes (primarily down-regulation); only *Gap junction protein beta 1* (*Gjb1*) was significantly downregulated (p=0.03; FR -4.59). CC011 male animals showed significant differential regulation of 3 genes: *Desmoglein-4 (Dsg4)* (p=0.01; FR 3.12), *Claudin-8 (Cldn8)* (p=0.02; FR -16.49), and *Gap junction protein alpha 1 (Gja1)* (p=0.0001; FR -3.35). Male animals had multiple differentially expressed genes when compared by strain. 5 of the 6 CC011 strain HF/HS diet female mice were moribund before week 20; endpoint analysis for this experimental group is currently ongoing. Sequencing and qPCR for fecal DNA microbial population analysis is also ongoing. Several in the 40-week experimental group were moribund before termination schedule.
- **Implications**
  - At present, there are no criteria to identify the population that is predisposed to NAFLD. The only current diagnostic method for these diseases is liver biopsy, an invasive and dangerous procedure that can lead to morbidity and possibly mortality. This study will potentially aide in determining non-invasive methods (such as microbial population distribution as a biomarker or intestine-specific permeability marker in fecal samples) for the predisposition to NAFLD.

8. **Abstract Title:** *Effect of the weight loss drug lorcaserin on DNA methylation in mammary glands of Sprague Dawley rats*

   Authors: Roudachevski, Ira, FDA/NCTR (Student); Pogribny, Igor P., FDA/NCTR (Mentor); Tryndyak, Volodymyr P., FDA/NCTR (Mentor)

   FDA Strategic Initiative: Unleashing the Power of Data

   Abstract:

   - **Synopsis**
     - The development of cancer in humans is caused by irreversible modifications of the genome through genetic and epigenetic alterations resulting in the acquisition of multiple heritable abnormal cellular phenotypes. The term "epigenetics" defines phenomena and mechanisms that cause alterations in the expression of genetic information that are not due to changes in the primary DNA sequence. The epigenetic phenomena include DNA methylation, posttranslational histone modifications, and nucleosome positioning, among which DNA methylation is the major and most-studied epigenetic mechanism. In the present study, we investigated the effect of the weight-loss

drug lorcaserin on the status of cellular DNA methylome in Sprague Dawley rats. Lorcaserin was withdrawn from the US market after an excess cancer risk was identified in a safety clinical trial.

- **Purpose**
  - The development of cancer in humans is caused by irreversible modifications of the genome through genetic and epigenetic alterations resulting in the acquisition of multiple heritable abnormal cellular phenotypes. The term "epigenetics" defines phenomena and mechanisms that cause alterations in the expression of genetic information that are not due to changes in the primary DNA sequence. The epigenetic phenomena include DNA methylation, posttranslational histone modifications, and nucleosome positioning, among which DNA methylation is the major and most-studied epigenetic mechanism. In the present study, we investigated the effect of the weight-loss drug lorcaserin on the status of cellular DNA methylome in Sprague Dawley rats. Lorcaserin was withdrawn from the US market after an excess cancer risk was identified in a safety clinical trial.
- **Methods**
  - Female Sprague-Dawley rats received lorcaserin by gavage 7 days/week for 24 weeks, at doses previously used in a 2-year cancer bioassay (0, 30, and 100 mg/kg/day). DNA methylation changes were investigated in mammary glands, a target organ for lorcaserin carcinogenicity in rats, by reduced representation bisulfite sequencing.
- **Results**
  - Lorcaserin exposure resulted in dose-dependent DNA alterations in the mammary glands, evidenced by the presence of 1591 and 1961 significantly differentially methylated CpG sites (a Benjamin-Hochberg adjusted $p < 0.05$ and 20% DNA methylation difference) in the treatment groups, as compared to the control group. Pathway enrichment analysis of these differentially methylated CpG sites demonstrated their strong representation in genes associated with cell morphology, cellular function and maintenance, cellular movement, and molecular transport. Importantly, among these differentially methylated CpG sites, 437 CpG sites were in common, with 401 being changed in the same direction in both treatment groups. A detailed analysis of differentially methylated CpG sites demonstrated that while a number of hypomethylated CpG sites did not differ between treatment groups, the number of hypermethylated CpG sites was 1.3 times greater in rats treated with 100 mg/kg/day of lorcaserin than in rats treated with 30 mg/kg/day, especially in sites located in genome intergenic regions.
- **Implications**
  - In summary, we have demonstrated that lorcaserin induced extensive DNA methylation changes in mammary glands at early preneoplastic stages of

lorcaserin-induced rat carcinogenesis. These findings provide a strong support of the importance of epigenetic alterations in the carcinogenicity of lorcaserin.

9. **Abstract Title:** *A Study of Tumor Mutational Burden using Targeted Panel Sequencing on Gene Regions of Interest*
Authors: Landon, Nguyen, FDA/NCTR (Student); Dan, Li, FDA/NCTR (Mentor)
FDA Strategic Initiative: Increasing Choice and Competition through Innovation
Abstract:

- **Synopsis**
  - Tumor mutation burden (TMB) is defined as the total number of somatic mutations found in the DNA of cancer cells. TMB is also used as an emerging biomarker associated with response to immune checkpoint inhibitor (ICI) therapy. For example, patients who have a higher number of mutations are more likely to be better candidates to certain types of treatment. Currently, the gold standard method of estimating TMB is Whole Exome sequencing (WES) which focuses only on protein-coding regions. A more recent and evolving method is Targeted Panel Sequencing (TS) which focuses on gene regions known to have strong associations with clinical relevance. With TS, patients are able to get their TMB estimations in a quicker turn-around time while keeping costs lower too. However, the tradeoff is that since TS focuses on reduced areas of the genome, this allows for some genes of interest not being included. Through simulation analysis of panels, we were able to better understand TS and make improvements in its accuracy. We concluded that panel size is a very important factor when using TS for TMB estimation. Although there are still many improvements needed with targeted panel sequencing, its future outlook of being routinely implemented in clinical use is something to look forward to.
- **Purpose**
  - This study aims to further explore targeted panel sequencing and its importance in tumor mutational burden. Currently, the gold standard method used to measure TMB is Whole Exon Sequencing (WES) which sequences all of the protein-coding regions. The method we are interested in TMB estimation is targeted panel sequencing. Targeted panel sequencing (TS) is a newer method of next generation sequencing that focuses on a select set of genes that have known association with the disease under study. With TS, we can reduce turn-around times as well as lower costs by specifying regions of interest. TS also allows for a greater depth of measurement while having less data burden. Given these benefits, medical clinics should try to implement more targeted panel sequencing into their practice.
- **Methods**
  - In this study, we evaluated targeted panel sequencing by comparing it to its gold-standard counterpart Whole Exon Sequencing (WES). Through simulation

and evaluation of data sets such as The Cancer Genome Atlas (TCGA) and Catalogue of Somatic Mutations in Cancer (COSMIC), we were able to better understand TS. We simulated hundreds of targeted panels with a large range of sizes from 0.2M to 3M. The mutations from TCGA database were used as the true set to estimate and compare the WES-based and panel-based TMB. RMSD was then used to measure the difference between our predicted and observed values. Statistical models were used to output an R-squared value which estimated the performance of each panel. The R-squared values of TS and WES were then compared to note any major performance differences.

- **Results**
    - Our findings indicted that TS is roughly consistent with WES for TMB estimation with an inherent variation being panel size. The relationship between TMB measure and panel size can be described in the equation 5*(panel-size-in-MB)^1/2. Smaller panels tended to overestimate the TMB values by 1.1 times plus a constant value around 1. As we increased the panel size, we found that the RMSD values decreased. The slope of the panels also differed in that a smaller panel had more outliers. In addition, smaller panels had more intercepts when plotted. We concluded that panel sizes 1M and above can provide for more reliable results when using targeted panel sequencing in TMB estimation. Consequently, this will allow for a better analysis of TMB estimation used in clinical practice for patients.
- **Implications**
    - Since targeted panel sequencing (TS) is more a next-generation method, there is still a lot to learn about this method. Currently, there is a lack of gold-standard pipelines as many technical obstacles still exist in implementing TS in routine clinical use. The transition of targeted panel sequencing in routine clinical use has yet to be widespread. One potential risk with targeted gene panels is that some genes of interest may not be included. As targeted panel sequencing only focuses on the reduced areas of the genome, that leaves other areas to be analyzed. However, the accuracy of TS is something that can gradually improve with advancing technology. That being said, targeted panel sequencing is an amazing tool for analyzing mutations in a given sample. We are continuing to develop new discoveries on TS in hopes to be able to incorporate it more often in clinical use.

10. **Abstract Title:** *Triclosan's effect on the intestinal epithelial cells*
    Authors: Benshel Bright, NCTR (Student); Kuppan Gokulan, NCTR (Mentor); Kumari Karn, NCTR (Fellow); and Sangeeta Khare, NCTR (Mentor)
    FDA Strategic Initiative: Public Health Emergency Preparedness and Response
    Abstract:

- **Synopsis**
    - Triclosan (TCS) is a xenobiotic compound that is used as antibacterial. This compound is found in many household items, including soaps, toothpastes, and toys. While this compound is not to be ingested, it can be introduced to the body via the accidental swallowing of toothpaste, the application of soaps on skin, and other methods. TCS introduced through the oral route interacts with the intestine's microbiome and intestinal mucosal layer before getting absorbed by the intestine. Through the intravenous route, triclosan skips this interaction before affecting the cells. The goal of my project is to determine the effects of triclosan on the intestinal cell permeability. To do so, T84 cells were chosen to stimulate the intestinal epithelial membrane because they mimic intestinal epithelial cells with a property of mucus secretion. The cells were harvested from the T75 flasks and transferred into wells designed for transepithelial electrical resistance (TEER) machine. The cells grow into a monolayer, stabilizing the TEER data and signifying that the wells are ready for the treatment. TCS was added; 1, 10, or 100 ug per well; on the apical side of the transwell and ran TEER for 72 hours. The apical portion represents an oral entry route. Pictures were taken of the cells post treatment. Afterwards, the apical and basal media was stored, and the membrane was removed for RNA processing. The apical media was stored to access lactate dehydrogenase activity to access cell death from the treatment, and the basal media was stored to determine if TCS permeated through the monolayer using an HPLC. Higher concentration of TCS showed changes in the morphology of cell monolayer with an indication of loosen cell-cell attachment (weaken intestinal permeability). Higher concentration of TCS also increased the number of mucus secreting cells. In conclusion, these preliminary results show that the higher concentration of TCS can have significant effect on gastrointestinal homeostasis.
- **Purpose**
    - Triclosan (TCS) is a xenobiotic compound that is used as antibacterial. This compound is found in many household items, including soaps, toothpastes, clothing, and toys. While this compound is not to be ingested, it can be introduced to the body via the accidental swallowing of toothpaste, the application of soaps on skin, and other methods. TCS has a half-life of 21 hours, meaning a one-time exposure can cause lasting effects. TCS gets processed mainly through the liver and partly through the skin. TCS introduced through the oral route interacts with the intestine's microbiome and intestinal mucosal layer before getting absorbed by the intestine. The goal of my project is to determine the effects of triclosan on the intestinal cell permeability.
- **Methods**
    - To determine the effects of TCS, T84 cells were chosen to stimulate the intestinal epithelial membrane because they mimic intestinal epithelial cells

with a property of mucus secretion. The cells were harvested from the T75 flasks and transferred into wells designed for transepithelial electrical resistance (TEER). The cells grow into a monolayer, stabilizing the TEER data and signifying that the wells are ready for the treatment. TCS was added 1, 10, or 100 ug per well from the apical side of the transwell and ran TEER for 72 hours. The apical portion represents an oral entry route while the basal portion represents an intravenous route. Pictures were taken of the cells post treatment. Afterwards, the apical and basal media was stored, and the membrane was removed for RNA processing. The apical media was stored to access lactate dehydrogenase activity to measure cell death post treatment, and the basal media was stored to run a HPLC to determine if TCS permeated through the monolayer.

- **Results**
  - Higher concentration of TCS showed changes in the morphology of cell monolayer with an indication of loosen cell-cell attachment (weaken intestinal permeability). Higher concentration of TCS also increased the number of mucus secreting cells. The mRNA expression of cell-cell junction related genes is currently ongoing. The apical and basal media will be processed for the LDH activity.
- **Implications**
  - Exposure of TCS to humans can occur via various consumer products, which can reach to intestinal tract. Based on our study, the higher concentration of TCS can have significant effect on gastrointestinal homeostasis.

11. **Abstract Title:** *Testing Mutagenicity of Nitrosamines Using the TK and HPRT Gene Mutation Assays*
    Authors: Zhang, Nathan, FDA/NCTR (Student); Li, Xilin, FDA/NCTR (Mentor); Le, Yuan, FDA/NCTR (Mentor); Mei, Nan, FDA/NCTR (Mentor)
    FDA Strategic Initiative: Public Health Emergency Preparedness and Response
    Abstract:
    - **Synopsis**
      - Nitrosamines are compounds that are carcinogenic in humans and experimental rodents. Nitrosamines act through a genotoxic mode of action, meaning that they directly interact with DNA. Several nitrosamines have been confirmed to be Ames-positive, indicating that they are mutagenic in bacteria. For example, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) are the two most well-studied nitrosamines and they are both potent rodent carcinogens and positive in the Ames test. However, little is known on the potential risk of many other nitrosamines, especially the newly identified nitrosamine drug substance-related impurities (NDSRIs), which are often formed in the drug manufacturing process. As such, we use N-nitrosonortriptyline as a case study to evaluate the mutagenicity of NDSRIs in human lymphoblastoid TK6 cells using

the thymidine kinase (TK) and Hypoxanthine phosphoribosyltransferase (HPRT) gene mutation assays. In a TK gene mutation assay, cells are seeded with and without trifluorothymidine (TFT), which selects for mutants. TFT is a pyrimidine analogue, which stops cell metabolism and cell division. Through this assay, any cell colonies that appear indicate the presence of a mutation. The HPRT assay works similarly. Cells are seeded with and without 6-thioguanine (6-TG), which is a purine analogue that can stop cell division. Any cell colonies that form with the presence of 6-TG indicate a mutation. The concentrations for the experiment were 0 μM, 1.25 μM, 2.5 μM, 5 μM, and 10 μM. Additionally, there was a group treated with 100 μM of N-nitrosodiethylamine, which served as a positive control. Four 96-well plates were seeded with the selective agent, and two plates were seeded without, which were colony efficiency plates. After incubating for three weeks, the number of cell colonies were counted, and the relative survival and mutation frequency were calculated. In the experiment, the higher concentrations had lower relative survivals as well as high mutation frequencies, suggesting mutagenicity.

- **Purpose**
    - Nitrosamines are compounds that are carcinogenic in humans and experimental rodents. Nitrosamines act through a genotoxic mode of action, meaning that they and their metabolites can directly interact with DNA. Several nitrosamines have been confirmed to be Ames-positive, indicating that they are mutagenic in bacteria. For example, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) are the two most well-studied nitrosamines and they are both potent rodent carcinogens and positive in the Ames test. However, little is known on the potential risk of many other nitrosamines, especially the newly identified nitrosamine drug substance-related impurities (NDSRIs), which are often formed in the drug manufacturing process. As such, we use N-nitrosonortriptyline as a case study to evaluate the mutagenicity of NDSRIs in human lymphoblastoid TK6 cells using the TK and HPRT gene mutation assays. The selective agents used in these assays (TFT for TK and 6-TG for HPRT) are toxic to normal cells, so cell colonies that appear indicate a mutation under that concentration.
- **Methods**
    - Cleansed human lymphoblastoid TK6 cells were recovered from a liquid nitrogen freezer and maintained at a density between 2x10^5 cells/mL and 1.5x10^6 cells/mL cells. For chemical treatment, 10x10^6 cells were washed and then treated with different chemicals for 4 hours in the presence of 5% hamster S9. After treatment, cells were plated at a density of 1.6 cells/mL to determine the colony efficiency. Cells were then maintained for 3 or 7 days in 10% horse serum for the TK or HPRT mutation assays, respectively. Subsequently, the treated cells were plated again, but in 20% horse serum and with trifluorothymidine (TFT) and 6-thioguanine (6-TG) selective agents, which tested

for TK6 and HPRT mutations, respectively. After incubating the plates at 37°C with 5% CO2 for 2 weeks, colonies (i.e., mutations) were counted. The relative survival (i.e., cytotoxicity) and mutation frequencies were calculated for each of the samples. Both TK and HPRT assays were repeated two times individually.)

- **Results**
  - Cells were treated with varying concentrations of N-nitrosonortriptyline. The concentrations were 0 (negative control), 1.25 µM, 2.5 µM, 5 µM, and 10 µM. Additionally, there was a group treated with 100 µM of N-nitrosodiethylamine (NDEA), which served as a positive control. The results of this experiment show that higher concentrations of N-nitrosonortriptyline can be more cytotoxic. In the TK gene mutation assay, N-nitrosonortriptyline induced higher mutation frequencies (MFs) from 2.5 µM to 10 µM in a concentration-dependent manner. N-nitrosonortriptyline showed mild induction on the MF at the concentration of 2.5 µM. For the HPRT gene mutation assay, N-nitrosonortriptyline induced concentration-dependently higher MFs from 1.25 µM to 10 µM. In addition, relative survival rate indicated high cytotoxicity of N-nitrosonortriptyline, which caused around 60-70% cell death at the high concentration.
- **Implications**
  - This experiment shows that N-nitrosonortriptyline may induce gene mutations and provides new information on the genotoxic potential of N-nitrosonortriptyline in human cells. Therefore, this impurity should be controlled tightly in the drug manufacturing process to reduce the potential carcinogenicity risk to patients, especially for those taking it chronically. Also, the observations from this study can support the risk assessment of nitrosamine impurities found in drugs.

Office of the Commissioner (OC)

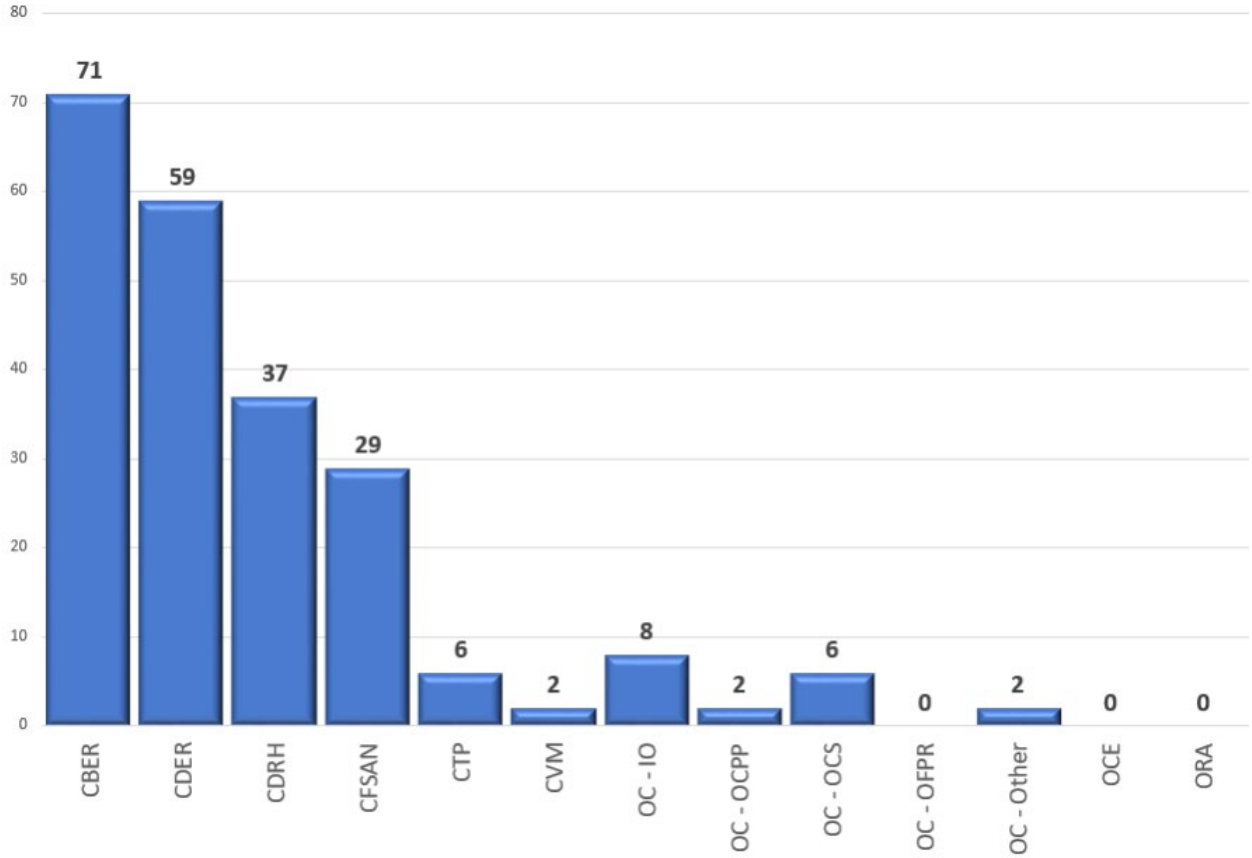
1. **Abstract Title**: *Top 5 Topics of Interest to Media in July*
   Authors: Neel Singh (Student), Dan Hetlage (Mentor)
   FDA Strategic Initiative: Empowering Patients and Consumers
   Abstract
   - **Synopsis**
     - In July 2022 (n=221 inquiries), Office of Media Affairs (OMA) received 221 inquiries. Majority (n=130) of these inquiries were triaged to CBER and CDER which is in line with top two areas/topics of inquiries being COVID-19 (n=74) and Monkey Pox (n=17). Most of the inquiries are from national print/online media which highlights the importance of print and online presence. OMA plays a pivotal role in getting the right information out to the American public in timey and efficient manner via the media actively and proactively.

- **Purpose**
  - One of the important functions of the Office of Media Affairs (OMA) is to respond to news media inquiries with timely and accurate information. OMA coordinates and triages inquiries internally to FDA centers based on jurisdiction/topic and for relevant subject matter experts to provide accurate information on the requested topic to the media covering FDA activities. As a summer intern within OMA, my role is to record and triage these inquiries internally. For the month of July, I helped OMA triage 261 media inquiries to various FDA sub-offices. The purpose in evaluating and analyzing this data is to gain better understanding of the topics asked to see if OMA can proactively communicate to get the information to the public.
- **Methods**
  - I analyzed the internal inquiry database that tracks the inquiry data to provide the below information for July inquiries in terms of center involved in the inquiry response, type of media outlet (National broadcast, National Print/online, local broadcast, local print, podcast, trade press, international press) requesting this, and general topic of the inquiry to see the top 5 topics for July.
- **Results**
  - July 1-31, 2022 – Total number of inquiries received = 221

Table1: Inquiries by Center

CBER	71
CDER	59
CDRH	37
CFSAN	29
CTP	6
CVM	2
OC - IO	8
OC - OCPP	2
OC - OCS	6
OC - OFPR	0
OC - Other	2
OCE	0
ORA	0

- **Chart 1: Bar Graph of – Inquiries by Center**



- **Table 2: Type of Media Outlet Inquiring**

National Broadcast	35
National Print/Online	138
Local Broadcast	8
Local Print	10
Podcast	0
Trade Press	21
International	14
Total Inquiries	**191**
Other	30
Total Inquiries	**221**

- **Table 3: Top 5 Topics for July**

Topic	# of Inquiries
COVID-19	74
Monkey Pox	17
Phillips Recall (CPAP or BiPAP)	7
JUUL/E-Cigarettes	6
Infant Formula	5

- **Implications**
  - CBER and CDER received the greatest number of inquiries related to biologics and drugs which is in line with the top 5 topic areas of inquiries. Also, most of the external inquiries came from national media source print/online and broadcast media which could make sense as national media contacts the national organization such as FDA to learn more about the key topics. The topic that is most top of mind of public health and media is COVID-19 which is not surprising considering the strong role of FDA and CBER in the vaccination effort. Second most requested topic was monkey pox, which has been emerging viral infection and it seems public is looking for more information on this topic. We can analyze the type of information requested on this newer viral infection and proactively get the information through FDA channels to the public.