U.S. FOOD & DRUG ADMINISTRATION

Listing of Abstracts

2024 Student Scientific Research Day

August 7, 2024

https://www.fda.gov/science-research/fda-stem-outreach-education-and-engagement/

FDA Annual Student Scientific Research Day

2024 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 2024 FDA summer students. Among these participants, OSPD received 13 abstracts from the Center for Biologics Evaluation and Research (CBER), 47 submissions from the Center for Drug Evaluation and Research (CDER), 20 from the Center for Devices and Radiological Health (CDRH), 14 from the Center for Food Safety and Applied Nutrition (CFSAN), 3 from the Center for Veterinary Medicine (CVM), 10 from the National Center for Toxicological Research (NCTR), and 1 from the Office of the Commissioner (OC) /Office of Digital Transformation.

There were 108 total public abstract submissions. Of the 108 total student abstract submissions, FDA's strategic initiatives below were supported with:

- 28 student projects related to Public Health Emergency Preparedness and Response.
- 22 student projects related to Increasing Choice and Competition through Innovation
- 51 student projects related to Unleashing the Power of Data.
- 7 student projects related to Empowering patients and consumers.

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.

Center for Biologics Evaluation and Research (CBER)

1. **Crawford, Caleb**
    - **Abstract title:** Microphysiological Systems for Potency Assays
    - **Authors:** Caleb Crawford (Student) FDA/CBER, Devin Vertrees, Evi B. Struble, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis:**
            - Two-dimensional mammalian cell cultures are often oversimplified systems that do not account for multiple cell types and blood supply of the living organs. We set out to design and build a lung microphysiological system (L-MPS) that more accurately simulates human lungs which can be used to test potency of antibody therapies against respiratory pathogens. Experimentation allowed for the determination of the operating pressure, TEER readings, the time necessary to achieve equilibration, and antibody concentrations that can be used in the experiment. In the future, lung cells will be infected with respiratory syncytial virus (RSV) and the infection measured in the presence and absence of the antibodies.
        - **Purpose:**
            - Potency is the amount of a drug that results in a specific desirable effect as measured with a potency assay. Two-dimensional mammalian cell cultures are commonly used to perform potency assays for antibody drugs. However, they are often oversimplified systems that do not account for multiple cell types and blood supply of the living organs. We set out to design and build a lung microphysiological system (L-MPS) that more accurately simulates human lungs which can be used to test potency of antibody therapies against respiratory pathogens.
        - **Method:**
            - The L-MPS is constructed by three components: a compressor, a controller, and an MPS chip. The compressor dries and pressurizes air before sending it to the controller, which regulates the pressure used to pump the media through the microfluidic channels in the chip. The chip is the component where model tissues are grown. We are using Humimic Chip-3 Plus, which has three compartments allowing for up to three model tissues/organs. The lung cells are grown on the apical side and endothelial cells on the basal side of a transwell ahead of time. An STX-2 Plus electrode attached to EVOM-3 instrument is used to measure trans-epithelial electrical resistance (TEER). When cells in the transwells are confluent, as indicated by TEER, they are placed into the center chamber of the chip. Antibodies are added to the first chamber and media is circulated on the basolateral side of the transwell. At different time points, aliquots of media are collected from the apical side of the transwell in the middle chamber and the third chamber. The concentration of the antibody is determined using ELISA. The distribution of the antibody to the lung compartment as a function of time is then calculated.

- **Results:**
    - We successfully installed and tested the three-component system using lung A549 cells. We determined the operating pressure, TEER readings, the time necessary to achieve equilibration, and antibody concentrations that can be used in the experiment. In the future, lung cells will be infected with respiratory syncytial virus (RSV) and the infection measured in the presence and absence of the antibodies.
- **Implications:**
    - Our data lays the foundation for building and testing a more complex L-MPS that includes endothelial cells and RSV infection. Such a system can be used to assess antibody therapies and determine their potency preventing respiratory viruses before being used in people.

2. **DeSilva, Minoli**
    - **Abstract title:** Understanding the effect of formulation changes on results of Dynamic Light Scattering analysis of IG products.
    - **Authors:** De Silva, Minoli, FDA/CBER (Student); Norton, Malgorzata, FDA/CBER (Co-Mentor); Eller, Nancy, FDA/CBER (Co-Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Protein aggregation has the potential to affect product safety and efficacy. These effects can include infusion reactions and formation of anti-drug antibodies that may reduce in vivo efficacy of the drug. Dynamic Light Scattering (DLS) is a qualitative method for determining the presence of subvisible ($\leq$1 micron) particles in a sample, that requires no sample preparation, and is relatively simple to run. DLS is a commonly used method to evaluate the level of particles in a product and has been used in lot release. Therefore, it is important to understand how various product attributes affect the final results. This information can assist in understanding and setting appropriate acceptance criteria for DLS.
        - **Purpose**
            - Protein biopharmaceuticals are prone to aggregation, which depends upon intrinsic protein stability, solute, and physical stresses during manufacturing that can include mixing, heating, and filtration. Protein aggregation has the potential to affect product safety and efficacy. Dynamic light scattering (DLS) is a commonly used method to evaluate particles from 1 nm to 1 micron. DLS is a qualitative method which calculates the hydrodynamic diameter based on Brownian motion. The goal of this study is to determine how various conditions affect these calculated results. This information should assist in interpreting DLS results and be helpful to CMC reviewers of DLS data contained in regulatory submissions.
        - **Methods**
            - Multiple lots from various Immune Globulin, Intravenous (IGIV) products were purchased for testing. Two different instruments by Malvern Panalytics, Zetasizer Nano ZS (software version 8.01) and the Zetasizer Ultra Red (software ZS XPLORER version 3), were used to test the samples. The Nano uses backscatter (173^{o}) only for measurements. The Ultra has the capability to use Multi-Angle

Dynamic Light Scattering (MADLS) which includes backscatter, or backscatter alone. All samples were tested at undiluted concentration of 10% (100 mg/mL) in product-specific formulation buffer. Product samples were diluted into each products' formulation buffer, dialyzed into various buffers which included differences in pH, sorbitol and/or polysorbate 80 (PS80) concentrations, and tested at different viscosities.

- **Results**
  - Since the hydrodynamic diameter includes ions associated with the protein, DLS results may be affected by changes in formulation buffer, as well as the presence of protein aggregates in the buffer. Additional DLS readouts are also affected. Therefore, it is necessary to look at all DLS readouts to determine the suitability of the data and product quality.
- **Implications**
  - Due to the relative simplicity of instrumentation, DLS has the potential to be a useful initial orthogonal method used to estimate the quantity and sizes of subvisible particles present in a sample, and to evaluate how changes to manufacturing or formulation influence DLS readouts. Understanding how product attributes affect DLS parameters should assist in setting appropriate acceptance criteria for DLS results. Therefore, it is necessary to look holistically at all DLS parameters to determine suitability of the data and product quality.
  - "My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate FDA."

3. **Deshpande, Ananya**
   - **Abstract title:** Verification of lentivirus-mediated transduction of Cas proteins into monoallelic cells
   - **Authors:** Deshpande, Ananya, FDA/CBER (Student); Bajgain, Pratima, FDA/CBER (Mentor); Sauna, Zuben, FDA/CBER (Mentor); Simhadri, Vijaya, FDA/CBER (Mentor)
   - **FDA Strategic Initiative:** Unleashing the Power of Data
   - **Abstract:**
     - **Synopsis**
       - CRISPR/Cas systems have the potential to revolutionize disease treatment. However, *in vivo* exposure to Cas proteins, which do not typically originate in humans, can induce immune response, and limit the clinical potential of this technology. Identifying Cas-derived peptides on MHC-I molecules is a key component of characterizing the potential immune risk of Cas proteins. For this complex assay to be successful, it is critical to first ensure that Cas proteins are being expressed in the appropriated cell lines. expressing has efficiently occurred by performing western blots. The characterization of transduced monoallelic cell lines described here, shows that these could be used to successfully perform assays to identify Cas-derived peptides.
     - **Purpose**
       - CRISPR/Cas systems have emerged as powerful gene editing tools and are poised to revolutionize the treatment of diverse human diseases. However, the Cas proteins can be perceived as foreign, elicit immune responses and limit the in vivo utility of the technology. Foreign

proteins are processed by antigen presenting cells (APCs) and presented by the Major Histocompatibility Complex (MHC) which are expressed on the surface of the APCs. There are two classes of MHC molecules: Class I (MHC-I) and Class II (MHC-II). MHC-I molecules present peptides from endogenously expressed proteins and MHC-II molecules present peptides derived from exogenous proteins. Here, we focus on the MHC-I mediated immune response to Cas proteins. This is because in most protocols, the Cas molecules are delivered as RNA using viral vectors and are expressed in the target cells. We used the Major histocompatibility complex (MHC)- Associated Peptide Proteomics (MAPPs) assay to identify Cas-derived peptides on MHC-I proteins using mass spectrometry. Before identification of regions via a MAPPs assay, it is essential to demonstrate successful transduction of the Cas proteins into cells. In this study we demonstrate successful transduction of Cas proteins in 9 cell lines that each express a unique MHC-I molecule.

- **Methods**
    - Here we used 9 monoallelic cell lines (i.e., each cell line expresses a unique MHC-I variant) to identify Cas-derived peptides on MHC-I molecules. We carried out lentiviral transduction of SaCas9 (Cas9 derived from *Staphylococcus aureus*) and Casϕ (Cas9 derived from Biggiephage) in each of the monoallelic cells. Monoallelic cells were transduced via spinfection using SaCas9 and Casϕ lentiviral vector with Internal Ribosome Entry Site2-enhanced Green Fluorescent Protein (IRES2-eGFP). Spinfection is a method to achieve efficient transduction of many cells. Cells are infected in the presence of polybrene, a polycation that neutralizes the charge repulsion between the virus and cell target surface and helps viral integration into the cells. Later, transduced cells were cultured and sorted twice for cells that were positive for both GPF and HLA A, B, C (MHC-I subtypes). Transduction efficiency is determined as the percentage of GFP-positive cells, via flow cytometry. The expression of SaCas9 and Casϕ proteins, was monitored on an immunoblot of the lysate of the sorted cells. After the verification of Cas expression in monoallelic cells, the transduced cells were further expanded in culture and used in the MAPPS assay.
- **Results**
    - The results demonstrate successful transduction of SaCas9 and Casϕ in all 9 monoallelic cell lines. On the SaCas9 immunoblot, each of the transduced samples had a band around 130 kDa which corresponds with the size of SaCas9. Similarly, for Casϕ, each of the transduced cell samples had a band around 76 kDa, which is the size of Casϕ. These bands were not observed in lysates from the control (non-transduced) cells.
- **Implications**
    - Based on the results obtained using flow cytometry, we conclude that spinfection resulted in efficient lentivirus transduction. This is because the cells continued to express the MHC-I proteins and were also positive for GFP (which is co-expressed with the Cas protein). The expression of the Cas9 and Casϕ was directly confirmed in an immunoblot. The characterization of the transduced monoallelic cell

lines demonstrates that these could be used to perform the MAPPs assay to identify Cas9 and Casϕ-derived peptides. Identification of Cas-derived peptides will be an important first step in the comprehensive immunological characterization of these Cas proteins. These results will have significant implications in the choice of Cas proteins to be used in clinical, in vivo gene editing.

4. **Hobson, Caroline and Alten, Kenta**
    - **Abstract title:** Evaluation of immunogenicity of live attenuated Leishmania parasites in a malnourished mouse model.
    - **Authors:** Hobson, Caroline, FDA/CBER (Student); Alten, Kenta, FDA/CBER (Student); Markle, Hannah, FDA/CBER (Mentor); Pacheco-Fernandez, Thalia, FDA/CBER (Mentor); Azodi, Nazli, FDA/CBER (Mentor); Pereira, Lais Da Silva, FDA/CBER (Mentor); Klenow, Laura, FDA/CBER (Mentor); Nakhasi, Hira L., FDA/CBER (Mentor); Gannavaram, Sreenivas, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **<u>Synopsis</u>**
            - Leishmaniasis, a parasitic infection that mainly impacts low to moderate income countries (LMICs), results in significant morbidity and mortality. Studies have shown that malnutrition has led to significantly poor clinical outcomes (1). BALB/C mice were fed two different diets, well nourished (WN) and poly nutrient deficient (PND). WN fed mice steadily gained weight throughout the entirety of the experiment. In comparison, the mice on the PND failed to gain weight. A comparison of immune response in the WN and PND fed mice was performed to analyze the differences in the vaccine immunogenicity by flow cytometry. PND fed mice also showed reduced recruitment of immune cell populations compared to WN group. Our data indicates that malnutrition plays a role in immune response development for the *LmCen*$^{-/-}$ vaccine candidate. Future studies will test the feasibility of reversing malnutrition-induced immune response deficiency by introducing a WN diet prior to vaccination. This can help us understand how to mitigate the effects of malnutrition on vaccine efficacy.
        - **<u>Purpose</u>**
            - *L*eishmaniasis, a parasitic infection that mainly impacts low to moderate income countries (LMICs), results in significant morbidity and mortality. Studies have shown that malnutrition has led to significantly poor clinical outcomes (1). Malnutrition can lead to increased risk in the development of Leishmaniasis in children primarily in developing nations where food security remains a challenge (6). Previous research showed that hosts that are protein deficient failed to recover from lesions caused by *Leishmania* (3, 5). Currently, there is no vaccine for human use in preventing Leishmaniasis. Our research group has been evaluating a live attenuated vaccine using a *Leishmania major* strain that lacks *centrin* gene (*LmCen*$^{-/-}$). The *centrin* gene plays a role in parasite replication and its deletion abrogates parasite proliferation. In this study, we examined the impact of malnutrition on the immune response following inoculation with the *LmCen*$^{-/-}$ vaccine.

- **Methods**
    - BALB/C mice were fed two different diets, well nourished (WN) and poly nutrient deficient (PND). Both diets consisted of equal caloric value and were provided ad *libitum*. The PND mice received food that was deficient in protein (3% protein), iron and zinc. WN mice received food containing 17% protein and normal levels of iron and zinc. Mice were fed the diets for 28 days and their malnutrition status was followed by measuring nutrient levels in the sera. Both groups of the mice were immunized with $1x10^{6}$ *LmCen*$^{-/-}$ in10 ul of PBS administered intradermally into the ear. Immune responses were assessed through flow cytometry using spleen and lymph node tissues.
- **Results**
    - WN fed mice steadily gained weight throughout the entirety of the experiment. In comparison, the mice on the PND failed to gain weight. A comparison of immune response in the WN and PND fed mice was performed to analyze the differences in the vaccine immunogenicity by flow cytometry. PND fed mice also showed reduced recruitment of immune cell populations compared to WN group. A reduction in $CD169^{+}$ macrophages (Mϕ) and $CD86^{+}$ dendritic cells (DCs) was observed in PND mice compared to WN group in the spleen.
- **Implications**
    - Our data indicates that malnutrition plays a role in immune response development for the *LmCen*$^{-/-}$ vaccine candidate. Reduction in the recruitment of activated Mϕ ($CD169^{+}$) and DCs ($CD86^{+}$) in the PND diet group suggests that immunogenicity is impaired under malnutrition. Future studies will test the feasibility of reversing malnutrition-induced immune response deficiency by introducing a WN diet prior to vaccination. This can help us understand how to mitigate the effects of malnutrition on vaccine efficacy.

5. **Jain, Ishaan and Bakaya, Vikrant**
    - **Abstract title:** Evaluation of The Leishmanin Skin Test using a Leishmania donovani antigen as a surrogate of latent infection and vaccine immunogenicity
    - **Authors:** Jain, Ishaan, FDA/CBER (Student); Bakaya, Vikrant, FDA/CBER (Student); Markle, Hannah, FDA/CBER (Mentor); Pacheco-Fernandez, Thalia, FDA/CBER (Mentor); Azodi, Nazli, FDA/CBER (Mentor); Pereira, Lais Da Silva, FDA/CBER (Mentor); Klenow, Laura, FDA/CBER (Mentor); Nakhasi, Hira L., FDA/CBER (Mentor); Gannavaram, Sreenivas, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - Leishmaniasis, caused by the Leishmania parasite, presents a significant global health challenge and is endemic to the United States. Despite its prevalence, there is no FDA-approved vaccine. The Leishmanin Skin Test (LST) serves as an effective surveillance tool for identifying individuals exposed to Leishmania by the delayed-type hypersensitivity (DTH) response resulting from cell-mediated immunity. The primary aim of this study is to evaluate a lyophilized LST formulation that elicits a robust DTH response in mice infected with Wild Type Leishmania major (LmWT) or a live-attenuated

Leishmania major centrin-deleted (LmCen-/-) strain by determining the composition of the cells recruited to the DTH site. The deletion of the centrin gene in the latter group renders the parasites unable to proliferate and cause leishmaniasis. The experiments involved intradermally inoculating C57BL/6 mice in their ears with 1x106 LmWT or LmCen-/- parasites. Following inoculation, the contralateral ear was inoculated with either the Good Laboratory Practices (GLP)-LST or the excipient 24 and 48 hours after the Leishmania antigen inoculation. The DTH response was measured, and the ear tissues were harvested. The primary outcome measure was the DTH response, defined by measurement of the area of ear around the injection site and histopathological examinations. Measurements of the lyophilized formulation confirmed a strong immune response, with marked inflammation and cellular infiltration at the test site. Histopathological analysis corroborated these observations, showing clear signs of immune activation and tissue remodeling. Flow cytometry analysis of the cells harvested from the organs also identified immune cell activation, with CD69+CD4+ as the most prominent memory T-cell marker in the region. The results of this study indicate that the LST formulation tested can serve as a reliable tool for detecting exposure to Leishmania and assessing cell-mediated immunity, thereby aiding in the surveillance and management of leishmaniasis. The findings from this study enable testing for prior exposure to Leishmania through a validated GMP-LST that can be readily deployed in disease-endemic regions.

- **Purpose**
    - Leishmaniasis, a disease caused by the Leishmania parasite, presents a significant global health challenge, and is endemic to the United States. The disease manifests in two major forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). The infection often remains asymptomatic for long periods of time. Despite an annual global burden of approximately 800,000 cases, there is no approved vaccine or diagnostic for disease surveillance (1). We are evaluating a live-attenuated Leishmania major centrin-deleted (LmCen-/-) strain as a potential vaccine candidate. The deletion of the centrin gene renders LmCen-/- parasites unable to proliferate and cause leishmaniasis. The Leishmanin Skin Test (LST) has existed for decades and is used to determine exposure to Leishmania; however, the antigen used in the test is not currently produced under Good Manufacturing Practice (GMP) conditions. LST serves as an effective surveillance tool for identifying individuals exposed to Leishmania by the delayed-type hypersensitivity (DTH) response resulting from cell-mediated immunity. The primary aim of this study is to evaluate a lyophilized LST formulation that elicits a robust DTH response in mice infected with low dose of Wild Type Leishmania major (LmWT) or LmCen-/- by determining the composition of the cells recruited to the DTH site.
- **Methods**
    - C57BL/6 mice were intradermally inoculated in the ear with either 1x105 LmWT parasites or LmCen-/- parasites. Following inoculation, the contralateral ear of each mouse was inoculated with either the

GLP-LST or the excipient. 24 and 48 hours after the Leishmania antigen inoculation, the DTH response was measured, and the ear tissues were harvested. Flow cytometry was performed using the CyTek Aurora to analyze the immune cells in the DTH response site. This analysis aimed to identify the cell populations involved in the DTH response to both LmWT and LmCen-/- infections. By comparing the immune responses between the two groups, the efficacy of the GLP-LST antigen in eliciting a measurable DTH response in both vaccinated and naturally infected individuals can be evaluated.

- **Results**
    - Measurement of the induration of the DTH site confirmed the specific immune response. The size of the DTH site in both the immunized and leishmanized mice exposed to the LST antigen was significantly larger than the size of the DTH site in the excipient group. Histopathological staining of the DTH site through hematoxylin and eosin (H&E) staining indicated recruitment of cells to the DTH site only in mice previously exposed to a Leishmania infection. Flow cytometry analysis revealed that the CD69+CD4+ T-cells were enriched in the DTH site. CD69+CD4+ T-cells represent the skin resident memory T-cells, indicating that LST recruits vaccine/infection induced cell populations to the DTH site. These results highlight the potential of the lyophilized formulation in providing a stable and effective LST.
- **Implications**
    - The results of this study indicate that the LST formulation tested can serve as a reliable tool for detecting exposure to Leishmania and assessing cell-mediated immunity, thereby aiding in the surveillance and management of leishmaniasis. The findings from this study enable testing for prior exposure to Leishmania through a validated GMP-LST that can be readily deployed in disease-endemic regions. The LST antigen is currently prepared from L. donovani promastigotes that can accurately detect prior exposure to L. major strain (3). Our data supports reintroduction of GMP-LST in the field towards surveillance and evaluating vaccine-induced immunogenicity.

6. **Jawa, Rayan**
    - **Abstract title:** A Machine Learning Approach for Identifying HLA Variants Associated with Symptomatic and Asymptomatic COVID-19
    - **Authors:** Jawa, Rayan, FDA/CBER (Student); Rawal, Atul, FDA/CBER (Mentor); Sauna, Zuben, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - Investigations of asymptomatic infection have provided new insights into patient-specific immunological features that protect patients from COVID-19 symptoms. Here we utilize machine learning in conjunction with explainable AI (XAI) to identify alleles in five HLA loci that can be either “protective” or “at-risk” for symptomatic COVID-19. From HLA-B, we determined that HLA-B*07:02, HLA-B*40:01, and HLA-B*15:01 are protective and that HLA-B*08:01, HLA-B*39:06, and HLA-B*51:01 place the individual at greater risk.

- **Purpose**
    - COVID-19 disease severity and symptoms can vary considerably among infected patients. Even though most studies investigating COVID-19 have focused on patients with a severe form of the disease, investigations of asymptomatic infection have provided new insights into patient-specific immunological features that protect patients from COVID-19 symptoms. A recent study showed an association between common human leukocyte antigen (HLA) alleles such as HLA-B*15:01 and asymptomatic COVID-19 infections [1].
- **Methods**
    - We utilized machine learning in conjunction with explainable AI (XAI) to identify alleles in five HLA loci that can be either "protective" or "at-risk" for symptomatic COVID-19. Data from the public online HLA-COVID database (composed of 3,238 samples) was used for training and validation of multiple ML classification models to identify the top performing model. The model was then further processed with XAI via SHAP (SHapley Additive exPlanations) to identify the "protective" and "at-risk" HLA alleles.
- **Results**
    - We determined that HLA-B*07:02, HLA-B*40:01, and HLA-B*15:01 are associated with asymptomatic disease, i.e., are protective and HLA-B*08:01, HLA-B*39:06, and HLA-B*51:01 are associated with symptomatic disease, i.e., place the individual at greater risk. Results were also obtained for four additional loci.
- **Implications**
    - These findings can be translated into algorithms that can help physicians better personalize COVID-19 treatment and achieve better clinical outcomes. A deeper understanding of human immune responses to the SARS-CoV-2 virus at the level of the individual can have implications for the design of vaccines and other treatment options for new viral epidemics and pandemics when they emerge.

7. **Jay, Nita**
    - **Abstract title:** Impact of Different Fetal Bovine Serum Sources on the Proliferation and Morphology of Mesenchymal Stromal Cells
    - **Authors:** Nita Jay (Student) FDA/CBER, Mona Mansouri (Mentor), Courtney Campagna (Mentor), Kyung Sung (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - Leishmaniasis, caused by the Leishmania parasite, presents a significant global health challenge and is endemic to the United States. Despite its prevalence, there is no FDA-approved vaccine. The Leishmanin Skin Test (LST) serves as an effective surveillance tool for identifying individuals exposed to Leishmania by the delayed-type hypersensitivity (DTH) response resulting from cell-mediated immunity. The primary aim of this study is to evaluate a lyophilized LST formulation that elicits a robust DTH response in mice infected with Wild Type Leishmania major (LmWT) or a live-attenuated Leishmania major centrin-deleted (LmCen-/-) strain by determining the composition of the cells recruited to the DTH site. The deletion of

the centrin gene in the latter group renders the parasites unable to proliferate and cause leishmaniasis. The experiments involved intradermally inoculating C57BL/6 mice in their ears with 1x106 LmWT or LmCen-/- parasites. Following inoculation, the contralateral ear was inoculated with either the Good Laboratory Practices (GLP)-LST or the excipient 24 and 48 hours after the Leishmania antigen inoculation. The DTH response was measured, and the ear tissues were harvested. The primary outcome measure was the DTH response, defined by measurement of the area of ear around the injection site and histopathological examinations. Measurements of the lyophilized formulation confirmed a strong immune response, with marked inflammation and cellular infiltration at the test site. Histopathological analysis corroborated these observations, showing clear signs of immune activation and tissue remodeling. Flow cytometry analysis of the cells harvested from the organs also identified immune cell activation, with CD69+CD4+ as the most prominent memory T-cell marker in the region. The results of this study indicate that the LST formulation tested can serve as a reliable tool for detecting exposure to Leishmania and assessing cell-mediated immunity, thereby aiding in the surveillance and management of leishmaniasis. The findings from this study enable testing for prior exposure to Leishmania through a validated GMP-LST that can be readily deployed in disease-endemic regions.

- **Purpose**
    - Leishmaniasis, a disease caused by the Leishmania parasite, presents a significant global health challenge, and is endemic to the United States. The disease manifests in two major forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). The infection often remains asymptomatic for long periods of time. Despite an annual global burden of approximately 800,000 cases, there is no approved vaccine or diagnostic for disease surveillance (1). We are evaluating a live-attenuated Leishmania major centrin-deleted (LmCen-/-) strain as a potential vaccine candidate. The deletion of the centrin gene renders LmCen-/- parasites unable to proliferate and cause leishmaniasis. The Leishmanin Skin Test (LST) has existed for decades and is used to determine exposure to Leishmania; however, the antigen used in the test is not currently produced under Good Manufacturing Practice (GMP) conditions. LST serves as an effective surveillance tool for identifying individuals exposed to Leishmania by the delayed-type hypersensitivity (DTH) response resulting from cell-mediated immunity. The primary aim of this study is to evaluate a lyophilized LST formulation that elicits a robust DTH response in mice infected with low dose of Wild Type Leishmania major (LmWT) or LmCen-/- by determining the composition of the cells recruited to the DTH site.
- **Methods**
    - C57BL/6 mice were intradermally inoculated in the ear with either 1x105 LmWT parasites or LmCen-/- parasites. Following inoculation, the contralateral ear of each mouse was inoculated with either the GLP-LST or the excipient. 24 and 48 hours after the Leishmania antigen inoculation, the DTH response was measured, and the ear

tissues were harvested. Flow cytometry was performed using the CyTek Aurora to analyze the immune cells in the DTH response site. This analysis aimed to identify the cell populations involved in the DTH response to both LmWT and LmCen-/- infections. By comparing the immune responses between the two groups, the efficacy of the GLP-LST antigen in eliciting a measurable DTH response in both vaccinated and naturally infected individuals can be evaluated.

- **Results**
    - Measurement of the induration of the DTH site confirmed the specific immune response. The size of the DTH site in both the immunized and leishmanized mice exposed to the LST antigen was significantly larger than the size of the DTH site in the excipient group. Histopathological staining of the DTH site through hematoxylin and eosin (H&E) staining indicated recruitment of cells to the DTH site only in mice previously exposed to a Leishmania infection. Flow cytometry analysis revealed that the CD69+CD4+ T-cells were enriched in the DTH site. CD69+CD4+ T-cells represent the skin resident memory T-cells, indicating that LST recruits vaccine/infection induced cell populations to the DTH site. These results highlight the potential of the lyophilized formulation in providing a stable and effective LST.
- **Implications**
    - The results of this study indicate that the LST formulation tested can serve as a reliable tool for detecting exposure to Leishmania and assessing cell-mediated immunity, thereby aiding in the surveillance and management of leishmaniasis. The findings from this study enable testing for prior exposure to Leishmania through a validated GMP-LST that can be readily deployed in disease-endemic regions. The LST antigen is currently prepared from L. donovani promastigotes that can accurately detect prior exposure to L. major strain (3). Our data supports reintroduction of GMP-LST in the field towards surveillance and evaluating vaccine-induced immunogenicity.

8. **Mandal, Pratyusha**
    - **Abstract title:** Advances in Adeno-Associated Virus (AAV) Gene Therapy: A Focus on Vector Production and Evaluation
    - **Authors:** Mandal, Pratyusha, FDA/CBER (Student); Abdelhamid, Leila, FDA/CBER (Mentor); Mazor, Ronit, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
    - **Abstract:**
        - **Synopsis**
            - The use of Adeno Associated Virus (AAV) in gene therapy has made significant advancements over time due to its non-pathogenic nature and broad tissue tropism. Recombinant AAV vectors serve as a pivotal platform for therapeutic gene delivery for the treatment of a variety of different diseases enabling long-term gene expression in various tissue targets. This project focused on two out of the 12 natural serotypes of AAV: AAV2 and AAV9. The FDA has currently approved five gene therapies. The first two approvals were for Luxterna and Zolgensma, which use AAV2 and AAV9 to help with retinal dystrophy and spinal muscular atrophy . In this project, we aimed to identify the difference in manufacturing processes between AAV2 and AAV9,

compare ELISA and qPCR quantification methods (capsid vs. viral transgene measurements), and measure purity of samples collected. To produce different serotypes of AAVs, a triple plasmid transfection system was used in HEK293 cells of two batches of each serotype and AAV particles were purified by density gradient. From this experiment, it was identified that there was consistency in yield within repeated batches of the same serotype, but not different serotypes with manufacturing methods used. It was also found that ELISA and qPCR quantification methods are comparable with AAV2 yield higher than AAV9 yield, and future testing with a silver stain and endotoxin assay will be used to identify purity of AAVs. This experiment has also successfully yielded validated AAV vectors to be used in future immunogenicity testing.

- **Purpose**
  - To manufacture and purify different serotypes of AAV (including AAV2 and AAV9) using a triple transduction method.
  - To quantify the AAV made using a qPCR and ELISA.
  - To identify purity of AAV collected.
- **Methods**
  - **1-. Producing different serotypes of AAVs using triple Plasmid transfection system**

    1.a. Expanding the QGibco™ Viral Production Cells 2.0 (VPCs 2.0) (a clonal derivative of the HEK293F cell line) in Gibco™ Viral Production Medium supplemented with 4 mM GlutaMAX™.

    1.b. Tri-transfection of Viral cells (6x10^8 cells in 200mL) using pHelper Vector (Part No. 340202, Cell biolab), pscAAV-GFP Control Vector (Part No. AAV-410, Cell biolab) and pAAV2/9n (Rep/cap)(Addgene). - Dependent on the concentration of the cell stocks growing, the respective amounts of Helper, Rep/Cap, and GFP plasmid were added to the cells and incubated for 72 hours. PEI MAX (Polysciences, Warrington, PA), at a polyethylenimine (PEI):DNA ratio of 2:1, was used as the transfection reagent.

    1.c. Harvesting of virus from cell pellets and supernatants.

    1.d. AAV vector purification using Iodixanol gradient.

    1.e. Concentrating AAV yield through buffer exchange using 100K PES concentrator.
  - **2- . AAV capsid titer determination**

    2.a. Detecting and Quantifying the native, non-denatured AAV capsids (full and empty) for the quantification of intact AAV particles using commercially available ELISA kit (Progen) and following the manufacturer procedures.
  - **3- Assessing the transgene expression**

    3.a. Quantitative Polymerase chain reaction (qPCR) Was utilized to detect and quantify GFP transgene using SsoAdvanced Universal Probes Supermix (1,725,281, Bio-Rad, Hercules, CA), and thermocycling conditions were 3 min at 95C and then 40 cycles of 95C for 30 s and 58C for 30 s on a Bio-Rad iCycler iQ Multicolor Real-Time PCR Detection System.
- **Results**
  - We produced a total of 2 batches of each AAV9 and AAV2 vectors each batch has around 1e12 capsids/ml or Vg/ml. AAV2 had a higher

yield compared to the AVV9 according to qPCR results.
    - Both results from the qPCR and ELISA are comparable.
    - ELISA values were relatively higher than qPCR since ELISA counts for both empty and transgene full capsids.
    - These findings were consistent among different serotypes.
- **Implications**
    - As previously reported in the literature, this project had comparable productivity of AAV vectors using the triple transfection system.
    - Both the qPCR and ELISA are rapid, relatively easy, and repeatable quantification methods of AAV evaluation.
    - To further enhance this project and ensure the quality of the produced AAV vectors, we will use an Endotoxin Assay before using these viral vectors in immunogenicity experimental studies.
    - Overall, this project successfully produced and quantified two serotypes of AAV- AAV2 and AAV9 which can be used for future immunogenicity experiments.

9. **Paidipally, Vedha**
    - **Abstract title:** Identification of a novel surrogate cell line for Anaplasma phagocytophilum propagation in vitro
    - **Authors:** Paidipally, Vedha, FDA/CBER (Student); Neerukonda, Sabarinath, FDA/CBER (Mentor); Elsworth, Brendan, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - Anaplasma phagocytophilum (Ap) is an obligate intracellular gram-negative bacterium that can be transmitted by ticks or via blood transfusion to cause Human Granulocytic Anaplasmosis (HGA). In its host, Ap infects and replicates in neutrophils, myeloid progenitors, and endothelial cells. Genome wide manipulation and identification of host factors involved in Ap infection require studies in tractable cell models that support Ap infection. In the present study, we compared cell lines of myeloid origin (HL60, K562, U937 and THP1) for their ability to support Ap replication. For this, cell lines were infected with cell-free Ap and the number of infected cells and number of bacterial genomes were quantified over the course of infection using Hema-3 staining and quantitative real time PCR respectively. In addition to HL60, a promyelocytic leukemia cell line that is widely used for Ap propagation and studies, we identified K562, a chronic myeloid leukemia-derived cell line, to support Ap propagation. K562 is an erythroleukemia cell line that can undergo spontaneous differentiation into early precursors of the monocytic, granulocytic, and erythroid series and therefore, may serve as an additional model to study Ap-infection of myeloid progenitors. Whether Ap uses its widely known receptor, sialylated and fucosylated P-selectin glycoprotein ligand 1 (PSGL-1) for entry into K562 is a subject of future investigations using receptor blocking or modification and gene deletion techniques.
        - **Purpose**
            - HL60 is a widely used cell model to propagate Anaplasma phagocytophilum (Ap) due to its neutrophil-like properties. In addition

to neutrophils, Ap also infects myeloid progenitors and endothelial cells of human host. Therefore, genome wide manipulation and identification of host factors involved in Ap infection require studies in additional tractable cell models that model wider cellular tropism of Ap infection. In the present study, we compared cell lines of myeloid origin (HL60, K562, U937 and THP1) for their ability to support Ap replication.

- **Methods**
  - We infected HL60, K562, U937 and THP1 cell lines with cell-free Ap to test their ability to support Ap replication. Cell-free Ap stock was generated by syringe lysis of Ap-infected (Strain: NCH1) HL60 cells. Post infection, cell lines were serially harvested on 3, 5 and 7 days post infection (dpi). Cells were spun onto microscope slides and stained with Hema-3 staining solutions to enumerate Ap-vacuole containing cells. Cellular DNA was isolated for the absolute quantification of Ap genome copies per cell using quantitative real time PCR (qRT-PCR). For absolute quantification, we generated standard curves using single copy gene plasmid standards for Ap (pleD) and host (beta-actin). Experimental sample threshold cycle values are normalized to standard curves to obtain gene copy numbers.
- **Results**
  - In addition to HL60, a promyelocytic leukemia cell line that is widely used for Ap propagation, we identified K562, a chronic myeloid leukemia-derived cell line, to support Ap propagation. K562 is an erythroleukemia cell line that can undergo spontaneous differentiation into early precursors of the monocytic, granulocytic, and erythroid series and therefore, may serve as an additional model to study Ap-infection of myeloid progenitors.
- **Implications**
  - Investigation of host cell subversion and host factors involved in A. phagocytophilum infection in its physiologically relevant target cell, the neutrophil, is challenging. Neutrophils pose several challenges for experimental studies ex vivo, due to their short ex vivo lifespan, inability to manipulate its genome or gene programs by transfection and transduction procedures. As a result, there is a need for additional tractable cell models that model Ap cellular tropism and allow genome-wide investigation of host factors involved in Ap infection. In addition to widely used neutrophil-like HL60 cell line for Ap infection studies, K562 cell line may model Ap-infection of myeloid progenitors.

10. **Simhadri, Sanath**
    - **Abstract title:** Harnessing Digital Twin Technology for Immunology Research
    - **Authors:** Simhadri, Sanath FDA/CBER (Student); Yogurtcu, Osman, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - Anaplasma phagocytophilum (Ap) is an obligate intracellular gram-negative bacterium that can be transmitted by ticks or via blood transfusion to cause Human Granulocytic Anaplasmosis (HGA). In its

host, Ap infects and replicates in neutrophils, myeloid progenitors, and endothelial cells. Genome wide manipulation and identification of host factors involved in Ap infection require studies in tractable cell models that support Ap infection. In the present study, we compared cell lines of myeloid origin (HL60, K562, U937 and THP1) for their ability to support Ap replication. For this, cell lines were infected with cell-free Ap and the number of infected cells and number of bacterial genomes were quantified over the course of infection using Hema-3 staining and quantitative real time PCR respectively. In addition to HL60, a promyelocytic leukemia cell line that is widely used for Ap propagation and studies, we identified K562, a chronic myeloid leukemia-derived cell line, to support Ap propagation. K562 is an erythroleukemia cell line that can undergo spontaneous differentiation into early precursors of the monocytic, granulocytic, and erythroid series and therefore, may serve as an additional model to study Ap-infection of myeloid progenitors. Whether Ap uses its widely known receptor, sialylated and fucosylated P-selectin glycoprotein ligand 1 (PSGL-1) for entry into K562 is a subject of future investigations using receptor blocking or modification and gene deletion techniques.

- **Purpose**
  - HL60 is a widely used cell model to propagate Anaplasma phagocytophilum (Ap) due to its neutrophil-like properties. In addition to neutrophils, Ap also infects myeloid progenitors and endothelial cells of human host. Therefore, genome wide manipulation and identification of host factors involved in Ap infection require studies in additional tractable cell models that model wider cellular tropism of Ap infection. In the present study, we compared cell lines of myeloid origin (HL60, K562, U937 and THP1) for their ability to support Ap replication.
- **Methods**
  - We infected HL60, K562, U937 and THP1 cell lines with cell-free Ap to test their ability to support Ap replication. Cell-free Ap stock was generated by syringe lysis of Ap-infected (Strain: NCH1) HL60 cells. Post infection, cell lines were serially harvested on 3, 5 and 7 days post infection (dpi). Cells were spun onto microscope slides and stained with Hema-3 staining solutions to enumerate Ap-vacuole containing cells. Cellular DNA was isolated for the absolute quantification of Ap genome copies per cell using quantitative real time PCR (qRT-PCR). For absolute quantification, we generated standard curves using single copy gene plasmid standards for Ap (pleD) and host (beta-actin). Experimental sample threshold cycle values are normalized to standard curves to obtain gene copy numbers.
- **Results**
  - In addition to HL60, a promyelocytic leukemia cell line that is widely used for Ap propagation, we identified K562, a chronic myeloid leukemia-derived cell line, to support Ap propagation. K562 is an erythroleukemia cell line that can undergo spontaneous differentiation into early precursors of the monocytic, granulocytic, and erythroid series and therefore, may serve as an additional model

to study Ap-infection of myeloid progenitors.

- **Implications**
  - Investigation of host cell subversion and host factors involved in A. phagocytophilum infection in its physiologically relevant target cell, the neutrophil, is challenging. Neutrophils pose several challenges for experimental studies ex vivo, due to their short ex vivo lifespan, inability to manipulate its genome or gene programs by transfection and transduction procedures. As a result, there is a need for additional tractable cell models that model Ap cellular tropism and allow genome-wide investigation of host factors involved in Ap infection. In addition to widely used neutrophil-like HL60 cell line for Ap infection studies, K562 cell line may model Ap-infection of myeloid progenitors.

11. **Simak, Joseph**
    - **Abstract title:** Long-term stability and in vitro prothrombin-converting activity neutralization potency of outdated antivenoms for treatment of envenomation by exotic snake species
    - **Authors:** Simak, Joseph, FDA/CBER (Student); Yan, Hailing, FDA/CBER (Mentor); Zhang, Pei, FDA/CBER (Mentor); Scott, Dorothy, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - To consider emergency use of expired antivenom (AV) products in life-threatening situations when no in date product is available, long-term stability of snake AV products beyond their expiry needs to be evaluated. We performed a pilot study evaluating the stability of physicochemical and microbiological parameters and in vitro potency of non-FDA licensed AV products indicated for envenomation by various epidemiologically important snakes nonnative in the USA. 42% (21) of evaluated AV vials passed the pre-screening based on evaluation of physicochemical parameters including opalescence, color, intrinsic and foreign particulates, protein content, pH, and endotoxin levels. Based on comprehensive visual inspection results correlating with protein degradation (HPLC) and particle (DLS) analysis, microbiological and AV neutralization of venom prothrombin-converting activity results, there is potential for long-term stability of AVs decades after expiration and for their qualification for emergency clinical use.
      - **Purpose**
        - Antivenoms (AV), immunoglobulin fractions from venom immunized animals, are crucial and potentially only lifesaving treatment for envenomation by venomous species. AVs are needed to treat patients bitten by local venomous species (WHO categorizes snakebite envenomation as the priority neglected tropical disease) as well as globally to treat bites by exotic pets, snakes kept in Zoos or for research. While there are FDA licensed AV products indicated to treat envenomation by native North American species, specific non-licensed AVs against exotic snake bites need to be imported through special process as experimental drugs. These products are needed to keep professionals (herpetologists, researchers, emergency

responders, deployed personnel) and public (private keepers, pet traders) safe. In the U.S., non-native envenomation by over 60 exotic venomous species were reported over 7 years. Availability of AVs has been affected by their shortages in many parts of the world, including the USA. Often, only expired specific AVs are available for treatment. Detailed information on AVs stability, including physiochemical and microbiological properties and neutralizing potency, is essential to assess their potential emergency use if no in date product is available.

- **Methods**
  - One monovalent and 5 polyvalent AV products (25lots/ 50 vials from 4 manufacturers) were obtained from the Viper Institute (Tucson, AZ) through donations from Zoos for research purposes. Appearance was evaluated by visual inspection (2000 – 3750 lux) assessing degree of opalescence (EU Pharm 2.2.2.1, hydrazine sulfate solution & hexamethylenetetramine solution reference Opalescence Suspensions, OS), color (Sigma Color Reference Solutions - Brown/Yellow standards, BY) and particulate matter content (foreign particles and intrinsic/proteinaceous particles). AV immunoglobulin components - molecular size distribution and purity were quantified by Size Exclusion High Performance Liquid Chromatography (SE-HPLC). Protein concentration (NanoDrop 2000 spectrophotometer, Thermo Scientific), Dynamic Light Scattering (DLS, Malvern) and pH were assessed. The endotoxin concentration was quantified by LAL (Limulus Amebocyte Lysate) assay (Endosafe Nexgen-PTS Reader/cartridge with lower limit of detection of 0.005 EU/ml, Charles River Laboratories). In vitro potency was assessed by neutralization of Echis carinatus venom (Sigma-Aldrich) prothrombin-converting activity (PCA) using human prothrombin (Abcam) and a chromogenic thrombin Substrate S-2238 (Chromogenix).
- **Results**
  - Overall, 42% (21) AV vials passed the pre-screening based on evaluation of 6 physicochemical parameters and endotoxin concentration. Protein concentration and pH were uniform in all 6 AV products. 24% (12) AV vials failed due to endotoxin level above 10 EU/mL, 24% (12) AV vials failed due to high opalescence, 8% (4) AV vials due to out of range color, 22% (11) AV vials due to the presence of foreign particulate and 10% (5) AV vials due to intrinsic particulates. SE-HPLC demonstrated decreased major AV product content {F(ab')2} while dimer + multimers fractions were increased in failed visual inspection/endotoxin pre-screening AV vials suggesting agglomeration process which results in subvisible, and visible particles, potentially causing adverse effects. DLS showed higher polydispersity index ($p<0.0001$) and aggregate content in failed pre-screened AV vials. Pilot results suggested significant potency of expired vials of the E. carinatus AVs to neutralize E. carinatus venom PCA. Vials which passed pre-screening showed significantly higher PCA neutralization potency as compared to failed vials ($p<0.017$). No correlations were found between stability of AVs and the time since their expiration nor temperature excursions during storage/shipping.
- **Implications**
  - Detailed visual inspection results correlate with protein degradation

(HPLC) and particle (DLS) analysis and may be useful for pre-screening under emergent conditions, particularly when instrumentation is not available. Comprehensive physicochemical and microbiological evaluation of expired AVs may predict their potency. AV neutralization of venom prothrombin-converting activity may serve as expired AV in vitro potency assay for several epidemiologically significant snake venom species. Based on our comprehensive physicochemical and microbiological evaluation of expired AVs for their qualification for emergency clinical use, some liquid expired AV products have potential for long-term stability even decades after their expiration.

12. **Vertrees, Devin**
    - **Abstract title:** Placenta Model for Investigating Viral Infection and Antibody Protection
    - **Authors:** Vertrees, Devin, FDA/CBER (Student); Li, Xiaohong, FDA/CBER; He, Yong, FDA/CBER; Struble, Evi FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - We aim to understand the mechanisms used by the human placenta to prevent viral infection, as well as the methods used by viruses to establish infection across this barrier. Using reporter-associated virus, we demonstrated that differentiated placental cells are less susceptible to infection by Zika virus. Furthermore, we showed that the expression of metalloprotease and putative viral receptors changes due to placental cell differentiation. These findings may elucidate how virus susceptibility changes over the course of pregnancy.
        - **Purpose**
            - Human placenta has evolved to be a barrier to infection, but the underlying mechanisms of this function are not fully elucidated. Using bioluminescent reporter-associated Zika virus (ZIKV), we aimed to characterize the susceptibility to infection of human placental cells compared to a commonly used cell line. We also aimed to assess ZIKV infection in differentiated versus un-differentiated placental cells, and investigate potential molecular pathways involved in the differences we observed in infection levels.
        - **Methods**
            - Vero and human placental (BeWo and Jeg3) cell lines were infected with two reporter Zika viruses and infection levels were assessed with both qRT-PCR and bioluminescence readout to show that virus infectivity was retained, and bioluminescence was reliably measurable. Forskolin-induced syncytialization of BeWo cells was then used as a model of placental differentiation and the markers of differentiation, including chorionic gonadotropin and E-Cadherin, were assessed. Subsequently, the differentiated and un-differentiated cells were infected with reporter-ZIKV and infection levels were measured. We also measured RNA and protein expression levels of various putative ZIKV receptors, including FcRn, Axl, and Tim1, as well as a placental metalloprotease, ADAM12.
        - **Results**

- We demonstrated that reporter-associated virus maintains infectivity using both commercially available bioluminescence kits and qRT-PCR. We also showed that Forskolin-differentiated BeWo cells are less susceptible to ZIKV infection. Differentiated BeWo cells exhibited lower Tim1 and FcRn mRNA levels and a higher expression of metalloprotease ADAM12. Inhibition of metalloprotease function resulted in lower proteolysis of E-cadherin. Proteolysis of other membrane receptors in presence and absence of the inhibitor are being investigated.

- **Implications**
  - Our research demonstrated that reporter Zika viruses are suitable for use in research and product development assays, such as potency assays. These types of experiments are necessary for preclinical analysis of potential ZIKV treatments and prophylaxis before they may be safely deployed in humans. Our research also provided new insights on the mechanisms underlying viral infections during pregnancy. The changes in expression of Tim1 could play a role in the observed decrease in viral susceptibility of differentiated BeWo cells. The increased expression of ADAM12 may contribute to functional decrease of this and other putative viral receptors via the cleavage of extracellular domains that recognize and mediate viral entry to cells. These findings have implications on the understanding of how Zika virus infections of the placenta and fetus establish and develop, and pathways that may be exploited for treatment. In the future, we aim to continue this research to further characterize viral infection during pregnancy and the immune mediators that offer protection against these infections.

13. **Wang, Jerri and Ou, Iris**
    - **Abstract title:** BSA for Stabilizing Influenza Viruses
    - **Authors:** Wang, Jerri, FDA/CBER (Student); Ou, Iris, FDA/CBER (Student); Plant, Ewan, FDA/CBER (Mentor); Ye, Zhiping, FDA/CBER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - Bovine serum albumin (BSA) is a protein stabilizer that has been recommended for use to store influenza viruses. We are testing to see if storing viruses with BSA can inhibit certain viruses from agglutinating to red blood cells. To begin testing the effects of BSA, we first grow SIAT and ECACC cells, both from the MDCK cell line and infect them with viruses. The viruses included in our study are two influenza A viruses, H1N1 and H3N2, and one influenza B virus. We will then conduct a hemagglutination (HA) assay to determine if virus has grown in cell culture. Viruses added to red blood cells (RBCs) causes the RBCs to agglutinate and stick to each other, forming a sheet. Virus will be stored with and without BSA. Additional HA assays will then be conducted in order to compare the two conditions and determine if the presence of BSA leads to virus inhibition. HA assays will be conducted with guinea pig and turkey RBCs. Results will be measured by plaque assays, HA titers, and virus particle counts. If BSA is an inefficient stabilizer, we expect that the plaque assay will show

more infectious virus for the samples stored without BSA, the HA titer assay will show more agglutination with the samples stored without BSA, and the virus counter will show approximately the same amount of virus for both samples stored with and without BSA. It is important confirming the validity of the recommendation for future virus storage and experimentation. Moreover, it's also possible that BSA may affect virus quality differently depending on the virus. Therefore, if this is the case for certain viruses, then this gives researchers something to think about when storing said viruses with BSA and help for future preparation of viruses.

- **Purpose**
    - Bovine serum albumin (BSA) is a protein stabilizer that has been recommended for use to store influenza viruses. This is because BSA protects the protein shell of the virus, thus preventing virus degradation as it is stored in cold temperatures. During a particular experiment in the lab, however, a sample of virus stored with BSA seemed to have a low hemagglutination (HA) titer. In hopes of increasing HA titer, a second aliquot on the same virus was added. The first aliquot was not stored with BSA, whereas the second was. In a typical setting, adding more virus should increase HA titer, but in this case, the HA titer decreased. Our assumption is that this occurred because the second sample of virus was stored with BSA. Therefore, we are testing to see if storing viruses with BSA can potentially inhibit certain viruses from agglutinating to red blood cells.
- **Methods**
    - To begin testing the effects of BSA, we first grow SIAT and ECACC cells, both from the MDCK cell line. Once these cells are confluent enough, the cell cultures will be infected with virus diluted to a 1 to 1000 ratio. The viruses included in our study are two influenza A viruses, H1N1 and H3N2, and one influenza B virus. We will then conduct a hemagglutinin (HA) assay to determine if virus is present has grown in cell culture: the virus will be serially diluted one in ten, and red blood cells (RBCs) are added. Virus binding causes RBCs to agglutinate and stick to each other, forming a sheet. If virus is present, we will harvest the virus and store it with BSA (to create a final concentration of 1%) and without BSA. Both samples, BSA and non-BSA, will be stored at -20 degrees Celsius and 4 degrees Celsius for one week. We will also stimulate a longer storage time by repeatedly thawing and refreezing samples. Additional HA assays will then be conducted in order to compare the two conditions and determine if the presence of BSA leads to virus inhibition. HA assays will be conducted with guinea pig and turkey RBCs.
- **Results**
    - Results will be measured by plaque assays, HA titers, and virus counter. The plaque assay is used to determine the amount of infectious virus present by counting the plaque unstained by the crystal violet staining. The more infectious virus present, the more plaques there will be. If the plaque assay results are the same for both BSA and without BSA, then it means that the available viable virus was not inhibited or degraded by the BSA. However, if the BSA sample shows more plaques than the one without BSA, then storing viruses

with BSA is effective and the recommendation should continue to be followed. If the opposite is true, then BSA may not be effective for storing the particular virus. For HA titer results, if there is enough virus, the RBCs and virus should bind and show agglutination. If the sample of virus stored with BSA does not show agglutination with the RBCs, this could mean that storing that virus with BSA causes binding inhibition. Finally, using the virus counter will show how much virus, regardless of its infectious abilities, is present in both samples. Even if BSA has inhibitory properties for some viruses, the virus amount should stay consistent with those viruses stored without BSA. If the BSA stored virus has less than the virus stored without, that could suggest that storing virus with BSA degraded the virus. On the other hand, if the BSA stored virus has more than the virus stored without, that could suggest the opposite, supporting the original recommendation. We expect that the plaque assay will show more infectious virus for the samples stored without BSA, the HA titer assay will show more agglutination with the samples stored without BSA, and the virus counter will show approximately the same amount of virus for both samples stored with and without BSA.

- **Implications**
    - It is important to confirm the validity of the recommendation for future virus storage and experimentation. Moreover, it's also possible that BSA may affect virus quality differently depending on the virus. Therefore, if this is the case for certain viruses, then this gives researchers something to think about when storing said viruses with BSA and help for future preparation of viruses. On a larger scale, it can be important for determining storage times for vaccine manufacturing and if virus is used to create potency standards.

Center for Drug Evaluation and Research (CDER)

14. **Acquah, Theophilus**
    - **Abstract title:** Assessment of low-resolution image reconstruction using generative adversarial network for spray dried particles.
    - **Authors:** Theophilus, Acquah, FDA/CDER (Student); Jayanti Das, FDA/CDER (Mentor), Yuan Zhang, FDA/CDER, Yang Yang, FDA/CDER (Mentor), Geng Tian, FDA/CDER (Supervisor), Ashraf Muhammad, FDA/CDER (Supervisor), Xiaoming Xu, FDA/CDER (Supervisor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Bovine serum albumin (BSA) is a protein stabilizer that has been recommended for use to store influenza viruses. We are testing to see if storing viruses with BSA can inhibit certain viruses from agglutinating to red blood cells. To begin testing the effects of BSA, we first grow SIAT and ECACC cells, both from the MDCK cell line and infect them with viruses. The viruses included in our study are two influenza A viruses, H1N1 and H3N2, and one influenza B virus. We will then conduct a hemagglutination (HA) assay to determine if virus has grown in cell culture. Viruses added to red blood cells (RBCs) causes the RBCs to agglutinate and stick to each other, forming a

sheet. Virus will be stored with and without BSA. Additional HA assays will then be conducted in order to compare the two conditions and determine if the presence of BSA leads to virus inhibition. HA assays will be conducted with guinea pig and turkey RBCs. Results will be measured by plaque assays, HA titers, and virus particle counts. If BSA is an inefficient stabilizer, we expect that the plaque assay will show more infectious virus for the samples stored without BSA, the HA titer assay will show more agglutination with the samples stored without BSA, and the virus counter will show approximately the same amount of virus for both samples stored with and without BSA. It is important confirming the validity of the recommendation for future virus storage and experimentation. Moreover, it's also possible that BSA may affect virus quality differently depending on the virus. Therefore, if this is the case for certain viruses, then this gives researchers something to think about when storing said viruses with BSA and help for future preparation of viruses.

- **Purpose**
    - Bovine serum albumin (BSA) is a protein stabilizer that has been recommended for use to store influenza viruses. This is because BSA protects the protein shell of the virus, thus preventing virus degradation as it is stored in cold temperatures. During a particular experiment in the lab, however, a sample of virus stored with BSA seemed to have a low hemagglutination (HA) titer. In hopes of increasing HA titer, a second aliquot on the same virus was added. The first aliquot was not stored with BSA, whereas the second was. In a typical setting, adding more virus should increase HA titer, but in this case, the HA titer decreased. Our assumption is that this occurred because the second sample of virus was stored with BSA. Therefore, we are testing to see if storing viruses with BSA can potentially inhibit certain viruses from agglutinating to red blood cells.
- **Methods**
    - To begin testing the effects of BSA, we first grow SIAT and ECACC cells, both from the MDCK cell line. Once these cells are confluent enough, the cell cultures will be infected with virus diluted to a 1 to 1000 ratio. The viruses included in our study are two influenza A viruses, H1N1 and H3N2, and one influenza B virus. We will then conduct a hemagglutinin (HA) assay to determine if virus is present has grown in cell culture: the virus will be serially diluted one in ten, and red blood cells (RBCs) are added. Virus binding causes RBCs to agglutinate and stick to each other, forming a sheet. If virus is present, we will harvest the virus and store it with BSA (to create a final concentration of 1%) and without BSA. Both samples, BSA and non-BSA, will be stored at -20 degrees Celsius and 4 degrees Celsius for one week. We will also stimulate a longer storage time by repeatedly thawing and refreezing samples. Additional HA assays will then be conducted in order to compare the two conditions and determine if the presence of BSA leads to virus inhibition. HA assays will be conducted with guinea pig and turkey RBCs.
- **Results**
    - Results will be measured by plaque assays, HA titers, and virus counter. The plaque assay is used to determine the amount of

infectious virus present by counting the plaque unstained by the crystal violet staining. The more infectious virus present, the more plaques there will be. If the plaque assay results are the same for both BSA and without BSA, then it means that the available viable virus was not inhibited or degraded by the BSA. However, if the BSA sample shows more plaques than the one without BSA, then storing viruses with BSA is effective and the recommendation should continue to be followed. If the opposite is true, then BSA may not be effective for storing the particular virus. For HA titer results, if there is enough virus, the RBCs and virus should bind and show agglutination. If the sample of virus stored with BSA does not show agglutination with the RBCs, this could mean that storing that virus with BSA causes binding inhibition. Finally, using the virus counter will show how much virus, regardless of its infectious abilities, is present in both samples. Even if BSA has inhibitory properties for some viruses, the virus amount should stay consistent with those viruses stored without BSA. If the BSA stored virus has less than the virus stored without, that could suggest that storing virus with BSA degraded the virus. On the other hand, if the BSA stored virus has more than the virus stored without, that could suggest the opposite, supporting the original recommendation. We expect that the plaque assay will show more infectious virus for the samples stored without BSA, the HA titer assay will show more agglutination with the samples stored without BSA, and the virus counter will show approximately the same amount of virus for both samples stored with and without BSA.

- **Implications**
    - It is important to confirm the validity of the recommendation for future virus storage and experimentation. Moreover, it's also possible that BSA may affect virus quality differently depending on the virus. Therefore, if this is the case for certain viruses, then this gives researchers something to think about when storing said viruses with BSA and help for future preparation of viruses. On a larger scale, it can be important for determining storage times for vaccine manufacturing and if virus is used to create potency standards.

15. **Alshammari, Suad**
    - **Abstract title:** Efficacy Endpoints for FDA-Approved Novel Drugs during 2023
    - **Authors:** Alshammari, Suad (Student) CDER, Zhang, Da (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - In 2023, the FDA approved 55 novel drugs. Biomarkers, surrogate endpoints, and clinical endpoints were utilized throughout the drug development processes to optimize the dosing regimens and support the approval decision of these drugs.
        - **Methodology**
            - Information about approved medications, efficacy endpoints, and biomarkers was collected from multiple resources including Document Archiving, Reporting, and Regulatory Tracking System (DARRTS), medication labels, Electronic Document Room (EDR). Dosing regimens and endpoints used in preclinical species, phase 1,

phase 2 and phase 3 studies were documented.

- **Results**
  - As this project is currently in the data collection stage, no definitive results can be reported at this time. We are actively gathering information from product labels, approval letters, DARRTS and EDR. This involves examining and documenting relevant details from each source to ensure a comprehensive dataset, focusing on the use of biomarkers, surrogate, and clinical endpoints, as well as investigating the doses tested in clinical development versus approved doses.
  - So far, we have extracted information for 29 out of the 55 drugs approved in 2023, including one drug used for diagnosis. Among these 29 drugs, 14 received accelerated approvals.
  - Once data collection is complete, the information will be meticulously organized, analyzed, and interpreted to draw meaningful conclusions. The forthcoming results will be based on a rigorous evaluation of the aggregated data.
- **Conclusion**
  - This study aims to gather comprehensive data from various sources, including product labels, approval letters, DARRTS and EDR to investigate efficacy endpoints of recently FDA-approved novel drugs. The results are expected to make meaningful contribution to the filed.

16. **Arri, Navpreet**
    - **Abstract title:** Evaluation of Vaginally Administered Long-Acting Polymer-Based ANDA Products: Inserts, Rings, and Drug Delivery Systems
    - **Authors:** Arri, Navpreet, FDA/CDER (Student); Li, Qi, FDA/CDER (Mentor); Wang, Yan, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Intravaginal products are designed for administration into the vagina. They encompass various dosage forms, such as cream, gel, inserts, suppository, tablets etc. The particular interest of this work is on extended-release inserts, rings, and systems. These formulations are specifically developed to provide both systematic and localized treatments for conditions such as contraception, vaginal atrophy, vaginal infections, infertility, and hormone replacement therapy. Generic intravaginal products are essential for women's health as they increase both affordability and accessibility. Product-Specific Guidances (PSGs) issued by the FDA are pivotal in facilitating the development and approval of such generic drug products. These PSGs outline the FDA's current thinking on what may be the most scientifically appropriate approaches for demonstrating the bioequivalence of test products to reference listed drugs (RLDs) in Abbreviated New Drug Applications (ANDAs). This study summarizes the formulation design used for long-acting intravaginal products and the current status of PSGs.
      - **Purpose**
        - This study aims to summarize the current polymer based extended release/long-acting intravaginal products, both brand and generic, on the US market and identify which NDAs still lack PSGs.

- **Methods**
  - Publicly available websites such as Drugs@FDA, OrangeBook and PSG for Generic Drug Development were used to complete the study. The FDALabel database, a web-based application that enables users to conduct customizable searches of the latest labeling of FDA-approved drugs, was used to first compile a list of the approved drug products. The search was filtered by specifying the route of administration to be vaginal. These initial results were then filtered to aim on dosage forms of inserts, extended-release inserts, rings, and drug delivery systems due to their long-acting nature. The following information about each drug was collected after reviewing the approved labeling to gain insight per product: product indication, frequency and duration, presentation, storage, active pharmaceutical ingredient (API), formulation, polymer (if used), clinical results, etc. Then correspondent PSG publication of these products was surveyed. Bioequivalence studies recommended for each drug product were collected and compared. In alignment with the mission of the FDA's Office of Generic Drugs (OGD), the approval of ANDA products were focused upon.
- **Results**
  - The initial search of FDA-approved products with a vaginal route of administration listed in the FDALabel database yielded 432 labeling results. Of the 16 products identified with the relevant dosage forms, the most frequent indications were for treating atrophic vaginitis due to menopause (7 products) and for contraception in females of reproductive age (6 products). Further analysis on dosage forms revealed 1 drug delivery system, 6 ring and 10 insert dosage forms with 3 classified as extended-release inserts. These extended-release inserts, used for contraception, required continuous vaginal insertion for three weeks followed by a one-week ring-free interval. These extended-release products contained two APIs: ethinyl estradiol, an estrogen, and etonogestrel, a progestin, while the other observed insert products contained only one API. Among the 6 ring products, 3 were indicated for contraception, in which only Annovera (NDA021367) provided contraception for thirteen 28-day cycles, following a regimen of three weeks of continuous use followed by a one-week dose-free interval during which it is cleaned, dried, and stored for reuse. Annovera contains two APIs: segesterone acetate, a progestin, and ethinyl estradiol, an estrogen, consistent with the pattern that long-acting products often have two APIs. Regarding the marketing categories of the 16 interested products, 10 were brand name products (NDAs) and 6 were generic name products (ANDAs). It was discovered that among these, five PSGs for long-acting products in the dosage forms of interest were posted for NDAs 020472, 201110, 020411, 021187, and 209627, with the PSG for NDA 021187 notably having 5 ANDA products approved. Analysis of the 16 products revealed the use of various polymers, including silicone polymers, hydrogel polymer comprised of polyethylene oxide (PEO) and urethane polymer, ethylene vinyl acetate (EVA) copolymers, polyethylene (PE), methyl siloxane-based polymers, cured silicone elastomer composed of dimethyl polysiloxane silanol, polyvinyl

acetate phthalate (PVAP), and polyethylene glycol.

- **Implications**
  - The results of the study provide a better understanding of the types of complex intravaginal long-acting products and the availability of PSGs in this area, which will subsequently facilitate planning for future PSG development. The development and publication of PSGs would facilitate innovative strategies for developing complex generic vaginal drug products, fostering exploration of new approaches to women's health.

17. **Asjid, Rabia**
    - **Abstract title:** The Use of Nonclinical Models as Mechanistic Insight in Rare Disease Drug Development
    - **Authors:** Asjid, Rabia, FDA/CDER (ORISE Fellow); Hayden, Sabrina, FDA/CDER (ORISE Fellow); Goel, Saryu, FDA/CDER (Mentor); Weis, Shawna, FDA/CDER (Mentor); Nugent, Bridget, FDA/CDER (Mentor); Lee, Kerry Jo FDA/CDER, (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Depending on scientific and therapeutic context, data from nonclinical models can sometimes be evaluated to provide mechanistic insights into the activities of the drug to inform regulatory review of new drug applications. Use of nonclinical models may be helpful in challenging rare disease drug development programs, where small patient populations, disease heterogeneity, and slow or variable disease progression may complicate clinical study design and analysis. Assessing the *in vitro* and animal models FDA has accepted as high-quality efficacy data supporting approvals for rare disease therapeutics can help inform our review of applications utilizing these approaches in future drug development programs for rare diseases.
      - **Purpose**
        - Clinical trials for novel rare disease drug development can be challenging due to small patient populations, slow or variable disease progression, and disease heterogeneity. Depending on scientific and therapeutic context, nonclinical data such as data from a relevant animal model or *in vitro* testing may be evaluated to provide mechanistic information to inform regulatory review of new drug applications. Nonclinical data provide information critical to the progression of a drug candidate through development. This study aims to review and assess *in vitro* and animal models that the FDA's Center for Drug Evaluation and Research (CDER) has recognized as providing mechanistic support for approvals for rare disease therapeutics in recent years. The goal of this research is to inform and support regulatory review of disease models in rare disease drug marketing applications.
      - **Methods**
        - Using the Document Archiving, Reporting, and Regulatory Tracking System (DARRTS) database, an internal CDER database that contains review documents for drug marketing applications, we reviewed and captured information from new drug applications (NDAs) and biologics license application (BLAs) approved by CDER for the

treatment of non-oncologic rare diseases between 2021-2023. If an *in vitro* or animal model was cited as mechanistic evidence in the review's Executive Summary, we completed a comprehensive analysis of the marketing application to evaluate the strengths of the nonclinical models. We focused on how translational each nonclinical model was in relation to human disease progression and clinical features, as well as whether biomarkers measured in animal models used to determine response to novel drugs were the same as those measured in human trials. Summary statistics and data visualizations will be used to determine trends in the use of nonclinical models in rare disease drug development.

- **Results**
    - There were 45 NDAs and BLAs with orphan product designation indicated for therapeutic (non-diagnostic), non-oncologic rare diseases approved by the FDA's CDER in 2021 through 2023. While data collection and analysis are ongoing, we identified eight novel drug approvals for the treatment of non-oncological rare diseases that utilized animal models and three approvals that utilized *in vitro* models as pharmacodynamic data to support approval. All approvals in our dataset that cited use of nonclinical mechanistic evidence had multiple sources of efficacy data in addition to pivotal trials that showed clinically meaningful results (e.g., mechanistic data from nonpivotal clinical studies and/or multiple sources of confirmatory evidence of effectiveness) to meet FDA's substantial evidence of effectiveness requirements.
- **Implications**
    - This research could inform further discussions about the utility of nonclinical data for regulatory purposes related to effectiveness. The study highlights the capability of nonclinical models to provide mechanistic data through demonstrating similarity in pathophysiology, pharmacology, and pharmacodynamics of the drug in the nonclinical model and human disease. The utilization of nonclinical models to support clinical efficacy data, may be helpful to bolster rare disease drug development programs. Utilizing nonclinical data for challenging rare disease drug development programs may improve efficiency, potentially accelerating access to new treatments for patients with unmet medical needs. Consistent and thorough evaluation of translational evidence across rare disease novel drug applications supports innovation while maintaining high standards of efficacy and safety.

18. **Azmal, Sharupa**
    - **Abstract title:** Impact of Injection Sites on the Pharmacokinetics of Monoclonal Antibodies Administered Intramuscularly
    - **Authors:** Azmal, Sharupa, FDA/CDER (Student); Choi, Su-Young, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - The intramuscular route of administration is a method of delivering medications directly into specific muscles (e.g., deltoid, glute, and thigh). Some of the benefits for intramuscular administration include

rapid and uniform absorption, quick onset of action, and delivery of large volumes of drug. Currently, however, there is limited data regarding the pharmacokinetics of monoclonal antibodies following intramuscular administration. In this project, we examined the labels of approved biologics and clinical protocols of Investigational New Drug (IND) submissions to determine whether they clearly stated or specified the site of intramuscular injection. Pharmacokinetic studies of antiviral monoclonal antibodies were also analyzed to evaluate trends in bioavailability. Through our findings, we hope to provide future sponsors considering an intramuscular route of administration for their drug, guidance on selecting an ideal IM injection site for optimal clinical use.

- **Purpose**
  - The intramuscular (IM) route of administration is a method of delivering medications directly into specific muscles (e.g., deltoid, glute, and thigh). Some of the benefits for intramuscular administration include rapid and uniform absorption, quick onset of action, and delivery of large volumes of drug. Currently, however, there is limited data regarding the pharmacokinetics of monoclonal antibodies following intramuscular administration. This study aims to survey the labels of approved biological products and clinical protocols of Investigational New Drug (IND) submissions to determine whether they clearly state or specify the site of intramuscular injection. The goal is to assess whether differences in IM injection site leads to differences in the pharmacokinetic profile.
- **Methods**
  - Initial data on approved biological products were extracted from the FDALabel database by using “BLA” and “intramuscular” as search terms. The data was further refined by excluding vaccines, botox, and immunoglobins. Once the list of biologics was finalized, the package insert for each product, which can be found on Drugs@FDA, was examined for the following information: site of IM injection, total volume per administration, volume per injection, bioavailability, and use in pediatric and/or adult populations. Data on antiviral monoclonal antibodies was sourced from clinical protocols of IND submissions. Descriptive statistics such as the monoclonal antibody indication, trial phases, use in pediatric and adult populations, injection site, total volume per administration, and volume per injection were accumulated. Bioavailability data was surveyed from pharmacokinetic studies conducted by sponsors. All collected data was summarized and analyzed using Excel to identify patterns and insights.
- **Results**
  - Nine approved biological products were identified that met our prespecified criteria. This includes three products which were verified as the same biologic with differing trade names, so they were counted as one product. 56% of biologics (5 out of 9 products) did not specify a site of IM injection. 33% of biologics (3 out of 9 products) specified the thigh as a site of IM injection. 11% of biologics (1 out of 9 products) specified the glute as a site of IM injection. According to key bioavailability findings from antiviral monoclonal antibody

pharmacokinetic studies, it is suggested that thigh injections have a greater bioavailability compared to gluteal injections. However, more clinical data is necessary to support this conclusion.

- **Implications**
    - The site of intramuscular injection may influence the pharmacokinetic profile of biologics. Our analysis revealed that more than half of approved biologics do not specify an injection site in the package insert, leaving the decision to trained healthcare providers to administer the drug where they believe is most appropriate. This variability can result in unintended effects on bioavailability leading to toxic adverse effects due to higher-than-expected drug concentrations or suboptimal efficacy due to lower than expected levels. These inconsistencies can adversely impact patient health outcomes. Our findings aim to inform future sponsors on selecting the optimal IM injection site for their drugs to ensure consistent and effective clinical use, ultimately improving patient safety and therapeutic efficacy.

19. **Butterfield, Natalie**
    - **Abstract title:** Evaluating the Suitability of FlowCam LO to Detect and Evaluate Subvisible Particulates in Biotherapeutic Drug Products
    - **Authors:** Butterfield, Natalie, FDA/CDER (Student); de Luna, Isabella, FDA/CDER (Student); Ilyushina, Natalia, FDA/CDER (Mentor); Bhirde, Ashwinkumar, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - Subvisible particulates (SVPs) in biotherapeutic drug products, including protein aggregates, pose concerns for product quality and immunogenicity. Current methods such as light obscuration (LO) may underestimate particulate counts due to the translucent nature of protein aggregates. Flow imaging (FI) offers morphological data potentially enhancing SVP characterization. However, FI is currently not included as a standard method for measuring subvisible particles by the USP due to insufficient data supporting its equivalency or similarity to LO. Standard microspheres, NIST standards (ETFE and mAb), and a therapeutic drug (Erbitux), were employed to compare FI with LO. Results show that FI is sensitive in detecting SVPs especially smaller particles compared to LO. Measurements of standard beads indicated an average 185% higher particle count with FI for 5 µm beads, decreasing with larger bead sizes. Analysis of Erbitux samples showed differences in particle size and count between expired and unexpired samples, with FI showing higher sensitivity and detecting smaller particles more effectively than LO. Similarly, NIST mAb standards demonstrated FI could measure particles that may be missed by LO, with a mean diameter of 2.7 µm and 188% higher particle counts per mL compared to LO. FlowCam LO shows promise in enhancing SVP quantification and characterization in therapeutic

proteins. The findings suggest a reconsideration of current standards to include FI, thus enhancing regulatory compliance and ensuring safer biopharmaceutical products through improved quality control.

- **Purpose**
  - Therapeutic proteins, such as monoclonal antibodies (mAbs), are susceptible to aggregation under chemical and physical stress. Protein aggregates, ranging from 100 nm to 10 mm, can induce immunogenicity. Subvisible particles (SVPs) in the size range of 2 to 100 µm, mostly protein aggregates, are of particular concern. Quantifying and characterizing SVPs in therapeutics is important for ensuring product quality. USP <1788> establishes light obscuration (LO) as the current standard method for measuring particles between10 µm and 25 µm. However, translucent protein aggregates smaller than 10 µm, which can also trigger immune responses, are not always detected by LO. Additionally, LO lacks the ability to characterize particles based on morphology. In contrast, flow imaging (FI) could provide detailed morphological data and superior sensitivity for transparent particles. FI captures high-speed images of particles as they flow, allowing for digital segmentation and characterization based on shape and grayscale. However, FI is currently not included as a standard method for measuring subvisible particles by the USP due to insufficient data supporting its equivalency or similarity to LO. Therefore, there is a need to evaluate the comparative assessment of FI and LO in the measurement of subvisible particles in therapeutics.
- **Methods**
  - To test the suitability of FlowCam LO (i.e., FI method), three different categories of industry standards polystyrene beads, NIST ETFE and NIST mAb were analyzed. Standard microsphere beads with diameters of 5 µm, 10 µm, 25 µm and 50 µm were used to determine the size measurement specificity of FlowCam LO. A total of 3 separate runs on different days were completed to ensure measurement reproducibility and repeatability. Initial samples were prepared by diluting suspended microsphere drops into filtered water. Samples with beads of 5 µm and 10 µm were prepared by adding 3 drops of solution to 1 mL of water. Beads of 25 µm and 50 µm were prepared by adding 4 drops of solution to 1 mL of water. The individual sample runs consisted of 500 µL total volume. Particles can get generated during storage of the DPs. Therefore, to evaluate the sensitivity of FI and LO in marketed therapeutic protein DPs samples of unexpired and expired Erbitux (Cetuximab) were tested following bead calibration. Three runs were performed for both conditions, with each sample consisting of 500 µL. Dynamic Light Scattering measurements were performed to quickly screen the drug products (DPs) and evaluate their signature peaks. Additionally, unexpired Cetuximab was run at

different sample volumes of 500 µL, 400 µL, and 300 µL with each volume being ran a total of three times. To evaluate the impact of particle counts and detection sensitivity, NIST mAb were also tested. NIST mAb standards were ran at volumes of 300 µL, followed by a 50% dilution to make three separate runs of 150 µL of NIST mAb and 150 µL of filtered water mixed for a total volume of 300 µL.

- **Results**
    - FI measured higher particle counts per mL when compared to LO. The standard bead runs showed that FlowCam was able to measure the diameters of standard polystyrene beads with an R^2 value of theoretical vs measured particle diameter in 0.9874 µm. The average difference between FI and LO particle measurements for 5 µm beads was about 185% with similar values on the two consecutive days. This percent difference for particle counts between FI and LO decreased with an increase in bead diameter size. Beads of 50 µm showed an average percent difference of 50% between FlowCam and LO. Unexpired Cetuximab showed an average lower particulate diameter size when compared to expired Cetuximab. Expired Cetuximab also showed a larger number of particulates with diameters of 25 µm and above, while unexpired samples showed an average of 0 particulates with diameters larger than 25 µm. Samples of unexpired Cetuximab with varying volumes showed a percent difference in particle count per mL between FlowCam and LO ranging from 160% to 180%. NIST Standard mAb had an average mean diameter of 2.7 µm, with FlowCam measuring 188% higher particle counts per mL when compared to LO.
- **Implications**
    - Our findings show the potential suitability of FlowCam LO as a method for the quantification and characterization of SVPs in therapeutic proteins. FI's higher sensitivity for smaller and translucent particles, shows capability in detecting and measuring particles that might be missed by LO. Morphological data provided by FlowCam LO can offer insights into the nature and behavior of protein aggregates, needed for understanding their impact on product quality and immunogenicity. The significant difference in particle counts and sizes observed between FI and LO, particularly for smaller particles, suggest that relying solely on LO may lead to an underestimation of SVP content in therapeutics. This underestimation could potentially compromise patient safety and product efficacy. Therefore, incorporating FI into standard particle measurement protocols could enhance the detection and characterization of SVPs, leading to improved quality control and better regulatory compliance. The reproducibility of FlowCam LO measurements across different sample volumes and conditions supports its robustness as an analytical tool.

These results can help advocate for a reevaluation of current USP standards to consider the inclusion of flow imaging methods like FlowCam LO. Adopting such advanced technologies could provide a better understanding of SVPs in biopharmaceutical products, contributing to safer therapeutics.

20. **Chen, Guannan**
    - **Abstract title:** Efficacy Evaluations for Clinical Trials Intended to Support Endometriosis-Associated Pain (EAP) Products
    - **Authors:** Chen, Guannan, FDA/CDER (Student); Chefo, Solomon, FDA/CDER (Mentor); Tang, Yun, FDA/CDER (Mentor); Lin, Daphne, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **<u>Synopsis</u>**
            - Endometriosis is one of the most common gynecological diseases, affecting 5 to 10 percent of reproductive age women (Taylor, Kotlyar, & Flores, 2021). Currently, there is no cure for endometriosis and the treatments have focused on managing pain symptoms, most commonly chronic pelvic pain, which may occur during menses (dysmenorrhea [DYS]), between menses (non-menstrual pelvic pain [NMPP]), or during sexual intercourse (dyspareunia) (Greene, et al., 2016). The efficacy evaluation of drug products intended for managing endometriosis-associated pain (EAP) relies on co-primary endpoints: improvement in pain scores during menses (DYS) and between menses (NMPP). Both endpoints use a patient-reported outcome scale (e.g., an 11-point Numerical Rating Scale [NRS; 0 = no pain, 10 = the worst pain imaginable]) to assess pain and take rescue analgesic use into account.
            - Previous drug approvals for EAP trials have been based on a responder analysis where a responder for DYS (or NMPP) is defined as achieving a reduction in mean DYS (or NMPP) scores by at least pre-determined threshold values without an increase in rescue analgesic use compared to baseline (a composite strategy). However, in the responder analysis, converting the actual numerical DYS and NMPP pain scores to a responder/non-responder status poses risks of potential loss of statistical power (especially when sample size is of concern) and misclassification errors (i.e., misclassification of patients who do not experience a meaningful change as responders due to the use of a specific responder threshold). Additionally, the composite strategy to address rescue analgesic use may provide an anti-conservative estimate, especially if more participants in the placebo group had increased rescue analgesic use compared to participants in the test group, and if the rescue effect only lasts a short duration as is common in pain management. In our work, we revisited one approved NDA for EAP indication and assessed DYS and NMPP pain scores as continuous efficacy outcomes while considering the impact of increased rescue analgesic use and the half-life of analgesics. We compare results when assessing pain scores as continuous outcomes against as binary responder outcomes using the NDA data and

through a simulation study.

- **Purpose**
  - The purpose of this project is to evaluate the current statistical analysis methods used in endometriosis-associated pain (EAP) trials and to compare them with an alternative statistical approach. The comparison will be based on an approved NDA data and a simulation study.
- **Methods**
  - We revisited one approved NDA for EAP indication and assessed the commonly used co-primary endpoints of dysmenorrhea (DYS) and non-menstrual pelvic pain (NMPP) pain scores as continuous efficacy outcomes while taking into account the impact of increased rescue analgesic use and the half-life of the analgesics in the analysis. Specifically, we compared results when assessing pain scores as continuous outcomes against as binary responder outcomes using the NDA data and through a simulation study. Logistic regression models for binary outcomes and a mixed-effects model for repeated measures (MMRM) and an Analysis of Covariance (ANCOVA) model with multiple imputation (MI) for continuous outcome were used to estimate the treatment effects. A tipping point analysis was conducted as a sensitivity analysis to assess the robustness of the MMRM approach under deviation from missing at random assumption.
  - In addition, in our analyses of DYS pain score using the NDA data, we applied two approaches to handle the subjects with no reported menstrual status during a pain assessment window: 1) the average score was assigned as 0 (sponsor's approach); 2) the average score was treated as missing.
  - We also adopted different approaches to account for the impact of increased rescue analgesic use in responder analysis and continuous outcomes analysis. In the responder analysis, a subject was considered as a non-responder in the final analysis if she was found to have increased use of analgesics at Week 24/EOT compared to baseline. In continuous outcomes analysis, we first determined the daily responder status for each subject and then censored the DYS or NMPP scores on the days when analgesic use non-responder status was identified.
- **Results**
  - Regarding the treatment effect on DYS and NMPP, our preliminary analysis of the NDA data suggests that both responder analysis and continuous outcomes analysis produced the same conclusions. We found that the impact of increased analgesic use was minimal due to the small percentage of affected pain scores in the NDA data. However, there were substantial differences in the treatment effect estimates on DYS between the two approaches for subjects with no reported menstrual status during a pain assessment window. Our approach resulted in a much smaller estimate compared to the sponsor's approach, indicating that the sponsor's treatment effect estimates on DYS could be anti-conservative.
  - Further comparisons of the responder analysis and continuous outcomes analysis in terms of operating characteristics will be

conducted through simulation. The simulation study is currently ongoing, and the results will be shared at the poster presentation.

- **Implications**
    - Dichotomizing continuous pain scores into binary responder outcomes can result in a significant loss of information. However, analyzing the data on its original continuous scale retains more information about its variability and distribution. Responder analysis may also have reduced statistical power, necessitating a larger sample size to achieve the same power as continuous outcomes analysis. Additionally, the choice of thresholds for dichotomization can be arbitrary and may influence results, potentially leading to different conclusions and introducing subjectivity and bias.
    - Furthermore, the findings from this research could guide better statistical practices for future EAP trials. For instance, they may provide insights on (i) accounting for increased analgesic use when analyzing continuous DYS and NMPP data, (ii) handling subjects with no reported DYS scores at a given visit, and (iii) determining the minimum number of reported DYS/NMPP scores needed to compute a reliable monthly score for analysis.

21. **Chen, Kevin**
    - **Abstract title:** Machine Learning Models and Monto Carlo Simulations Applied to CMC Data Analysis on Crystallinity Monitoring and Control Strategy During Drug Development, Manufacturing, and Regulatory Assessment
    - **Authors:** Chen, Kevin, FDA/CDER (Student); Wu, Huiquan, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation; Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Along with advancements in data management and standardization practices, advanced machine learning could be an important tool to address significant and evolving challenges in the CMC domain. These challenges include the potential for missing data related to crystallinity monitoring and control strategies in the CMC area. Missing data could result due to crystallinity concerns being accounted for in formulation and process design or coming about through omissions in data submissions. This situation may present significant obstacles in CMC assessment including statistical analyses of CMC data when needed. In this work, we demonstrate the feasibility of using machine learning techniques to illustrate these challenges including associated risk and highlight the importance of rational process design and drug manufacturing process controls for ensuring product quality and risk mitigation.
        - **Purpose**
            - During pharmaceutical drug product development and manufacturing, polymorphism and associated crystallinity change are important process phenomena which may pose potential risk to drug product manufacturing, critical quality attributes (CQAs), efficacy, and safety under certain circumstances. Typically, such potential risks can be mitigated via efforts during drug development, manufacturing, and regulatory assessment prior to commercial manufacturing, etc. This

often requires extensive expertise across the board and appropriate oversight from regulatory authorities. While a great deal of scientific literatures is available for crystallinity and polymorphism characterization for certain specific high-risk drugs, to the best of our knowledge, commonality analysis and methodology development for crystallinity monitoring and control strategy across different drugs from CMC submission data have been barely reported. This study aims to explore the application of advanced machine learning models and Monto Carlo simulations to CMC submission data analysis on Crystallinity Monitoring and Control Strategy across Drug Development, Manufacturing, and Regulatory Assessment.

- **Methods**
  - Through data mining of approved NDAs and ANDAs by the FDA during 2017-2022 which had polymorphism and/or crystallinity keywords, we established a dataset which contained 148 approved NDAs and ANDAs and involved crystallinity monitoring and control strategy development of high-risk drug product manufacturing processes.
  - We applied clustering technique to the crystallinity CMC dataset generated for exploratory pattern recognition.
  - We applied several machine learning techniques and Monto Carlo simulations to the crystallinity CMC data set for risk classification in the pharmaceutical manufacturing CMC domain.
- **Results**
  - Exploratory clustering results using the KAMILA algorithm were obtained. Clusters formed by the k-prototypes algorithm were found to be similar to those found by the KAMILA algorithm.
  - An optimal cluster size of 3 was chosen and the distribution of covariate characteristics within each cluster is displayed.
  - This visualization provides a demonstration in how patterns in cluster characteristics can be discovered which, combined with external data or expert domain knowledge, may provide deeper insights into areas potentially requiring further scrutiny in CMC practice. 100% of drug products in cluster 1 have a milling manufacturing step, while the same is true for approximately 75% of drug products in clusters 2 and 3.
- **Implications**
  - We have demonstrated a few of the potential applications for statistical missing data and machine learning methodology in analyses of crystallinity monitoring and control strategies of high-risk pharmaceutical manufacturing processes.
  - We have provided an exploratory cluster analysis to discover patterns in approved drug submissions.
  - We have also conducted simulations under a generated risk-related outcome of interest using several common machine learning classification approaches (i.e., logistic regression, decision trees, random forest, and support vector machines).
  - Some scientific and technical considerations from FDA regulatory perspective will be provided to stimulate more interests in this challenging and evolving interdisciplinary area, and to facilitate implementation of innovative technologies across drug development, manufacturing, regulatory submission and assessment for ultimately

fulfilling public health mission, and making safe, effective, and high-quality medicines to the public.
    - Acknowledgements: Yingjie Chen, Ph.D., FDA CDER 2022 Summer ORISE Fellow; Daniel Obrzut, Ph.D, Division of Pharmaceutical Manufacturing Assessment II, OPMA, OPQ, CDER, FDA

22. **Cho, Ginny**
    - **Abstract title:** Review of Supplemental BLA for the Addition of New Drug-Linker Manufacturing Sites for Antibody-Drug Conjugate Products
    - **Authors:** Cho, Ginny, FDA/CDER (Student); Rivera-Rosado, Leslie, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract**:
        - **<u>Synopsis</u>**
            - Supplemental biologics license applications (sBLA) for antibody drug conjugate (ADC) products are reviewed by both small molecule and biologics reviewers at OPQ. A review of the content of all post-approval supplements submitted for ADC products was completed to identify common factors among the sBLA submitted to the Agency for the addition of new drug-linker intermediate manufacturing sites. Information gathered from this study will enable consistent expectations and assessment of data submitted to support the addition of new manufacturing sites for drug-linker intermediates of an ADC product.
        - **<u>Purpose</u>**
            - To review the content of all supplemental Biologics License Applications (sBLAs) submitted to the Agency for antibody drug conjugate (ADC) products and identify those sBLAs that specifically proposed the addition of a new manufacturing site for drug-linker intermediates (DL). The sBLA will be reviewed to identify the type and amount of Chemistry, Manufacture, and Controls data provided to support the DL manufacturing site addition. This information will be used to develop a recommended practice document to help assessors effectively and consistently review this type of sBLA for ADCs.
        - **<u>Methods</u>**
            - Using FDA regulatory platforms, DARRTS and CDER Informatic Platform (Panorama), identify all sBLA submitted to the Agency for all eleven (11) FDA-approved ADCs. A total of 186 sBLAs were identified; of which six (6) sBLA supplements were for the addition of a new manufacturing (and testing) site for DL and one (1) was for a Post-Approval Change Management Protocol (PACMP) for a future addition of a new DL manufacturing site. A comparison of the type and amount of data submitted in the sBLA to support the DL manufacturing site addition and the Information Requests (IR) sent by the Agency for the 7 supplements was completed.
        - **<u>Results</u>**
            - According to the review of the 7 ADC supplements, the following type and amount of data were identified as common among the submissions: (1) release and comparability data for three process performance qualification (PPQ) batches for the DL; (2) at least 3 months of accelerated DL stability data comparing at least 3

commercial scale batches of pre- and post-change DL; and (3) at least 3 months of long-term DL stability data.
    - Among the reviewed supplements, 4 supplements had the same DL manufacturing process at the new site when compared to the approved DL manufacturing site while 2 supplements had modified DL manufacturing processes.
    - After determination of pre- and post-change DL comparability, 5 out of 7 sBLA submissions provided drug substance (DS) release data for a minimum of 3 DS batches (DS is the result of conjugating the DL to the antibody intermediate); while one submission was refused to file due to lack of DS data. Furthermore, 5 submissions included at least 3 months of long term and some accelerated stability data for DS. A review of the PACMP for the addition of an alternative DL manufacturing site revealed that if adequate data and risk assessment are provided for the DL, that release data for DS batches manufactured with post-change DL might not be needed to support the change and that a commitment to place the first DS batch manufactured with the post-change DL into the stability program might be sufficient.
    - The most common Information Requests (IR) sent out by the Agency during the assessment of the sBLA were for the request of additional stability data, which was later submitted by the applicants.
  - **Implications**
    - The information gathered from this study will be used to write a Recommended Practice document to ensure consistent review and expectations amongst small molecule and biologics reviewers at OPQ for this type of sBLA. In addition, the information from this study was used to support an Agency presentation titled: *Regulatory Considerations for Bioconjugates from the Small Molecule Perspective* to be presented at the CASSS CMC Strategy Forum Summer 2024.

23. **Chopra, Pranshu**
    - **Abstract title:** Determination of Tolbutamide and Warfarin in buffer using ultra high-performance liquid chromatography–tandem mass spectrometry for in vitro assays
    - **Authors:** Chopra, Pranshu FDA/CDER (Student); Qusa, Mohammed FDA/CDER; Mistry, Sabyasachy FDA/CDER; DePalma, Ryan FDA/CDER (Mentor); Matta, Murali FDA/CDER; Volpe, Donna FDA/CDER; Rouse, Rodney FDA/CDER.
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response and Empowering patients and consumers
    - **Abstract:**
      - **Synopsis**
        - Warfarin and tolbutamide are index substrates for CYP2C9 metabolism and are currently recommended by the FDA to assess clinical drug-drug interactions (DDI). Thus, it is important to determine the concentration of tolbutamide (TOB) and warfarin (WAR) and their primary metabolites, 4-hydroxytolbutamide (4-OH-TOB), 7-hydroxywarfarin (7-OH-WAR), respectively, within potassium phosphate buffer used in metabolism assays. A robust selective and sensitive UHPLC–MS/MS methods for determination TOB and WAR and their metabolites was developed and validated. A reversed phase

UHPLC C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile mobile phases were used for chromatographic separation. Analytes and labeled internal standards, were extracted from the phosphate buffer using a simple protein precipitation method and analyzed with a total chromatographic run time of 2.2 min. The UHPLC column provided baseline separation between target analytes and matrix peaks. The optimized chromatographic conditions provided optimal retention and excellent peak shape for analytes and internal standards. The assay was linear over a concentration range of 3.0-384.0 nM, inter- and intra-assay precision and accuracy were less than ±10.0% for both drugs and their corresponding metabolites. Recovery was more than 90.0% for all analytes. Matrix effects were studied by spiking quality control samples at concentrations of 9.0 nM and 325.0 nM of each analyte into 6 different sets. No significant matrix effects were observed, precision and accuracy were within ±15.0%. The methods for TOL and WAR and their metabolites in HLM solution have been validated and used to support of in vitro metabolism studies.

- **Purpose**
  - The primary purpose of the study is to provide analytical support for two in vitro metabolism assays. These assays are designed to provide insights on additional clearance mechanisms, such as OAT2 hepatic uptake, for warfarin and tolbutamide which are utilized as index substrates of CYP2C9 for clinical DDI studies. To that end, we developed and validated selective and sensitive UHPLC–MS/MS methods to detect and quantify tolbutamide (TOB) and warfarin (WAR) and their metabolites, 4-hydroxy tolbutamide (4-OH-TOB), 7-hydroxy warfarin (7-OH-WAR), respectively, within the human liver microsomes (HLM) buffer.
- **Methods**
  - All samples will be analyzed using the LC-MS/MS method. The method was linear over a concentration range of 3.0-384.0 nM. Back-calculated concentrations were determined using a least squares regression analysis employing a weighted (1/x2) linear regression (y=mx+b). Reversed phase UHPLC C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile mobile phases were used for separation with a total chromatographic runtime of 2.2 minutes. Extraction of analytes from HLM buffer involved adding 120 µL of acetonitrile containing internal standards to 96-well filter plates. An aliquot of 30 µL of HLM media was added to each well. Samples were shaken for 5 mins at 500 rpm and centrifuged at 4000 rpm for 2 min. The collection plate containing supernatant was placed in autosampler and 5 µL was injected into a mass spectrometer.
- **Results**
  - The method validation results are as follows: Inter- and intra-assay precision and accuracy were less than ±10.0% for both drugs and their corresponding metabolites. Recovery was more than 90.0% for all analytes. Matrix effects were studied by spiking quality control samples at concentrations of 9.0 nM and 325.0 nM of each analyte into 6 different sets. No significant matrix effects were observed, precision and accuracy were within ±15.0%. The methods for TOB

and WAR and their metabolites in HLM buffer has been validated and used to support of in vitro studies.

- **Implications**
    - The outcome of this study will be validated bioanalytical methods for the precise and accurate simultaneous detection method for tolbutamide, warfarin, and their active metabolites- 4-hydroxy tolbutamide and 7-hydroxy warfarin. Additionally, data generated from these methods will help to evaluate the in vitro inhibition of WAR and TOB met.

24. **Chun, Rachel**
    - **Abstract title:** Penetration of Anti-infective Agents into Pulmonary Epithelial Lining Fluid
    - **Authors:** Chun, Rachel, FDA/CDER (Student); Wu, Kunyi, FDA/CDER (Mentor); Yang, Xiaoxia, FDA/CDER (Mentor); Nicasio, Anthony, FDA/CDER (Mentor); Zhang, Lainey, FDA/CDER (Student)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - Warfarin and tolbutamide are index substrates for CYP2C9 metabolism and are currently recommended by the FDA to assess clinical drug-drug interactions (DDI). Thus, it is important to determine the concentration of tolbutamide (TOB) and warfarin (WAR) and their primary metabolites, 4-hydroxytolbutamide (4-OH-TOB), 7-hydroxywarfarin (7-OH-WAR), respectively, within potassium phosphate buffer used in metabolism assays. A robust selective and sensitive UHPLC–MS/MS methods for determination TOB and WAR and their metabolites was developed and validated. A reversed phase UHPLC C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile mobile phases were used for chromatographic separation. Analytes and labeled internal standards, were extracted from the phosphate buffer using a simple protein precipitation method and analyzed with a total chromatographic run time of 2.2 min. The UHPLC column provided baseline separation between target analytes and matrix peaks. The optimized chromatographic conditions provided optimal retention and excellent peak shape for analytes and internal standards. The assay was linear over a concentration range of 3.0-384.0 nM, inter- and intra-assay precision and accuracy were less than ±10.0% for both drugs and their corresponding metabolites. Recovery was more than 90.0% for all analytes. Matrix effects were studied by spiking quality control samples at concentrations of 9.0 nM and 325.0 nM of each analyte into 6 different sets. No significant matrix effects were observed, precision and accuracy were within ±15.0%. The methods for TOL and WAR and their metabolites in HLM solution have been validated and used to support of in vitro metabolism studies.
        - **Purpose**
            - The primary purpose of the study is to provide analytical support for two in vitro metabolism assays. These assays are designed to provide insights on additional clearance mechanisms, such as OAT2 hepatic uptake, for warfarin and tolbutamide which are utilized as index substrates of CYP2C9 for clinical DDI studies. To that end, we

developed and validated selective and sensitive UHPLC–MS/MS methods to detect and quantify tolbutamide (TOB) and warfarin (WAR) and their metabolites, 4-hydroxy tolbutamide (4-OH-TOB), 7-hydroxy warfarin (7-OH-WAR), respectively, within the human liver microsomes (HLM) buffer.

- **Methods**
  - All samples will be analyzed using the LC-MS/MS method. The method was linear over a concentration range of 3.0-384.0 nM. Back-calculated concentrations were determined using a least squares regression analysis employing a weighted (1/x2) linear regression (y=mx+b). Reversed phase UHPLC C18 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile mobile phases were used for separation with a total chromatographic runtime of 2.2 minutes. Extraction of analytes from HLM buffer involved adding 120 µL of acetonitrile containing internal standards to 96-well filter plates. An aliquot of 30 µL of HLM media was added to each well. Samples were shaken for 5 mins at 500 rpm and centrifuged at 4000 rpm for 2 min. The collection plate containing supernatant was placed in autosampler and 5 µL was injected into a mass spectrometer.
- **Results**
  - The method validation results are as follows: Inter- and intra-assay precision and accuracy were less than ±10.0% for both drugs and their corresponding metabolites. Recovery was more than 90.0% for all analytes. Matrix effects were studied by spiking quality control samples at concentrations of 9.0 nM and 325.0 nM of each analyte into 6 different sets. No significant matrix effects were observed, precision and accuracy were within ±15.0%. The methods for TOB and WAR and their metabolites in HLM buffer has been validated and used to support of in vitro studies.
- **Implications**
  - The outcome of this study will be validated bioanalytical methods for the precise and accurate simultaneous detection method for tolbutamide, warfarin, and their active metabolites- 4-hydroxy tolbutamide and 7-hydroxy warfarin. Additionally, data generated from these methods will help to evaluate the in vitro inhibition of WAR and TOB met.

25. **Cionfolo, Haley**
    - **Abstract title:** Non-Approval Outcome Trends for CDER New Drug and Biologic License Applications, 2013-2022
    - **Authors:** Cionfolo, Haley, FDA/CDER (Student); Bugin, Kevin, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - It has been reported that the issuance of Complete Response Letters for novel new drugs reviewed by CDER has increased over the last decade. This study sought to characterize non-approval outcomes occurring between 2013 to 2022 to enhance the existing regulatory knowledgebase and inform the successful planning of drug development. A master database of the outcomes for novel drug and biologic applications in CDER occurring between 2013 to 2022 was

developed. Descriptive analysis was used to determine the distribution of outcomes across the dataset by variables like year, pharmaceutical company size, orphan drug designation, and outcome type. This mixed-methods review included 517 initial submissions and 140 non-approval outcomes. Of the 202 initial submissions from 2013-2017, 45 were not approved; while 95 of the 315 initial submissions in 2018-2022 were not approved. Of all non-approval outcomes from 2013-2022, 106 were of applications from small businesses, with 37 of these designated for orphan drugs. Non-approvals from non-small businesses comprised 34 non-approval outcomes, with only 5 pertaining to orphan designated drugs. Applications from small pharmaceutical companies disproportionately represented the non-approval outcomes occurring in 2013-2022. Non-approval outcomes were also disproportionately distributed across the five-year periods, with greater occurrences in 2018-2022. A rise in non-approval outcomes for novel new drugs and biologics could represent an opportunity for FDA and applicants to work together to better prepare and guide programs through the regulatory process.

- **Purpose**
    - It has been reported that the issuance of Complete Response Letters for novel new drugs reviewed by CDER has increased over the last decade. This study sought to characterize non-approval outcomes occurring between 2013 to 2022 to enhance the existing regulatory knowledgebase and inform the successful planning of drug development. What are the key trends for non-approval outcomes of CDER NMEs and original BLAs from 2013 to 2022? What are the differences in trends in non-approval outcomes between small business and non-small businesses applicants, and differences between orphan and non-orphan drug designated programs?
- **Methods**
    - This study is a mixed method review of reports from the Data Analysis Search Host (DASH). Between 2013 and 2022, 602 applications were submitted to the Center for Drug Evaluation and Research, 517 of which were initial submissions. Of these, 140 leading to non-approval outcomes. A master database of these outcomes for novel drug and biologic applications in CDER occurring between 2013 to 2022 was developed. Descriptive analysis was used to determine the distribution of outcomes across the dataset by variables like year, pharmaceutical company size, orphan drug designation, and outcome type.
- **Results**
    - This study sought to describe the distribution of non-approval outcomes across variables over the last decade and included 517 initial submissions and 140 non-approval outcomes. Of the 202 initial submissions from 2013-2017, 45 were not approved; while 95 of the 315 initial submissions in 2018-2022 were not approved. Of all non-approval outcomes from 2013-2022, 106 were of applications from small businesses, with 37 of these designated for orphan drugs. Non-approvals from non-small businesses comprised 34 non-approval outcomes, with only 5 pertaining to orphan designated drugs. Applications from small businesses disproportionately represented

the non-approval outcomes occurring in 2013-2022. Non-approval outcomes were also disproportionately distributed across five-year periods, with a greater number in 2018-2022.

- **Implications**
    - Applications from small pharmaceutical companies disproportionately represented the non-approval outcomes occurring in 2013-2022. Non-approval outcomes were also disproportionately distributed across the five-year periods, with greater occurrences in 2018-2022. A rise in non-approval outcomes for novel new drugs and biologics could represent an opportunity for FDA and applicants to work together to better prepare and guide programs through the regulatory process.

26. **Clayman, Samantha**
    - **Abstract title**: Long-Acting Injectable Antipsychotics: Review of Delivery System and Generic Product Development
    - **Authors**: Clayman, Samantha, FDA/CDER (Student); Zhang, Qiangnan, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - The FDA has approved twelve New Drug Applications (NDAs) for long-acting injectable (LAI) antipsychotic products for the treatment of schizophrenia and bipolar disorder. These LAI antipsychotics are crucial for enhancing patient compliance, improving adherence, increasing quality of life, and reducing healthcare costs. However, these products are often expensive, limiting their accessibility. Introducing generic counterparts would make these medications more affordable and widely available, benefiting a larger patient population. Currently, only two generic LAI products are on the market, which could be attributed to several factors: patent issues, complex formulation and excipients, complicated bioequivalence (BE) study design, etc. This study summarizes the formulation technologies used for extended antipsychotic drug release and discusses the challenges in developing generic products.
        - **Purpose**
            - This study aims to summarize the dosage and administration, dosage forms and strengths, and formulation for LAI antipsychotic new drug products. It also discusses the challenges associated with developing generic LAI antipsychotic drug products.
        - **Methods**
            - An in-depth analysis of scientific literature and FDA databases (e.g., Orange Book and Drugs@FDA) on LAI antipsychotic products.
        - **Results**
            - The twelve approved LAI products are based on different delivery systems for the drug substances aripiprazole, aripiprazole lauroxil, olanzapine pamoate, paliperidone palmitate, and risperidone. There are three different mechanisms in which these antipsychotic products work, including dopamine D2 receptor antagonists (i.e., olanzapine pamoate, paliperidone palmitate, and risperidone), partial D2 agonists (i.e., aripiprazole and aripiprazole lauroxil), partial serotonin 5-HT1A

receptor agonists (i.e., aripiprazole and aripiprazole lauroxil), and 5-HT2A receptor antagonists (i.e., aripiprazole, aripiprazole lauroxil, olanzapine pamoate, paliperidone palmitate, and risperidone).

- In addition, the approved LAI new products are categorized based on our scientific understanding of the formulation technologies utilized for achieving extended drug release:*PLGA Microsphere*: This technology was the first delivery system for LAI antipsychotic drug products. It has been used in two biweekly risperidone products (Risperdal Consta (NDA 021346) approved in 2003 and Rykindo (NDA 212849) approved in 2023). The recently approved product, Rykindo, offers a shorter oral supplement phase when initiating risperidone treatment.
- *In-Situ Forming Depot*: This method is also used in risperidone products. It provides extended-release via three phases (i.e., initial drug release, drug diffusion, and implant erosion) and allows for once-monthly or every two-month dosing intervals (i.e., Perseris (NDA 210655) approved in 2018, Uzedy (NDA 213586) approved in 2023, and Risvan (NDA 214835) approved in 2024), with no oral supplement phase.
- *Drug Substance Injectable Suspension*: The drug substance is the only insoluble component in the formulation, and therefore, the extended release is achieved by the slow dissolution of the insoluble drug substance. This formulation method has been used for aripiprazole (i.e., Abilify Maintena (NDA 202971) approved in 2013 and Abilify Asimtufii (NDA 217006) approved in 2023) allowing once a month and once every two-month dosage intervals with an oral supplementation phase, respectively; aripiprazole lauroxil (i.e., Aristada (NDA 207533) approved in 2015 and Aristada Initio (NDA 209830) approved in 2018), allowing every six-week dosage interval and initial loading dosage with an oral supplementation phase, respectively; olanzapine pamoate (i.e., Zyprexa Relprevv (NDA 022173) approved in 2009), allowing biweekly or monthly dosing intervals; and paliperidone palmitate (i.e., Invega Sustenna (NDA 022264) approved in 2009, Invega Trinza (NDA 207946) approved in 2015, and Invega Hafyera (NDA 207946) approved in 2021) allowing once monthly, once every three-month, and once every six-month dosing, respectively.
- The complexity of these formulation technologies presents significant challenges for generic product development, given small process and raw material manufacturing and control differences may result in significant product performance deviations. Generally, in vivo steady-state pharmacokinetic (PK) studies on patients are recommended for demonstrating BE of generic products. The BE study design is challenging due to the limited subject pool, the long duration of the BE study, and the high drop-out rate among schizophrenia patients.
- Additionally, patent issues are a significant consideration in developing generic products. For example, Teva's generic paliperidone palmitate injectable suspension was approved in 2021 and then discontinued until 2024 after winning the patent lawsuit

against Janssen, the NDA holder of Invega Sustenna (NDA 022264), returning to the market.

- **Implications**
    - The overview of approved LAI new drug products provides insight into current LAI antipsychotic product development direction to improve patient compliance, such as eliminating the oral supplement phase and extending dosing intervals. Recognizing the challenges associated with the development of generic LAI antipsychotic drug products are helpful for addressing scientific gaps for the FDA to evaluate generic drug equivalence and for the industry to efficiently develop generic products.

27. **deLuna, Isabella**
    - **Abstract title:** Insulin drug product quality in pump system – Assessment of subvisible particles
    - **Authors:** Isabella de Luna, FDA/CDER (Student); Natalie Butterfield, FDA/CDER (Student); Nicholas Trunfio, FDA/CDER; Qiong Fu, FDA/CDER; Suvajyoti Guha, FDA/CDRH (Mentor); Ashwinkumar Bhirde, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - The presence of subvisible particles (SVPs) in therapeutic protein drug products, such as insulin, is a critical quality attribute (CQA) in drug product (DP) development due to risks to patient safety. Changes in SVP content, including size, concentration, and type, can result from mechanical and thermal stress during insulin pump use. This research employed emerging techniques to detect and characterize SVPs. For SVP detection, flow imaging microscopy (FIM) was utilized, while characterization was performed using morphologically directed Raman spectroscopy (MDRS) and high-throughput dynamic light scattering (HT-DLS). These methods were applied to evaluate the quality of insulin drug products (DP) under various stresses and pump system configurations. 3 insulin analogs and 1 follow-on insulin analog will be utilized to assess their SVP stability with various techniques under stressed conditions. Preliminary data with Humalog and Admelog show that both control and stressed samples exhibited various particle types, including spherical silicone oil particles and globular protein aggregates. However, stressed samples contained much larger silicone oil particles and a notable increase in particle concentration compared to control samples. The size distribution differed, with stressed samples having a higher proportion of particles greater than 10 µm. Additionally, light obscuration (LO) detected a small fraction of the particles that FIM detected, highlighting FIM's potential as a more sensitive SVP detection method. Results from this study will assist reviewers from CDER and CDRH in making science-based review decisions when assessing insulin and insulin pump-related submissions.
        - **Purpose**
            - Diabetic patients rely on insulin drug products (DPs) to maintain blood glucose levels, typically self-administering these medications. Insulin

pumps are popular for their near-physiologic, programmable doses and lifestyle compatibility. However, patients using tubed pumps may expose their insulin and device to extreme that are not typically addressed in current guidance documents, posing a risk to clinical outcomes. Evidence suggests insulin aggregation in the pump reservoir and infusion set tubing can lead to occlusions and compromise effectiveness. This research studies the effects of both mechanical and thermal stress, tube length, and pump flow rate on insulin subvisible particle (SVP) content, a critical quality attribute (CQA). We used flow imaging microscopy (FIM), light obscuration (LO), high-throughput dynamic light scattering (HT-DLS), and morphologically directed Raman spectroscopy (MDRS) to comprehensively characterize SVPs in stressed insulin samples. Addressing these regulatory concerns supports improved manufacturing, testing, and surveillance practices, enhancing insulin pump therapy safety and effectiveness.

- **Methods**
  - Light obscuration (LO) and flow imaging microscopy (FIM), as indicated in USP <788> for detecting particulate matter in injectable therapeutics, were primarily employed in this study. High-throughput dynamic light scattering (HT-DLS) and morphologically directed Raman spectroscopy (MDRS) were also used to further characterize the insulin drug products (DPs) and their particulates for signature peaks and morphology and chemical identity. This study evaluated four insulin analogs Humalog and Admelog (insulin lispro DPs), Novolog (insulin aspart DP), and Apidra (insulin glulisine DP) under various mechanical (100-300 RPM) and thermal (45 °C) stresses. A popular insulin pump system from Medtronic was used to test the quality of the insulin DPs under these stress conditions.
- **Results**
  - Various particle types were observed in Humalog and Admelog samples, including silicone oil, fibrous particles, globular protein aggregates, and other miscellaneous particles. Both Admelog and Humalog controls displayed comparable particle counts (Admelog: 9,873.2 ± 117.7, Humalog: 12,907.6 ± 1,653.1) and exhibited HT-DLS signature peaks at 5.3 ± 0.5 nm and 6.7 ± 0.1 nm, respectively. Stressed Admelog samples (100 RPM, 45 °C, 14 days) from pump systems had SVP concentrations 14 times higher (190,783 ± 24,590 P/mL) than the control (12,907 ± 1,653 P/mL) when measured using FIM. Stressed samples also displayed a wider silicone oil particle diameter range (1-60 µm) compared to control samples (1-7 µm). In control samples, 90% of particles were within the 2-10 µm range, whereas in stressed samples, this proportion decreased to 60%, with the remaining particles exceeding 10 µm. Additionally, LO detected only 5-20% of the particles that FIM detected, emphasizing FIM's superior sensitivity in detecting SVPs. Future steps will involve testing additional insulin analogs, integrating MDRS analysis, and assessing further parameters within the pump system. This includes examining the tube and cannula length of infusion sets and the flow rate to explore their potential impact on insulin quality.

- **Implications**
    - The implications of this research will aid in the development, regulation, and use of insulin drug products (DPs) in insulin pumps. Our preliminary findings indicate that there are high levels of silicone oil particles in the stressed samples prompting further investigation to address any quality issues., Pump system parameters such as infusion set tube length and flow rate under worst case scenarios may need investigation to ensure insulin quality when in contact with the pump components. The study underscores the importance of including both FIM and LO data assessments in regulatory submissions to detect SVP formation that may impact insulin DP quality. This research also demonstrates the utility of advanced characterization techniques like FIM (e.g., FlowCam LO) and HT-DLS in detecting and analyzing SVPs, providing a more comprehensive understanding of insulin DP quality in pump systems. Ultimately, improving the robustness of insulin formulations and pump systems can enhance therapeutic outcomes, reduce adverse events, and support better patient adherence and quality of life for individuals managing diabetes.

28. **Dong, Anna**
    - **Abstract title:** Standardization of Reporting PK Parameters in USPI Labels
    - **Authors:** Dong, Anna FDA/CDER (Student) and Bi, Youwei (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Purpose**
            - Specifically, this research will focus on:
            - Scoping all the USPI labels for new molecular entities and new therapeutic biological products approved in the past 5 years
            - Summarize the reporting of PK parameters
        - **Methods**
            - We extracted the new drugs used to treat oncology (cancer) / hematologic malignancies approved in the past 5 years from Drugs@FDA, focusing on New Molecular Entities for drugs and New Therapeutic Biological Products (NMEs). The units of PK parameters from these drugs and biologics were captured from USPI labels, including, but not limited to, clearance (CL), volume of distribution (V), half life (Thalf), area under the concentration-time curve (AUC), maximum concentration (Cmax), trough concentration (Ctrough). Further, how these units were described in the USPI label were also summarized. The summary statistics was conducted in the Excel.
        - **Results**
            - A total of 69 NMEs approved in the past 5 years for the treatment of oncology/ hematologic malignancies were summarized in this research, including the treatment of various cancers and solid tumors, including breast cancer, prostate cancer, etc. The reported units for the PK parameters in USPI varied both within and across drug products. Among the PK parameters surveyed, the unit of AUC has the most variations: µg.day/mL (1.7%), µg*h/mL (3.4%), µg·h/L (1.7%), µg·h/mL (1.7%), µg•hr/mL (3.4%), day*µg/mL (24%), day·mcg/mL (1.7%), day•µg/mL (1.7%), h*µM (1.7%), h*mg/mL (1.7%), h*ng/mL (1.7%), h*nM (1.7%), h·ng/mL (6.8%), mcg*h/mL (3.4%), mcg·h/mL

(1.7%), ng • day/mL (1.7%), ng*h/mL (8.0%), ng.day/mL (1.7%), ng.h/mL (6.8%), ng.hr/mL (5.1%), ng·h/mL (11.9%), ng·hr/mL (1.7%), nM·h (1.7%). The unit of V has the least variations: L (97%) and L/m2 (3%). CL has the following units: L/d (1.5%), L/h/m2 (3%), L/hour (4.5%), L/day (24%), L/h (42%), L/hour/m2 (1.5%), L/hours (1.5%), L/hr (17%), mL/h (3%), and mL/min (1.5%). Thalf has the following units: days (30%), h (6%), hours (58%), hr (1.5%), hrs (1.5%), and months (1.5%). Cmax has the following units: µg/mL (21%), U/mL (1.7%), nM (3.4%), ng/mL (60%), mcg/mL (10.5%), µM (1.7%). We also found inconsistency within product. For example, for most USPI, the unit of V is in L, whereas as volume units in AUC, Cmax and Cmin are in mL.

- **Implications**
    - In conclusion, the reported units for the PK parameters in USPI varied and are not consistent. For example, for time hour, there are a few illustrations including h, hr, hour and hours. Therefore, it is necessary to standardize the nomenclature for the units for the PK parameters in USPI. Our work provides a landscape for the PK parameter units that helps facilitating the unit standardization in the future.

29. **Fishstein, Michael**
    - **Abstract title:** Reduction of cell-surface sialic acid enhances fusion of filovirus GP-psudotyped virus-like particles in primary human monocyte-derived macrophages (MDM)
    - **Authors:** Fishstein, Michael, FDA/CDER (Student); Stantchev, Tzanko FDA/CDER (Mentor); Shapiro, Marjorie, FDA/CDER (Supervisor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - Sialic acid(s) terminating glycans are ubiquitously displayed on vertebrate cells and have been shown to serve as specific binding sites and/or receptors of different viruses. Alternatively, sialic acids on the virion surface may be recognized by members of the siglec family of cellular glycoproteins, thus facilitating virus attachment and cell fusion. This latter mechanism has been shown to play a role in Ebola virus (EBOV) infection. However, the role of cell surface sialic acid(s) in filovirus entry remained largely understudied. Using fusion competent, but replication incapable virus-like particles (VLP) we established that in contrast to many other viruses, reduction of cell surface sialic acid(s) significantly enhanced filovirus GP-mediated fusion and these effects were due to increased virion binding and endocytosis. Our findings contribute to better understanding of the mechanisms of filovirus cellular entry and may provide insights regarding the development of potential new anti-filovirus therapies.
        - **Purpose**
            - Investigate the role of cell-surface sialic acid(s) in the cell entry of filovirus (e.g. Ebola virus) surface glycoproteins (GP) pseudotyped virus-like particles (VLP).
        - **Methods**
            - Primary human macrophages were generated from elutriated monocytes by 7 to 10 days differentiation in DMEM supplemented with pooled human serum. Beta lactamase (BlaM)-containing VLP

were used to investigate the effects of neuraminidase and/or a sialyltranferase inhibitor(s) on the filovirus GP-mediated cell fusion. VLP containing the red fluorescent protein mCherry were used to study the effects of these treatments on VLP cell binding and endocytosis. The VLP were generated by co-transfecting 293T cells with plasmids encoding different filovirus GPs and a vector encoding the VP40-BlaM or VP40-mCherry chimeric proteins, respectively. In addition, biochemical methods were used to quantify the levels of cell-surface sialic acid.

- **Results**
  - Neuraminidase and/or sialyltransferase inhibitor pre-treatment of primary human MDM significantly enhance the fusion and entry of BlaM containing filovirus GP pseudotyped VLP into these cells. Experiments with analogous mCherry containing VLPs imply that these effects are due to increased VLP binding and endocytosis.
- **Implications**
  - Our findings contribute to better understanding of the mechanisms of filovirus entry into cells. In addition, they may provide new approaches for the development of new anti-filovirus therapies and methods for characterization and release testing of these therapies.

30. **Fulkerson, Gwendolyn**
    - **Abstract title:** Original BLA Complete Response Letter Product Quality Keyword Trend Analysis
    - **Authors:** Fulkerson, Gwendolyn, FDA/CDER (Student); Roelofs, Brian, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - We present an ongoing linguistic keyword analysis of Biologics License Application (BLA) Complete Response (CR) letters from the past ten years (2014-2024). Using a preexisting corpus, keywords were extracted, categorized, and analyzed; a database was created with identified words, and larger-scale keyword trends were quantified. Trends in product quality, facilities and microbiology related CR terms have varied by year. Specific keywords identified include data, control strategy, acceptance criteria, and process validation. This database will serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.
      - **Purpose**
        - We seek to generate a database of the terminology used in product quality items from Biologics License Application (BLA) Complete Response (CR) letters from 2014-2024. Using the content of past CR letters, the Agency can assess trends in CR actions, track deficiencies related to product quality (PQ), microbiology, and facility inspections that preclude approval of a BLA, and provide additional pre-BLA submission feedback for items of higher frequency where merited. This database will also inform and validate future automated data mining approaches for further refinement of CR letter analysis.

- **Methods**
    - A database of 108 CR letters from 2014 through 2024 was compiled. This corpus was further refined to a subset of 60 letters with headers and therefore sections pertaining to PQ, microbiology, and facilities. These were read in their entirety and keyword(s) were cataloged for each section of each letter. These keywords were sorted by topic, with up to two ranked subtopics. These topics were chosen based on recurring issues in the BLA PQ CR sections. The topics were analyzed by year and by frequency, and the letters themselves were analyzed by the contents of the PQ header. Counts of each topic were graphed in a scatter plot to assess frequency, and trend lines were fitted to analyze patterns for topics at each level and sub-level.
- **Results**
    - The proportion of CR letter headers corresponding to PQ, microbiology, and facilities has varied annually. The observed trends will be discussed. The individual PQ topics that have the greatest frequency over time are data, control strategy, acceptance criteria, and process validation. However, statistical analyses of these topics demonstrated no clear correlation in change over the years studied. For more complex CR issues that included a subtopic, the subtopics with the greatest frequency are manufacture, quality attributes, and qualification. Again, no clear correlation of increase or decrease over the time period studied emerged.
- **Implications**
    - CR letters with facility inspections related comments showed a gradual increase over the period of time studied, with a higher increase in the date range of 2021-2024 relative to 2014-2020. Of note, on March 13, 2020, the President of the United States issued a COVID-19 Emergency Declaration. The effects of the COVID-19 pandemic and the emergency had widespread impacts on travel and global supply chains. Our analysis of the frequency of individual PQ CR topics by keywords and their occurrence over time did not demonstrate any statistically significant increase or change in frequency. Our database will now serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.

31. **Glenn, Amber**
    - **Abstract title:** Predicting Pediatric Adverse Drug Events Through Secondary Pharmacology and Juvenile Animal Studies
    - **Authors:** Glenn, Amber, FDA/CDER (Student); Abulwerdi, Gelareh, FDA/CDER (Mentor); Burckart, Gilbert, FDA/CDER (Mentor); Racz, Rebecca, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - We present an ongoing linguistic keyword analysis of Biologics License Application (BLA) Complete Response (CR) letters from the past ten years (2014-2024). Using a preexisting corpus, keywords were extracted, categorized, and analyzed; a database was created with identified words, and larger-scale keyword trends were quantified. Trends in product quality, facilities and microbiology related CR terms

have varied by year. Specific keywords identified include data, control strategy, acceptance criteria, and process validation. This database will serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.

- **Purpose**
  - We seek to generate a database of the terminology used in product quality items from Biologics License Application (BLA) Complete Response (CR) letters from 2014-2024. Using the content of past CR letters, the Agency can assess trends in CR actions, track deficiencies related to product quality (PQ), microbiology, and facility inspections that preclude approval of a BLA, and provide additional pre-BLA submission feedback for items of higher frequency where merited. This database will also inform and validate future automated data mining approaches for further refinement of CR letter analysis.
- **Methods**
  - A database of 108 CR letters from 2014 through 2024 was compiled. This corpus was further refined to a subset of 60 letters with headers and therefore sections pertaining to PQ, microbiology, and facilities. These were read in their entirety and keyword(s) were cataloged for each section of each letter. These keywords were sorted by topic, with up to two ranked subtopics. These topics were chosen based on recurring issues in the BLA PQ CR sections. The topics were analyzed by year and by frequency, and the letters themselves were analyzed by the contents of the PQ header. Counts of each topic were graphed in a scatter plot to assess frequency, and trend lines were fitted to analyze patterns for topics at each level and sub-level.
- **Results**
  - The proportion of CR letter headers corresponding to PQ, microbiology, and facilities has varied annually. The observed trends will be discussed. The individual PQ topics that have the greatest frequency over time are data, control strategy, acceptance criteria, and process validation. However, statistical analyses of these topics demonstrated no clear correlation in change over the years studied. For more complex CR issues that included a subtopic, the subtopics with the greatest frequency are manufacture, quality attributes, and qualification. Again, no clear correlation of increase or decrease over the time period studied emerged.
- **Implications**
  - CR letters with facility inspections related comments showed a gradual increase over the period of time studied, with a higher increase in the date range of 2021-2024 relative to 2014-2020. Of note, on March 13, 2020, the President of the United States issued a COVID-19 Emergency Declaration. The effects of the COVID-19 pandemic and the emergency had widespread impacts on travel and global supply chains. Our analysis of the frequency of individual PQ CR topics by keywords and their occurrence over time did not demonstrate any statistically significant increase or change in frequency. Our database will now serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.

32. **Godse, Sandip**
    - **Abstract title:** Smart Wearables: Transforming Clinical Assessments in Duchenne Muscular Dystrophy Trials
    - **Authors:** Godse, Sandip, FDA/CDER (Student); Bhattaram, Atul, FDA/CDER (Mentor); Sharma, Vishnu, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - We present an ongoing linguistic keyword analysis of Biologics License Application (BLA) Complete Response (CR) letters from the past ten years (2014-2024). Using a preexisting corpus, keywords were extracted, categorized, and analyzed; a database was created with identified words, and larger-scale keyword trends were quantified. Trends in product quality, facilities and microbiology related CR terms have varied by year. Specific keywords identified include data, control strategy, acceptance criteria, and process validation. This database will serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.
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            - We seek to generate a database of the terminology used in product quality items from Biologics License Application (BLA) Complete Response (CR) letters from 2014-2024. Using the content of past CR letters, the Agency can assess trends in CR actions, track deficiencies related to product quality (PQ), microbiology, and facility inspections that preclude approval of a BLA, and provide additional pre-BLA submission feedback for items of higher frequency where merited. This database will also inform and validate future automated data mining approaches for further refinement of CR letter analysis.
        - **Methods**
            - A database of 108 CR letters from 2014 through 2024 was compiled. This corpus was further refined to a subset of 60 letters with headers and therefore sections pertaining to PQ, microbiology, and facilities. These were read in their entirety and keyword(s) were cataloged for each section of each letter. These keywords were sorted by topic, with up to two ranked subtopics. These topics were chosen based on recurring issues in the BLA PQ CR sections. The topics were analyzed by year and by frequency, and the letters themselves were analyzed by the contents of the PQ header. Counts of each topic were graphed in a scatter plot to assess frequency, and trend lines were fitted to analyze patterns for topics at each level and sub-level.
        - **Results**
            - The proportion of CR letter headers corresponding to PQ, microbiology, and facilities has varied annually. The observed trends will be discussed. The individual PQ topics that have the greatest frequency over time are data, control strategy, acceptance criteria, and process validation. However, statistical analyses of these topics demonstrated no clear correlation in change over the years studied. For more complex CR issues that included a subtopic, the subtopics with the greatest frequency are manufacture, quality attributes, and

qualification. Again, no clear correlation of increase or decrease over the time period studied emerged.

- **Implications**
    - CR letters with facility inspections related comments showed a gradual increase over the period of time studied, with a higher increase in the date range of 2021-2024 relative to 2014-2020. Of note, on March 13, 2020, the President of the United States issued a COVID-19 Emergency Declaration. The effects of the COVID-19 pandemic and the emergency had widespread impacts on travel and global supply chains. Our analysis of the frequency of individual PQ CR topics by keywords and their occurrence over time did not demonstrate any statistically significant increase or change in frequency. Our database will now serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.

33. **Gorospe, Jordan**
    - **Abstract title:** Development of a novel in vitro binding assay using tumor-derived exosomes (TDEs) to facilitate the development of novel products and biosimilars.
    - **Authors:** Gorospe, Jordan, FDA/CDER (Student); Shapiro, Marjorie, FDA/CDER (Mentor); Simhadri, Venkateswara, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - We present an ongoing linguistic keyword analysis of Biologics License Application (BLA) Complete Response (CR) letters from the past ten years (2014-2024). Using a preexisting corpus, keywords were extracted, categorized, and analyzed; a database was created with identified words, and larger-scale keyword trends were quantified. Trends in product quality, facilities and microbiology related CR terms have varied by year. Specific keywords identified include data, control strategy, acceptance criteria, and process validation. This database will serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.
        - **Purpose**
            - We seek to generate a database of the terminology used in product quality items from Biologics License Application (BLA) Complete Response (CR) letters from 2014-2024. Using the content of past CR letters, the Agency can assess trends in CR actions, track deficiencies related to product quality (PQ), microbiology, and facility inspections that preclude approval of a BLA, and provide additional pre-BLA submission feedback for items of higher frequency where merited. This database will also inform and validate future automated data mining approaches for further refinement of CR letter analysis.
        - **Methods**
            - A database of 108 CR letters from 2014 through 2024 was compiled. This corpus was further refined to a subset of 60 letters with headers and therefore sections pertaining to PQ, microbiology, and facilities. These were read in their entirety and keyword(s) were cataloged for each section of each letter. These keywords were sorted by topic,

with up to two ranked subtopics. These topics were chosen based on recurring issues in the BLA PQ CR sections. The topics were analyzed by year and by frequency, and the letters themselves were analyzed by the contents of the PQ header. Counts of each topic were graphed in a scatter plot to assess frequency, and trend lines were fitted to analyze patterns for topics at each level and sub-level.

- **Results**
    - The proportion of CR letter headers corresponding to PQ, microbiology, and facilities has varied annually. The observed trends will be discussed. The individual PQ topics that have the greatest frequency over time are data, control strategy, acceptance criteria, and process validation. However, statistical analyses of these topics demonstrated no clear correlation in change over the years studied. For more complex CR issues that included a subtopic, the subtopics with the greatest frequency are manufacture, quality attributes, and qualification. Again, no clear correlation of increase or decrease over the time period studied emerged.
- **Implications**
    - CR letters with facility inspections related comments showed a gradual increase over the period of time studied, with a higher increase in the date range of 2021-2024 relative to 2014-2020. Of note, on March 13, 2020, the President of the United States issued a COVID-19 Emergency Declaration. The effects of the COVID-19 pandemic and the emergency had widespread impacts on travel and global supply chains. Our analysis of the frequency of individual PQ CR topics by keywords and their occurrence over time did not demonstrate any statistically significant increase or change in frequency. Our database will now serve as a platform for validating automated data mining approaches and ongoing assessments of frequency of individual topics.

34. **Howlader, Md Sariful Islam**
    - **Abstract title:** Emerging targeted lipoprotein(a) lowering therapeutics: Is it a promising strategy to reduce the risk of atherosclerotic cardiovascular disease and cardiovascular events?
    - **Authors:** Md Sariful Islam Howlader, FDA/CDER (student), Jihye Ahn, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis:**
            - This review examines the emerging landscape of targeted therapies aimed at lowering Lipoprotein(a) (Lp(a)) levels and their potential to mitigate the risk of atherosclerotic cardiovascular disease (ASCVD) and cardiovascular events. Elevated Lp(a) levels have consistently been associated with increased ASCVD risk, contributing to inflammation, oxidative stress, and arterial plaque formation. Despite robust observational evidence and mechanistic insights into Lp(a)'s role in cardiovascular pathogenesis, the clinical efficacy of reducing Lp(a) to lower major adverse cardiovascular events (MACE) remains uncertain. A comprehensive literature search spanning studies from 2010 to 2024 identified 45 relevant studies from diverse global

populations, highlighting significant reductions (80-90%) in Lp(a) levels with novel therapies like antisense oligonucleotides and small interfering RNA. Challenges include variability in Lp(a) measurement and population heterogeneity impacting threshold determinations and the relationship between Lp(a) and ASCVD. Moving forward, further research is essential to establish the long-term safety and efficacy profiles of Lp(a)-lowering therapies, crucial for refining treatment guidelines and improving patient outcomes. Enhancing awareness among healthcare providers and researchers about Lp(a)'s role in ASCVD risk stratification is pivotal for optimizing therapeutic approaches and advancing global cardiovascular health.

- **Purpose**
  - Epidemiological studies have consistently shown an association between elevated Lipoprotein(a) [Lp(a)] levels and an increased risk of atherosclerotic cardiovascular disease (ASCVD). Lp(a), characterized by its composition of low-density lipoprotein (LDL) particle and apolipoprotein(a) [apo(a)], is known to play a significant role in atherosclerosis and cardiovascular disease (CVD) pathogenesis. Elevated Lp(a) levels contribute significantly to inflammation, oxidative modifications, and foam cell formation, which are pivotal processes in the development of arterial plaque and progression to conditions such as coronary heart disease and stroke. Despite the established link supported by observational studies and pathogenesis evidence, the clinical benefit of lowering Lp(a) levels in terms of reducing major adverse cardiovascular events (MACE) has yet to be established. The objective of this study is to summarize current knowledge of Lp(a) in cardiovascular diseases, to conduct literature review of association between elevated Lp(a) and risk of ASCVD, and to survey the drugs that lower Lp(a) including targeted Lp(a) lowering therapies, lipid-modifying drugs, and other drugs that have shown to alter Lp(a) levels, and their impact on clinical cardiovascular outcomes.
- **Methods**
  - A comprehensive search of electronic databases including PubMed, MEDLINE, and Embase was conducted to investigate the relationship between lowering Lp(a) levels and ASCVD and major adverse cardiovascular events (MACE). Keywords such as "Lipoprotein(a)", "Lp(a)", "cardiovascular events", "atherosclerosis", and "clinical trials" were employed to identify relevant studies published from 2010 to 2024. Inclusion criteria encompassed original research articles, systematic reviews, meta-analyses, and randomized controlled trials focusing on the impact of elevated Lp(a) on cardiovascular outcomes and interventions targeting Lp(a) reduction in both ASCVD and non-ASCVD populations. Data extraction involved capturing details on study design, participant characteristics, Lp(a) measurement methods, intervention strategies, follow-up duration, and cardiovascular outcomes. Quality assessment tools adapted for observational studies and clinical trials were utilized to assess the methodological quality and reliability of the included studies.
- **Results**
  - Forty-five studies that met our initial criteria included diverse

populations from North America, Europe, and Asia. These studies revealed that elevated levels of Lp(a) above 65 nmol/L correlates with an increased risk for experiencing ASCVD events. Studies investigating novel Lp(a)-lowering therapies such as antisense oligonucleotides and small interfering RNA reported substantial reductions in circulating Lp(a) levels by 80-90%. In a Phase 2 study of olpasiran, it was reported that over 90% reduction in Lp(a) levels was achieved with a single dose administration and the reduction sustained 3 to 6 months. This review also highlights challenges including intra-individual and inter-assay variability of Lp(a) measurement and heterogeneity in study population to determine Lp(a) thresholds and the magnitude of relationship between Lp(a) and ASCVD which may differ among different patient populations.

- **Implications**
    - While managing elevated Lp(a) levels is recognized as a potential strategy to reduce the risk of ASCVD, there has been limited experience to validate whether lowering Lp(a) can be translated to cardiovascular risk reduction. Further research is necessary to elucidate the long-term effectiveness and safety profiles of Lp(a)-lowering therapies. Meanwhile, increasing awareness among healthcare providers and researchers about the current knowledge of Lp(a) and potential role of L(p) lowering in reducing ASCVD risk is an important step to identify patients at increased risk for ASCVD and MACE, to optimize therapeutics and to improve patient outcomes.

35. **Huang, Peirung**
    - **Abstract title:** A Landscape Survey for the Presentations Approved for Subcutaneous Protein Products
    - **Authors:** Huang, Peirung, FDA/CDER (Student); Wang, Yow-Ming, FDA/CDER (Mentor); Li, Zhe, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract**:
        - **Synopsis**
            - Drug-device combination products often have different presentations, such as vial, prefilled syringe (PFS), autoinjector (AI), etc., in their product development stages. The use of AI is expected to provide less pharmacokinetic (PK) variability between injections comparing to hand-held syringes like PFS, hence, improves the safety profile. It is also more convenient to self-administer, which can help patient compliance with subcutaneous (SC) drugs. However, due to the complexity of engineering, AI presentations are usually introduced after the pivotal phase 3 study is conducted where product safety and efficacy has been demonstrated. Therefore, different development strategies were utilized to support the approval of the later device presentations. In this study, we conducted a landscape survey of approved therapeutic protein products with AI presentations and the progression of product presentation availability based on the Center for Drug Evaluation and Research (CDER)- approved biological license applications (BLAs) in the Purple Book.

- **Purpose**
    - To investigate the development strategies of product presentations for approved SC therapeutic protein drugs, we began with a landscape survey for the presentations approved for SC products in this study. Our aim was to create a database to identify the products that warranted further investigation of the device/presentation development strategy to inform future new device development.
- **Methods**
    - To identify BLAs with vials, PFS, and AI presentations for SC administration, we obtained a list of CDER-approved BLAs from FDA's Purple Book Database of License Biological Products. From the list, we selected products with the license type of 351(a) and products that has SC in their listed route of administrations (ROAs). From the selected 351(a) BLAs between 1975 to 2024, we extracted relevant information on each device presentation, such as the protein structure, product concentration, other ROAs if any, size of syringe and volume of injection from the first approved drug labels and later approved labels on Drugs@FDA website. Information on device name were obtained from the Container Closure System document in the regulatory submission packages (internal database). Then, we summarized our findings on different product presentations of all these products.
- **Results**
    - Based on our survey of all the CDER-approved BLAs, we identified 125 products with the presentations of vial (n=66, 52.8%), PFS (n=31, 24.8%), or AI (n=23, 18.4%). There were 39, 15, and 16 products that only had vial, AI, or PFS presentation, respectively. Eight of the 125 products had vial and PFS, 15 had AI and vial, and 17 had AI and PFS. Five had all three of these presentations (vial, AI, and PFS). These products were categorized into 6 therapeutic areas: cardiometabolic and endocrine disorders (n=60), cancer (n=9), infectious diseases (n=2), immunology and inflammation (n=34), neuropsychiatric (n=9), genetic disorders (n=2), and others (n=9). Most of these PFS and AI products used 1 mL syringes, and some used 2.25 mL syringes.
- **Implications**
    - The findings from this study can contribute to our understanding of the current landscape in device development of approved SC therapeutic protein product presentations. This database will provide insights for our further investigation of the development strategies for vials, PFS, and AI and may help us identify the products that worth further following-up about their future new devices, such as the ones that already have approved PFS, but no AI presentation approved yet.

36. **Jennings, Reese**
    - **Abstract title:** Developing Open-Source Software Tools for Cross-Study Analysis of Structured Toxicology Study Datasets via R Shiny Application
    - **Authors:** Jennings, Reese FDA/CDER(Student); Snyder, Kevin FDA/CDER (Mentor); Ahmed, Sabbir FDA/CDER, Ali, Yousif, MD, FDA/CDER, Butler, Susan FDA/CDER, L. Quinn, Stephanie FDA/CDER
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**

- **Synopsis**
    - The Office of New Drugs (OND) is engaged in developing open-source software tools to facilitate cross-study analyses of CDISC-SEND-formatted electronic standardized toxicology study datasets. The project seeks to improve the functionality of an R Shiny application that was developed to generate interactive visualizations that enable users to easily compare and contrast the results of multiple repeat dose toxicity studies. Despite adhering to SEND standards, many submitted datasets often present inconsistencies in how the fields are populated; there are also multiple acronyms, capitalizations, and phrasings used separately that correspond to the same word or idea, which complicates both querying the data and cross-comparison. Currently, the application is only compatible with a limited number of datasets, but the project seeks to expand the compatibility of the querying process in order to be able to work with all available datasets.
- **Purpose**
    - The project seeks to improve the functionality of an R Shiny application that allows users to perform cross-study analyses of CDISC-SEND formatted standardized toxicology study data. The application generates interactive visualizations that enable users to easily compare and contrast the results of multiple repeat dose toxicity studies.
- **Methods**
    - Data normalization and scoring procedures were implemented in order for the application to produce outputs incorporating numerical and categorical data from multiple studies. An interactive user interface dashboard was then developed to allow users to select and group test studies to make customizable toxicology profiles based on user specified criteria. Concurrently, R code was developed to standardize the querying of these datasets, ensuring consistency and enabling seamless integration of a broader application of the app to multiple different datasets for the purpose of compare and contrasting.
- **Results**
    - The project involved developing open-source software tools to improve the toxicology review program at the OND. The R Shiny application is aimed at comparing various factors within a study simultaneously, e.g. hepatotoxicity, renal toxicity, etc. Moreover, users can visualize relationships across multiple toxicology studies. Trends in body weight and increases in liver enzymes are key indicators of toxicity in groupings. The application incorporates a z-scoring method normalize signal strength across studies, highlighting the degree of toxicity observed in each of five major organ systems. During development, the application was designed to load and analyze a small selection of datasets; it is currently in the process of being optimized for compatibility with additional datasets.
- **Implications**
    - This work will facilitate more efficient and reliable cross-comparison of current toxicology data. The application allows flexibility as it enables users to customize and define selections of data and their thresholds. Using cross-study analysis, users can efficiently produce specific findings relating to their target. This application enables the user to produce visual comparisons of drug toxicity across multiple studies, providing an

integrated understanding of the toxicological profile of a given compound under various testing conditions, e.g. species, route of administration, dosing duration. This application will ultimately enhance the OND's toxicology review program.

37. **Jung, Dahee**
    - **Abstract title:** Biomarker Utilization in Neurological Drug Products Approved by FDA (2008-2023): A Landscape Analysis
    - **Authors:** Jung, Dahee, FDA/CDER (Student); Zhang, Yifei, FDA/CDER (Mentor); Sabarinath, Sreedharan, FDA/CDER; Uppoor, Ramana, FDA/CDER; Mehta, Mehul, FDA/CDER
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - This study provides a landscape analysis of biomarker usage in the regulatory approval of neurological drug products during the years 2008-2023. A total of 65 CDER-approved New Molecular Entities (NME) for the treatment of neurological diseases were summarized. More than one third of these products had biomarker data described in the regulatory reviews available from FDA website. Biomarkers have played various roles in drug development and approval, such as serving as surrogate endpoint for accelerated approval, providing confirmatory evidence for demonstrating effectiveness, and supporting clinical trial design and dose selection. Potential challenges that limited the use of biomarker data were noted, such as demonstration of clinical relevance, bioanalytical assay validation, and the quality of biomarker data. This analysis provides a basis for better leveraging the biomarker information to facilitate drug development and regulatory decision making. The findings are expected to facilitate the development and approval of novel therapeutics for neurological diseases, and to offer insights for advancing regulatory research on biomarkers in other therapeutic areas, towards the goal of unleashing the power of biomarker data.
        - **Purpose**
            - Neurological diseases have devastating effects on patients and their caregivers, posing a significant global health burden. However, these diseases are often characterized by complex disease pathophysiology and heterogeneous progression, posing significant challenges for drug development. Biomarkers, which provide information about biological mechanisms, disease progression, and response to treatment, have the potential to serve as the basis for drug development and regulatory decision making, particularly in the field of neurology. Consequently, biomarkers have been increasingly used in regulatory decision-making in recent years. The purpose of this landscape analysis is to provide an in-depth review of biomarker usage in the development and approval of neurological drugs during the years 2008-2023, and to identify the opportunities and challenges associated with utilizing biomarker data more effectively, thereby supporting future drug development and regulatory decisions.
        - **Methods**
            - A complete list of NMEs approved in 2008-2023 was obtained from the

FDA public website (https://www.fda.gov/drugs/nda-and-bla-approvals/new-molecular-entity-nme-drug-and-new-biologic-approvals), and the approvals in neurology were filtered based on the FDA internal database "CDER Novel Drug Approvals Dashboard". The analysis was conducted using a survey method to evaluate FDA CDER-approved neurology drugs classified as New Molecular Entity (NME) with at least one biomarker measured by the sponsor and included in the NDA/BLA review. Biomarker-related information was manually extracted from the documents available on Drugs@FDA website, focusing on clinical pharmacology review, clinical review, multidisciplinary integrated review, and labeling. The documents were scanned for the keywords relevant to biomarkers. The utility of biomarkers for each product were summarized to illustrate the trends of biomarker usage, as well as the roles of biomarkers in drug development and regulatory decision making.

- **Results**
  - The biomarker data were summarized from a total of 65 NMEs approved between 2008 and 2023 for the treatment of neurological diseases. More than one third of these approved drugs had biomarker data described in the FDA regulatory reviews. Over the years, the utilization of biomarkers in drug applications has increased significantly. Biomarkers have been playing various roles in drug development and regulatory decision-making, such as (1) serving as surrogate endpoint for accelerated approval; (2) providing confirmatory evidence for demonstrating effectiveness; (3) supporting clinical trial design and dose selection. The challenges for effective use of the biomarker data arose from multiple aspects, such as insufficient information to demonstrate clinical relevance, inadequate bioanalytical assay validation, and poor quality of biomarker data.
- **Implications**
  - This study provides a comprehensive landscape analysis to illustrate the increasing impact of biomarkers in the drug development and regulatory approval, focusing on neurological NMEs. The major roles of biomarker data were summarized, and potential challenges were elaborated. A solid understanding on the current progress and knowledge gap in this field will maximize the potential to better leverage the biomarker data and to facilitate the development and approval of effective treatments for neurological diseases. The study approach and insights from this analysis will also shed light on the regulatory research on biomarkers in other therapeutic areas for the benefit of patients.

38. **Khedr, Mohammed**
    - **Abstract title:** An Analysis of Postmarketing Requirements and Commitments Related to Demographic Subpopulations for New Drugs Approved in CDER Between 2019-2021
    - **Authors:** Khedr, Mohammed, FDA/CDER (Student); Poddar, Atasi, FDA/CDER (Mentor); Chege, Wambui
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Clinical trials to support approval of new drugs should include

demographically diverse groups to ensure the collection of comprehensive data on populations that reflect individuals who will use the drugs, if approved. FDA requires and promotes such data collection for the Agency's regulatory decision making. FDA's increasing efforts to promote diversity in clinical trials, including the requirement for submission of Diversity Action Plans for certain clinical studies, are expected to result in more prospective data collection on diverse populations. Historically, under certain circumstances, FDA required that sponsors collect these data following drug approval through PMR/PMCs, which may change due to ongoing efforts to modify the paradigm, signaling a preference for adequate premarketing data collection. Our study of the postmarketing requirements and commitments to ensure inclusion of diverse participants indicate that, for about two-fifths (42%) of NMEs approved between 2019-2021, a diversity-related PMR/PMC was issued. The majority of the diversity-related PMRs/PMCs were related to sex and gender. Our analyses identified an increased trend in issuing race-related PMRs/PMCs. The majority of these PMRs/PMCs are for oncology indications. For non-oncology indications, sex/gender related PMR/PMCs were most frequent. For about half of the PMR/PMCs, less than ten years was allowed for data collection. Analysis of trends of issuance of PMR/PMCs for inclusion of diverse populations in clinical trials can be potentially valuable for assessing the impact of the efforts of FDA and sponsors to ensure individuals who will use the approved therapeutic products, are included in clinical trials prior to approval of the new drugs.

- **Purpose**
    - FDA regulations require sponsors to present information from premarket clinical trials on the safety and effectiveness of drugs in terms of sex/gender, age, and racial subgroups. FDA has issued several guidance documents with recommendations to increase diversity in clinical trials. In a recently published draft guidance titled Diversity Action Plans to Improve Enrollment of Participants from Underrepresented Populations in Clinical Studies (June 2024), FDA describes the format and content of Diversity Action Plans that sponsors are required to submit for certain medical products to ensure enrollment of participants from underrepresented populations. The purpose of this study is to characterize recent postmarketing requirements (PMR) and commitments (PMC) issued by FDA (2019-2021) for sponsors to collect information on the safety and efficacy of new drugs in demographic subpopulations after the drug is approved. More prospective data collection by the sponsors, following Diversity Action Plans guidance, may impact the current practice of collecting the information following approval or the time allotted for such data collection.
- **Methods**
    - The new molecular entities (NMEs) approved between 2019-2021 were identified from FDA's internal database. The information about PMRs and PMCs were manually abstracted from approval letters and summary reviews posted on FDA's website. FDA's Postmarketing Requirements and Commitments database

(https://www.accessdata.fda.gov/scripts/cder/pmc/index.cfm) was leveraged to identify the original projected completion date and the status of the study (as of April 8, 2024). Use of expedited pathway or orphan status were identified from CDER Novel Drug Therapy Approval Report. Categories and subcategories of demographic sub-populations were identified from the PMR/PMCs. The time allotted for data collection was estimated by calculating the difference between the date of approval and the "report submission date" for the PMR/PMCs obtained from FDA's approval letters.

- **Results**
  - Between 2019-2021, FDA approved 151 NMEs; for 42% of NMEs at least one PMR/PMC included collecting additional data on three demographic subpopulations. The PMR/PMCs were issued exclusively for sex/gender (26.5%), age (9%), and race (7%). For 3% of the NMEs, subpopulations related to both sex/gender and age were included. An increase in race-related PMR/PMCs from none (2019) to 4% (2020) and 16% (2021) was identified. There was no ethnicity related PMR/PMC. The majority of PMR/PMCs for these three subpopulations were for the following groups: a) sex/gender: pregnant individuals (73%), b) age: children (18 years or below) (68%), c) race: all races (69%). For oncology indications, the majority of PMR/PMCs were related to race (60%) and for non-oncology indications, the majority were related to sex/gender (80%). For non-orphan drugs, sex/gender-related PMR/PMCs were predominant (90%). No such trends for a specific demographic subpopulation were identified for orphan drugs. The time allotted for data collection by FDA at the time of approval ranged from less than one year (2%), one to ten years (52%), and more than 10 years (46%). Data collection was ongoing for 33% of PMR/PMCs and delayed or pending for 55% of PMR/PMCs.
- **Implications**
  - The preliminary findings from this study could serve as a benchmark to evaluate the impact of FDA policies such as the Diversity Action Plans guidance and other ongoing FDA initiatives to enhance diversity of clinical trials. This study's findings can also serve as a benchmark for evaluation of the impact of legislative actions (e.g., Food and Drug Omnibus Reform Act (2022)). Also, this study can assist with identifying demographic attributes of populations that are more likely to be evaluated in postmarketing studies so that they can be a focus of assessments for barriers preventing their inclusion in premarketing studies. Furthermore, our study findings provide preliminary insight into the average time allotted for fulfillment of PMR/PMCs, which can be useful for assessing if steps should be taken to understand and limit delays.

39. **Kim, Yurim**
    - **Abstract title:** Inclusion of Information on Diverse Populations in the Labeling of New Molecular Entities Approved in 2018
    - **Authors:** Kim, Yurim, FDA/CDER (Student); Elimika Pfuma Fletcher, FDA/CDER; Sarah Ridge, FDA/CDER; Ramamoorthy, Anuradha, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**

- **Synopsis**
  - Clinical trials to support approval of new drugs should include demographically diverse groups to ensure the collection of comprehensive data on populations that reflect individuals who will use the drugs, if approved. FDA requires and promotes such data collection for the Agency's regulatory decision making. FDA's increasing efforts to promote diversity in clinical trials, including the requirement for submission of Diversity Action Plans for certain clinical studies, are expected to result in more prospective data collection on diverse populations. Historically, under certain circumstances, FDA required that sponsors collect these data following drug approval through PMR/PMCs, which may change due to ongoing efforts to modify the paradigm, signaling a preference for adequate premarketing data collection. Our study of the postmarketing requirements and commitments to ensure inclusion of diverse participants indicate that, for about two-fifths (42%) of NMEs approved between 2019-2021, a diversity-related PMR/PMC was issued. The majority of the diversity-related PMRs/PMCs were related to sex and gender. Our analyses identified an increased trend in issuing race-related PMRs/PMCs. The majority of these PMRs/PMCs are for oncology indications. For non-oncology indications, sex/gender related PMR/PMCs were most frequent. For about half of the PMR/PMCs, less than ten years was allowed for data collection. Analysis of trends of issuance of PMR/PMCs for inclusion of diverse populations in clinical trials can be potentially valuable for assessing the impact of the efforts of FDA and sponsors to ensure individuals who will use the approved therapeutic products, are included in clinical trials prior to approval of the new drugs.
- **Purpose**
  - FDA regulations require sponsors to present information from premarket clinical trials on the safety and effectiveness of drugs in terms of sex/gender, age, and racial subgroups. FDA has issued several guidance documents with recommendations to increase diversity in clinical trials. In a recently published draft guidance titled Diversity Action Plans to Improve Enrollment of Participants from Underrepresented Populations in Clinical Studies (June 2024), FDA describes the format and content of Diversity Action Plans that sponsors are required to submit for certain medical products to ensure enrollment of participants from underrepresented populations. The purpose of this study is to characterize recent postmarketing requirements (PMR) and commitments (PMC) issued by FDA (2019-2021) for sponsors to collect information on the safety and efficacy of new drugs in demographic subpopulations after the drug is approved. More prospective data collection by the sponsors, following Diversity Action Plans guidance, may impact the current practice of collecting the information following approval or the time allotted for such data collection.
- **Methods**
  - The new molecular entities (NMEs) approved between 2019-2021 were identified from FDA's internal database. The information about PMRs and PMCs were manually abstracted from approval letters and summary reviews posted on FDA's website. FDA's Postmarketing

Requirements and Commitments database (https://www.accessdata.fda.gov/scripts/cder/pmc/index.cfm) was leveraged to identify the original projected completion date and the status of the study (as of April 8, 2024). Use of expedited pathway or orphan status were identified from CDER Novel Drug Therapy Approval Report. Categories and subcategories of demographic sub-populations were identified from the PMR/PMCs. The time allotted for data collection was estimated by calculating the difference between the date of approval and the "report submission date" for the PMR/PMCs obtained from FDA's approval letters.

- **Results**
  - Between 2019-2021, FDA approved 151 NMEs; for 42% of NMEs at least one PMR/PMC included collecting additional data on three demographic subpopulations. The PMR/PMCs were issued exclusively for sex/gender (26.5%), age (9%), and race (7%). For 3% of the NMEs, subpopulations related to both sex/gender and age were included. An increase in race-related PMR/PMCs from none (2019) to 4% (2020) and 16% (2021) was identified. There was no ethnicity related PMR/PMC. The majority of PMR/PMCs for these three subpopulations were for the following groups: a) sex/gender: pregnant individuals (73%), b) age: children (18 years or below) (68%), c) race: all races (69%). For oncology indications, the majority of PMR/PMCs were related to race (60%) and for non-oncology indications, the majority were related to sex/gender (80%). For non-orphan drugs, sex/gender-related PMR/PMCs were predominant (90%). No such trends for a specific demographic subpopulation were identified for orphan drugs. The time allotted for data collection by FDA at the time of approval ranged from less than one year (2%), one to ten years (52%), and more than 10 years (46%). Data collection was ongoing for 33% of PMR/PMCs and delayed or pending for 55% of PMR/PMCs.
- **Implications**
  - The preliminary findings from this study could serve as a benchmark to evaluate the impact of FDA policies such as the Diversity Action Plans guidance and other ongoing FDA initiatives to enhance diversity of clinical trials. This study's findings can also serve as a benchmark for evaluation of the impact of legislative actions (e.g., Food and Drug Omnibus Reform Act (2022)). Also, this study can assist with identifying demographic attributes of populations that are more likely to be evaluated in postmarketing studies so that they can be a focus of assessments for barriers preventing their inclusion in premarketing studies. Furthermore, our study findings provide preliminary insight into the average time allotted for fulfillment of PMR/PMCs, which can be useful for assessing if steps should be taken to understand and limit delays.

40. **Kilaparthi, Meghna**
    - **Abstract title:** An overview of pediatric oncology drug approvals from 2013 to 2023 with a focus on extrapolation and dosage selection
    - **Authors:** Meghna Kilaparthi (Student), Suryatheja Ananthula FDA/CDER (Mentor), Ritu Chadda, Lauren Price, Ruby Leong, Gilbert Burckart, Stacy Shord, Rosane Charlab
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response

- **Abstract:**
  - **Synopsis**
    - Based upon the review of publicly and non-publicly available data of oncology drug approvals, we summarized how safety, effectiveness and dosing regimens were established for pediatric oncology approvals for 33 oncology drugs (2013 to 2023).
  - **Purpose**
    - Conducting clinical trials in pediatric patients with cancer is challenging due to several limitations including physiological and pathological differences within the pediatric subpopulations as well as between pediatric and adult populations, rarity of the cancers across these populations, the need to develop age-appropriate pediatric formulations, and pediatric clinical trial access. We reviewed pediatric oncology drug approvals to understand how the recommended dosages for pediatric patients were identified in pediatric patients for their subsequent approval.
  - **Methods**
    - Oncology drugs with a pediatric approval granted from 2013 to 2023 were identified from the FDA Pediatric Oncology Drug Approvals list. Data were then collected from FDA approved labeling, study reports, and clinical and clinical pharmacology reviews. We reviewed the data to identify which oncology drugs were approved for pediatric patients, how the recommended dosage was selected for pediatric patients and whether an age-appropriate formulation was developed for marketing.
  - **Results**
    - A total of 33 drugs with a total of 46 pediatric oncology indications were approved from 01/2013 to 05/2023. The 33 drugs consisted of 16 small molecule drugs, 1 radioligand and 16 biological products. Amongst the 46 pediatric indications, 18 were approved with the initial adult marketing applications and remaining 28 were from supplemental applications. While efficacy and safety were extrapolated for 25 pediatric indications based upon the available adult clinical data, clinical trials were conducted to establish the efficacy and safety for the remaining 21 pediatric indications. For 15 of these 21 indications, more than 1 dose level was investigated in the pediatric studies to establish the recommended dosage for pediatric patients. To-be marketed age-appropriate formulations were available for 9 of the 13 orally administered drugs. For the remaining 4 drugs, the recommended dosage for pediatric patients differs from that evaluated in clinical trials because the formulation administered in the trials was not made commercially available; these recommended dosages for pediatric patients using the commercially available dosage forms and strengths were selected to provide drug exposure within range of the values observed in pediatrics from the clinical study. Further, these commercially available dosage forms cannot be administered to pediatric patients unable to swallow capsules or tablets whole.
  - **Implications**
    - Extrapolation of data from clinical trials conducted in adults supported approval for 54% (25/46) of the pediatric oncology

indications. For 33% (15/46) of the indications, a dose escalation/de-escalation strategy was utilized to identify the recommended pediatric dosage and establish efficacy and safety. Further evaluation of the collected data may help to identify factors which supported successful extrapolation or dose-finding approaches for pediatric patients. One identified challenge in pediatric oncology drug development is the need to develop to-be marketed age-appropriate formulation(s). Due to lack of a to-be marketed age appropriate formulation, 31% (4/13) of the orally administered drugs cannot be administered to pediatric patients who are unable to swallow capsules or tablets whole and dosage recommendations were made by rounding up the doses based upon available capsule or tablet strengths. These findings highlight the need for discussions focused on age-appropriate formulation development during milestone meetings.

41. **Liu, Ellen**
    - **Abstract title:** Summary of Studies for a Subset of NDAs Approved via the 505(b)(2) Pathway
    - **Authors:** Liu, Ellen, FDA/CDER (Student); Lee, Angela, FDA/CDER (Orise Fellow); Muneer, Naiha, FDA/CDER (ORISE Fellow); Stier, Ethan, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Based upon the review of publicly and non-publicly available data of oncology drug approvals, we summarized how safety, effectiveness and dosing regimens were established for pediatric oncology approvals for 33 oncology drugs (2013 to 2023).
        - **Purpose**
            - Conducting clinical trials in pediatric patients with cancer is challenging due to several limitations including physiological and pathological differences within the pediatric subpopulations as well as between pediatric and adult populations, rarity of the cancers across these populations, the need to develop age-appropriate pediatric formulations, and pediatric clinical trial access. We reviewed pediatric oncology drug approvals to understand how the recommended dosages for pediatric patients were identified in pediatric patients for their subsequent approval.
        - **Methods**
            - Oncology drugs with a pediatric approval granted from 2013 to 2023 were identified from the FDA Pediatric Oncology Drug Approvals list. Data were then collected from FDA approved labeling, study reports, and clinical and clinical pharmacology reviews. We reviewed the data to identify which oncology drugs were approved for pediatric patients, how the recommended dosage was selected for pediatric patients and whether an age-appropriate formulation was developed for marketing.
        - **Results**
            - A total of 33 drugs with a total of 46 pediatric oncology indications were approved from 01/2013 to 05/2023. The 33 drugs consisted of 16 small molecule drugs, 1 radioligand and 16 biological products. Amongst the 46 pediatric indications, 18 were approved with the

initial adult marketing applications and remaining 28 were from supplemental applications. While efficacy and safety were extrapolated for 25 pediatric indications based upon the available adult clinical data, clinical trials were conducted to establish the efficacy and safety for the remaining 21 pediatric indications. For 15 of these 21 indications, more than 1 dose level was investigated in the pediatric studies to establish the recommended dosage for pediatric patients. To-be marketed age-appropriate formulations were available for 9 of the 13 orally administered drugs. For the remaining 4 drugs, the recommended dosage for pediatric patients differs from that evaluated in clinical trials because the formulation administered in the trials was not made commercially available; these recommended dosages for pediatric patients using the commercially available dosage forms and strengths were selected to provide drug exposure within range of the values observed in pediatrics from the clinical study. Further, these commercially available dosage forms cannot be administered to pediatric patients unable to swallow capsules or tablets whole.

- **Implications**
  - Extrapolation of data from clinical trials conducted in adults supported approval for 54% (25/46) of the pediatric oncology indications. For 33% (15/46) of the indications, a dose escalation/de-escalation strategy was utilized to identify the recommended pediatric dosage and establish efficacy and safety. Further evaluation of the collected data may help to identify factors which supported successful extrapolation or dose-finding approaches for pediatric patients. One identified challenge in pediatric oncology drug development is the need to develop to-be marketed age-appropriate formulation(s). Due to lack of a to-be marketed age appropriate formulation, 31% (4/13) of the orally administered drugs cannot be administered to pediatric patients who are unable to swallow capsules or tablets whole and dosage recommendations were made by rounding up the doses based upon available capsule or tablet strengths. These findings highlight the need for discussions focused on age-appropriate formulation development during milestone meetings.

42. **Low, Yuki**
    - **Abstract title:** Information Guide on Key Elements of Comparative Clinical Endpoint Biosimilar Studies
    - **Authors:** Low, Yuki FDA/CDER (Student); Kim, Jessica FDA/CDER (Mentor); Choi, Nam Hee FDA/CDER (Mentor); Alosh, Mohamed FDA/CDER (Mentor); Grosser, Stella FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
      - **Synopsis**
        - Based upon the review of publicly and non-publicly available data of oncology drug approvals, we summarized how safety, effectiveness and dosing regimens were established for pediatric oncology approvals for 33 oncology drugs (2013 to 2023)
      - **Purpose**
        - Conducting clinical trials in pediatric patients with cancer is

challenging due to several limitations including physiological and pathological differences within the pediatric subpopulations as well as between pediatric and adult populations, rarity of the cancers across these populations, the need to develop age-appropriate pediatric formulations, and pediatric clinical trial access. We reviewed pediatric oncology drug approvals to understand how the recommended dosages for pediatric patients were identified in pediatric patients for their subsequent approval.

- **Methods**
    - Oncology drugs with a pediatric approval granted from 2013 to 2023 were identified from the FDA Pediatric Oncology Drug Approvals list. Data were then collected from FDA approved labeling, study reports, and clinical and clinical pharmacology reviews. We reviewed the data to identify which oncology drugs were approved for pediatric patients, how the recommended dosage was selected for pediatric patients and whether an age-appropriate formulation was developed for marketing.
- **Results**
    - A total of 33 drugs with a total of 46 pediatric oncology indications were approved from 01/2013 to 05/2023. The 33 drugs consisted of 16 small molecule drugs, 1 radioligand and 16 biological products. Amongst the 46 pediatric indications, 18 were approved with the initial adult marketing applications and remaining 28 were from supplemental applications. While efficacy and safety were extrapolated for 25 pediatric indications based upon the available adult clinical data, clinical trials were conducted to establish the efficacy and safety for the remaining 21 pediatric indications. For 15 of these 21 indications, more than 1 dose level was investigated in the pediatric studies to establish the recommended dosage for pediatric patients. To-be marketed age-appropriate formulations were available for 9 of the 13 orally administered drugs. For the remaining 4 drugs, the recommended dosage for pediatric patients differs from that evaluated in clinical trials because the formulation administered in the trials was not made commercially available; these recommended dosages for pediatric patients using the commercially available dosage forms and strengths were selected to provide drug exposure within range of the values observed in pediatrics from the clinical study. Further, these commercially available dosage forms cannot be administered to pediatric patients unable to swallow capsules or tablets whole.
- **Implications**
    - Extrapolation of data from clinical trials conducted in adults supported approval for 54% (25/46) of the pediatric oncology indications. For 33% (15/46) of the indications, a dose escalation/de-escalation strategy was utilized to identify the recommended pediatric dosage and establish efficacy and safety. Further evaluation of the collected data may help to identify factors which supported successful extrapolation or dose-finding approaches for pediatric patients. One identified challenge in pediatric oncology drug development is the need to develop to-be marketed age-appropriate formulation(s). Due to lack of a to-be marketed age appropriate

formulation, 31% (4/13) of the orally administered drugs cannot be administered to pediatric patients who are unable to swallow capsules or tablets whole and dosage recommendations were made by rounding up the doses based upon available capsule or tablet strengths. These findings highlight the need for discussions focused on age-appropriate formulation development during milestone meetings.

43. **McCoy, Kevin**
    - **Abstract title:** Leveraging Machine Learning to Characterize Relationships Among Product Quality Attributes and Clinical Performance
    - **Authors:** McCoy, Kevin (Student) CDER, Stella Grosser, Jinhui Zhang, Mack Shih, Xiaoming Xu,
      Junghi Kim (Mentor) CDER
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **<u>Synopsis</u>**
            - Under the Biosimilar User Fee Act (BsUFA) III, a regulatory research pilot program has been established to pursue various research areas of interest. One research priority is to increase reliance on analytical data for a demonstration of biosimilarity to a reference product. Defining and standardizing analytical approaches relevant to clinical performance could help support demonstrations of no clinically meaningful differences using less resources than clinical studies require. However, despite improvements in analytical techniques, there is an incomplete understanding of the relationship between a product's quality attributes and its clinical performance. We employ unsupervised machine learning techniques such as dimensionality reduction and hierarchical clustering to answer questions 1) whether there is any pattern in quality attributes' behaviors in the demonstration of biosimilarity and 2) whether differences in analytical assessments in biosimilars relate to clinical outcomes. The relationship across analytical data, pharmacokinetic data, and clinical efficacy data was explored for 9 approved adalimumab and 5 approved trastuzumab biosimilar products.
        - **<u>Purpose</u>**
            - Under the Biosimilar User Fee Act (BsUFA) III, a regulatory research pilot program has been established to pursue various research areas of interest. One research priority is to increase reliance on analytical data for a demonstration of biosimilarity to a reference product.
        - **<u>Methods</u>**
            - We employ unsupervised machine learning techniques such as dimensionality reduction and hierarchical clustering to answer questions 1) whether there is any pattern in quality attributes' behaviors in the demonstration of biosimilarity and 2) whether differences in analytical assessments in biosimilars relate to clinical outcomes.
        - **<u>Results</u>**
            - The relationship across analytical data, pharmacokinetic data, and clinical efficacy data was explored for 9 approved adalimumab and 5 approved trastuzumab biosimilar products.

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- **Implications**
    - Defining and standardizing analytical approaches relevant to clinical performance could help support demonstrations of no clinically meaningful differences using less resources than clinical studies require. However, despite improvements in analytical techniques, there is an incomplete understanding of the relationship between a product's quality attributes and its clinical performance.

45. **Mada, Sahil**
    - **Abstract title:** Dose Selection and Optimization for Neurology and Psychiatry Drugs Approved from 2020-2024
    - **Authors:** Mada, Sahil FDA/CDER (Student); Sabarinath, Sreedharan FDA/CDER; Uppoor, Ramana FDA/CDER; Metha, Mehul FDA/CDER; Du, Ping FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - This study investigates dose selection and optimization strategies in neurological and psychiatric drug products approved in the U.S. between 2020 and 2024. It aims to understand the rationales behind these strategies using Model Informed Drug Development (MIDD) methodologies across various patient populations. By analyzing clinical trial designs, pharmacokinetics, pharmacodynamics, efficacy/safety data, and exposure-response relationship of selected novel drugs, the study seeks to identify trends and relationships in dose optimization.
        - **Purpose**
            - As of 2024, neurological drug products represented the second-largest share of approved drugs in the United States, following oncology products. Drugs in neurological and psychiatric areas often overlap and involve complex central nervous system (CNS) interactions. Dose selection and optimization in these areas are crucial for preserving clinical benefits while ensuring optimal tolerability. This study aims to systematically assess and compare dose selection and optimization strategies in neurology and psychiatry products. It seeks to understand the rationales behind dose selection and optimization for different modalities and the use of Model Informed Drug Development (MIDD) methodologies across various patient populations (e.g., pediatrics, adults) from premarket stages to FDA approval.
        - **Methods**
            - A comprehensive search of neurology and psychiatry drug products approved between 2020 and 2024 was conducted using public resources (Drug@FDA) and FDA internal resources (Dartts and Docubridge). This search identified novel drugs with varying indications, clinical trial designs, mechanisms of action, routes of administration, target populations, modalities, and dosing regimens. Of the novel drugs identified, we analyzed submitted Clinical Pharmacology reports, briefing meeting packages, and FDA Clinical Pharmacology reviews. Specifically, we reviewed clinical trial designs, pharmacokinetics (PK) and pharmacodynamics (PD) modeling, population PK modeling, efficacy and safety data, exposure-response relationship, and dose selection and optimization both premarket and as recommended and approved by the FDA.

- **Results**
    - Up to now, we have reviewed 9 NDA/BLA applications, focusing on Duchenne Muscular Dystrophy (n=3), Alzheimer's Disease (n=2), Myasthenia Gravis (n=3), and Friedrich's Ataxia (n=1). Six drugs had pivotal Phase 3 trials, while three had pivotal Phase 2 studies. Among these, four required dose optimizations during review stages. One Clinpharm review conducted PK simulation in different weight groups for pediatrics. Registration trial of another application had dose reductions due to adverse effects. The dose reductions were reviewed, optimized and approved. Two BLAs for Alzheimer's Disease were approved via accelerated pathway using biomarker (e.g., Aβ). One NDA for Duchenne Muscular Dystrophy was also approved via accelerated pathway using biomarker (e.g., dystrophin in skeletal muscle). Completing the review of all identified datasets is essential to identify any trends or relationships, which could provide valuable insights into regulatory challenges and dose optimization strategy.
- **Implications**
    - This study systematically assesses dose selection and optimization strategies in the related fields of neurology and psychiatry. The insights gained from this research will offer valuable guidance to reviewers, aiding them in providing informed comments and making pivotal regulatory decisions.

46. **Mei, Andrew**
    - **Abstract title:** The Distribution of Drug Substance Related Major Deficiencies In FY 2023 First Cycle Complete Response Letters for ANDAs
    - **Authors:** Andrew Mei (student), Pinaki Desai, Rongzuo Xu, Xiang Yu, Deborah Johnson (Mentor), Fang Yuan (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - The response to a complete response letter (CRL) that contains major deficiencies will be classified as a major amendment. According to the Guidance for Industry, "ANDA Submissions — Amendments to Abbreviated New Drug Applications Under GDUFA," such major amendments result in a longer review period and may delay the timely access to quality, affordable generic medicines. Among the 284 CRLs issued in FY 2023 for the 1st cycle ANDA submissions, 31 of them contained major deficiencies related to Drug Master File (DMF) assessments. The primary objective of this study is to identify each major deficiency related to the DMF manufacturing process. The second objective is to categorize all DMF-related major deficiencies based on the Guidance for ANDA submissions. The third objective is to collect the corresponding response times for each major CRL and their respective review times. The final objective is to determine which DMF-related major deficiencies may contribute to bottlenecks in the generic drug 1st cycle approval process.
        - **Purpose**
            - The response to a complete response letter (CRL) that contains major deficiencies will be classified as a major amendment. According to the Guidance for Industry, "ANDA Submissions — Amendments to

Abbreviated New Drug Applications Under GDUFA," such major amendments result in a longer review period and may delay the timely access to quality, affordable generic medicines. This study will identify which DMF-related major deficiencies may contribute to bottlenecks in the generic drug approval process.

- **Methods**
  - The major deficiencies, CR response time, and review time are gathered from Panorama, EDR, and internal data base (WRAP).
- **Results**
  - Among the 284 Complete Response Letters (CRLs) issued in FY 2023 for the 1st cycle ANDA submissions, 31 of them contained major deficiencies related to Drug Master File (DMF) assessments. Each DMF related major deficiency is evaluated and classified based on the Guidance for ANDA submissions. The corresponding response times for each major CRL and their respective review times are collected and compared.
- **Implications**
  - The findings will assist the agency to identify opportunities of improvements for the pharmaceutical industry in the life cycle drug substance DMFs, to improve the quality of DMFs, shorten the review timeline and eventually enhance the 1st cycle approval rate of ANDAs.

47. **Nana, Andre**
    - **Abstract title:** Chemistry, Manufacturing and Control Post-Approval Changes – Potential Global Considerations for Generic Drug Products
    - **Authors:** Nana, Andre, FDA/CDER/OGD/OB/DBIII (ORISE Fellow); Braddy, April C., FDA/CDER/OGD/OB/DBIII (Advisor); Gong, Li, FDA/CDER/OGD/OB/DBIII (Co-Mentor); Dandamudi, Suman, FDA/CDER/OGD/OB/DBIII (Co-Mentor). Acknowledgements: Ren, Ke, FDA/CDER/OGD/OB/DBIII; Sayeed, Vilayat A., FDA/CDER/OPQ/OPQAI/DPQAII; Hughes, Jonathan, FDA/CDER/OGD/OGDP
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
      - **Synopsis**
        - Generic drugs are designed to be equivalent to the reference listed drug (RLD), which has received approval through a new drug application (NDA). Generic drug applicants should demonstrate that their product is pharmaceutically equivalent and bioequivalent to the RLD, as the abbreviated new drug application (ANDA), relies on the FDA's recognition of the safety and efficacy of the RLD. Consequently, availability of RLDs drug products is crucial for developing generic drugs. RLDs are usually chosen as the Reference Standard (RS) for in vivo bioequivalence (BE) studies. However, if an RLD is unavailable under certain circumstances, the FDA can designate another drug product as RS for comparative purposes in the BE studies. In the United States, only FDA-approved drug products are used as comparators for BE studies. Elsewhere, in the absence of a local comparator, some regulators may consider the use of a foreign comparator drug product, under certain conditions, as shown in the "Survey of the Regulatory Requirements for the Acceptance of Foreign Comparator Products by Participating Regulators and Organizations of the International Generic Drug Regulators Programme." The use of a foreign comparator drug product

raises concerns about its comparability to the local reference product in terms of quality, safety, and efficacy. Indeed, there is a potential for differences in bioequivalence that may result from even slight differences between the foreign comparator and the United States approved RLD. Factors such as regulations governing chemistry, manufacturing, and control (CMC) post-approval changes can affect the foreign comparator drug product. There have been recent international efforts, led by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), to establish harmonization of drug product lifecycle management, resulting in the release of the ICH guideline Q12 (November 2019). The ICH guideline Q12 provides a foundation for CMC post-approval management, covering aspects such as the categorization of post-approval changes, identification of Established Conditions (ECs), and Post-Approval Change Management Protocols (PACMP). This abstract aims to compare current CMC post-approval guidelines among the United States and some ICH members, namely the European Union and Canada.

- **Purpose**
  - Chemistry, Manufacturing, and Control (CMC) post-approval changes significantly contribute to the quality of the drug substances and drug products that patients access. Those changes are typically made by the application holders to enhance their capability to meet market demand and are assessed to meet the quality, safety and efficacy requirements. For various reasons, including supply chain disruptions, a drug manufacturer may need to make changes such as introducing a new drug product manufacturing site, a new Active Pharmaceutical Ingredient (API), manufacturing process, or specifications. These post-approval changes may have the potential to adversely affect the quality, safety and/or efficacy of any drug product, included comparator drug products (RLD and RS). Consequently, to mitigate these concerns, regulatory agencies provide guidelines to industry on how to handle such circumstances. This abstract reports a comparison of the CMC post-approval changes guidelines of the United States and the European Union and Canada.
- **Methods**
  - We collected from April to June 2024, publicly available guidelines related to CMC post-approval changes, sourced from regulators' websites. These guidelines include the “Guidance for Industry - Changes to an Approved NDA or ANDA (FDA - 2004),” the “Post-Notice of Compliance (NOC) Changes: Quality Document (Health Canada - 2019),” and the “Guidelines on the details of the various categories of variations (European Commission - 2013).” We extracted information related to the reporting categories, the established conditions/items affected by the changes, and the regulatory process applicable to each reporting category. Pertinent information is subsequently compared to the United States’ guideline.
- **Results**
  - All three regulations use a risk-based approach to evaluate post-approval modifications, which is reflected in the reporting categories. High-risk changes, which have a significant potential to affect the drug safety and efficacy, require the submission of a supplement, and

approval by the regulatory authority prior to their implementation. These are “Major changes” in the United States, “Type II variations” in the European Union and “Level I – Supplement” in Canada. For instance, in the United States, such modifications require the submission of a prior approval supplement (§ 314.70(b)). Examples of high-risk changes include modifications that can affect the sterility of a sterile drug product, and changes in specifications outside of the range of acceptance criteria. Moderate risk changes are handled through a notification (immediate or within a certain-time frame). Low risk changes are included in annual reports in the United States (Minor changes) and Canada (Level III). The European Union recognizes minor variations of Type IB (‘Tell, Wait and Do’ procedure), and Type IA (‘do and tell’ procedure, with immediate notification or in the annual report). Of note, the United States implemented the ICH Q12 guideline since 2021, but the guideline was still in the process of implementation by the European Union, and Canada at the moment of submission of this abstract (ICH website).

- **Implications**
    - CMC post-approval changes are vital in maintaining the quality, safety, and efficacy of drug products. Regulations governing these changes aim to ensure compliance with the conditions established during the approval process without compromising the quality, safety and effectiveness of the drug substance or the drug product. This comparison between the United States and other jurisdictions provides insight of the similarities and divergences in the regulations. The ongoing comparison as well as the expert discussions will provide more details and clarifications, particularly regarding the dosage forms of interest in the project, which are otic, ophthalmic, and parenteral solutions. Combined with other aspects such as the drug approval process, bioequivalence/biowaivers, manufacturing regulations, CMC post-approval changes could serve as reference criteria when considering a foreign comparator if an FDA approved drug product is not available. However, further exploration on both regulatory and scientific aspects is needed.

48. **Osei, Wilberforce**
    - **Abstract title:** The Application of PBPK Modeling for Development and Approval of Therapeutic Proteins
    - **Authors:** Osei, Wilberforce, FDA/CDER(Student); Wang, Ting, FDA/CDER (ORISE fellow); Wang, Yow-Ming FDA/CDER(Mentor); Sun, Qin FDA/CDER (Mentor)
    - **FDA Strategic Initiative**: Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Purpose**
            - The application of physiologically based pharmacokinetic (PBPK) modeling for therapeutic proteins (TPs) is limited, and it is crucial to understand the opportunities and challenges to enhance their contribution to model-informed drug development. We conducted this study to: 1) identify novel TPs with PBPK modeling application in labeling or FDA reviews; 2) characterize the identified TPs; 3) assess different application category and summarize examples; 4) investigate additional applications in literature; 5) summarize current practice and

provide insights for future strategy.

- **Methods**
  - We searched FDA's Purple Book, labeling, and FDA reviews to identify TPs with PBPK modeling application and characterized them by approval year, disease area, and TP type. We assessed FDA review to categorize PBPK application types and summarize examples. Furthermore, we searched PubMed and Google for additional applications in literature. Finally, we summarized current practice and provided insights on opportunities and challenges.
- **Results**
  - For TPs approved by FDA as of June 2024, only 10/257 (4%) had PBPK modeling application in labeling or review. The number of PBPK applications increased over time, and the majority is for cancer pharmacology (80%) and monoclonal antibody products (60%). The application categories include TP DDI (n=8), pediatrics PK (n=1, olipudase alfa), and dose selection (n=1, epcoritamab). The TP DDI application can be further categorized into T cell engager (TCE) cytokine DDI (e.g., glofitamab), antibody-drug conjugate (ADC) payload DDI (e.g., polatuzumab vedotin), and cytokine modulator DDI (e.g., satralizumab). PBPK modeling approaches were used to support ADC payload DDI assessment in labeling without clinical DDI studies. However, their applications for TCE cytokine DDI and cytokine modulator DDI were deemed inadequate due to multiple limitations. Other applications were supportive in addition to clinical data. The main application types in literature are like those in labeling or review, with some additional uses (e.g., dosing route change, organ impairment).
- **Implications**
  - Our research provides an update of current knowledge about PBPK modeling application for TPs. Although PBPK modeling approaches have been used successfully for ADC payload DDI assessment to support labeling, their applications in other areas were supportive or inadequate. Future efforts should focus on addressing knowledge gaps (e.g., collecting physiological change data in special populations, improving the understanding of exposure-response relationship between clinically relevant cytokine levels and CYP suppression) to further advance PBPK modeling application for TPs.

49. **Perera, Hansana**
    - **Abstract title:** Impact of Galactosylation on the Pharmacokinetics of Monoclonal Antibodies
    - **Authors:** Perera, Hansana, FDA/CDER (Student); Swisher, Jennifer, FDA/CDER (Mentor); Mindaye, Samuel, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Monoclonal antibodies (mAbs) are crucial in therapeutic biologics due to their high specificity and efficacy. Despite identical amino acid sequences, post-translational modifications, like galactosylation, may impact pharmacokinetics (PK), affecting duration and access to targets in the body. This study investigates the effects of galactosylation on mAb PK profiles to ensure consistency between clinical trials and market

performance. Data were sourced from PK studies in approved biosimilar programs and 351a programs with major manufacturing changes. Galactosylation differences (roughly 10% or more) were identified, and PK profiles were analyzed. Lot and batch numbers in PK studies were matched to glycosylation levels, measuring glycans such as G0, G1, G2, and high mannose. Three products exhibited significant galactosylation differences. PK profile analyses were conducted with the Office of Clinical Pharmacology (OCP). Findings emphasize the potential need for controlled galactosylation to ensure consistent clinical performance. Differences in galactosylation may affect clearance rates and efficacy, challenging the assumption that mAbs with different glycan profiles exhibit the same PK. These insights guide the engineering of mAbs with optimized glycan profiles, enhancing effectiveness and minimizing adverse effects. Understanding specific glycans' roles, such as mannose and galactose residues, can improve manufacturing processes for consistent product quality. The study also underscores the regulatory need for stringent guidelines on controlling glycosylation during mAb production, supporting future research in biologics, and leading to more effective and reliable therapeutics for patients.

- **Purpose**
  - Monoclonal antibodies (mAbs) are crucial in therapeutic biologics due to their high specificity and efficacy. Even though mAbs may share identical amino acid sequences, post-translational modifications can significantly impact their pharmacokinetics (PK), influencing their duration and access to targets within the body. Among these modifications, galactosylation, the addition of galactose to the Fc region of antibodies, is highly variable and generally uncontrolled. Recent experience suggests that for some products, differences in galactosylation may impact PK behavior. A process change in one product caused a 14% galactosylation difference, resulting in 50% lower drug exposure for the higher G0 material. Another product showed G0 cleared 1.3-2 times faster than other glycoforms. These findings prompted investigation into whether galactosylation impacts PK broadly in protein therapeutics or is influenced by other factors. Understanding these effects can ensure consistency, ensuring that drugs work just as well in the market as in clinical trials.
- **Methods**
  - This study utilized two sources of data to determine the impact of galactosylation on pharmacokinetics. The first source included PK studies from approved biosimilar programs (biosimilar vs. US/EU reference product), while the second source comprised PK studies from 351a programs where a major manufacturing change occurred in late development. Cases with significant galactosylation differences (roughly 10% or more) were identified, and the corresponding PK profiles were analyzed, despite the expectation that these PK profiles should be similar. Lot and batch numbers used in the PK studies were matched to those used to determine glycosylation levels. The glycans measured included G0, G1, G2, and mannose. High mannose was included due to its association with faster clearance rates.
- **Results**
  - Three additional products were found thus far that exhibited significant

differences in galactosylation between lots used for head-to-head PK studies, exceeding 10%. The first product had 11.36% more galactosylation than the EU-approved product. The second product was compared between Process 1 and Process 2, with Process 1 containing 15.05% more G0F than Process 2. The third product was analyzed by comparing its two cell lines. The CHO cell line had 23% more G0F and 26% less G1F than the Sp2/0-derived cell line. The analysis of the PK profiles for these three products was conducted in close collaboration with the Office of Clinical Pharmacology (OCP). There is preliminary evidence that differences in galactosylation lead to different variations in the PK of different classes of antibody-based products.

- **Implications**
    - The findings from this study on the impact of galactosylation on the PK of mAbs suggest significant implications for the field of pharmaceutical development. If it is determined whether and when galactosylation matters, it will highlight the need for more control of this attribute to ensure consistency of clinical performance. Differences in galactosylation appear to affect clearance rates and overall efficacy, challenging the assumption that mAbs that are different in this sense will exhibit the same PK profile. These insights can lead to more precise engineering of mAbs with optimized glycan profiles, possibly enhancing their effectiveness and minimizing adverse effects. More specifically, understanding the role of specific glycans such as mannose and various galactose residues can lead the manufacturing process to ensure consistent product quality. This research also reveals how manufacturing changes can alter glycosylation which could significantly impact patient outcomes. Consequently, regulatory agencies may need to develop more stringent expectations for controlling glycosylation during mAb production. Overall, this study provides support for future research and development in the biologics field, leading to more effective and reliable therapeutics for patients.

50. **Qian, Ethan**
    - **Abstract title:** Developing an Interactive Dosing Interface Platform
    - **Authors:** Ethan Qian FDA/CDER (Student), Brett Martini, Sarah Schrieber, Jianmeng Chen, Suresh Doddapaneni, Chandrahas Sahajwalla, Ping Ji FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract**
        - **Synopsis**
            - A pharmacokinetic (PK)-based bridging strategy is essential in dosing regimens and the route of administration changes during drug development and regulatory review. Using this strategy has been limited by the lack of decision support tools allowing the integration pharmacokinetic data, modeling, and treatment recommendations. Our project aims to develop a user-friendly tool to integrate PK data, modeling, simulation, dosing, and route of administration to provide an accessible platform to facilitate drug development and regulatory decision-making. This tool employs R packages such as rxode2 and PKNCA for modeling and parameter calculation. With the Shiny package, the interface allows users to input information, such as study design, dosing information, and other model specific inputs, and visualize the

resulting PK profiles, as well as provide outputs of summary statistics for the PK parameters. This platform helps facilitate drug development and regulatory decision-making by providing users with customizable simulations to evaluate and support changes to dose regimens and routes of administration for drugs and biologics.

- **Purpose**
  - The purpose of this project is to create a real-time, interactive, user-friendly tool to integrate PK data, modeling, simulation, dosing, and route of administration to provide an accessible platform to facilitate drug development and regulatory decision-making.
- **Methods**
  - The input information for the database, including dosing and PK modeling information was obtained from the FDA Purple Book database and literature. To build the dosing interface platform, RStudio was linked to Git as the source code version control system. In RStudio multiple R packages were used including: rxode2, used for model simulation and PKNCA, used to calculate PK parameters and create summary statistics. To create the platform, the Shiny package was used.
- **Results**
  - The platform is composed of one input panel and two output panels. In the input panel, users enter information, such as the dose, number of doses, dosing intervals, and study duration. The user also includes model specific inputs, such as the absorption rate for a SC model and infusion rate for an IV model. Users can then visualize the PK profiles within the interface, and graphics can be generated showing the concentration over time profiles based on the adjustable input variables. Additionally, the interface creates summary statistics of the PK parameters based on the adjusted inputs.
- **Implications**
  - By providing real-time, interactive simulations, the platform we developed provides an easy way to simulate PK profiles for Mabs with different dosing regimens. The platform is interactive, searchable, and user controlled. Users input information about the study design, dosing information, and PK parameters into the platform and, visualization of the simulation outputs are generated. Our project successfully improved the readability of data by including graphics and charts as part of the output. In summary, our project created a tool that adds to understanding the impact of dose/dosing regimens on the PK. The database we created will be an essential tool for FDA reviewers in supporting regulatory decision-making about changes to dosing regimens and route of administrations.

51. **Quddos, Fatima**
    - **Abstract title:** A Survey Assessing the Effect of Gastric Emptying Caused by GLP-1 Receptor Agonists on Concomitantly Administered Oral Products
    - **Authors:** Fatima Quddos (Student), Sila Yandarg (Student), Yanhui Lu, Edwin Chow, Mohamad Kronfol, & Mohamed Ismail Nounou (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - The study has two aims: a) to report on gastric emptying using clinical

trials and studies examining changes in pharmacokinetics of oral contraceptives from the regulatory documents, and b) to investigate the effects of GLP-1 receptor agonists on gastric emptying from literature. Based on the review conducted, the labels of all approved GLP-1 products (14/14) contained information about delayed gastric emptying. Furthermore, majority of clinical trials and drug interaction studies of oral contraceptives reported a change in endpoints related to gastric emptying for the former and change in pharmacokinetic properties for the latter. By evaluating approved GLP-1 receptor agonists on gastric emptying delay, we gain an understanding on the differences in their potential for drug-drug Interactions. This can help make an informed decision for GLP-1s DDI study designs and products' labels. This survey provides initial insights on how GLP-1 drugs may affect the pharmacokinetics of other drugs.

- **Purpose**
  - This study investigated the methods and results of gastric emptying assessments used in literature and US Food and Drug Administration (FDA) clinical pharmacology reviews and labels in marketed GLP-1 products approved by the FDA. The study has two aims: a) to report on gastric emptying using clinical trials and studies examining changes in pharmacokinetics of oral contraceptives from the regulatory documents, and b) to investigate the effects of GLP-1 receptor agonists on gastric emptying from literature.
- **Methods**
  - Based on products' label from 2005 until 2023, 14 GLP-1 drug products approved by the FDA were reviewed. Information collected from the clinical pharmacology reviews and labels included: information or warnings about gastric emptying, clinical trials conducted specifically to investigate gastric emptying and any change in pharmacokinetics of oral contraceptives. PubMed, public FDA clinical pharmacology reviews and clinicaltrials.gov were used along with products' FDA labels for data collection.
- **Results**
  - Based on the review conducted, the labels of all approved GLP-1 products (14/14) contain information about delayed gastric emptying. In the clinical pharmacology reviews of the 14 approved GLP-1s, 24 clinical trials examined gastric emptying, employing acetaminophen pharmacokinetics or scintigraphy as primary or secondary endpoints. Nineteen trials documented a delay in gastric emptying based on the defined endpoints. In two scintigraphy studies, the time taken for half-emptying of stomach contents was delayed by 1.09 to 2 hours. Additionally, eleven studies evaluating gastric emptying via acetaminophen absorption tests observed reduced AUC (Range: 11.6 – 38%) and Cmax (Range: 14-63%). In the clinical pharmacology reviews, there were eight detailed studies of interaction with oral contraceptives, with five out of eight studies reporting a reduced peak serum concentration (Cmax) and delayed time to maximum concentration (tmax) for the active components of the oral contraceptives (Levonorgestrel (Cmax reduction: 13-46%, tmax delay: 1-3.5 hours), Ethinyl Estradiol (Cmax reduction: 12-59%, tmax delay: 0.3-4.5 hours) and Norelgestromin (Cmax reduction: 26-55%, tmax

delay: 2-2.5 hours)). Next, Pubmed returned 19 clinical trial results between 2005 and 2024 for gastric emptying and GLP-1 related terms (e.g., semaglutide, exenatide, etc.). Only 2 studies showed no change in defined endpoints related to gastric emptying with GLP-1 administration. Finally, Pubmed returned 14 case studies between 2005 and 2024 for search terms including gastric emptying and GLP-1 related terms. Of these, three case studies did not report on gastric emptying. The remaining 11 case studies reported 17 cases of retained stomach contents or regurgitation following approved fasting protocols when the patient was taking a GLP-1 receptor agonist.

- **Implications**
  - In summary, the gastric emptying effect of GLP-1 receptor agonists on the pharmacokinetics of oral contraceptives is investigated based on the review of regulatory documents and literature. By evaluating approved GLP-1 receptor agonists on gastric emptying delay, we gain an understanding on the differences in their potential for drug-drug Interactions. This can help make an informed decision for GLP-1s DDI study designs and products' labels. This survey provides initial insights on how GLP-1 drugs may affect the pharmacokinetics of other drugs.

52. **Sahin, Timur**
    - **Abstract title:** Assessing Inconsistencies Within Warnings & Precautions for Antipsychotic Labeling
    - **Authors:** Timur Sahin, FDA/CDER/OND (Student); Kimberly Updegraff, FDA/CDER (Mentor); Bernard Fischer, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - The study has two aims: a) to report on gastric emptying using clinical trials and studies examining changes in pharmacokinetics of oral contraceptives from the regulatory documents, and b) to investigate the effects of GLP-1 receptor agonists on gastric emptying from literature. Based on the review conducted, the labels of all approved GLP-1 products (14/14) contained information about delayed gastric emptying. Furthermore, majority of clinical trials and drug drug interaction studies of oral contraceptives reported a change in endpoints related to gastric emptying for the former and change in pharmacokinetic properties for the latter. By evaluating approved GLP-1 receptor agonists on gastric emptying delay, we gain an understanding on the differences in their potential for drug-drug Interactions. This can help make an informed decision for GLP-1s DDI study designs and products' labels. This survey provides initial insights on how GLP-1 drugs may affect the pharmacokinetics of other drugs.
      - **Purpose**
        - This study investigated the methods and results of gastric emptying assessments used in literature and US Food and Drug Administration (FDA) clinical pharmacology reviews and labels in marketed GLP-1 products approved by the FDA. The study has two aims: a) to report on gastric emptying using clinical trials and studies examining changes in pharmacokinetics of oral contraceptives from the regulatory documents, and b) to investigate the effects of GLP-1 receptor

agonists on gastric emptying from literature.

- **Methods**
  - Based on products' label from 2005 until 2023, 14 GLP-1 drug products approved by the FDA were reviewed. Information collected from the clinical pharmacology reviews and labels included: information or warnings about gastric emptying, clinical trials conducted specifically to investigate gastric emptying and any change in pharmacokinetics of oral contraceptives. PubMed, public FDA clinical pharmacology reviews and clinicaltrials.gov were used along with products' FDA labels for data collection.
- **Results**
  - Based on the review conducted, the labels of all approved GLP-1 products (14/14) contain information about delayed gastric emptying. In the clinical pharmacology reviews of the 14 approved GLP-1s, 24 clinical trials examined gastric emptying, employing acetaminophen pharmacokinetics or scintigraphy as primary or secondary endpoints. Nineteen trials documented a delay in gastric emptying based on the defined endpoints. In two scintigraphy studies, the time taken for half-emptying of stomach contents was delayed by 1.09 to 2 hours. Additionally, eleven studies evaluating gastric emptying via acetaminophen absorption tests observed reduced AUC (Range: 11.6 – 38%) and Cmax (Range: 14-63%). In the clinical pharmacology reviews, there were eight detailed studies of interaction with oral contraceptives, with five out of eight studies reporting a reduced peak serum concentration (Cmax) and delayed time to maximum concentration (tmax) for the active components of the oral contraceptives (Levonorgestrel (Cmax reduction: 13-46%, tmax delay: 1-3.5 hours), Ethinyl Estradiol (Cmax reduction: 12-59%, tmax delay: 0.3-4.5 hours) and Norelgestromin (Cmax reduction: 26-55%, tmax delay: 2-2.5 hours)). Next, Pubmed returned 19 clinical trial results between 2005 and 2024 for gastric emptying and GLP-1 related terms (e.g., semaglutide, exenatide, etc.). Only 2 studies showed no change in defined endpoints related to gastric emptying with GLP-1 administration. Finally, Pubmed returned 14 case studies between 2005 and 2024 for search terms including gastric emptying and GLP-1 related terms. Of these, three case studies did not report on gastric emptying. The remaining 11 case studies reported 17 cases of retained stomach contents or regurgitation following approved fasting protocols when the patient was taking a GLP-1 receptor agonist.
- **Implications**
  - In summary, the gastric emptying effect of GLP-1 receptor agonists on the pharmacokinetics of oral contraceptives is investigated based on the review of regulatory documents and literature. By evaluating approved GLP-1 receptor agonists on gastric emptying delay, we gain an understanding on the differences in their potential for drug-drug Interactions. This can help make an informed decision for GLP-1s DDI study designs and products' labels. This survey provides initial insights on how GLP-1 drugs may affect the pharmacokinetics of other drugs.

53. **Sayyed, Arsalaan**

- **Abstract title:** Assessment of efficacy and toxicity profile by treatment of Immune checkpoint inhibitor and antibody-drug conjugate combination therapy across solid cancers: a systematic review and metanalysis
- **Authors:** Sayyed, Arsalaan (Student) FDA/CDER; Caroline Taylor, Shiowjen Lee, Tao Wang (Mentor) FDA/CDER
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
    - **<u>Purpose</u>**
        - The purpose of this study is to evaluate the efficacy and toxicity of combination therapy for cancer treatment using antibody-drug conjugates (ADC) and immune checkpoint inhibitors (ICI) in solid cancers. Although there is some success with the single treatments of ADCs and ICIs, many patients develop a resistance to the treatment. Combination therapy is one way to address this resistance as indicated by a recent study aimed at treating urothelial cancer with a combination of enfortumab vedotin and pembrolizumab. This remains largely unknown for many other solid cancers due to limited clinical trial data. In addition, immune checkpoint inhibitor (ICI) therapy often shows a correlation between efficacy and toxicity in clinical studies. Moreover, accumulating evidence suggests that anti-cancer immune responses are a major attribute for ADC-mediated anticancer efficacy. Therefore, we hypothesize that combining ADCs with ICIs may leverage their complementary mechanisms, potentially enhancing treatment efficacy synergistically. This study aimed to determine if the efficacy and toxicity of ADC and ICI combination therapy, as demonstrated in many different phase trials, warrant their use in clinical practice. Specifically, our study sought to assess whether there is an association between treatment efficacy and treatment related adverse events (TRAEs).
    - **<u>Methods</u>**
        - A systematic review of the literature was conducted following the PRISMA guidelines. The databases included were Medline, Embase, and Cochrane, which were searched from their conception until July 1, 2024. The search terms included all possible combinations of FDA-approved ICIs and ADCs. Our study was limited to solid cancers, studies conducted in English, and Phase I, II, and III trials. The primary outcomes of our study included objective response rate (ORR), progression-free survival (PFS), discontinuation rate, duration of response (DoR), median overall survival (mOS), any-grade adverse events, and grade ≥3 adverse events. Correlations between variables were estimated using the pairwise method to account for missing data.
    - **<u>Results</u>**
        - A total of 12 trials including 982 patients were analyzed. Age was strongly correlated and significant with discontinuation rate (r=0.6815, p=0.0209). The ORR showed strong positive correlations and significance with PFS (r=0.7197, p=0.0125) and mOS (r=0.9367, p=0.0059). Serious grade ≥3 adverse events were strongly correlated and significant with any adverse events (r=0.7675, p=0.0158).
    - **<u>Implications</u>**
        - The study underscores the potential of combination therapy using ADCs and ICIs in enhancing cancer treatment efficacy while also presenting

considerable toxicity challenges. The patients' age should be considered in clinical practice, as our results have shown significant correlations between age and discontinuation rate (r=0.6815, p=0.0209). Therefore, age-specific management strategies should be considered to optimize treatment adherence and outcomes. The efficacy of combination therapy is highlighted by strong positive correlations and significance between ORR with PFS (r=0.7197, p=0.0125) and mOS (r=0.9367, p=0.0059). This may help sponsors and clinicians deliberate their decision on moving forward with the next stages of clinical trials, as the data supports that ORR might be a potential indicator for assessing PFS and mOS. Moreover, any-grade treatment-related adverse events were significantly correlated with grade ≥3 adverse events (r=0.7675, p=0.0158). These findings highlight the importance for clinicians and sponsors to carefully consider and manage the symptoms and effects associated with these therapies. Management of toxicity is crucial to ensure that the benefits of combination therapy are not overshadowed by severe TRAEs.

54. **Tang, Qian**
    - **Abstract title:** Evaluation of Win Ratio Methods utilized in Rare Hematological Diseases Clinical Trials for Composite Endpoints
    - **Authors:** Tang, Qian, FDA/CDER (Student); Cai, Xiaoyu, FDA/CDER (Mentor); Chen, Yeh-Fong, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - In rare hematological diseases clinical trials, where patients are likely heterogeneous, an increasing number of applications intend to increase the chance of detecting treatment effect by utilizing a composite endpoint derived from the measurements of multiple outcomes, due to the infeasibility to adequately power any of the single outcome. Commonly used composite endpoints are based on different binary or survival endpoints, sometimes in rare disease clinical trials patient reported outcomes are needed to measure patients' feel, function and survives, thus continuous endpoints based on patients' change from baseline are needed to be considered. This project explores the pros and cons of various methods for analyzing composite endpoints under different criteria for defining success and interpreting findings. Statistical methods include the O'Brien rank sum test (O'Brien, 1984), Finkelstein-Schoenfeld (F-S) method (Finkelstein and Schoenfeld, 1999), and the win ratio method (Pocock et al., 2012). Luo et al. (2015) and Dong et al. (2016) have proposed variance estimators and extensions for the win ratio method, with Dong also introducing the stratified win ratio method in 2018. Our study evaluates these methods alongside the win proportion and weighted win ratio approaches using numerical simulations across various data types and parameter settings.
        - **Purpose**
            - In rare hematological diseases clinical trials, where patients are likely heterogeneous, an increasing number of applications intend to increase the chance of detecting treatment effect by utilizing a

composite endpoint derived from the measurements of multiple outcomes, due to the infeasibility to adequately power any of the single outcome. Commonly used composite endpoints are based on different binary or survival endpoints, sometimes in rare disease clinical trials patient reported outcomes are needed to measure patients' feel, function and survives, thus continuous endpoints based on patients' change from baseline are needed to be considered. This project explores the advantages and disadvantages of different methods for analyzing composite endpoints under various circumstances when knowing or prioritizing different winning criteria as well as how to interpret the findings.

- **Methods**
  - Several statistical methods have been proposed for analyzing composite endpoints, including the O'Brien rank sum test (O'Brien, 1984), Finkelstein-Schoenfeld (F-S) method (Finkelstein and Schoenfeld, 1999), and the win ratio method (Pocock et al., 2012). For the win ratio method, Luo et al. (2015) introduced a closed-form variance estimator for a specific definition of wins, losses, and ties, while Dong et al. (2016) extended this approach to accommodate any definition of winners and losers. Dong further proposed the stratified win ratio method in 2018. In our study, in addition to these established methods, we also explore the performance of composite endpoints using the win proportion and weighted win ratio approaches.
- **Results**
  - The operating characteristics of the aforementioned methods are compared in numerical simulation under various data types of the components and parameters settings.
- **Implications**
  - The project was prompted by recent submissions employing novel methods for designing and analyzing composite endpoints. It aims to deepen our understanding of the advantages and disadvantages of using composite endpoints in small-scale clinical trials through literature review, theoretical derivation, numerical simulation, and case studies. This effort will enable reviewers to offer informed guidance and recommendations for applications involving composite endpoints.

55. **Qi, Tong**
    - **Abstract title:** De-noising digital health signals from continuous glucose monitoring device using wavelet-based analysis
    - **Authors:** Qi, Tong, FDA/CDER (Student); Jung, Tae Hyun, FDA/CDER (Mentor); Kim, Yoonhee, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
      - **Synopsis**
        - Digital health technologies leveraging sensor-generated data enable frequent and objective remote monitoring of patients, yet they often face challenges such as extraneous noise. This study investigates the regulatory application of wavelet transform in processing these data to ensure their suitability for clinical decision-making. We applied Discrete

Wavelet Transform (DWT) to continuous glucose monitoring (CGM) data collected from 50 children aged 10 to 18 with type 1 diabetes. Our methodology included a comprehensive grid search across 33 wavelets spanning 5 families and 4 decomposition levels to optimize noise reduction while preserving signal integrity. Simulation experiments validated DWT's effectiveness in reducing noise, particularly evident during hypoglycemic and hyperglycemic periods and across various time intervals (daily, 8-hour, 6-hour, and 4-hour segments). Key metrics such as Mean Squared Error (MSE), Signal-to-Noise Ratio (SNR), and Peak Signal-to-Noise Ratio (PSNR) were employed to assess denoising performance. This research underscores DWT's critical role in preprocessing sensor data, enhancing the quality of healthcare management data, and preparing it for more sophisticated analyses.

- **Purpose**
  - Endpoints using digital health technology (DHT) which utilize sensor-generated data allow more frequent and objective remote monitoring of patients. When integrated into clinical studies, however, these data using DHT technologies face challenges like the capture of extraneous data or noise. To effectively manage the influx of DHT based endpoints in regulatory process and ensure that the data collected is meaningful for decision-making, sophisticated data processing and analysis techniques are essential for filtering out noise. However, few statistical methods are introduced and rarely utilized to process the DHT based endpoints to be fit-for-purpose. The aim of this project is to assess the regulatory utility of wavelet transform in processing DHT based endpoints. We will apply multiple wavelet transforms methods in a continuous glucose monitoring (CGM) data to enhance precise detection and removal of noise while preserving the essential characteristics of the original signal.
- **Methods**
  - We implemented the Discrete Wavelet Transform (DWT) to denoise the original continuous glucose monitoring (CGM) data. Our grid search involved 33 different wavelets from 5 separate wavelet families and 4 different levels of decomposition to identify the most suitable wavelet and decomposition level for the specific CGM data. To evaluate the performance of denoising, we added varying levels of noise, particularly targeting the hypoglycemia and hyperglycemia periods during the day. Using simulations, we tested whether the DWT could significantly reduce the noise. We applied this technique not only to daily records but also to segmented records of 8-hour, 6-hour, and 4-hour intervals to investigate which time ranges during the day had higher rates of hypoglycemia events. Metrics such as mean squared error (MSE), signal-to-noise ratio (SNR), and peak signal-to-noise ratio (PSNR) were used to assess the denoising performance.
- **Results**
  - The data used in this project is derived from a public dataset featuring 50 children aged 10 to 18 with type 1 diabetes. Glucose levels were continuously monitored over two separate 24-hour periods using the OneTouch Ultra Meter, with readings taken at 5-minute intervals. According to clinical standards, a hypoglycemia event is defined as having at least three consecutive glucose records (equivalent to 15

minutes) below 70 mg/dL. After rigorous data cleaning, we processed 4753 valid samples. The Discrete Wavelet Transform (DWT) played a crucial role in refining glucose records that were slightly above 70 mg/dL, especially in cases where there were fewer than 5 instances between two hypoglycemia events. The DWT was also effective in handling records precisely at or just below 70 mg/dL with three consecutive points. Wavelet types and decomposition levels were chosen based on criteria such as minimizing Mean Squared Error (MSE) while maximizing Peak Signal-to-Noise Ratio (PSNR) and Signal-to-Noise Ratio (SNR). For analytical purposes, we segmented the data into 6-hour intervals, resulting in a comprehensive dataset of 9513 samples. Initially, 1951 samples were identified as hypoglycemic. Following the application of DWT, this number increased to 2012, significantly enhancing our ability to detect hypoglycemia and identify individuals at risk.

- **Implications**
    - The Discrete Wavelet Transform (DWT) offers a robust method for preprocessing and denoising raw sensor data collected by digital health devices. This refined data is pivotal for enhancing subsequent analytical procedures, including the application of Continuous Wavelet Transform (CWT) and Wavelet Scattering for classification tasks. By reducing noise and improving signal clarity, DWT not only enhances the accuracy of glucose monitoring and hypoglycemia detection but also facilitates more precise and reliable insights into patient health trends over time. This refined data can potentially lead to advancements in personalized healthcare management and therapeutic interventions, thereby providing informative data for furthermore sophisticated analysis.

56. **Wang, Neng**
    - **Abstract title:** Evaluating Data Imputation Methods for Acute Postoperative Pain Management in the Presence of Frequent Rescue Medication Use
    - **Authors:** Neng Wang FDA/CDER (Student), Jing Han (Mentor) FDA/CDER, Sue Jane Wang
    - **FDA Strategic Initiative:** Empowering Patients and Consumers
    - **Abstract:**
        - **Synopsis**
            - This study aims to investigate the effectiveness of products for managing acute postoperative pain, with a particular focus on evaluating data imputation methods used in the face of frequent rescue medication use. Rescue interventions can introduce significant complexities in assessing treatment efficacy, particularly when their use varies considerably across different study groups. Drawing on comprehensive datasets from multiple approved New Drug Applications and simulated data, we systematically compare different imputation approaches applied to the placebo groups. We also compare those approaches in different treatment arms within the same study using consistent pain models. We will contrast these results on potential patterns and informativeness. The finding may facilitate guidance on efficiently evaluating acute pain treatment efficacy.
        - **Purpose**
            - This study aims to investigate the effectiveness of products for managing acute postoperative pain, with a particular focus on

evaluating data imputation methods used in the face of frequent rescue medication use.

- **Methods**
  - Rescue interventions can introduce significant complexities in assessing treatment efficacy, particularly when their use varies considerably across different study groups. Drawing on comprehensive datasets from multiple approved New Drug Applications and simulated data, we systematically compare different imputation approaches (Truncated, Observed, LOCF, BOCF, WOCF, windowed LOCF) applied to the placebo groups. We also compare those approaches in different treatment arms within the same study using consistent pain models.
- **Results**
  - The Observed approach may exhibit a tendency to overestimate the treatment effect in all the treatment groups especially the placebo group, due to the confounding influence of rescue medication. On the other hand, the Truncated, LOCF, BOCF, and WOCF approaches may lean towards underestimating the treatment effect in all the treatment groups especially the placebo group, as they disregard the effects of subsequent active treatment.
- **Implications**
  - We will contrast these results on potential patterns and informativeness. The finding may facilitate guidance on efficiently evaluating acute pain treatment efficacy. This, in turn, enhances the accuracy and reliability of clinical trial outcomes, informs regulatory decisions, and contributes to standardizing imputation practices in clinical research. Ultimately, the study supports more robust and consistent evaluations of pain management products, addressing the complexities introduced by rescue medication and improving the overall assessment of treatment effectiveness.

57. **Zhang, Siwei**
    - **Abstract title:** The use of a Generalized Linear Model for Testing Proportionality of Average Release Rates for Topical Dermatological Products.
    - **Authors:** Zhang, Siwei FDA/CDER (Student); Rantou, Elena, FDA/CDER (Mentor); Kim, Jessica, FDA/CDER (Mentor); Yap, John, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
      - **Synopsis**
        - In vitro release testing (IVRT) is a crucial component of the regulatory approval process for tgeneric topical dermatological products, ensuring product quality and performance over time. IVRT is used to compare the release rates between test and reference products. Traditionally, the Wilcoxon Rank Sum test is employed to assess the equivalence of release rates between products with no significant formulation differences. However, this method may not be suitable for comparing products with different formulations. Our study aims to quantitatively assess whether the proportion of release rates between high and low strengths of the test product is similar to that of the reference product. This comparison is critical for bioequivalence (BE) study waivers. If the high strength of the test product is BE to the reference, a BE waiver could be applied to the in vitro permeation

tests or in vivo pharmacokinetic studies for the lower strength. Conversely, if the low strength of the test product is bioequivalent to the reference, the waiver could apply to the higher strength. We employed a Generalized Poisson regression model, incorporating the interaction term between treatment and strength, to quantify the relationship between the proportionalities. A meta-analysis was conducted to estimate the effect size of the interaction term. Hypothesis testing was performed to determine whether 90% confidence interval for the exponential of interaction term includes 1. Simulation studies were conducted to estimate the power and type I error across different margins for rejecting the null hypothesis. Additionally, a Bayesian Poisson regression model was utilized to mitigate the influence of small sample size in IVRT. Our findings provide a roust framework for assessing release rate proportionality, guiding bioequivalence decisions in IVRT studies.

- **Purpose**
  - In vitro release testing (IVRT) is a crucial component of the regulatory approval process for topical dermatological products. IVRT is used to assess product sameness between test and reference products, and is often used to ensure that product quality and performance are maintained over time and in the presence of challenges, especially when in vivo clinical studies are impractical for routine quality control. The main purpose of this study is to quantitatively assess whether the proportion of the release rates between high and low strengths of the test product is equivalent to the proportion between high and low strengths of the reference product. This comparison is critical for supporting bioequivalence (BE) study waivers for different strength versions of the topical formulation.
- **Methods**
  - In the traditional characterization-based bioequivalence study, the Wilcoxon Rank Sum test is employed to assess the equivalence of release rates between products with no significant formulation differences. However, this method may not be suitable for comparing products with different formulations. In this study, we adapted a generalized Poisson regression model to include an interaction term between treatment and strength, to quantify the relationship of the high to low proportionality between the two products. Additionally, we conducted a meta-analysis to estimate the effect size of the interaction term. Hypothesis testing was performed to determine whether 90% confidence interval for the exponential of interaction term includes 1. Simulation studies were conducted to estimate the power and type I error across different margins for the interaction term for rejecting the null hypothesis. To address the small sample sizes typically encountered in IVRT, a Bayesian Poisson regression model was employed as well.
- **Results**
  - The meta-analysis provided a combined estimate and confidence interval for the interaction term, which will be of interest to explore options for setting a regulatory margin. The simulation studies demonstrated the power and type I error rate for different margin limits of the interaction term, helping to determine the appropriate

thresholds for concluding the ratio of proportionality is equal between test and reference. The Bayesian Poisson regression model offered an alternative approach, particularly beneficial in IVRT studies with small sample sizes and low averaged product release rates.

- **Implications**
  - The study provides a statistical framework to quantitatively determine whether the proportional relationship of the release rates of the active ingredient from two strengths of the test product is similar to that of the reference product. By quantifying the proportionality of release rates, this approach can support regulatory decisions for BE waivers.

58. **Yanardag, Sila**
    - **Abstract title:** A survey of prevalence of pediatric dosing for GLP-1s indicated for T2DM and weight management.
    - **Authors:** Sila Yanardag, Fatima Quddos, Edwin Chow, Mohamad Kronfol, Shirley Seo, Mohamed Ismail Nounou (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - This study aims at evaluating approved GLP-1s on the use of pharmacometrics tools to support pediatric dosing for different indications. This could aid in understanding the patterns of how different populations responds to GLP-1s in different indications, formulations, and dosage, through which we can predict and make an informed decision for further GLP-1s pediatric study designs.
      - **Purpose**
        - This study assessed the prevalence of pediatric dosing in marketed GLP-1 products in the US approved by the US Food and Drug administration (FDA) and evaluated the extent of population pharmacokinetics (PopPK) data usage to support dosing. The study aims to understand the tools used for dosing strategies, such as pharmacometrics analyses, population pharmacokinetics (PopPK) analyses, pharmacokinetic (PK) modeling, modeling & simulation (M&S) and pharmacokinetics/pharmacodynamics (PK/PD) modeling that contribute to the derivation of pediatric dosing.
      - **Methods**
        - Based on products' labelling from 2005 until 2023, 14 GLP-1 drug products approved by the FDA were reviewed. Information collected from the label included: the indication, inclusion of pediatric population, dosing regimen based on age groups, dosing strategy, the use of PopPK and the clinical trials conducted in pediatrics. PubMed, public FDA clinical pharmacology reviews and clinicaltrials.gov were used along with products' FDA labels for data collection.
      - **Results**
        - A total of 14 products approved by the FDA between 2005 and 2023 were reviewed. Based on the review conducted, 7 of the 14 (46.6%) products have an approved indication for pediatric use and contain dosing recommendations in the label. For the seven products approved in pediatrics, there was a total of 5 clinical trials conducted with pediatric patients (age range 10-18), all of which were phase 3

trials. All 7 pediatric approved products utilized PopPK analysis to support dosing. PopPK analysis were used to verify the dose selection in pediatrics that was already studied in adult and pediatric population, and PK of the products. In total, there were 26 pharmacometrics analyses performed for the 14 products. 20 of 26 (76.9%) were multi-study PopPK analyses, whereas 6 out of 26 (23.1%) were single-study exposure-response pharmacometrics population pharmacokinetics analyses.

- **Implications**
  - Population PK analysis and modeling & simulation are frequently used tools to guide drug development and inform recommendations on therapeutic individualization. By evaluating approved GLP-1s on the use of pharmacometrics tools to support pediatric dosing for different indications, we gain an understanding on the patterns of how different populations responds to GLP-1s in different indications, formulations, and dosage, through which we can predict and make an informed decision for further GLP-1s pediatric study designs.

59. **Yu, Qi**

- **Abstract title:** Missing Data Handling for CGM data for Regulatory Submission
- **Authors:** Yu, Qi (Student) CDER, Wenda Tu (Mentor), Yoonhee Kim, Yun Wang, Roberto Crackel, Hye Soo Cho
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
  - **Synopsis**
    - Continuous glucose monitoring (CGM) is a digital health technology (DHT) that allows people to monitor real-time blood glucose (BG) values with a wearable medical device. With an increasing use of CGM in clinical practice for diabetes management, the high-frequency time series CGM readings (i.e., BG values collected by CGM every five minutes) from clinical trials can be further synthesized into clinical endpoints (i.e., CGM derived endpoints) and used to support efficacy and safety evaluation in the drug development of anti-diabetic products. However, a prevailing issue of utilizing CGM data for regulatory decision making is missing CGM data, an almost inevitable problem due to reasons such as occasional device errors, subject noncompliance, and premature study dropouts, etc. Ignoring or incorrectly handling missing CGM data can lead to a biased evaluation of the treatment effect. To date, there is no regulatory consensus on how to handle missing CGM data. In this project, we use simulation studies to investigate different methods to address missing CGM data at both raw reading level and endpoint level, and to evaluate the performance of different missing data handling methods, and their impact on the estimation of a treatment effect. We aim to provide guidance on appropriate missing data handling methods for regulatory purposes.
  - **Purpose**
    - This project evaluates and compares the performance of a variety of statistical methods for managing missing continuous glucose monitoring (CGM) data in a clinical trial setting via simulation studies. We aim to provide guidance on appropriate CGM missing data

handling methods for regulatory purposes.

- **Methods**
  - To address the challenge of missing CGM data, our project employs extensive simulation studies. These simulations evaluate various methods for handling missing data at both the raw reading level and the endpoint level. We explore statistical models and multiple imputation techniques to understand their performance for treatment effect estimation. Each method is rigorously tested to assess its ability to mitigate bias introduced by missing data. Our approach includes comparing the results of these methods under different scenarios, such as varying degrees of missing data and different patterns of data loss. By simulating various missing scenarios commonly encountered in a clinical trial setting, we aim to identify the most robust and reliable strategies for handling missing CGM data.
- **Results**
  - The simulation studies reveal differences in the performance of various missing data handling methods. For example, methods that account for the time-series nature of CGM data tend to demonstrate superior accuracy in estimating mean time-in-range (TIR) compared to simpler approaches like mean substitution in raw data reading level. Our findings highlight the importance of choosing appropriate techniques based on the specific characteristics of the missing data to ensure unbiased and reliable clinical trial outcomes.
- **Implications**
  - The implications of our study are profound for the regulatory landscape of diabetes drug development. By providing evidence-based recommendations for handling missing CGM data, we aim to inform and shape regulatory guidelines. Our findings advocate for the adoption of advanced imputation and statistical methods that can accurately address missing data challenges, thereby enhancing the validity and reliability of clinical trial results. This guidance can lead to more informed regulatory decisions, ultimately improving the development and approval process of anti-diabetic therapies. Furthermore, our research underscores the need for ongoing evaluation and refinement of missing data handling practices as CGM technology and clinical methodologies continue to evolve.

60. **Zhao, Andrew**

- **Abstract title:** A Retrospective Analysis of Dose Finding and Optimization Strategies of Novel Fix-Dose Combination Drug Products in 2021-2023
- **Authors:** Andrew Zhao FDA/CDER (Student), Ellen Liu FDA/CDER(Student), Xin Wei FDA/CDER (Mentor), Jiang Liu FDA/CDER (Mentor)
- **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation & Unleashing the Power of Data
- **Abstract:**
  - **Synopsis**
    - The FDA initiative Project Optimus, which aimed to optimize cancer drug dosages earlier in development, began with single-agent therapies and has successfully shifted the oncological drug development paradigm. Combination therapy, with its potential additive or synergistic

effects, is now central to cancer treatment due to its ability to address tumor heterogeneity and resistance. Extending Project Optimus to combinations is a natural progression, yet determining optimal dosages for combination therapies remains challenging due to potential drug interactions affecting both efficacy and toxicity. This research project summarizes and compares strategies for selecting first-in-human (FIH) starting doses and dose escalation ranges for combination therapies approved from 2018 through 2023. Data from public and FDA internal sources were used to collect preclinical data, clinical starting doses, dose escalation steps, biomarker studies, benefit-risk analyses, and model-informed drug development (MIDD) applications. Among the 302 novel drugs approved from 2018 to 2023, 58 were combination therapies, with 24 targeting cancer and 14 targeting infectious diseases. For combinations with one novel and one approved drug, dose selection focused on the novel component, while rule-based interval, assisted, or model-based approaches were used for oncology Phase I dose selection. For novel-novel combinations, dose optimization aimed to maximize efficacy while adhering to toxicity constraints. Model-informed approaches integrating drug-drug interaction data, toxicities, and dose/exposure-response relationships were employed to improve dose selection. Findings from this research will inform trial design and policy development, enhancing a holistic, multidisciplinary approach to dose finding and optimization.

- **Purpose**
  - The FDA initiative Project Optimus aimed at optimizing cancer drug dosages earlier in development, initially focusing on single-agent therapies, has successfully shifted the oncological drug development paradigm. Combination therapy, with its potential additive or synergistic effects, is now central to cancer treatment, addressing tumor heterogeneity and resistance. Thus, extending Project Optimus to combinations is a natural progression. However, determining optimal dosages for combination therapies is challenging due to potential drug interactions affecting both efficacy and toxicity. Study design and model-informed drug development (MIDD) play pivotal roles in overcoming these challenges by providing structured approaches to explore and optimize dosing regimens. This project compiles comprehensive evidence to characterize the landscape of combination therapy drug development, particularly focusing on dosage selection and optimization strategies. The assessment covers strategies for determining overlapping dose-limiting toxicities (DLTs) and pharmacodynamic interactions, selecting appropriate starting and escalation doses, assessing 'acceptable' toxicity thresholds, and evaluating drug activities and clinical efficacy. Such analyses will streamline regulatory recommendations regarding dose finding and optimization for oncological combination therapies and assist in regulatory policy development.
- **Methods**
  - The FDA initiative Project Optimus aimed at optimizing cancer drug dosages earlier in development, initially focusing on single-agent therapies, has successfully shifted the oncological drug development paradigm. Combination therapy, with its potential additive or synergistic

effects, is now central to cancer treatment, addressing tumor heterogeneity and resistance. Thus, extending Project Optimus to combinations is a natural progression. However, determining optimal dosages for combination therapies is challenging due to potential drug interactions affecting both efficacy and toxicity. Study design and model-informed drug development (MIDD) play pivotal roles in overcoming these challenges by providing structured approaches to explore and optimize dosing regimens. This project compiles comprehensive evidence to characterize the landscape of combination therapy drug development, particularly focusing on dosage selection and optimization strategies. The assessment covers strategies for determining overlapping dose-limiting toxicities (DLTs) and pharmacodynamic interactions, selecting appropriate starting and escalation doses, assessing 'acceptable' toxicity thresholds, and evaluating drug activities and clinical efficacy. Such analyses will streamline regulatory recommendations regarding dose finding and optimization for oncological combination therapies and assist in regulatory policy development.

- **Results**
  - From 2018 to 2023, 302 novel drug products were approved, with 83 for cancer treatment. Of these, 58 were combination therapies, and 17 were fixed-dose combinations. Among the approved combination therapies, 24 targeted cancer and 14 targeted infectious diseases. Most combinations involved one novel drug combined with approved drugs, except for one (binimetinib and encorafenib) developed directly as a combination without prior individual approval. For combination therapies with one novel and one approved drug, dose selection typically focused on the novel component, with the approved drug's dosage fixed at its approved level unless significant pharmacokinetic interactions or overlapping toxicities were expected. Dose selection for the novel drug was based on single- and multiple-ascending dose studies for the novel component first, drug-drug interaction studies, and dose-ranging and POC studies in the combination setting. For oncology combinations, the maximum tolerated dose (MTD) paradigm was commonly used. Rule-based, interval-assisted, or model-based parametric/nonparametric approaches were used for Phase I dose selection. For novel-novel combinations, dose optimization aimed to maximize efficacy while adhering to toxicity constraints (e.g., < 30% DLT). Model-informed approaches integrating pharmacokinetic (PK) and pharmacodynamic (PD) data efficiently supported dose selection (e.g., for Sarclisa (isatuximab), Opdualag (nivolumab and relatlimab FDC), Inqovi (decitabine and cedzuridine FDC), etc.).
- **Implications**
  - This study characterizes the landscape of novel combination therapies approved from 2018 through 2023, with a focus on cancer and infectious diseases due to their potential to overcome disease heterogeneity and resistance. The findings on dose selection and optimization strategies from approved combinations can inform clinical trial design, conduct, and policy development. The MTD paradigm is becoming less appropriate for novel oncology products targeting molecular alterations driving cancer progression. Instead, a pragmatic,

holistic, and multidisciplinary approach to dose finding and optimization is needed. Identifying optimal doses for combination treatments requires evaluating multiple dose levels in sufficient numbers of targeted patients. To enhance efficiency at each stage of drug development, model-informed approaches should be employed, utilizing dose-exposure-pharmacodynamic (PD) response relationships with up-to-date nonclinical and clinical data. These approaches provide quantitative support for dose selection.

61. **Zidan, Yousof**
    - **Abstract title:** Validation of an in vitro assay that can monitor and control cell-free protein synthesis manufacturing technologies.
    - **Authors:** Zidan, Yousof, FDA/CDER (Student); Huang, Bruce, FDA/CDER (researcher); Ortega-Rodriguez, Uriel, FDA/CDER (researcher); Houchens, Tylee, FDA/CDER (researcher); Flores, Matthew, FDA/CDER (researcher); Ju, Tongzhong, FDA/CDER (Mentor); Biel, Thomas, FDA/CDER (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **<u>Synopsis</u>**
            - Cell-free protein synthesis (CFPS) is an innovative synthetic biology technique that utilizes cellular extracts to manufacture proteins ex-cyto to circumvent the inherent complexities and limitations associated with traditional cell-culture-based methods. The CFPS manufacturing process for drug substance is an advanced technology that is highly advantageous for biologic drug manufacturers and commercial protein manufacturers, because the reaction conditions can be precisely controlled to improve product quality, non-standard amino acids can be incorporated into the primary amino acid sequence at specific sites, and the manufacture of the protein can be rapid (<24 hours) as compared to traditional cell based biomanufacturing. The aim of this study is to develop a laboratory scale Escherichia coli based CFPS manufacturing process at the Agency that will be used to address current manufacturing and regulatory science gaps associated with the manufacture of drug products using CFPS platform technologies. Here, we propose to validate an in vitro assay that can monitor and control the quality of the crude cell extracts used for E. coli based CFPS manufacturing to minimize the variability in the productivity between drug substance batches manufactured using CFPS technologies. Over the summer, we developed an E. coli-based super folded Green Fluorescent protein (sfGFP) biomanufacturing process that generated sfGFP with >95% purity and began development of the in vitro assay that will be used to monitor the translational and transcriptional variability of the crude cell extract.
        - **<u>Purpose</u>**
            - Consistent productivity remains a challenge for industrial protein manufacturers that are using CFPS platform technologies. Productivity of the CFPS manufacturing process is known to be associated with the transcriptional and translational capability of the cell extracts. Bacterial cultivation conditions, harvest times, and ineffective cell lysis are known to have an impact on the transcriptional and translational activity of the cell extracts. The purpose of this study is to establish the first laboratory

scale CFPS manufacturing system at the Agency and develop a validated sfGFP protein assay that can monitor for changes in the productivity of the cell extracts prior to CFPS manufacturing.

- **Methods**
  - E. coli (BL21-DE3 strain) was transformed to express sfGFP, cultured to mid-log phase using 2XYT Broth, harvested by centrifugation, and lysed using a chemical reagent. The protein extract was subjected to sterile filtration prior to sequential chromatography: His-trap, anion exchange, and cation exchange. Purity of the sfGFP was determined using SDS-PAGE, SEC-UPLC, and CE-SDS. Identity was assessed by immunoblotting and fluorescence (Ex:488/ Em:520 nm) detection. The protein concentrations were determined using BCA protein assay which were then used to associate fluorescence measurements obtained by spectroscopy.
- **Results**
  - We have established an upstream and downstream bioprocess that manufactures sfGFP with >95% purity. Over a 12-hour cultivation period, the transformed BL21-DE3 strain exhibited rapid growth and produced bright green pellets suggesting the sfGFP was produced. The identify of the purified protein was confirmed based on fluorescence measurements (Ex: 488 nm/Em: 520 nm), as well as the consistency between the observed and theoretical molecular weight of the protein. To demonstrate biomanufacturing reproducibility, four different sfGFP campaigns were characterized. To initiate the development and validation of an in vitro assay that can monitor transcriptional and translational activity of cell extracts, we began by establishing that 0.5 µg/mL was the limit of detection for monitoring the fluorescence of the purified sfGFP, while the lower limit of quantitation was 0.9 µg/mL. Linearity between the protein concentration and fluorometric measurements of the sfGFP was determined using an 8-point curve that had a correlation coefficient of 0.99 over a concentration range between 1 and 600 µg/mL.
- **Implications**
  - This research will be used to educate assessors that regulate drug products manufactured using CFPS technologies, to improve understanding of potential critical process parameters for CFPS manufacturing processes, and to provide a validated sfGFP assay for controlling the variability of the extracts used during CFPS manufacturing. The demonstrated cost-effectiveness and scalability of the CFPS method, particularly through mechanical cell lysis techniques, open several avenues for future research. This study will contribute to improving the control of critical raw materials used for CFPS manufacturing by industry in the following fields: biotechnology, synthetic biology, and pharmaceutical science.

Center for Devices and Radiological Health (CDRH)

62. **Arteaga, Eduardo**
    - **Abstract title:** Development of Digital Biomarkers for Mild Traumatic Brain Injury (mTBI)
    - **Authors:** Eduardo, Arteaga (Student), Prathapan, Smriti and Civillico, Eugene (Mentor) CDRH

- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
    - **<u>Synopsis</u>**
        - Mild traumatic brain injury (mTBI) comprises 95% of the estimated 4.8 million head injuries each year in the United States. It is believed to be caused by an initial physical trauma which compresses brain tissue. CT scans and MRI are traditionally used to diagnose most head injuries, but they are not capable of detecting all TBIs, especially mTBIs. Although our brain is protected by cerebrospinal fluid and the skull, mTBI may lead to long-lasting neurodegenerative symptoms. mTBI is most commonly diagnosed via a neurological exam, which examines cognitive and motor function, optometric reflexes, sensory function, and coordination. Here we present a partial analysis of data from ongoing neurology Regulatory Science Tool development in CDRH's Office of Science and Engineering Laboratories. In this study, participants with and without diagnosed mTBI perform 4 tasks in a virtual reality (VR) environment while 4 streams of data are collected. The data streams are i) weight distribution and balance obtained from insole sensors in the participant's shoes; ii) eye tracking data (pupil position and size) from the head-mounted VR; iii) 22-channel electroencephalography (EEG) data from an array of electrodes on the head; iv) task performance. Data from participants without a history of brain injury and one confirmed mTBI participant were included in this analysis. Additional data collection is planned but was not part of this work. The goal of the study is to determine what classifier accuracy can be achieved with these data streams, as a step towards the development of digital biomarkers.
    - **<u>Purpose</u>**
        - We hypothesize that a machine learning classifier can be trained on task and physiological data to distinguish between mTBI and non-mTBI datasets. Our custom VR environment enables tasks that are not feasible in real world settings, allowing for more controlled experimentation. Quantitative sensor data such as balance, pupillometry, and brain electrical activity have become increasingly attractive in determining mTBI status, as they are not subject to the same biases and interpretation variability as self-report and other qualitative measures. These types of measurements could drastically improve the diagnosis of mTBI. By determining and detecting events in physiological biomarkers, this research aims to preliminiarily construct a modely for determining whether subjects show tendencies of mTBI or healthy populations.
    - **<u>Methods</u>**
        - Data collection for this study was approved by the FDA's Institutional Review Board (HSR Protocol 2019-CDRH-107). All participants completed the study on FDA's White Oak Campus (Human-Device Interaction Lab, Building 62). The mTBI participant was recruited by our collaborators at MedStar Health. Going forward, additional mTBI subjects will be recruited by the R Adams Cowley Shock Trauma Center at the University of Maryland Medical Center, under a contract with CDRH. An HTC Vive VR head-mounted display (HMD) with ProEye was used to run a Unity environment which generated the 4 tasks. The four

tasks were: i) lean right or left to collect or avoid a virtual object; ii) distinguish whether a tone was higher or lower than the previous tone; iii) remember and repeat back a sequence of letters; and iv) track a target by movement of the eyes. The HMD recorded pupil diameter, location, gaze, and spatial distribution. The Moticon insole sensors were placed in the participant's shoes. The EEG cap was placed on the participant's head prior to performing the VR tasks to get a baseline reading and recorded a second time for the tasks. Mean and standard deviation for all spatial and temporal aspects of the test (i.e. leaning force, eye movement, power spectral density, etc.) were computed for all parameters for the entire system per participant. The MNE Python package was used to analuze and visualize EEG data.

- **Results**
  - We created and analyzed biomarker profiles for the 29 subjects. We generated Python scripts to syncrhonize file types acquired on different time bases. A custom .xdf file type facilitated migration from a MATLAB environment to Python. We have established a framework for feature extraction from new participnt data that is expected in the future. Within the subset of data analyzed, the greatest difference between mTBI and non-mTBI participants was seen in the insole data. Less overall leaning direction change occurred in the single mTBI participant than the average of the non-mTBI group, up to half the amount when using a 60% threshold value for total left and right leaning pressure to determine leaning. This is in line with reports in the literature of a reduction in sharp movements in mTBI patients.
- **Implications**
  - The mTBI VR assessment is a promising way of creating a base profile for biomarkers of healthy and mTBI individuals to use as a method for training ML models. However, it is not possible currently to draw definitive conclusions due to data collection still being incomplete. The uncertainties that come from the current pretrained models to validate the model using the current dataset can be fixed by running the scripts and analysis on the complete data set which will include many new mTBI participants.

63. **Avila, Anna**
    - **Abstract title:** Mechanistic Assessment of Cardiac Contractility Modulation Device Signals in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes
    - **Authors:** Avila, Anna, FDA/CDRH (Student); Feaster, Tromondae K., FDA/CDRH (Mentor); Maura, Casciola, FDA/CDRH; Blinova, Ksenia FDA/CDER; Narkar, Akshay, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
      - **Synopsis**
        - For the first time, mutant loss-of-function human induced pluripotent stem cell cardiomyocytes are used to evaluate clinical cardiac contractility modulation signals in vitro. This work provides a robust cardiac model to elucidate mechanisms of emergent cardiac electrophysiology medical device signals to support safety and effectiveness studies.
      - **Purpose**

- Non-excitatory electrical stimulations including Cardiac Contractility Modulation (CCM) are medical device-based therapies delivered to the heart during the absolute refractory period to enhance cardiac function. We previously evaluated the acute (i.e., seconds) effects of CCM in 'healthy' normal 2D human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) monolayers, on flexible substrate, and found enhanced calcium and contractility. In the present study, we sought to develop an acute CCM assay to evaluate the mechanistic contribution of key cardiac-excitation contraction (ECC) coupling proteins (i.e., L-type calcium channels and ryanodine receptors) in vitro using hiPSC-CMs as a model. Clinical CCM devices have been shown to modulate gene and protein expression however the functional effects are largely unknown.

- **Methods**
  - Custom platinum electrodes were fabricated to electrically stimulate hiPSC-CMs. Control (i.e., normal 'healthy' and isogenic), and mutant hiPSC-CMs were cultured for 2 days on flexible substrate then morphology, contractility, and intracellular calcium handling were quantified in parallel.
- **Results**
  - We found L-type calcium channel and ryanodine receptor loss-of-function mutant hiPSC-CMs displayed blunted response during CCM relative to control.
- **Implications**
  - This study provides a comprehensive characterization of the acute effects of CCM in multiple hiPSC-CMs with various ECC mutations. Future studies will investigate additional mutant hiPSC-CMs and non-excitatory electrical stimulation signals. These data provide an in vitro model to assess physiologically relevant mechanisms and evaluate safety and performance of future cardiac electrophysiology medical devices.

64. **Aseem Milind, Pradhan**
    - **Abstract title:** Design and development of digital phantoms structures as a guide for selecting spatial resolution in simulations of electrophysiological activity in tissue
    - **Authors:** Pradhan, Aseem Milind FDA/CDRH (Student); Kaboudian, Abouzar, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Cardiovascular disease remains a leading cause of mortality in the United States and developed nations worldwide. A significant proportion of these deaths stem from hemodynamic collapse caused by disruptions in normal cardiac electrical activity. Numerical simulations of cardiac dynamics play an increasingly pivotal role in the context of advancing patient-specific interventions, digital twin technologies, and conducting in-silico clinical trials. Given the computational cost associated with such simulations, the digital models are optimized by employing coarser grids for anatomical tissue representation. In this study, we present a method to determine the minimum spatial resolution necessary for numerical simulations to replicate critical electrophysiological wave dynamics in anatomically realistic tissue

models, ensuring both the desired fidelity and computational efficiency, through development and use of digital phantoms. We use these phantoms to quantify the effects of key structural features such as curvature and thickness on the minimum spatial resolution necessary.

- **Purpose**
  - In this study, we aim to establish the minimum spatial resolution necessary for numerical simulations to replicate critical electrophysiological wave dynamics in anatomically realistic tissue models, ensuring both the desired fidelity and computational efficiency using digital phantom structures.
- **Methods**
  - We modeled a series of digital phantom structures, including simple rings, and helices of constant and diminishing radii, characterized by a range of thicknesses and spatial resolutions to mimic delicate cardiac tissue components such as pectinate muscles. We conducted simulations of excitation waves within these phantom structures by utilizing the Minimal Atrial/Ventricular, Tusscher-Panfilov, and O'Hara-Virág-Varró-Rudy cellular models. The behavior of the waves in these structures were compared against benchmarks to assess the accuracy of the simulations in replicating key electrophysiological parameters such as wave propagation velocity.
- **Results**
  - Our initial findings indicate that, contrary to intuition, structures with lower curvatures require higher resolution meshes for a resolved numerical simulation.
- **Implications**
  - Digital phantoms provide an effective means to evaluate the accuracy of the computational models in thin structures which is essential to ensure efficacy and safety of numerical methods. They can be instrumental in developing computational models that are safe and effective in areas such as in-silico clinical trials, patient specific interventions, and digital twin technologies.

65. **Campbell, Breanna**
    - **Abstract title:** A Novel Technique to Quantify Sacroiliac Joint Motion
    - **Authors:** Campbell, Breanna FDA/CDRH, (Student); Shetye, Snehal, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - The increased use of novel in-line devices for sacroiliac joint fusion devices has raised questions regarding their mechanism of action as to how joint fixation and stabilization is realized. To this end, cadaver testing has been leveraged to verify the device's ability to restrict joint motion. However, characterization of range of motion of the joint has proven difficult since the SI joint inherently exhibits little movement (can be less than 0.2 degrees). This project aims to validate a novel technique to characterize relative joint rotation and translations with appropriate resolution for measurement of sacroiliac joint motion. Virtual markers were created to outline an area of interest, and the locations of the virtual markers were tracked during application of rotations about each axis. The coordinates of the virtual markers at each

frame were used to create best-fit planes, and the angular displacement of the planes was analyzed and compared to the known applied rotation. It was observed that angular displacement from the planar analysis aligned well with the applied rotation, suggesting that the virtual marker technique is a reliable method for tracking rotational motion. Further studies will be done to determine the error of the motion tracking system and the smallest angular change that can be measured. The virtual marker best-fit plane method also provides a more comprehensive characterization of joint range of motion compared to single probe methods, since it can also be used to determine distraction of the joint and visually view joint motion when plotted. This can be beneficial in future cadaver testing of in-line fusion devices by providing the opportunity for additional measures to demonstrate joint fixation.

- **Purpose**
  - The sacroiliac joint has been identified as the primary source of low back pain in approximately 30% of the patient population. If pain relief is not obtained from conservative treatments, surgical treatment can be pursued, such as fusion of the joint achieved via implantation of a motion stabilizing device. Medical device companies have recently developed a minimally invasive posterior approach to device implantation, wherein the device is inserted parallel and inside the joint space. This procedure is less invasive and spares the stabilizing muscles surrounding the sacroiliac joint. However, it remains unclear how these novel devices provide sufficient fixation and stabilization to the sacroiliac joint to promote fusion when compared to traditional screw-based devices. To this end, human cadaver testing can be utilized to verify the device's ability to restrict joint motion. However, the sacroiliac joint exhibits low mobility even in the intact state and proper characterization of the sacroiliac joint range of motion requires specialized measurement hardware. The aim of this study was to develop a novel technique to assess relative rotations and translations of the sacrum and ilium joint faces and assess its accuracy and repeatability under dynamic loading conditions.
- **Methods**
  - First, digital markers were created surrounding the area of interest using an Optotrak motion capture system. The locations of the virtual markers were tracked during rotation by an MTS hydraulic spine simulator, which performs rotations in the three cardinal axes. The coordinates of the virtual markers were used to create best fit planes, and the relative rotations and translations of the planes was determined through analysis of normal and in-plane vectors to the surface. This output can be compared to the known angles applied by the spine simulator, which allowed for determination of accuracy and repeatability of the motion tracking system.
- **Results**
  - Initial experiments showed that virtual marker plane motion aligned with the rotations applied by the spine simulator in each axis of rotation. When plotting angular displacement from the virtual marker plane data and from the spine simulator output, the curves exhibited a similar shape and amplitude, which suggests that the use of digital

marker planes is a reliable method for tracking rotational motion. Further investigation will be done to quantify the error of the system, as well as determine the smallest angular change that can be accurately measured.

- **Implications**
    - The outcome of this project can improve quantification of joint range of motion during sacroiliac joint cadaver testing and will help ensure observed lack of motion is due to the presence of the fusion device and not an inadequate resolution of the system. The use of planes also offers a more comprehensive assessment of joint movement, providing data on joint distraction at different regions of the joint and a visual plot of motion, which cannot be obtained from a single motion tracking probe. This new technique provides additional parameters to joint motion and device fixation performance. Finally, this system can be used in future studies to determine optimal cadaver testing protocols of the sacroiliac joint, as no consensus currently exists regarding proper loading and boundary conditions for a worse case testing scenario.

66. **Chen, Zixi**
    - **Abstract title:** Diffusion Coefficient Measurement of Vaporized Hydrogen Peroxide through Single and Dual Layers of Polymeric Membranes
    - **Authors:** Chen, Zixi, FDA/CDRH (Student); Linden, Sara, FDA/CDRH (Mentor); Liu, Yunzhi, FDA/CDRH (Mentor); Week, Jon, FDA/CDRH (Supervisor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - As concerns grow over the environmental and health risks associated with ethylene oxide (EtO) sterilization, vaporized hydrogen peroxide (VHP) is a promising alternative for medical device terminal sterilization. This study investigates VHP diffusion through polymeric materials that are commonly used in medical devices. Herein, we carried out VHP diffusion coefficient measurements of polymeric membranes of varied thicknesses and polymer compositions. Diffusion (D), permeation (P), and partition (K) coefficients were calculated based on Fick's law, the results demonstrated that VHP effectively permeates these polymeric materials, with varying coefficients. For instance, 0.8 mm thick silicone and 0.2 mm thick TPU membranes exhibited distinct diffusion characteristics, as 2.1 x 10-6 cm^2/s and 2.8 X 10-8 cm^2/s, respectively. This project lays the foundation for the improvement of reliability of VHP sterilization in complex medical devices.
        - **Purpose**
            - Terminal sterilization of medical devices is important to ensure that bioburden present during the manufacturing process has been adequately inactivated, rendering the devices safe for use. Currently, ethylene oxide (EtO) is a gaseous, chemical sterilant and it is used for approximately 50% of device sterilization. However, concerns of environmental and health safety has led government agencies to limit the use and emission of EtO. Vaporized hydrogen peroxide (VHP) is another chemical sterilant that has recently recognized by FDA as a Category A sterilant. Despite the FDA's recognition of VHP as a Category A sterilant, research on its ability to traverse single or multi-layer

materials and affect internal interfaces of leads and catheters, remains limited. Herein, this project aims to address a knowledge gap by determining diffusion coefficient measurements through polymeric membranes of varying thickness. The findings of this study could facilitate VHP sterilization simulation in manufacturing applications.

- **Methods**
  - An acrylic glove box with two chambers connected by a side door is employed in this project. In the main chamber, a 100 mL solution of 50% hydrogen peroxide is evaporated at room temperature until the vapor concentration of VHP reaches 400 ± 25 mg/m³, where the absolute concentration of VHP is monitored by a VHP probe located in the main chamber. Afterward, the side door between chambers is opened, exposing a diffusion cell placed in the small chamber to the VHP. This cell is sealed at one end with the polymeric membrane facing the main chamber. A second VHP probe, enclosed within the diffusion cell, records the absolute concentration of VHP that diffuses through the polymeric membrane. The diffusion coefficient (D), permeation coefficient (P) and partition coefficient (K) are then calculated based on Fick's law by analyzing the relationship between elapsed time and the absolute concentrations recorded by both probes. To enhance the accuracy of the diffusion data and mitigate errors caused by non-uniform membrane thickness and observation time discrepancies, diffusion coefficient uncertainty is also considered in this project.
- **Results**
  - During the four-hour evaporation period of a 50% hydrogen peroxide solution, the concentration in the main chamber exhibited a biphasic increase. It rapidly rose to approximately 200 mg/m³ within the first hour, followed by a slower rate of growth at approximately 1 mg/m³ per minute for the subsequent 3 hours, ultimately reaching about 400 mg/m³. Upon connecting the two chambers, the absolute VHP concentration in the main chamber abruptly decreased, while the VHP concentration in the secondary chamber began a gradual increase after a lag time. The observed lag time and calculated diffusion coefficients varied as a function of membrane thickness and polymer composition. Consistent with previous experiments, longer lag times and smaller diffusion coefficients were generally observed for thicker membranes of one certain polymer composition. The results indicated that diffusion coefficient (D), permeation coefficient (P), and partition coefficient (K) for a 0.8 mm thick silicone membrane were determined to be averages of 2.1 x 10-6 cm²/s, 1.8 x 10-4 cm²/s, and 90, respectively. In contrast, for a 0.2 mm thick Pellethane® thermoplastic polyurethane (TPU) membrane, the averages of values were 2.8 X 10-8 cm²/s, 4.2 X 10-6 cm²/s, and 133, respectively.
- **Implications**
  - This research aims to collect reproducible and reliable results based on VHP diffusion through polymer membranes which are commonly used in medical devices, including leads and catheters. These can be utilized for the development of a computational model to predict VHP diffusion through polymeric materials. Additionally, it seeks to challenge the common misconception that VHP is only suitable for surface sterilization. By quantifying VHP penetration through polymeric

membranes, this research provides evidence that VHP can effectively permeate materials, suggesting its ability to sterilize internal surfaces and complex geometries in medical devices.

67. **Eich, Brandon**
    - **Abstract title:** Quantifying Consistency and Resilience of Explainability Heatmaps in AI Models
    - **Authors:** Eich, Brandon, FDA/CDRH/OSEL/DIDSR (Student); Zamzmi, Ghada, FDA/CDRH/OSEL/DIDSR (Mentor); Delfino, Jana, FDA/CDRH/OSEL/DIDSR (Mentor); Lago, Miguel, FDA/CDRH/OSEL/DIDSR (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **<u>Synopsis</u>**
            - AI-enabled medical devices do not typically provide explanations for their decisions. Heatmaps generated using post-hoc methods such as Smooth Grad-CAM (Gradient-weighted Class Activation Mapping) are often used as surrogate explanations for how convolutional neural networks (CNNs) make conclusions by highlighting areas of interest in an image. Evaluating the consistency of heatmap based explanations is an important step to assess the safety and effectiveness of AI-enabled imaging devices with heatmap-like explanations. We examined the ability of heatmaps to manage variations in the input and assess their effectiveness in providing consistent explanations. To test for consistency, we manipulated input images (e.g., rotation, adding noise, etc.) from the MedMNIST dataset and measured the similarity between heatmaps for the same unmanipulated images via the structural similarity index measure (SSIM) and mean squared error (MSE). Overall, large disruptions to the input images were required to alter the heatmaps. Additionally, some image categories, like chest x-rays, were significantly more effected by disruptions to the initial input images.
        - **<u>Purpose</u>**
            - We aim to develop a framework to quantify consistency and robustness of heatmaps. Objective evaluation metrics aid in understanding black box models, allowing them to be trusted, ensuring safety and effectiveness, and making them more likely to be used and aid human experts. Objective evaluation metrics can be used to test models and their resistance to changes in test input, allowing researchers, developers, regulators, and practitioners to rely on and trust in model output from different cases.
        - **<u>Methods</u>**
            - AbdomenCT, BreastMRI, ChestCT, ChestXR, Hand, and HeadCT. After the model achieved high accuracy (99.22% overall; AUC: 100.00%), we used this trained model to re-categorize both altered and unaltered test images. Altered images were subjected to manipulation by different means, such as rotation, added noise, flipping, random shuffle of the image pixels, and luminance changes. After model categorization, we used Smooth Grad-CAM to generate heatmaps to understand how the model determined which categories the images belonged to. We then compared heatmaps from altered and unaltered images. We used structural similarity index measure (SSIM) and mean squared error (MSE) to determine the similarity between the altered and unaltered

heatmaps and objectively quantify differences in heatmaps by the different manipulations. These changes in SSIM and MSE reflect how susceptible models are to varied input and could also reflect how human observers may change their decision based on varied input or examining images in ways they are not used to.

- **Results**
    - Smooth Grad-CAM produced heatmaps reasonably resistant to disruptions. Large variations in noise and rotation were required to disrupt heatmaps. For example, rotating an image by 50° led to substantial changes (e.g., decrease of ~25% in SSIM) with larger rotations decreasing accuracy further (e.g., ~40-60%). Additionally, chest x-rays were more susceptible to disruptions from noise, while chest x-rays, hands, and head CTs were more susceptible to rotation. An increase in noise might have been problematic as chest x-rays pick up on more information (i.e., non-background information), with an increase in noise making more information detrimental. An increase in rotation may have made the less symmetrical categories more susceptible to this disruption and suggests that the explanation (and model) is biased towards the standard orientation. This finding goes against the spatial invariance characteristic of CNN models. However, heatmaps were reasonably resistant to disruptions, with some categories producing heatmaps that only decreased by ~20% in SSIM. Nevertheless, disruptions lead to alterations to the heatmaps and increase with disruption level.
- **Implications**
    - Objective evaluation of heatmap consistency can aid in interpretation and evaluation of AI explainability features, allowing future users, developers, and regulatory scientists to better trust and rely on model output. If heatmaps are robust to manipulation this would suggest that the model is picking up on specific information relevant to the image and providing stable explanations, even in response to these variations.

68. **Filienko, Daniil**
    - **Abstract title:** Knowledge-based synthetic data simulation for obtaining pixel-level annotations in digital mammography
    - **Authors:** Filienko, Daniil, FDA/CDRH (Student); Sizikova, Elena, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Breast cancer has long been afflicting people worldwide: according to the most recent report from International Agency for Research on Cancer, breast cancer has been one of the most widespread cancers diagnosed worldwide both in the number of cases and associated deaths (Bray, 2024). Approximately two million new cases and over six hundred thousand deaths occurred during 2022 alone (Sung, 2021). In the United States, breast cancer is expected to account for 29% of all new cancers in women (DeSantis, 2015). However, research into breast cancer detection and prevention has not been progressing as rapidly as it could, due to the scarcity of publicly available medical data. One of the key limiting factors in development and assessment of robust algorithms intended to help with mammography medical devices has

been limited access to large-scale datasets with suitable annotations. Synthetic data generated with plausible physical and biological constraints may address some of the data limitation issues. Unlike examples generated with data-hungry generative AI, synthetic data created using simulations with knowledge-based digital 3D models exploits clinical domain expertise and does perpetuate existing data biases. In particular, we propose to generate pixel-level annotations using physics simulations, which are notoriously difficult to obtain for medical images. Our preliminary experiments show that synthetic mammography examples created using the proposed approach demonstrate segmentation and detection performance trends similar to those in real patient examples.

- **Purpose**
  - Data is the lifeblood of AI, and hence, quantity and quality of data may directly influence the quality of the resulting AI models within medical devices. Very few digital mammography (DM) datasets are available publicly, and only a fraction of these contain pixel-level annotations for relevant tissues, since pixel-level annotations typically require a time-consuming, costly and laborious annotation process conducted by a specialist. A potential way to mitigate such data imbalance is to utilize synthetic data generation. The proposed simulation of synthetic data relies on knowledge-based models which inherently incorporate domain expertise (``knowledge'' of physics and biology) to create physically realistic images and annotations. The proposed method of creating synthetic data does not rely on seeing similar examples previously, unlike more main-stream generative AI methods, and is therefore less prone to propagating data biases and other limitations of real patient datasets.
- **Methods**
  - In this project, we create pixel-level annotations for breast lesions, and assess the ability of the resulting large-scale synthetic dataset in identifying performance trends in lesion segmentation and detection, both common mammography analysis tasks. We follow the conventions set in M-SYNTH (Sizikova, 2023) and analyze models trained on the full training dataset as well as subgroups to analyze performance across different subsets of data. For segmentation, we analyze performance using U-Net (Ronneberger, 2014), focusing on examining the trends within three subgroups of interest: breast density, lesion density, and lesion size. We vary the property of interest in the training dataset and evaluate a model on held-out test dataset. We measure performance using Dice and Hausdorff distance metrics, evaluating ability of the synthetically trained model to produce proper segmentation masks. For lesion bounding box detection, we rely on the Faster R-CNN (Girshick, 2015) architecture and follow a similar experimental setup. We measure detection performance using Intersection over Union (IoU) and Area under the Curve (AUC) metrics.
- **Results**
  - We have observed similar trends in our segmentation and detection results to previous established research employing classification task when evaluating the data. Despite limited performance on real-world dataset, the results indicate that the dataset exhibits similar properties

as real dataset while also permitting to generate unique occurring conditions that rarely occur in real-world datasets. Models trained on DM images with larger lesion sizes tend to perform worse than the models trained on images with smaller lesions, potentially because of greater variability within the latter training datasets, since small lesions can occur in more unique places within the breast. Increase in lesion density led to consistent improvement in the models' performance across breast densities, most likely due to the model being able to distinguish the lesion from breast tissues more easily when the lesion is more dense, and hence has higher contrast with the breast tissue. We also noticed that the models trained on different breast densities tend to exhibit different behavior in terms of generalization across breast densities and sizes. Models trained on fatty and scattered breasts tend to generalize better than the models trained on dense and heterogeneous breasts, exhibiting more consistent and higher performance across other breast densities.

- **Implications**
    - The ability to generate realistic breast mammograms with pixel-level annotation is important for development and assessment of mammography AI. This project takes a first step in this direction by providing pixel-level annotations for lesions. Such annotations are necessary to establish reference standards for development and assessment of algorithms, but are extremely challenging to obtain from already very limited patient datasets. The proposed pipeline of generating pixel-level annotations using knowledge-based models does not rely on patient data, and therefore does not perpetuate existing biases. On the other hand, the proposed approach is general, and can be applied to many applications of medical AI where knowledge-based models and associated models of acquisition devices are available.

69. **Jayaswal, Daksh**
    - **Abstract title:** Preliminary study to Identify Worst-case Mechanical Parameters for Assessing Durability of Anterior Vertebral Body Tethering (AVBT) Systems
    - **Authors:** Jayaswal, Daksh, FDA/CDRH (Student); Palepu, Vivek, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - Breast cancer has long been afflicting people worldwide: according to the most recent report from International Agency for Research on Cancer, breast cancer has been one of the most widespread cancers diagnosed worldwide both in the number of cases and associated deaths (Bray, 2024). Approximately two million new cases and over six hundred thousand deaths occurred during 2022 alone (Sung, 2021). In the United States, breast cancer is expected to account for 29% of all new cancers in women (DeSantis, 2015). However, research into breast cancer detection and prevention has not been progressing as rapidly as it could, due to the scarcity of publicly available medical data. One of the key limiting factors in development and assessment of robust algorithms intended to help with mammography medical devices has been limited access to large-scale datasets with suitable annotations. Synthetic data generated with plausible physical and biological

constraints may address some of the data limitation issues. Unlike examples generated with data-hungry generative AI, synthetic data created using simulations with knowledge-based digital 3D models exploits clinical domain expertise and does perpetuate existing data biases. In particular, we propose to generate pixel-level annotations using physics simulations, which are notoriously difficult to obtain for medical images. Our preliminary experiments show that synthetic mammography examples created using the proposed approach demonstrate segmentation and detection performance trends similar to those in real patient examples.

- **Purpose**
  - Data is the lifeblood of AI, and hence, quantity and quality of data may directly influence the quality of the resulting AI models within medical devices. Very few digital mammography (DM) datasets are available publicly, and only a fraction of these contain pixel-level annotations for relevant tissues, since pixel-level annotations typically require a time-consuming, costly and laborious annotation process conducted by a specialist. A potential way to mitigate such data imbalance is to utilize synthetic data generation. The proposed simulation of synthetic data relies on knowledge-based models which inherently incorporate domain expertise (``knowledge'' of physics and biology) to create physically realistic images and annotations. The proposed method of creating synthetic data does not rely on seeing similar examples previously, unlike more main-stream generative AI methods, and is therefore less prone to propagating data biases and other limitations of real patient datasets.
- **Methods**
  - In this project, we create pixel-level annotations for breast lesions, and assess the ability of the resulting large-scale synthetic dataset in identifying performance trends in lesion segmentation and detection, both common mammography analysis tasks. We follow the conventions set in M-SYNTH (Sizikova, 2023) and analyze models trained on the full training dataset as well as subgroups to analyze performance across different subsets of data. For segmentation, we analyze performance using U-Net (Ronneberger, 2014), focusing on examining the trends within three subgroups of interest: breast density, lesion density, and lesion size. We vary the property of interest in the training dataset and evaluate a model on held-out test dataset. We measure performance using Dice and Hausdorff distance metrics, evaluating ability of the synthetically trained model to produce proper segmentation masks. For lesion bounding box detection, we rely on the Faster R-CNN (Girshick, 2015) architecture and follow a similar experimental setup. We measure detection performance using Intersection over Union (IoU) and Area under the Curve (AUC) metrics.
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  - We have observed similar trends in our segmentation and detection results to previous established research employing classification task when evaluating the data. Despite limited performance on real-world dataset, the results indicate that the dataset exhibits similar properties as real dataset while also permitting to generate unique occurring conditions that rarely occur in real-world datasets. Models trained on

DM images with larger lesion sizes tend to perform worse than the models trained on images with smaller lesions, potentially because of greater variability within the latter training datasets, since small lesions can occur in more unique places within the breast. Increase in lesion density led to consistent improvement in the models' performance across breast densities, most likely due to the model being able to distinguish the lesion from breast tissues more easily when the lesion is denser, and hence has higher contrast with the breast tissue. We also noticed that the models trained on different breast densities tend to exhibit different behavior in terms of generalization across breast densities and sizes. Models trained on fatty and scattered breasts tend to generalize better than the models trained on dense and heterogeneous breasts, exhibiting more consistent and higher performance across other breast densities.

- **Implications**
    - The ability to generate realistic breast mammograms with pixel-level annotation is important for development and assessment of mammography AI. This project takes a first step in this direction by providing pixel-level annotations for lesions. Such annotations are necessary to establish reference standards for development and assessment of algorithms but are extremely challenging to obtain from already very limited patient datasets. The proposed pipeline of generating pixel-level annotations using knowledge-based models does not rely on patient data, and therefore does not perpetuate existing biases. On the other hand, the proposed approach is general, and can be applied to many applications of medical AI where knowledge-based models and associated models of acquisition devices are available.

70. **Kanakaraj, Praitayini**
    - **Abstract title:** Decoding diverging representations in AI model for improved transparency.
    - **Authors:** Kanakaraj, Praitayini, FDA/CDRH (Student); Burgon, Alexis, FDA/CDRH (ORISE Fellow); Petrick, Nicholas, FDA/CDRH (Mentor); Samala, Ravi K., FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Artificial intelligence (AI) models excel at identifying patterns, allowing them to learn complex tasks but making them susceptible to spurious patterns within data. Models may create spurious correlations between non-clinical features -- such as those related to demographic subgroups -- and a clinical task, which results in AI performance bias and poor generalizability to their intended population. Additionally, AI performance bias can arise from inherent differences in the task difficulty between subgroups. Evaluating and addressing sources of bias during the testing phase is challenging due to lack of sufficient data within subgroups. Our work provides a novel approach to support meaningful subgroup analyses during the AI model evaluation phase when only modest subgroup data is available. Hyperdimensional computing (HDC) encodes data into high-dimensional vectors allowing for efficient representation of complex relationships, like those in the AI feature space. Using HDC, we encode the extracted AI model features

and examine the cosine similarity between subgroup feature representations. This process helps identify the presence of dissimilar representations, referred as diverging characteristics (DCs). In this study, we identify DCs and investigate their underlying relationship to AI bias through the analysis of related dissimilarity scores. We demonstrate this relationship in a case study in which we train models for breast cancer classification and examine the differences in representation of subgroups defined by breast density. Identifying the specific sources of bias that impact the model performance is not trivial. Thus, to validate our approach, we train these models on systematically sampled datasets to amplify bias through the promotion of spurious correlations. Our results support our hypothesis that different sources of bias present differently in the model feature space, as captured by the DC score. The identified DCs indicate potential sources of bias in AI models, thus can help increase model transparency and assist with AI device evaluation.

- **Purpose**
  - There are many sources of artificial intelligence (AI) bias. Spurious correlations between patient subgroups, such as those defined by demographic attributes, and the output of AI models can be formed during the AI device development phase. These spurious correlations, often a result of 'shortcut learning', perform well on training datasets but fail to transfer to real-world test conditions. Additionally, fundamental differences in task difficulty between subgroups can result in AI performance bias. Both sources of bias lead to AI bias in the clinical deployment phase and can exacerbate health inequity. Identifying sources of bias during the premarket evaluation phase is critical. However, it is challenging because the collection of test data for every patient subgroup is often unreasonable, making it difficult to ensure meaningful subgroup analysis for AI bias assessment. Thus, there is a need to develop a regulatory science tool (RST) that can identify the potential sources of AI bias with a limited number of samples during the evaluation phase. The outputs generated by this RST across different subgroups could provide additional information about AI models to end users, thereby enhancing transparency, ensuring the safe and effective deployment of AI models, and promoting reliability in AI-assisted decision support systems.
- **Methods**
  - Spurious correlations and subgroup difficulty during the AI development process are represented as differences in the model feature space resulting in AI performance bias. These 'diverging characteristics' (DCs) are subtle, which makes them difficult to identify. We address this by encoding the features into hyperdimensional vectors (HVs), the data representation used in hyperdimensional computing (HDC). HVs can capture intricate patterns, retain subtle characteristics, and efficiently represent modest subgroup data. In our approach, subgroups in the testing data are represented by HVs encoded from the final dense layer features. Low cosine similarity (high dissimilarity) between HVs indicates an abundance of DCs. We trained a ConvNeXt AI model for cancer detection in mammography images using the Emory BrEast Imaging Dataset (EMBED). Model bias was systematically amplified by

varying the prevalence of breast density categories in the positive class during the AI development phase, which enables us to study the DCs in AI models at varying levels of AI performance bias. We present three sets of results at varying levels of AI performance bias: (1) 100% breast density subgroup A&B and 0% from subgroup C&D, (2) 50% and 50% split and (3) 0% from A&B and 100% from C&D.

- **Results**
  - In addition to providing a potential learning shortcut by promoting correlation between breast density and cancer status, skewing the prevalence across experiments (1), (2) and (3) impacts the task difficulty, as it is well established that the AI classification of C&D is difficult compared to A&B. Thus, subgroup disease distribution and difficulty are two distinct sources of bias. The dissimilarity scores from the HDC analysis are 0.0052 ± 0.003 [low], 0.0209 ± 0.008 [medium] and 0.0502 ± 0.0143 [high] for (1), (2) and (3) respectively. DCs are subtle and need to be assessed relative to each other. The medium DC scores in (2) show that even with equal density subgroup prevalence there is potential for AI bias, which is likely explained by the subgroup task difficulty. This also applies to (1) where the training task was easy, resulting in the model learning only features related to the easy A&B subgroup, which leads to low DC scores but poor performance on the C&D subgroup. However, in case of (3), highest DC scores were observed due to the combination of systematically amplified bias as well as increased task difficulty due to only training on C&D category for cancer positive data.
- **Implications**
  - Evaluating AI performance bias before deployment is crucial for ensuring trustworthiness and reliability in AI-assisted decision support systems. However, challenges remain, including the lack of sufficient test data for meaningful subgroup performance analysis and the absence of robust methods to identify sources of bias. In this study we developed a novel approach to identify DCs, potential differences in patient subgroup characteristics, by analyzing the features extracted from the AI models during the testing phase. Understanding these DCs can identify potential sources of bias in an AI model and pinpoint areas where the model may not perform equitably across subgroups. This knowledge of AI bias provides improved transparency in the deployed AI models for AI users. This approach could also benefit the post-market monitoring of deployed AI models to ensure they continue to operate as intended.

71. **Lakkasetter Chandrashekar, Bhuvana**
    - **Abstract title:** Fatigue Behavior of Additively Manufactured Polymeric Materials: An Insight to Medical Device Durability
    - **Authors:** Lakkasetter Chandrashekar, Bhuvana, FDA/CDRH/OSEL/DAM (Student); Porter, Daniel, FDA/CDRH/OSEL/DAM (Mentor); Di Prima, Matthew, FDA/CDRH/OSEL/DAM (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data & Empowering Patients and Consumers
    - **Abstract:**

- **Synopsis**
  - Additive Manufacturing popularly known as 3D printing has not only led to innovations in the field of mechanical and automobile engineering, but also motivated many novel ides in the field of bioengineering. Gaining popularity due to ease of use and customizable patient needs, additive manufacturing has also intrigued scientists to study material properties that are essential for medical device functioning. While much has already been discovered, there is much more to be studied and understood in terms of life of the material, to cater to the needs and better performance of medical devices. Hence this study is aimed at evaluating one of the most important material properties, fatigue behavior of polymeric materials to better predict additively manufactured device performance.
- **Purpose**
  - Increasing need for efficient product development and personalized medicine has inspired the modern medical device industry to develop and adapt novel fabrication techniques such as Additive Manufacturing (AM). Although the past decade has witnessed significant development in this field, there are many factors that need further research such as AM device durability and variability. These factors can greatly impact device performance, especially for materials such as AM polymers. Prior studies have focused on investigating static tensile properties. However, there has not been enough characterizing AM device fatigue behavior. Therefore, the current study aims to evaluate the fatigue performance of additively manufactured polymers using experimental and computational methods. Furthermore, these efforts will help FDA and industry better understand the potential of computational modeling to predict AM polymer device performance.
- **Methods**
  - Rotary bend and tensile coupons were printed with polyamide12 material (Nylon PA2200) on an EOS P396 printer in the horizontal and vertical build orientations. Previous literature and preliminary tensile tests provided the necessary static material properties for Finite Element Analysis (FEA) modeling. Completely reversed rotary bend fatigue tests were conducted on an ADMET eXpert 9300 system at various stress amplitudes. The stress levels calculated from these experiments were verified by FEA. Axial tensile fatigue tests were conducted with an Instron ElectroPuls E3000 system. Load levels were chosen to produce similar stress levels as those targeted in rotary bend fatigue. The Stress-Life (S-N) curves were generated and various mean-stress theory models were applied to understand the fatigue behavior. Additionally, optical profilometry and microscopy provided surface roughness and optical images of all the specimens to understand sample topography.
- **Results**
  - Amongst various samples tested, increased sample diameter gave more consistent and reliable results. Samples experiencing higher forces and lower life did not reach expected stress values when compared to those at lower forces. At stress values less than 23.5 MPa, the samples ran for more than one million cycles without fracture. FEA for rotary bend tests depicted a 10% discrepancy between the stress values calculated with

the analytical formula and computational methods. Tensile parameters such as the Young's modulus obtained from experiments agreed with literature values. Future axial tensile fatigue tests with the same material will provide better insight into the fatigue behavior of the polymer which will be compared to rotary fatigue tests.

- **Implications**
    - The samples fractured at various stress levels depicting plastic deformation failures. FEA simulation consistently indicated the fracture area on the samples experiencing maximum stresses and strains. Although there were no significant differences between horizontal and vertical build samples, at lower stresses the horizontal build orientation produced longer bending fatigue life indicating better longevity for this AM polymeric material. This might be due the material exhibiting lower elastic modulus with horizontal build orientations compounded with the bending based mechanism of the tests. It could also be due to Z-build direction layer effects which have weaker shear and tensile planes. The discrepancies between analytical and FEA values could be due to modeling simplifications and boundary conditions. Additionally, the current fatigue modeling techniques involved using preliminary stress-life curves that might not be representative of the precise fatigue behavior. Upcoming experiments and modeling will incorporate more precise input data and specimen design to better simulate and understand the fatigue behavior of Nylon PA2200 material.

72. **Mamiya, Yubi**
    - **Abstract title:** Comparison of synthetic skin generation approaches
    - **Authors:** Mamiya, Yubi, FDA/CDRH (Student); Saharkhiz, Niloufar, FDA/CDRH (Research Scientist); Sizikova, Elena, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
    - **Abstract:**
        - **Synopsis**
            - This project seeks to compare the synthetic images generated by S-SYNTH, a knowledge-based, adaptable skin simulation framework, with those generated by state-of-the-art generative AI models. We will create synthetic skin images using a conditional latent diffusion model, Synthetic Derm, that generates images with lesions of specific skin disease presentations and skin colors (Sagers et al. "Augmenting medical image classifiers with synthetic data from latent diffusion models."). The two types of synthetic image generation methods will be evaluated by comparing downstream lesion segmentation performance.
        - **Purpose**
            - The development of machine learning models to assist with the diagnosis of skin diseases has prompted the need for more image samples of skin diseases. However, a lack of samples from patients with diverse skin colors and disease presentations has hindered the improvement of these models. Researchers in the Division of Imaging, Diagnostics, and Software Reliability (DIDSR/OSEL/CDRH/FDA) developed the S-SYNTH pipeline, a knowledge-based, adaptable skin simulation framework to rapidly generate synthetic skin, 3D models and digitally rendered images using an anatomically inspired multi-layer, multi-component skin and growing lesion model (Kim, MICCAI 2024). It

is of interest to understand how the synthetic images generated by S-SYNTH compare in terms of lesion segmentation performance to synthetic images generated by state-of-the-art generative AI models. This project intends to implement a diffusion model that generates synthetic skin images, obtain segmentation masks and compare the lesion segmentation performance on the images generated using the two approaches.

- **Methods**
  - We will implement a conditional latent diffusion model that generates synthetic skin images with lesions either unconditionally, or conditioned on prompts, including skin disease and skin color (our approach is based on Sagers et al. "Augmenting medical image classifiers with synthetic data from latent diffusion models."). In order to cover a broad scope of skin diseases and skin colors, the model will be trained on multiple public real patient datasets. Once the synthetic images are generated, they will be segmented to obtain lesion mask predictions. Finally, datasets with different ratios of synthetic and real images will be compiled and evaluated using downstream lesion segmentation (Bozorgpour et al., MICCAI 2023). We will evaluate the performance of lesion segmentation between the datasets with images from S-SYNTH pipeline and images from the diffusion model.
- **Results**
  - In S-SYNTH, we observed an improvement in lesion segmentation performance when evaluated datasets of real images were supplemented with synthetic images. The sample of synthetic images with controlled melanosome fraction, blood fraction, hair artifact, and lesion shape variation exhibited performance trends comparable to those found in the literature. We hypothesize that diffusion models will be able to more accurately replicate complex lesion shapes and skin patterns but may be limited in addressing darker skin tones and artifacts subgroups, since these examples may be limited in the training set used to create the diffusion model. In the preliminary experiments with synthetic images from diffusion models, we found that the lesion boundaries were less defined, which may pose a challenge in lesion segmentation tasks. These images also exhibited unnatural artifacts and hallucinations which may pose an additional challenge.
- **Implications**
  - The results of our study will offer qualitative and quantitative performance comparisons of synthetic data created using two approaches: simulations using knowledge-based models and diffusion models, a type of generative AI model. The results of this project will provide insight into the strengths and weaknesses of the two approaches as tools for augmented limited patient datasets in skin imaging AI.

73. **Miller, Dickerson**
    - **Abstract title:** Development of a Regulatory Science Tool for Optimizing Recovery of Extractables from Medical Devices
    - **Authors:** Dickerson, Miller, FDA/CDRH (Student); Young, Joshua, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**

- **Synopsis**
  - Chemical characterization of medical devices is used to address some biocompatibility endpoints instead of animal testing. However, certain challenges in conducting chemical characterization, such as selection of appropriate extract preparation methods, require refinement to ensure reliable chemistry data is obtained. A framework for predicting recovery has been developed to assist this which includes four steps: 1. Define a chemical subspace: all the possible chemicals that are of interest for the analysis. 2. Choose a model that describes that recovery of the sample preparation technique. 3. Choose surrogate chemicals with different values for a property of interest that governs recovery. 4. Measure the experimental recovery to verify the model. In order to assist chemists with an appropriate choice of standards to demonstrate adequate recovery, a regulatory science tool (RST) with relevant properties of interest from the chemical subspace as well as the ability for the user to input their own data is being developed. This tool will perform important calculations regarding recovery, partition coefficients, and solubility factors, provide graphical representations, and an interactive chemical space. The tool will allow chemists to optimize solvent exchange methods and will be publicly accessible to chemists and researchers. The flexibility of the approach avoids recommending a best practice approach that may not be applicable to all scenarios. Additionally, the framework of this approach can be extended to a variety of other sample preparation techniques, such as sample evaporation to increase concentration.
- **Purpose**
  - The purpose of this project is to develop a publicly-accessible regulatory science tool (RST) designed to optimize the recovery of extractables from medical devices and mitigate challenges in conducting chemical characterization. This tool aims to assist chemists in selecting appropriate extract preparation methods by providing a framework for predicting recovery, performing essential calculations, and offering graphical and interactive representations. By addressing challenges in chemical characterization and enabling customization based on user input, the tool seeks to improve the reliability of chemistry data and support diverse sample preparation techniques while aiding chemists in consistent recovery of extractables from medical devices.
- **Methods**
  - Shifts in the sigmoidal recovery distribution (recovery vs. partition coefficient) were demonstrated for various solvent exchange scenarios such as dichloromethane to water volume ratio and exchange iterations. Evaporation was evaluated in several solvents including hexanes, isopropyl alcohol, and dichloromethane using multiple temperature conditions. The experimental measurements of recovery were compared to predicted recoveries to demonstrate efficacy of the model and a 80% recovery cut-off was selected as the minimum recovery for an analyte to be considered successfully solvent exchanged. From these results, a web application was developed using Flask, SQL, HTML, and hosted with pythonanywhere.
- **Results**
  - Predicted recoveries agreed well with experimental recovery (within

20% RMSE). The models used were incorporated into the web app which allows for rapid assessment of many different chemicals simultaneously. This approach demonstrates the ability to predict the recovery of a wide range of chemicals using different solvent exchange methods. The flexibility of the approach avoids recommending a best practice approach that may not be applicable to all scenarios. Additionally, the framework of this approach can be extended to a variety of other sample preparation techniques, such as sample evaporation to increase concentration. The web application allows chemists to optimize their solvent exchange methods in a virtual, interactive space.

- **Implications**
  - The flexibility of the approach avoids recommending a best practice approach that may not be applicable to all scenarios. Additionally, the framework of this approach can be extended to a variety of other sample preparation techniques, such as sample evaporation to increase concentration. The web application will perform important calculations regarding recovery, partition coefficients, and solubility factors, provide graphical representations, and an interactive chemical space. The tool will allow chemists to optimize solvent exchange methods and will be publicly accessible to chemists and researchers.

74. **Mahashetty, Saanika**
    - **Abstract title:** Sex- and Gender-Specific Subgroup Analysis in Approved Pre-Market Approval (PMA) Applications for Calendar Years 2022-2023
    - **Authors:** Saanika Mahashetty (Student) FDA/CDRH, Antoinette Hazlett (Mentor), Virginia Mensah (Mentor), Terri L. Cornelison (Sponsor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Since 2017, a review of Post Market Applications (PMA) applications received following the 2014 Guidance "Evaluation of Sex-Specific Data in Medical Device Clinical Studies" has been conducted to determine the rate of reporting of sex- and gender-specific data in submissions. This review was conducted for Calendar Year (CY) 2022 and Calendar Year (CY) 2023. This effort is one of the priorities of the CDRH Health of Women Program's Research Roadmap to optimize CDRH for consistent sex- and gender-specific data collection, analysis, and reporting, and to promote advancement of regulatory science related to health of women.
      - **Purpose**
        - Advances in science have shown that sex and gender differences may play a significant role in the performance of medical devices in both men and women. To better understand these unique performance issues and how they affect safe and effective use of devices, we must improve the availability and communication of sex- and gender-specific data. The 2014 CDRH Guidance Evaluation of Sex-Specific Data in Medical Device Clinical Studies outlined recommendations for sex-specific patient enrollment, data analysis, and public reporting of study information. This poster updates previous years analyses of sex- and gender-specific data in PMAs submitted after the issuance of the

Guidance. This poster also includes review of the completeness and quality of sex- and gender-specific subgroup data collection, reporting, and analysis, stratified by device indications from multiple clinical specialties.

- **Methods**
  - Demographic subgroup analyses data were examined from the Summary of Safety and Effectiveness Data (SSED) of approved PMA applications found in the public PMA database. The PMA database search function was used to find publicly available approved PMAs for calendar years 2022 and 2023 by selecting original CDRH applications with a decision date between January 1st, 2022, and December 31st, 2023. Data was reviewed from the demographic subgroup analyses included in the publicly available Summary of Safety and Effectiveness Data (SSED) for PMAs submitted in CY2022 and CY2023. If information was not present in the SSED, the original application was reviewed.
- **Results**
  - Data were evaluated from all 22 approved PMA applications from CY2022. For CY2023, 31 of 36 total approved PMA applications were evaluated; one PMA did not include clinical studies and four PMAs included only single sex studies. For CY2022 and CY2023, 100% of PMA applications reported sex/gender-specific data. For CY2022, 64% of PMA applications reported sex/gender-specific analysis, 59% publicly reported the analysis. For CY2023, 71% of PMA applications reported sex/gender-specific analysis and made the analysis publicly available.
- **Implications**
  - Since 2017, on average 52% of study participants were female, 65% of PMA applications reported sex/gender-specific analysis, and 58% publicly shared those data. In CY2022-2023, 100% of approved PMA applications reported sex/gender-specific data. When compared to prior years, there was a similar percentage of approved PMA applications that reported sex/gender-specific data analysis and that shared results of those analyses publicly.
  - The results for CY2022-2023 suggest that continuous effort is needed to increase and improve the completeness and quality of sex/gender-specific subgroup data collection, reporting, and analysis in PMA applications for our collective improved understanding of sex/gender-specific influences in medical device performance and safety, and to make data more available and transparent to the public.

75. **Milton, Philip**
    - **Abstract title:** Effect of Species, Anticoagulant, and Temperature on in vitro Dynamic Hemolysis Testing Using a Rheoemter
    - **Authors:** Milton, Philip, FDA/CDRH (Student); McLeod, David, FDA/CDRH (Post-doc); Ponnaluri, Sailahari, FDA/CDRH (Mentor); Malinauskas, Richard, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Hemolysis is an important measure in the safety evaluation of blood-contacting medical devices and materials. Currently, blood contacting medical devices are tested in accordance with ASTM F1830/F1841 standards to assess in vitro dynamic hemolysis. These standards allow

for flexibility in the blood properties used in testing, such as species, anticoagulant, and temperature. However, blood parameters can impact the sensitivity of hemolysis testing, making it difficult to interpret or compare results. To better comprehend how sensitive mechanical hemolysis testing is to changes in blood properties, a rotational rheometer was used to simulate damaging forces that red blood cells might experience as they pass through medical devices. Testing on the rheometer allowed for an efficient evaluation of multiple blood parameters. First, rheometer conditions, including shear rate, test duration, and waveform type, were optimized on both Double Gap (DG) and Cone and Plate (CP) fixtures. Next, to evaluate the ability of the rheometer to distinguish differences in blood parameters, three parameters often adjusted by companies (species, anticoagulant, and temperature) were tested at the optimized rheometer conditions. Preliminary results indicated that the rheometer could be used to assess the hemolytic sensitivity of these parameters. Further characterization of both the DG and CP rheometer fixtures will be performed to reach a more repeatable, clinically accurate test simulation that mimics the periodic forces experienced by blood cells as they flow through medical devices.

- **Purpose**
  - A thorough understanding of blood damage potential, such as hemolysis, is essential in the evaluation of blood-contacting medical devices to reduce patient risk to anemia or thrombosis. Currently, in vitro dynamic hemolysis testing best practices are outlined in ASTM standards F1830-19 and F1841-19e1. However, to be least burdensome to companies, these standards allow for flexibility in selecting blood testing parameters, such as species, anticoagulant, temperature, and the blood adjustment protocol, all of which may affect the hemolysis results. To efficiently gain an understanding of how sensitive hemolysis is to a multitude of blood parameters, a rotational rheometer was used to simulate fluid forces in medical devices while allowing for shorter experimental times and using small blood volumes. The aims of this study are two-fold: (1) develop a protocol for performing blood damage sensitivity analysis using an Anton Paar MCR 102e rheometer with both the Double Gap (DG) and Cone and Plate (CP) fixtures, and (2) evaluate the ability of the rheometer to distinguish the effect of blood parameters on hemolysis.
- **Methods**
  - To optimize in vitro dynamic hemolysis testing on the rheometer using both the DG and CP fixtures, test duration, steady and pulsatile rotational conditions, and shear rates at both laminar and turbulent flow conditions were tested. The optimal condition would ideally produce repeatable plasma free hemoglobin (pfHb) levels greater than 100 mg/dL to allow for distinguishing hemolytic sensitivity differences and trends. Once optimized, blood from bovine, porcine, ovine, and human donors was tested approximately 24 hours after blood was drawn. Blood was drawn into either anticoagulant citrate dextrose solution A (ACDA) or heparin (used in porcine and human blood only). Blood hematocrit was adjusted to 35% ± 2% via hemodilution with phosphate buffered saline (PBS) or hemoconcentrated using gentle

centrifugation in accordance with ASTM F1830. Testing was conducted on the rheometer at the optimized condition at 23 and 37°C in triplicate, blood samples were collected, and the plasma was isolated via two rounds of centrifugation. Using a microvolume spectrophotometer (Thermo Scientific NanoDrop One), a direct optical absorbance method (Cripps) was utilized to determine the concentration of pfHb in each sample. Samples were corrected by their background static hemolysis levels.

- **Results**
  - The DG system was tested at shear rates of 2000 and 8500 1/s, for durations of 5, 10, and 15 minutes, under steady and pulsatile rotational conditions. The turbulent shear rate of 8500 1/s was chosen to ensure blood mixing and 5 minute tests were selected due to minimal differences in hemolysis at greater durations. Low hemolysis levels obtained using the DG fixture made it difficult to identify hemolysis differences between parameters, so testing transitioned to the CP fixture. With the CP fixture, shear rates of 8500, 11,000, and 13,500 1/s were tested at 5 and 15 minutes. Ultimately 11,000 1/s at 5 minutes was selected, as this shear rate generated more consistent results and turbulent mixing. Tests were run under steady conditions for 5 minutes at 11,000 1/s at both 37°C and 23°C. Initial results indicated reduced hemolysis levels at 23°C compared to 37°C. ACDA yielded greater hemolysis than heparin at both 37 and 23°C for porcine blood, but the opposite for human. Differences in species were also observed: at 37°C, bovine blood was generally more sensitive than porcine, followed by ovine, and lastly human; at 23°C, porcine blood was generally most sensitive, followed by bovine, ovine, and lastly human.
- **Implications**
  - This study shows that rheometric testing, used to simulate shear conditions of blood pumps and similar blood contacting devices, may be utilized to examine blood factors that may impact the sensitivity of hemolysis testing. However, further characterization of the CP system is needed to examine repeatability and the effect of blood drying at the plate edge at higher temperatures (37°C), which may lead to higher viscosity readings and more variable hemolysis results. Additionally, further examination of the DG fixture should be performed to determine if optimal test conditions can achieve comparable hemolysis levels to the CP fixture when operated under steady and pulsatile rotational conditions (allowing for stress relaxation of red blood cells over sustained durations). While this study explored only species, anticoagulant, and temperature differences, many other blood parameters that affect hemolysis (such as pH and glucose) will also be tested. This and future work could be used to update medical device testing in ASTM F1830 and F1841 by standardizing blood properties to provide optimally sensitive hemolysis results.

76. **Sabogal, Bryan**
    - **Abstract title:** A Comparison of Gait Events Derived By Smartphone and Walkway Sensors
    - **Authors:** Sabogal, Bryan, FDA/CDRH (Student); Kontson, Kimberly FDA/CDRH (Mentor)
    - **FDA Strategic Initiative**: Unleashing the power of Data

- **Abstract:**
  - **Synopsis**
    - Gait abnormalities are a hallmark of neurodegenerative disease and many other neurological conditions, necessitating precise monitoring to inform treatment strategies and track disease progression. While walkway sensors provide high accuracy, they are often costly, cumbersome, and impractical for continuous monitoring outside clinical settings. Conversely, smartphone sensors offer a portable, cost-effective alternative but their reliability and precision in capturing critical gait events like heel strikes and toe-offs remain underexplored. The goal of this research is to compare the effectiveness of smartphones and reference walkway sensors in gait analysis. By leveraging filtering techniques and statistical analyses, we aim to determine if smartphone sensors can provide a feasible solution for remote gait analysis and monitoring.
  - **Purpose**
    - The purpose of this research is to observe the impact and effectiveness of smartphones in gathering data for gait analysis as compared to larger, less portable walkway sensor systems. Gait abnormalities are a hallmark of neurodegenerative disease and many other neurological conditions, necessitating precise monitoring to inform treatment strategies and track disease progression. While walkway sensors provide high accuracy, they are often costly, cumbersome, and impractical for continuous monitoring outside clinical settings. Conversely, smartphone sensors offer a portable, cost-effective alternative but their reliability and precision in capturing critical gait events like heel strikes and toe-offs remain underexplored. This study aims to evaluate the performance of smartphone sensors in detecting these events compared to walkway sensor systems. By leveraging filtering techniques and statistical analyses, we aim to determine if smartphone sensors can provide a feasible solution for remote gait analysis and monitoring.
  - **Methods**
    - Self-selected pace walking gait data was previously collected by the FDA, using smartphones (iPhone X and Samsung Galaxy S22) placed at the lower back and right thigh to gather acceleration signals at 100 Hz. The dataset also includes walkway sensor measurements, indicating with high accuracy the timing of each footfall on the walkway. Previous studies (Tao et. al, 2024) have used algorithms to plot certain gait events such as toe-off and heel strike. To evaluate these events and when they occur, we employ a similar method in which the raw sensor data is passed through Gaussian and Kalman filtering to remove noise. Gait events were detected using the peaks and troughs of the smartphone acceleration data. Walkway data was evaluated by graphing the left and right foot contact periods, and the time difference in detection from each method was assessed.
  - Results
    - Initial results revealed a discrepancy between the number of events detected by both smartphones compared to the walkway. There were, on average, 10 additional events detected by the smartphone via peaks and troughs compared to the walkway. Also, due to these additional

events, oftentimes the gait event detected by the walkway split the difference between two peaks or troughs found in the smartphone acceleration data. However, the detection difference in toe-off events was 24.5 ± 20.1 frames, and 27.8 ± 9.8 frames for heel strike events.

- Implications
    - These findings could reveal the reliability of using smartphones to assess and monitor clinical populations with mobility and gait disorders. If smartphone sensors match the accuracy of reference walkway sensors in detecting gait events, they offer a cost-effective, portable solution for continuous monitoring outside clinical settings. This can lead to timely, personalized interventions, improved patient engagement, and reduced healthcare costs. Moreover, the accessibility of smartphone sensors allows for broader patient monitoring and the collection of valuable data to advance research on various gait disorders. Integrating this technology into routine clinical practice supports the goals of personalized medicine, ultimately enhancing the quality of life for patients with gait abnormalities.

77. **Shannon, Tate**
    - **Abstract title:** Steady Flow Characterization of Venous Valve Devices
    - **Authors:** Shannon, Tate, FDA/CDRH (Student); Ibarra, Bryan, FDA/CDRH; Ponnaluri, Sailahari, FDA/CDRH; Carr, Ian, FDA/CDRH; Weidenhamer, Nathan, FDA/CDRH; D'Souza, Gavin, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition and Response
    - **Abstract:**
        - **Synopsis**
            - FDA is seeing an increasing number of pre-market submissions for venous valve devices that are being proposed as a novel and alternate treatment option for severe cases of chronic venous insufficiency (CVI). Due to the novelty of this product area and breakthrough technological features, there is a lack of standard and well-established test guidelines for effectively evaluating these devices. This regulatory science project aims to develop a standardized non-clinical test method for characterizing the steady-flow hydrodynamic performance of venous valve devices. By adapting and modifying the existing cardiac valve standard, ISO 5840, the study establishes a new Regulatory Science Tool (RST) specifically tailored for venous valve devices. The methodology encompasses three key components: (1) scaling and modifying steady-flow test equipment based on ISO 5840-1, (2) validating the test methodology using the standard nozzle design, and (3) designing a representative vein valve test sample. This project produces a standard steady-flow test system including a standard nozzle design for venous valves, along with steady flow characteristic datasets for test system validation. This research has significant implications for streamlining device testing and regulatory review processes for a novel product, potentially reducing time and cost associated with bringing venous valve devices to market.
        - **Purpose**
            - Venous reflux due to incompetent vein valves in the lower limbs is one cause of chronic venous insufficiency (CVI). CVI results in poor circulation, varicose veins, skin changes, swelling, edema, and recurrent

and/or chronic ulcers. Current treatment options have associated shortcomings including the lack of compliance by patients to adhere to the prescribed therapy (e.g., graduated compression stockings) and known complications with surgical techniques (e.g., vein valve transposition). Over the past 5 years, FDA has seen an increasing number of pre-market submissions for venous valve devices that are being proposed as a novel and alternate treatment option for severe cases of CVI. The novelty of this product area, breakthrough technological features, and lack of standard test guidelines present challenges to both device manufacturers and FDA reviewers. These challenges often result in increased deficiencies during regulatory review and increased Pre-Submissions to discuss proposed bench verification and validation testing. To address some of the challenges, this project aims to develop a standard non-clinical test method in the form of a Regulatory Science Tool (RST) for characterizing the steady-flow hydrodynamic performance of venous valve devices based on the well-established ISO 5840 standard for cardiac valve prostheses.

- **Methods**
  - We first developed a benchtop steady-flow test loop for cardiac valves per the ISO 5840-1 specifications. Using this loop, we validated the test methodology by comparing the experimental a) pressure drop vs. flow rate and b) leakage vs. back pressure characteristics of the ISO 5840-1 standard nozzles against the standard published data. Subsequently, we scaled the steady-flow cardiac valve testing apparatus including the standard nozzles, to produce flow characteristics relevant to the venous physiology. Using the steady-flow venous valve test loop, we are in the process of obtaining the experimental a) pressure drop vs. flow rate and b) leakage vs. back pressure characteristics for the standard vein valve nozzles. Further, we are also testing the standard vein valve nozzles in the steady-flow cardiac valve test system to quantify its hydrodynamic performance in the ISO 5840-1 standard system.
  - In addition to the standard nozzles, we also designed and fabricated a silicone-based test vein valve sample representative of a native deep femoral vein valve. The steady-flow performance of the sample valve is being characterized using the standard cardiac valve and scaled venous valve steady flow test systems described above.
- **Results**
  - We established detailed instructions for replicating the steady-flow test system in ISO 5840-1 (Annex I). We validated the test system with both forward and reverse steady flows using the standard cardiac valve nozzles. Our test results matched the standard pressure drops for forward flow with minimal differences in the 0-5 L/min range—the range most relevant to venous valves—and differences of less than 1 mmHg for flow rates up to 15 L/min (maximum tested flow rate). Similarly, for backflow, the measured leakage volumes also matched the standard within an acceptable range of error.
  - The steady-flow hydrodynamic performance characterization described in this project is critical to understanding the venous valve device's performance from a fundamental fluid mechanics standpoint under a well-controlled and highly reproducible setting prior to conducting more complex pulsatile flow assessment.

- **Implications**
    - This project is expected to address some of the regulatory challenges for venous valve devices by providing standardized non-clinical test recommendations for effective characterization of their hydrodynamic performance. The published Regulatory Science Tool (RST) from this project is expected to provide device innovators and manufacturers with a standard and reproducible non-clinical test methodology for characterizing the steady-flow hydrodynamic performance of their venous valve devices in a controlled benchtop environment. This will streamline device testing and regulatory review for this novel product, thus reducing the burden, time, and cost to both device manufacturers and FDA reviewers for getting devices quickly to the market and to patients.

78. **Sherekar, Mukul**
    - **Abstract title:** The evaluation of AI-enabled computer-aided rule-out devices
    - **Authors:** Mukul Sherekar, FDA/CDRH (Student/ORISE Fellow); Michelle Mastrianni, FDA/CDRH (Post Doctoral Research Associate); Elim Thompson, FDA/CDRH(Mentor); Weijie Chen, FDA/CDRH (Mentor); Frank Samuelson, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation & Unleashing Power of Data
    - **Abstract:**
        - **Purpose**
            - Mammography is a low-dose X-ray imaging technique for screening and/or diagnosing breast cancer. Mammography screening has been shown to reduce breast cancer morbidity and mortality, but it also generates a large reading volume for radiologists. AI imaging devices can provide valuable assistance to radiologists, saving time and resources. One such avenue of assistance can be an AI enabled computer aided rule-out device. It analyzes mammograms for presence/absence of cancer. Mammograms deemed 'cancer-negative' by a rule-out device are not passed onto a radiologist for review. While such devices are promising in improving the clinical workflow in mammography screening, evaluation metrics to assess their diagnostic performance do not yet exist. Therefore, by extending conventional ROC analysis, our project goal is to demonstrate how these AI-enabled algorithms can be evaluated if used in a rule-out setting.
        - **Methods**
            - We use a winning algorithm from the 2023 Screening Mammography Breast Cancer Detection AI Challenge hosted by the Radiological Society of North America (RSNA) as our rule-out algorithm and the open-source Emory Breast Imaging Dataset (EMBED) for training and testing. We improve the algorithm performance by adding more training data from other databases and tuning the hyperparameters. With an independent testing dataset, we evaluate both the AI standalone performance using conventional ROC analysis, as well as the predicted performance in a rule-out setting using utility theory and an extended ROC analysis that considers both radiologist and AI-alone diagnostic performance. By comparing the radiologist-alone (standard of care without the AI) diagnostic performance with the predicted performance in a rule-out setting, we study situations when such a rule-out device

can benefit the patients and situations when the device would lower the overall diagnostic performance. We stratify our results by subgroups including breast density, women's age, etc. to investigate the generalizability of the algorithm performance.

- **Results**
  - Data collection is on-going. Results will be presented in the poster at the conference.
- **Implications**
  - As AI-enabled computer-aided medical devices continue to improve their performance and achieve a diagnostic performance like that of radiologists, researchers have been actively investigating the use of rule-out devices in mammography screening to help reduce radiologists' workloads while maintaining a non-inferior (or even a superior) diagnostic performance compared to the standard-of-care workflow without the AI. As the FDA receives more pre-submissions on rule-out devices, our project helps the FDA prepare for any future submissions of AI-based, semi-autonomous medical devices by demonstrating how these devices should be evaluated using retrospective data, which may provide scientific justifications for future FDA guidance documents on AI-based rule-out devices. The methodology from our project can be applied to any AI-enabled rule-out devices such as mammography screening or other semi-autonomous AI devices that remove AI-negative cases without human supervision.

79. **Tynan, Ava**
    - **Abstract title:** How to Simulate a Skin Image Acquisition Device?
    - **Authors:** Tynan, Ava, FDA/CDRH (Student); Saharkhiz, Niloufar, FDA/CDRH (Mentor); Sizikova, Elena, FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - Current skin image datasets utilized for developing and evaluating accurate and unbiased artificial intelligence (AI) algorithms often are small and do not contain the necessary diverse range of subgroups needed to properly train or evaluate an AI algorithm. Synthetic skin image datasets may be an option in filling the gaps that current patient skin image datasets are not able to fulfill. The S-SYNTH pipeline introduced by researchers in the Division of Imaging, Diagnostics, and Software Reliability (DIDSR/OSEL/CDRH/FDA) and presented in S-SYNTH: Knowledge-Based, Synthetic Generation of Skin Images (Kim, MICCAI 2024) is a skin simulation framework that generates synthetic skin using 3D models and digitally rendered images. The pipeline generates a 3D skin model, subsequently rendered using a camera model to create each synthetic skin image. In this project, we survey literature to identify key parameters needed to model a realistic smartphone camera acquisition system, and implement them into the S-SYNTH pipeline.
      - **Purpose**
        - Current skin image datasets utilized for developing and evaluating accurate and unbiased artificial intelligence (AI) algorithms often are small and do not contain the necessary diverse range of subgroups needed to properly train or evaluate an AI algorithm. Synthetic skin

image datasets may be an option in filling the gaps that current patient skin image datasets are not able to fulfill. The S-SYNTH pipeline introduced by researchers in the Division of Imaging, Diagnostics, and Software Reliability (DIDSR/OSEL/CDRH/FDA) and presented in S-SYNTH: Knowledge-Based, Synthetic Generation of Skin Images (Kim, MICCAI 2024) is a skin simulation framework that generates synthetic skin using 3D models and digitally rendered images. The pipeline generates a 3D skin model, subsequently rendered using a camera model to create each synthetic skin image. A limitation of the currently implemented camera model is that the standard version does not necessarily take into account the complexities of a real camera. The goal of this project is to offer a better understanding and an initial exploration of key camera parameters and features to implement a realistic smartphone camera model.

- **Methods**
  - We will start with a literature review to identify the key features necessary for an accurate camera, and then change parameters on the existing camera model to address these aspects. After optimizing parameters, the newly rendered images will be compared to the skin images generated by the standard camera model. Within the initial literature review, we found that camera models are often evaluated by comparing renderings of a known digital scene (e.g., Cornell box) to a real photo of the box acquired using a smartphone camera. While some camera parameter values (e.g., pixel size, blur, focus, and others) may be inferred from literature, others may need to be estimated.
- **Results**
  - Through the preliminary literature review ((Lyu, Arxiv 2022), (Hetz, Arxiv 2024)), we identified some of the initial components needed for developing an improved camera model. One of the most emphasized features in camera models is ray tracing, which is used to simulate how lighting interacts with a generated 3D scene. The spectral response function (SRF) that defines the spectral sensitivity of the sensor will directly impact how accurately the camera model will display shadows and scene colors. The location of camera focus and magnification are also important, since blurry or unfocused lesion images may impact subsequent analysis. The camera geometry (e.g., lens thickness, perspective or orthographic projection) affects how the camera rays intersect with the scene and the realism of the resulting 3D projection into a two-dimensional image. Finally, the field of view (FoV) of a camera describes the viewable area and affects the type of fine- and large-scale variation seen. We will identify parameter ranges that represent a realistic smartphone sensor, and implement them into the existing S-SYNTH pipeline.
- **Implications**
  - After identifying the features and parameters that are most important for simulating a smartphone camera and rendering a skin image, the next step is to test the camera implementation by comparing resulting images to real examples. We plan to compare images qualitatively, as well as quantitatively, by evaluating down-stream segmentation performance in public datasets.

80. **Yuan, Emilee**

- **Abstract title:** Effects of Test Duration on the In Vitro Flow Loop Thrombogenicity Assessment of Biomaterials and Medical Devices
- **Authors:** Emilee Yuan (Student) FDA/CDRH, Carlos Serna III, Miriam Danzis, Mehulkumar Patel, Megan Jamiolkowski, Qijin Lu (Mentor) FDA/CDRH
- **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
- **Abstract:**
    - **<u>Synopsis</u>**
        - As part of an FDA-led interlaboratory study to develop standardized best practices for dynamic in vitro thrombogenicity testing and to reduce the need for acute animal studies, we investigated the effects of blood recirculation time (1 vs. 2 hours) on the comparative thrombogenicity results produced by an in vitro flow loop system. For this test system, clinical cardiopulmonary bypass roller pumps were used to simultaneously run four in vitro flow loops. Each roller pump was manually adjusted using a modified dynamic occlusion method previously developed in our lab to ensure similar flow conditions across all of the flow loops. Then, bovine blood anticoagulated to a donor specific heparin concentration was recirculated at 15 rpm (~200 mL/min) through a polyvinyl chloride tubing loop containing a test material at room temperature for 1 or 2 hours. Four materials with varying thrombogenic potentials were used in this study: polytetrafluoroethylene (PTFE), silicone, latex, and BUNA. To characterize the thrombogenicity of the materials, platelet count reduction and thrombus surface coverage were measured. Both the 1-hour and 2-hour tests were able to effectively differentiate a historically thromboresistant material (PTFE) from known thrombogenic materials (Latex, BUNA). However, results for silicone were inconsistent between the two recirculation times. For the 1-hour test, the results for silicone were very similar to PTFE, whereas they were comparable to Latex and BUNA at the 2-hour test. Thus, these preliminary results suggest that increasing the circulation time to 2-hours does not substantially increase the test sensitivity but, it is possible that the relative thrombogenicity potential of silicone, and potentially other materials with an intermediate thrombogenicity, may be affected by the recirculation time. However, due to the low sample size and large variation in the thrombogenicity results for silicone, additional testing is needed to verify this observation.
    - **<u>Purpose</u>**
        - Acute in vivo animal studies, such as the non-anticoagulated venous implant (NAVI) assay, are commonly used to evaluate the thrombogenicity potentials of blood-contacting medical devices. However, the NAVI study has several challenges. The device size relative to the vessel diameter, implantation technique, and inherent blood coagulability differences between individual animals can affect thrombus formation and cause large variability in test results. Additionally, this assay is resource demanding and has ethical issues since it requires the animals to be sacrificed. To address some of the limitations of the NAVI assay, in vitro flow loop systems have been developed. However, due to the lack of standardized test protocols and the inherent variability of blood, the results produced by these dynamic

flow systems tend to differ greatly. In an effort to develop standardized best practices for performing such testing, an FDA-led interlaboratory study is currently underway to investigate different test parameters that could affect the sensitivity of the in vitro flow loop assay. As part of this interlaboratory study, we are investigating the effects of blood recirculation time (1 vs. 2 hours) on the comparative thrombogenicity results produced by an in vitro flow loop system.

- **Methods**
  - Four clinical cardiopulmonary bypass (CPB) roller pumps were used to simultaneously run four in vitro flow loops in parallel. Each roller pump was manually adjusted using the modified dynamic occlusion setting method previously developed in our lab to ensure similar flow conditions across all of the flow loops. Donor bovine blood was drawn into containers with Anticoagulant Citrate Dextrose Solution A (ACDA), shipped to our laboratory overnight, and testing was completed within 24-36 hours of the blood draw. Blood from different donors was used for the 1-hour (n= 3 donors) and 2-hour tests (n=2 donors). Immediately before starting each dynamic flow test, the blood was recalcified and heparinized to a donor-specific concentration. For dynamic testing, blood was recirculated at 15 rpm (~200 mL/min) through a 6.4 mm inner diameter polyvinyl chloride (PVC) tubing loop containing a test material at room temperature for 1 or 2 hours. Four materials with varying thrombogenic potentials are used in this study: polytetrafluoroethylene (PTFE), silicone, latex, and BUNA. To characterize the thrombogenicity of the materials, the platelet count reduction in the blood and the percentage of thrombus surface coverage on the test materials at the end of the 1-hour and 2-hour tests were measured.
- **Results**
  - The donor specific heparin concentrations for the 2-hour recirculation tests (1.0-1.2 U/mL) were found to be slightly higher than that for the 1-hour tests (0.8 U/mL). Both the 1-hour and 2-hour tests were able to effectively differentiate a historically thromboresistant material (PTFE) from known thrombogenic materials (Latex, BUNA). In the 1-hour test, there was a significant difference in platelet count reduction between the negative control PTFE (thromboresistant material) and all the other test materials ($p < 0.05$, $n = 3$). However, the thrombus surface coverage between the negative control (PTFE) and silicone was not significantly different. This suggests that the 1-hour test may not be sensitive enough to differentiate thromboresistant materials from materials with an intermediate thrombogenic potential (silicone) based on thrombus surface coverage. Similarly, the preliminary data for the 2-hour test (n=2) shows a substantial difference in platelet reduction and thrombus surface coverage between the thromboresistant (PTFE) and thrombogenic (latex and BUNA) materials. Compared to the 1-hour recirculation time, it appears that silicone produced greater platelet count reduction and thrombus formation in the 2-hour test. However, the preliminary results suggest that increasing the test duration from 1 hour to 2 hours may not substantially improve the test sensitivity.
- **Implications**
  - Successful development of in vitro flow loop thrombogenicity test

systems to replace acute animal studies has a broad impact on the medical device industry, as thrombogenicity evaluation is needed for all blood-contacting devices. To ensure the effectiveness of an in vitro test method, it is important to investigate how the key test parameters impact the test results. In this particular study, the preliminary results suggest that both the 1-hour and 2-hour tests were able to effectively differentiate between thromboresistant and thrombogenic materials and that increasing the circulation time to 2-hours does not substantially increase the test sensitivity. This suggests the 1-hour test may be a viable option because it saves time and increases test efficiency. However, it is possible that the relative thrombogenic potential of silicone, and potentially other materials with an intermediate thrombogenicity, may be affected by the recirculation time. Due to the low sample size (n=2 for 2-hour test) and large variation in the thrombogenicity results for silicone, additional testing is needed to verify this observation. The information gathered from this study will be used in the interlaboratory study to develop standardized best practices, such as the optimal blood recirculation time, for performing dynamic thrombogenicity testing.

81. **Zuccaroli, Isabella**
    - **Abstract title:** Analysis of the Relationship Between Timed Up and Go Test Transition Times and Balance Tasks in Patients with Parkinson's Disease and Age-Matched Controls
    - **Authors:** Zuccaroli, Isabella, FDA/CDRH (Student); Kontson, Kimberly FDA/CDRH (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **<u>Synopsis</u>**
            - The Timed Up and Go (TUG) Test is a commonly used clinical test to evaluate balance, mobility, and fall risk among patients with movement disorders such as Parkinson's Disease. By dividing the Timed Up and Go (TUG) test into distinct transitions and integrating wearable sensors to measure movement, we can potentially gain deeper insights beyond what is achievable solely through TUG completion time. Methods: The "Wearables for gait in Parkinson's Disease and age-matched controls" Version 1.0 dataset was used. Frame-by-frame annotations of participant actions during the TUG and balance tests as well as 3 degree of freedom acceleration data from ankle-worn sensors were extracted from a control cohort (n=28) and PD cohort (n=41). The duration for each of six transitions (Sit to Stand, Turn, Turn to Sit, Walk 1, Walk 2, and Total TUG Duration) in the TUG task was extracted. Average acceleration, peak acceleration, root mean square of acceleration, and integral of acceleration were derived from the magnitude of acceleration for six balance tasks. Pearson's correlation coefficient (alpha = 0.05) was calculated between the TUG transition duration and the acceleration data from the Balance tasks. Results: In the Parkinson's Disease cohort there exist two correlations in the fourth balance task (B4)—one between B4 average magnitude of acceleration and Turn time (correlation: -0.331; p-value: 0.037) and one between B4 average acceleration and Turn to Sit time (correlation: -0.345; p-value: 0.029). Moreover, in the Age-Matched Control cohort (n=25), there is a

correlation of 0.392 (p-value: 0.048) between B4 and peak acceleration of the Walk 1 portion of the TUG test. There is also a relationship between the fifth balance task (B5) and the average acceleration for Walk 1 time (correlation: -0.529; p-value: 0.005), along with peak acceleration for Walk 2 time (correlation: -0.472; p-value: 0.015). Implications: With these observed relationships in two of the balance tasks, these results serve as a high level finding to show that the TUG test does in fact have a relationship with balance. Moreover, it demonstrates that analyzing the TUG test according to transition phases can strengthen the assessment of a patient's balance, and consequently, their fall risk and general mobility.

- **Purpose**
  - The Timed Up and Go (TUG) Test is a commonly used clinical test to evaluate balance, mobility, and fall risk among patients with movement disorders such as Parkinson's Disease (PD). The TUG Test is performed by having a patient sit in a chair and, when prompted, stand up, walk 3 meters, make a 180° turn, and return to a seated position in the chair. By using movement and observational data from an individual's performance in the TUG test, clinicians and healthcare providers can utilize this test to evaluate disease progression and inform consequent treatment. When individuals with PD take their medication, the TUG completion time does not significantly differ between those with balance issues and those without. By dividing the Timed Up and Go (TUG) test into distinct transitions (such as sit-to-stand, walking, turning, and stand-to-sit) and integrating wearable sensors to measure movement, we can potentially gain deeper insights beyond what is achievable solely through TUG completion time. Therefore, the goal of this work was to conduct a preliminary analysis of the relationship between wearable-derived balance metrics and TUG test results to supplement the existing evidence that demonstrates the clinical utility of the TUG test.
- **Methods**
  - To conduct this analysis, we used the "Wearables for gait in Parkinson's Disease and age-matched controls" Version 1.0 dataset collected through an FDA partnership with Johns Hopkins Medicine and VA Ventures. Comma-separated value files containing data for the TUG and Balance tests were extracted from the dataset for control (n=28) and PD (n=41) cohorts. These files contained 3 degree of freedom acceleration sensor data for two sensors located on the left and right ankles as well as frame-by-frame annotations of the participants actions. Using Python, the duration (in seconds) for each transition in the TUG task was extracted. There were six total transition labels: Sit to Stand, Turn, Turn to Sit, Walk 1, Walk 2, and Total TUG Duration. For the acceleration data captured during the Balance tasks, average acceleration, peak acceleration, root mean square of acceleration, and integral of acceleration were derived from the magnitude of acceleration for each of the six balance tasks. Pearson's correlation coefficient (alpha = 0.05) was calculated between the TUG transition duration and the acceleration data from the Balance tasks.
- **Results**
  - Results show weak/moderate but significant correlations between

wearable-derived balance metrics and TUG transition duration in two of the balance tasks. In the Parkinson's Disease Participant cohort (n=41) there exist two correlational relationships in the balance task (B4) in which the participant stood for ten seconds with their eyes closed and feet together. There was a correlation of -0.331 (p-value: 0.037) between average magnitude of acceleration and Turn time during this balance task. Additionally, there was a correlation of -0.345 (p-value: 0.029) between the same average acceleration data and the Turn To Sit time. Where the PD cohort revealed significant correlations during turning transitions, analysis of the control cohort (n=25) revealed moderate correlations during the walking transitions. A positive correlation of 0.392 (p-value: 0.048) was calculated between the same balance task (B4) and peak acceleration during the Walk 1 portion of the TUG test. There was also a relationship between the fifth balance task (B5)—right foot front tandem stance—and the average acceleration for Walk 1 time (correlation: -0.529; p-value: 0.005), along with peak acceleration for Walk 2 time (correlation: -0.472; p-value: 0.015).

- **Implications**
    - With these observed correlational relationships in two of the balance tasks in this dataset, these results serve as a high level finding to show that the TUG test does in fact have a relationship with balance. Moreover, it demonstrates that analyzing the TUG test according to transition phases can strengthen the assessment of a patient's balance, and consequently, their fall risk and general mobility. Results showed that significant correlations between balance metrics and turning transition times exist in the PD cohort but not in the control cohort. There were also no significant correlations between the total TUG duration and the wearable-derived balance metrics. This suggests that the turn and turn-to-sit transitions may be a stronger indicator of a PD patient's overall balance than the total TUG duration.

Center for Food Safety and Applied Nutrition (CFSAN)

82. **Chang, Dylan**
    - **Abstract title:** Validation of iSentry Microfluidics Devices to Screen Pathogenic Phenotype
    - **Authors:** Chang, Dylan, FDA/CFSAN (Student); Librizzi, Victoria, FDA/CFSAN (Mentor); Sharma, Shashi, FDA/CFSAN (Mentor); Allard, Marc, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - The Center for Food Safety and Applied Nutrition (CFSAN) division of the Food and Drug Administration (FDA) has the responsibility of protecting the nation's public health through preventing food contamination and regulating food safety. CFSAN is partnering with the Defense Applied Research Projects Agency (DARPA) Friend or Foe project to test a novel biosurveillance device that can detect pathogenicity of unknown microorganisms before they become a public health threat. As pathogens have become more widespread due to globalization, climate change, and increasing population density, the need for biosurveillance of undiscovered pathogens that may impact the food supply becomes

greater than ever. The novel microfluidics iSENTRY devices plan to address these challenges through high-throughput sorting of bacterial samples. The iSENTRY features an innovative platform to detect pathogenicity or danger that a microorganism may pose to public health. First, a single bacterial E. coli cell will be encapsulated within a droplet, which after incubating, will give rise to form a colony of identical daughter cells. Then, the iSENTRY will merge a bacterial droplet with a secondary droplet containing mammalian cells to create an enlarged droplet holding both the mammalian cells with a bacterial colony. Once a laser is emitted on the droplet, the iSENTRY will direct the droplet to a "hit" outlet, which indicates toxicity, or "waste" outlet depending on the excitation of fluorescence proteins detected in the mammalian cell after exposure to the bacteria. Currently, CFSAN is testing the functionality of the iSENTRY microfluidic devices using a catalog of strains of E. coli with known pathogenicity to evaluate the accuracy of the iSENTRY sorting system and create a baseline toxicity level for future experimental work involving classifying unknown bacteria as a "friend" or "foe". The DARPA Friend or Foe project and iSENTRY offers a quicker and more accurate alternative to traditional methods of measuring pathogenicity, enabling regulatory agencies to promptly implement necessary preventative measures to combat the threat of emerging pathogens. The high-throughput nature of the iSENTRY has the potential to significantly impact regulatory science by offering a method to determine pathogenicity without prior information, such as biomarkers or genetic reference data that other screening methods rely on.

- **Purpose**
  - The Center for Food Safety and Applied Nutrition(CFSAN) division of the Food and Drug Administration(FDA) has the responsibility of protecting the nation's public health through preventing food contamination and regulating food safety. CFSAN is partnering with the Defense Applied Research Projects Agency (DARPA), Friend or Foe project to test a novel biosurveillance device that can detect pathogenicity of unknown microorganisms before they become a public health threat. As pathogens have become more widespread due to globalization, climate change, and increasing population density, the need for biosurveillance of undiscovered pathogens that may impact the food supply becomes greater than ever. The novel microfluidics iSENTRY devices plan to address these challenges through high-throughput sorting of bacterial samples. By monitoring the toxic effect of bacteria on a mammalian cell line, the iSENTRY can sort the bacteria as a "friend" or "foe". Bacteria marked as "foe" can be further explored through biomarkers or genetic sequencing in order to decide whether preventative measures are needed. Current research is focused on the functionality and performance of classifying known bacteria such as Escherichia coli (E. coli) O157:H7 in order to generate a baseline toxicity level for future tested strains.
- **Methods**
  - The iSENTRY features an innovative platform to detect pathogenicity or danger that a microorganism may pose to public health. First, a single bacterial E. coli cell will be encapsulated within a droplet, which after

incubating, will give rise to form a colony of identical daughter cells. Then, the iSENTRY will merge a bacterial droplet with a secondary droplet containing mammalian cells to create an enlarged droplet holding both the mammalian cells with a bacterial colony. The iSENTRY will direct the droplet to a "hit" outlet, which indicates toxicity, or "waste" outlet depending on the excitation of fluorescence dye detected in dead mammalian cells after exposure to the bacteria. The "hits" will then be dispensed onto an agar plate or can be sequenced and compared to add genotypic context.

- **Results**
  - Currently, CFSAN is testing the functionality of the iSENTRY microfluidic devices using a catalog of strains of E. coli with known pathogenicity to evaluate the accuracy of the iSENTRY sorting system and create a baseline toxicity level for future experimental work involving classifying unknown bacteria as a "friend" or "foe". In this classification, Shiga toxin producing E. coli such as the O157:H7 strain can be identified and can be further investigated using genetic tools such as genome sequencing in order to decide whether public health safety measures should be implemented.
- **Implications**
  - The DARPA Friend or Foe project and iSENTRY offers a quicker and more accurate alternative to traditional methods of measuring pathogenicity, enabling regulatory agencies to promptly implement necessary preventative measures to combat the threat of emerging pathogens. The high-throughput nature of the iSENTRY has the potential to significantly impact regulatory science by offering a method to determine pathogenicity without prior information, such as biomarkers or genetic reference data that other screening methods rely on. Because iSENTRY microfluidic devices only require a mammalian cell line, animal testing often used in regulatory science can also be avoided, reducing the cost and time necessary for pathogenic screening. Once current research is done regarding the functionality of the iSENTRY device, a faster, cheaper, and more accurate alternative to pathogenic screenings of bacteria may be available.

83. **Chegireddy, Nihar**

- **Abstract title:** Enhancement of the Food Application Regulatory Management (FARM) System's Backfiles Utility Tool to Optimize the Searchability of Historical Food Safety Records
- **Authors:** [1]Chegireddy, Nihar (Student); Ahmed, Nafisa, FDA/CFSAN (Contractor); Girmay, Berhane, FDA/CFSAN (Mentor), [1]Joint Institute for Food Safety and Applied Nutrition (JIFSAN), University of Maryland, College Park, MD 20742
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
  - **Synopsis**
    - The Food Application Regulatory Management (FARM) system's Backfiles Utility Tool is designed to optimize the searchability of historical regulatory food safety records to support informed decisions regarding the pre- and post-market safety assessment of food ingredients and packaging materials. The tool allows the user to manually index records; updating metadata such as page numbers,

document authors, and record dates. The volume of files (500K+ document records) and the functional design of the Backfiles Utility Tool makes the manual process a tedious task. The current implementation requires that the user re-download large PDF files multiple times to identify page numbers associated with “child” documents. To alleviate the inefficient manual process, various enhancements were made to the Backfiles Utility Tool that include user interface design and automation steps. New features are also being explored to expedite the process.

- **Purpose**
  - The FARM system contains over 1.2 million documents, with 500K+ legacy files, i.e., historical physical documents scanned into the system with stamped page numbers. After scanning these documents, many stamped page numbers did not match their auto-assigned PDF page numbers. To address this, the FARM team created the Backfiles Utility Tool. This tool allows users to search a document for a stamped page number and enter the PDF page number on which it appears. Once saved, FARM users can select a specific document from a submission’s roadmap (a directory of folders and documents within a submission), and the system will link it to the correct PDF and navigate the user to the correct page. While effective, this tool is inefficient, causing delays despite efforts by various users. The main purpose for enhancing the tool is to reduce the time users spend downloading records and inputting metadata, allowing for the efficient correction of data fields and optimization of the submission’s searchability.
- **Methods**
  - Since the Backfiles Utility Tool is a self-contained module, the enhancements were all updated using native FARM components. The technologies involved are Java Server Faces (JSF), J2EE for the FARM backend, Oracle database, and Documentum Document repository. The FARM User Interface (UI) uses the Prime Faces framework on the presentation layer to create a user-friendly interface with a Java Enterprise Java Beans (EJB) services backend. The Oracle database is where the metadata is stored. The Documentum document repository is a Commercial Off The Shelf (COTS) product to store and manage documents related to the CFSAN OFAS submissions.
- **Results**
  - Enhancement 1: Automatically correct duplicate records
    a. This enhancement allows users to enter the correct PDF page for one record with a duplicate stamped page, and the system automatically updates the PDF page for each additional record with the same stamped page.
  - Enhancement 2: Combine the submission and document detail interface
    a. Users can toggle between viewing all records in a submission or records within a specific page range. This allows the user to load a PDF file only once, enabling multiple records to be updated simultaneously.
  - Enhancement 3: Additional commenting capabilities
    a. This expands capabilities for commenting and recording errors/abnormalities, creating new methods of identifying documents that cannot be satisfactorily updated using the Backfiles Utility Tool.

- Enhancement 4: Export feature
    a. This provides the ability to export Backfile Lists to Excel, which will be useful for potential enhancements with automated functionalities such as Machine Learning and Artificial Intelligence technologies.

- **Implications**
    - Enhancements to the FARM Backfiles Utility Tool have significantly increased the efficiency of curating files. Efficiency has been improved by roughly five times. However, due to the large volume of files and records, future enhancements are being explored. Research is ongoing for potential automation solutions to further increase efficiency. The team is exploring the development of automated text extraction and identification methods (e.g., PyTorch, Range-based OCR) and models to scan documents for stamped pages and create a corresponding link to the original document. Traditional character recognition software often results in inconsistencies, making a Machine Learning model a suitable solution for enhancing file curation. We are also testing a potential feature in which the Backfiles Utility Tool imports a spreadsheet with metadata and automatically updates the corresponding fields in the FARM system.

84. **Clement, Peace**
- **Abstract title:** Comparison of Two Analytical Methods Regarding the Detection of 30 Per- & Polyfluoroalkyl (PFAS) in Eggs
- **Authors:** Clement, Peace, FDA/CFSAN (Student); Genualdi, Susan, FDA/CFSAN (Mentor); DeJager, Lowri, FDA/CFSAN (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
    - **Synopsis**
        - Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals of increasing concern due to their persistence in the environment and potential health impacts. Regulatory agencies like the Food and Drug Administration (FDA) have developed methods to detect and quantify PFAS in commonly consumed foods. This study compares the FDA's single laboratory validated method with a newly developed method, for detecting 30 PFAS and specifically for investigating interferences with perfluorooctane sulfonic acid (PFOS) in eggs. The FDA's current method involves QuEChERS extraction and solid phase extraction (SPE) followed by analysis on a SCIEX 6500+ Triple Quadrupole Mass Spectrometer and serves as a benchmark in this comparison. The new method incorporates a newly developed matrix removal cartridge which lessens the duration while effectively separating the analyte from the matrix. This method uses a SCIEX 7500 Triple Quadrupole Mass Spectrometer. Both methods were evaluated for their accuracy and results were compared across different egg samples. Preliminary results indicate that the new method offers improvements in time efficiency, while the FDA's method remains highly robust. This comparison aims to highlight the strengths and limitations of each method, providing valuable insights for regulatory applications and further method development. The findings suggest that integrating the advanced techniques of the new method could be

applicable in situations where only specific matrices are of interest and provide rapid analysis times.

- **Purpose**
  - Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals of increasing concern due to their persistence in the environment and potential health impacts. Regulatory agencies like the Food and Drug Administration (FDA) have developed methods to detect and quantify PFAS in commonly consumed foods. This study compares the FDA's single laboratory validated method with a newly developed method, for detecting 30 PFAS and specifically for investigating interferences with perfluorooctane sulfonic acid (PFOS) in eggs.
- **Methods**
  - The FDA's current method involves QuEChERS extraction and solid phase extraction (SPE) followed by analysis on a SCIEX 6500+ Triple Quadrupole Mass Spectrometer and serves as a benchmark in this comparison. The new method incorporates a newly developed matrix removal cartridge which lessens the duration while effectively separating the analyte from the matrix. This method uses a SCIEX 7500 Triple Quadrupole Mass Spectrometer. Both methods were evaluated for their accuracy and results were compared across different egg samples.
- **Results**
  - Preliminary results indicate that the new method offers improvements in time efficiency, while the FDA's method remains highly robust. This comparison aims to highlight the strengths and limitations of each method, providing valuable insights for regulatory applications and further method development.
- **Implications**
  - The findings suggest that integrating the advanced techniques of the new method could be applicable in situations where only specific matrices are of interest and provide rapid analysis times.

85. **Duncan, Laishawn**
    - **Abstract title:** *Development of an In-House Baby Food Reference Material*
    - **Authors:** Duncan, Laishawn, FDA/CFSAN (Student); Barber, Chuck, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - FDA's Closer to Zero approach is to reduce the risk of dietary exposure to contaminants. To achieve this, measurement of contaminants with matrix-matched reference materials are essential. A matrix-matched reference material is used to improve measurement accuracy, verify the results of analytical measurements, and validate analytical methods. There are no matrix-matched reference materials for thallium; therefore, CFSAN is working with NIST to develop an in-house kale/beet powder reference material. The method used for elemental analysis consisted of using microwave assisted digestion for decomposing samples, inductively coupled plasma mass spectrometry (ICP-MS) for quantification, and direct mercury analyzer (DMA) for analytical comparison.

- **Purpose**
  - Arsenic, lead, cadmium, thallium, and mercury occur in the environment and in some foods naturally. The FDA regulates toxic elements in foods and aims to reduce consumer dietary exposure, with a special emphasis on foods commonly eaten by babies and young children as their smaller metabolisms and body masses put them at a higher risk. FDA's Closer to Zero Action Plan aims to reduce dietary exposure to contaminants to as low as possible. To improve measurement accuracy, verify the results of analytical measurements, and validate analytical methods, well characterized matrix-matched quality control materials are essential. Thallium is a toxic element of emerging concern, but current food reference materials do not include thallium with certified values. Along with the National Institute of Standards and Technology, CFSAN is developing an in-house, matrix-matched reference material for arsenic, lead, cadmium, thallium, and mercury.
- **Methods**
  - The ten bottles of the in-house kale/beet powder reference material analyzed were a stratified random set based on packaging order and duplicate 0.5 g aliquots were taken from each bottle. The analytical measurements were completed using microwave-assisted acid digestion and inductively coupled plasma mass spectrometry (ICP-MS). The mass fraction of each analyte was determined by a method of standard additions. In addition, Hg was determined by direct mercury analyzer (DMA) for use as an analytical comparison. The in-house kale/beet powder reference material and quality control materials, NIST SRM 1568b Rice Flour, and NIST SRM 1643f Trace Elements in Water were analyzed by ICP-MS with standard additions, and NIST SRM 1568b Rice Flour and NIST SRM 1515 Apple Leaves were used as quality controls for DMA measurements.
- **Results**
  - The in-house reference materials mass faction values were 98.1 ± 3.13 ng/g As (RSD of 3 %), 180 ± 4.1 ng/g Cd (RSD of 2 %), 159.4 ± 36.3 ng/g Pb (RSD of 23 %), 35.7 ± 1.0 ng/g Hg (RSD of 3 %), and 949 ± 16 ng/g Tl (RSD of 2 %). The high RSD for Pb measurements were due to sample inhomogeneity across the production lot. The DMA mass fraction results for Hg were 38.3 ± 1.7 ng/g Hg (RSD of 4 %) which were in agreement with the ICP-MS mass fractions.
- **Implications**
  - Reference materials allow researchers to validate methods, verify laboratory performance, and assess the accuracy of their results. The goal for developing a characterized, matrix-matched reference material is to provide quality controls that closely match the sample composition (fat, protein, carbohydrates) thus experiencing similar matrix effects during analysis. FDA's Closer to Zero Action Plan to reduce dietary exposure of contaminants in food requires an iterative approach to achieve continual improvements over time. Analytical methods and data collection are involved in all 4 stages of the FDA's approach. Reference materials play a significant role to ensure measurement accuracy when collecting data to reduce dietary exposure to contaminants.

86. **Irizawa, Sachi**

- **Abstract title:** Development of a Sequencing-Based Strategy as a Confirmatory Method for Detection of Cyclospora cayetanensis
- **Authors:** Sachi Iriawa, FDA/CFSAN (Student); Laura Ewing-Peeples FDA CFSAN, Susan Leonard FDA CFSAN, Mark Mammel FDA CFSAN, Mauricio Durigan FDA CFSAN (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
    - **Synopsis**
        - Cyclospora cayetanensis is a protozoan parasite that causes foodborne and waterborne outbreaks of a diarrheal illness named cyclosporiasis. A new detection method based on the mitochondrial genome was developed. Two targets designed for detection by conventional PCR, Mit3 and Mit4cox1, were compared with the currently validated real-time PCR method for detection. The Mit3 and Mit4cox1 targets were designed with the goal of sequencing the PCR amplicons of positive samples even when the amount of input DNA is very low. The new detection method presented a similar sensitivity as the real-time PCR. The new marker, Mit4cox1 is more sensitive and more specific than the previously published marker Mit3
    - **Purpose**
        - Cyclospora cayetanensisis a coccidian parasite that causes a gastrointestinal illness called Cyclosporiasis. This parasite is an important cause of foodborne outbreaks and is associated with the consumption of fresh produce and contaminated water. The development of specific and sensitive detection methods for C. Cayetanensis is crucial to effectively address data gaps and provide regulatory support during outbreak investigations. The inclusion of new C. cayetanensis genome sequences in public databases has enabled the exploration of multicopy mitochondrial genome-based markers and expanded screening strategies with sensitive and more specific molecular markers. The aim of this study was to develop a sequence-based confirmatory method for the detection of Cyclospora cayetanensis in addition to Mit1C and to compare it with previous published methods.
    - **Methods**
        - In this study, DNA purified from fecal clinical samples was used. First, 34 samples were tested using the Mit1C real-time PCR method for the detection of C. cayetanensis. Then, two conventional PCR reactions, targeting molecular markers Mit3 (previously published) and Mit4cox1, were performed using a touchdown-PCR protocol. The Mit4cox1 target was determinedin silico to have greater sequence specificity for C. cayetanensis than Mit3. Amplicons were visualized in the QIAxcel Advanced System, then purified using the QIAquick PCR Purification Kit, and concentrations were obtained with Qubit. All samples underwent Sanger-sequencing, were assembled using SeqMan Ultra 17, and subjected to phylogenetic analysis against reference sequences utilizing MEGA11. To maximize the chances of obtaining high-quality assembled sequences, two forward and two reverse sequences were performed in all samples with Ct values ≥ 30.
    - **Results**
        - Utilizing the validated Mit1C real-time PCR method for C. cayetanensis

detection, all 34 samples yielded positive results, with Ct values between 28-38. Sample DNA concentrations ranged from 0.176 to 23 ng/µl. High-quality sequences were obtained from the PCR amplicons even when no band was observed in QIAxcel. C. cayetanensis sequence confirmation was obtained for 25 and 29 samples using the Mit3 and Mit4cox1 targets, respectively. Phylogenetic analysis provided further confirmation that the Mit4cox1 sequences were specific to C. cayetanensis.

- **Implications**
  - Complementary methods can be used to support findings using the C. cayetanensis regulatory method; however, these methods should be based on different gene targets and/or different molecular detection technologies that provide further information. In this study, we were able to provide sequence confirmation using a new mitochondrial genome target of clinical samples that were positive for C. cayetanensis by the standard Mit1C detection method (BAM Chapter 19c). The new marker, Mit4cox1, is more sensitive and more specific than the previously published Mit3 marker. PCR using Mit4cox1 resulted in higher quality sequences, which led to more successful assembly. The new detection method presented a similar sensitivity as the currently validated method based on real-time PCR.

87. **Jiang, Selena**
    - **Abstract title:** Determination of Type B trichothecenes in cereals using LC-MS/MS
    - **Authors:** Jiang, Selena, FDA/CFSAN (Student); Zhang, Kai FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - Mycotoxins are compounds that are produced by certain fungi that are toxic to both humans and animals. Various cereal grains, such as barley, wheat, oats, and maize, are susceptible to mycotoxin contamination and can often be contaminated with multiple mycotoxins at the same time. Using LC-MS/MS to separate, identify, and determine multiple mycotoxins in the food matrix would be time-saving in regulating the presence of mycotoxins in food matrices as this would allow for the analysis of multiple mycotoxins simultaneously. Additionally, using 13C mycotoxin standards to perform stable isotope dilution allows for a simple LC-MS/MS procedure, making it more user-friendly and allowing for easier replication.
      - **Purpose**
        - The objective of the study is to develop and validate a method that can determine five type B trichothecenes, nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and deoxynivalenol-3-glucoside, in rice-, multigrain-, and corn-based baby foods. The developed method will be used for a quantitative occurrence study of these analytes in cereal products.
      - **Methods**
        - Samples were fortified with five corresponding isotopically labeled 13C mycotoxin standards (13C-IS) and then extracted with acetonitrile/water, centrifuged, filtered, and/or concentrated, followed by LC-MS/MS analysis. The method development and validation include

the analysis of rice-, corn-, and multigrain-based cereals fortified with the five mycotoxins at concentrations ranging from 20 to 1000 ng/g. The method performance will be evaluated using accuracy, precision, and within- and between-matrix variability as well as analysis of certified matrix reference materials.

- **Results**
  - Preliminary data show that the majority of analyte recoveries range from 80 to 120% with relative standard derivations (RSDs) <20%. We expect that using 13C-IS eliminated the need for matrix-matched calibration standards for quantitation, simplified sample preparation, and achieved simultaneous identification and quantitation of multiple mycotoxins in a simple LC-MS/MS procedure.
- **Implications**
  - An important aspect of the FDA Mycotoxin Program is the analysis of various food products for the purpose of collecting prevalence data. There are limited data on the degree of type B trichothecenes contamination in U.S. cereals products, particularly the presence of deoxynivalenol and its modified forms (e.g., 3/15A-DON) in cereal products, making it difficult to determine whether there are mycotoxin-related risks associated with the consumption of these food products. The development of the method and subsequent occurrence study will be used to collect data for exposure and risk assessment purposes.

88. **Li, Jolie**
    - **Abstract title:** Development of Rapid Species-Specific Molecular Methods for Detecting Cronobacter from Critical Foods and Environmental Samples
    - **Authors:** Li, Jolie, FDA/CFSAN (Student); Mammel, Mark, FDA/CFSAN; Kwon, Hee Jin; Deng, Xiaohong; Pillai, Segaran; Binet, Rachel; Chen, Yi; Gopinath, Gopal (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - Mycotoxins are compounds that are produced by certain fungi that are toxic to both humans and animals. Various cereal grains, such as barley, wheat, oats, and maize, are susceptible to mycotoxin contamination and can often be contaminated with multiple mycotoxins at the same time. Using LC-MS/MS to separate, identify, and determine multiple mycotoxins in the food matrix would be time-saving in regulating the presence of mycotoxins in food matrices as this would allow for the analysis of multiple mycotoxins simultaneously. Additionally, using 13C mycotoxin standards to perform stable isotope dilution allows for a simple LC-MS/MS procedure, making it more user-friendly and allowing for easier replication.
      - **Purpose**
        - The objective of the study is to develop and validate a method that can determine five type B trichothecenes, nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and deoxynivalenol-3-glucoside, in rice-, multigrain-, and corn-based baby foods. The developed method will be used for a quantitative occurrence study of these analytes in cereal products.
      - **Methods**
        - Samples were fortified with five corresponding isotopically labeled 13C

mycotoxin standards (13C-IS) and then extracted with acetonitrile/water, centrifuged, filtered, and/or concentrated, followed by LC-MS/MS analysis. The method development and validation include the analysis of rice-, corn-, and multigrain-based cereals fortified with the five mycotoxins at concentrations ranging from 20 to 1000 ng/g. The method performance will be evaluated using accuracy, precision, and within- and between-matrix variability as well as analysis of certified matrix reference materials.

- **Results**
  - Preliminary data show that the majority of analyte recoveries range from 80 to 120% with relative standard derivations (RSDs) <20%. We expect that using 13C-IS eliminated the need for matrix-matched calibration standards for quantitation, simplified sample preparation, and achieved simultaneous identification and quantitation of multiple mycotoxins in a simple LC-MS/MS procedure.
- **Implications**
  - An important aspect of the FDA Mycotoxin Program is the analysis of various food products for the purpose of collecting prevalence data. There are limited data on the degree of type B trichothecenes contamination in U.S. cereals products, particularly the presence of deoxynivalenol and its modified forms (e.g., 3/15A-DON) in cereal products, making it difficult to determine whether there are mycotoxin-related risks associated with the consumption of these food products. The development of the method and subsequent occurrence study will be used to collect data for exposure and risk assessment purposes.

89. **Lin, Jefferny**
- **Abstract title:** Evaluation of Neogen® Molecular Detection Assay for Rapid Screening of Cronobacter
- **Authors:** Lin, Jefferny, FDA/CFSAN (Student); Kwon, Hee Jin, FDA/CFSAN (Mentor); Wang, Aidan, FDA/CFSAN (Student); Deng, Xiaohong, FDA/CFSAN (Mentor); Chen, Yi, FDA/CFSAN (Mentor)
- **FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
- **Abstract:**
  - **Synopsis**
    - Mycotoxins are compounds that are produced by certain fungi that are toxic to both humans and animals. Various cereal grains, such as barley, wheat, oats, and maize, are susceptible to mycotoxin contamination and can often be contaminated with multiple mycotoxins at the same time. Using LC-MS/MS to separate, identify, and determine multiple mycotoxins in the food matrix would be timesaving in regulating the presence of mycotoxins in food matrices as this would allow for the analysis of multiple mycotoxins simultaneously. Additionally, using 13C mycotoxin standards to perform stable isotope dilution allows for a simple LC-MS/MS procedure, making it more user-friendly and allowing for easier replication.
  - **Purpose**
    - The objective of the study is to develop and validate a method that can determine five type B trichothecenes, nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and deoxynivalenol-3-glucoside, in rice-, multigrain-, and corn-based baby foods. The

developed method will be used for a quantitative occurrence study of these analytes in cereal products.

- **Methods**
  - Samples were fortified with five corresponding isotopically labeled 13C mycotoxin standards (13C-IS) and then extracted with acetonitrile/water, centrifuged, filtered, and/or concentrated, followed by LC-MS/MS analysis. The method development and validation include the analysis of rice-, corn-, and multigrain-based cereals fortified with the five mycotoxins at concentrations ranging from 20 to 1000 ng/g. The method performance will be evaluated using accuracy, precision, and within- and between-matrix variability as well as analysis of certified matrix reference materials.
- **Results**
  - Preliminary data show that the majority of analyte recoveries range from 80 to 120% with relative standard derivations (RSDs) <20%. We expect that using 13C-IS eliminated the need for matrix-matched calibration standards for quantitation, simplified sample preparation, and achieved simultaneous identification and quantitation of multiple mycotoxins in a simple LC-MS/MS procedure.
- **Implications**
  - An important aspect of the FDA Mycotoxin Program is the analysis of various food products for the purpose of collecting prevalence data. There are limited data on the degree of type B trichothecenes contamination in U.S. cereals products, particularly the presence of deoxynivalenol and its modified forms (e.g., 3/15A-DON) in cereal products, making it difficult to determine whether there are mycotoxin-related risks associated with the consumption of these food products. The development of the method and subsequent occurrence study will be used to collect data for exposure and risk assessment purposes.

90. **Khanna, Saara**
- **Abstract title:** The Determination of Melatonin and its Metabolites in Dietary Supplements
- **Authors:** Khanna, Saara, FDA/CFSAN (Student); Coppin, Julia, FDA/CFSAN (Mentor); Pawar, Rahul, FDA/CFSAN (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
  - **Synopsis**
    - The goal of the work presented in this poster was to use a targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure the melatonin content and its metabolites N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), and N1-acetyl-5-methoxykynuramine (AMK) in products marketed as dietary supplements in the US, containing melatonin.
  - **Purpose**
    - In the US, products containing melatonin are marketed as dietary supplements and sold primarily as a sleeping aid for children and adults. These products are available to consumers through conventional and online retailers and are offered in a variety of forms, such as tablets, capsules, and gummies. The goal of the work presented in this poster was to use a targeted liquid chromatography-tandem mass

spectrometry (LC-MS/MS) method to measure the melatonin content and its metabolites N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), and N1-acetyl-5-methoxykynuramine (AMK) in these products. A stability study was also conducted to examine potential degradation of melatonin in tested products over a set timeframe.

- **Methods**
  - In preparation for extraction of the products labeled as dietary supplements, approximately 10 pieces of the gummy, tablet, or other edible products were weighed, frozen overnight at -80 oC, and then composited using an IKA MT 40 grinding chamber until the sample was ground into a fine powder. For capsules, the contents of 10 capsules were emptied and mixed. For extraction, 0.5 ± 0.05 g of sample was weighed in a test tube, 5.0 mL of water and 10.0 mL of acetonitrile were added, and then the tubes were vortexed for approximately15 seconds. Test tubes were shaken for 3 minutes at 1700 rpm, QuEChERS salts were added, and then the tubes were shaken again for 3 minutes at 1700 rpm. The tubes were centrifuged for 5 minutes for collection of the supernatant extract. Extracts were then diluted, filtered, and placed in autosampler vials for LC-MS/MS analysis. One-hundred ten (110) products labeled as supplements were analyzed for the quantitation of melatonin, AFMK, and AMK. For the stability study, 27 of the 110 products were selected based on product type, melatonin label claim, and expiration date. Samples were composited and reanalyzed every three (3) months.
- **Results**
  - The QuEChERS extraction and LC-MS/MS method was validated for melatonin, AFMK, and AMK with spike recoveries between 80-135%. In this survey study, 110 products marketed as dietary supplements labeled to contain melatonin and intended for children were randomly selected and analyzed to quantitatively determine the presence of each analyte in matrices including gummies, liquids, capsules, tablets, and miscellaneous edibles. Melatonin was identified in 108 of 110 products with concentrations ranging between 0.017 – 130 mg/g (0.042 – 50 mg/serving) or 0 – 667% of the label claim. In tested products, melatonin content was measured to be greater than product label claims in 27 of 54 (50%) of gummy products and 4 of 4 (100%) other edible products. To date, data collected on a sub-set of products do not suggest significant degradation of melatonin over time.
- **Implications**
  - The determination of melatonin content in this study highlights the need for quality control and accurate labeling in dietary supplements.

91. **Moreno-Santiago, Maria**
    - **Abstract title:** Single Laboratory Validation and Optimization of the FDA's Method for PFAS in food regarding Cholic Acids Interferences
    - **Authors:** Maria Moreno Santiago, FDA/CFSAN (Student); Susan Genualdi, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative**: Public Health Emergency Preparedness and Response
    - **Abstract:**
      - **Synopsis**
        - Cholic acid compounds, which can be found in food matrices such as

egg, beef liver, and seafood, present a challenge as they interfere in the analysis of perfluorooctane sulfonic acid (PFOS). Taurodeoxycholic Acid (TDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA), are bile acids prevalently found in these matrices. Because of their interference with PFOS at the m/z 499 → 80 transition, these can lead to inaccurate quantification results. For this reason, it is important to eliminate the interference of cholic acids to minimize any erroneous data interpretations. In this project, the FDA's method for PFAS in food was optimized to eliminate cholic acids interferences and validated through a single laboratory validation for specific food matrices. This was done by incorporating a hybridized weak anion exchange (WAX)/graphitized carbon black Solid Phase Extraction (SPE) cartridge to ensure the removal of cholic acids during sample preparation. Samples from three food matrices (eggs, liver, and cod) were extracted using the new hybridized cartridge in place of the WAX cartridge in the current FDA method. The analysis of PFAS components and monitoring of cholic acid interferences in these matrices was performed using liquid chromatography/mass spectrometry. This presentation will discuss the outcomes, challenges, and results associated with this project.

- **Purpose**
  - Optimize the FDA's method for PFAS in food to minimize cholic acids interferences and validate the new modification through a single laboratory validation for specific food matrices.
- **Methods**
  - Samples from three food matrices (eggs, liver, and cod) were extracted using a hybridized weak anion exchange (WAX)/graphitized carbon black Solid Phase Extraction (SPE) cartridge in place of the WAX cartridge in the current FDA method. The analysis of PFAS components and monitoring of cholic acid interferences in these matrices was performed using liquid chromatography/mass spectrometry.
- **Results**
  - Cholic acid interferences were eliminated using the new cartridge and the method was validated for three food matrices (eggs, liver, and cod).
- **Implications**
  - Validation of an optimized method for PFAS in eggs, liver, and cod through the use of the new hybridized cartridge will remove cholic acid interferences and reduce analysis time by combining two separate clean-up steps into one.

92. **Parimi, Manasvini**
    - **Abstract title:** Gluten quantitation in fermented sourdough using a multiplex-competitive ELISA
    - **Authors:** Parimi, Manasvini, FDA/CFSAN/JIFSAN (Student); Galanis, Christina, FDA/CFSAN (Student); Panda, Rakhi, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Empowering Patients and Consumers
    - **Abstract:**
      - **Synopsis**
        - Gluten, found in wheat, barley, and rye, is avoided by individuals with celiac disease due to adverse effects caused by the consumption of gluten. Regulations set in place by the FDA allow gluten-free products to

be labeled properly. Accurate quantitation of gluten, especially in fermented and hydrolyzed products, is necessary to comply with the requirements. A multiplex-competitive ELISA was used to quantify gluten in fermented sourdough. The sourdough was prepared by combining different starters with rice flour and water, and then incurring with varying amounts of gluten. Sourdough was then fermented for 72 hours, and samples were taken every 24 hours for analysis. Samples were analyzed using the multiplex-competitive ELISA with a gluten-incurred yogurt calibrant, and six gluten-specific antibodies. The average % gluten recovery, and % CV (coefficient of variation) were calculated from the results. Average gluten recovery was between 55-195%, and the % CV was between 2-31%, with most samples resulting in % CV of ≤ 20%. The multiplex-competitive ELISA can provide accurate and precise quantitation of wheat gluten in fermented sourdough prepared with different starter cultures. Effects of heat treatment and several other variations in sourdough preparation on quantitation are currently being evaluated.

- **Purpose**
  - Gluten is a protein found in wheat, barley, and rye which can cause adverse reactions in individuals with celiac disease. As a precaution, these individuals need to follow a strict gluten-free diet. There are FDA regulations in place that define gluten-free foods. Accurate quantification of gluten in different foods, including fermented and hydrolyzed foods, is necessary to comply with these regulations. In this study, we report a multiplex-competitive ELISA for the quantification of gluten in fermented sourdough.
- **Methods**
  - Gluten-incurred sourdough was prepared using four types of sourdough starters. The starter cultures were combined with rice flour and water. Then, the mixtures were incurred with 8, 20, and 100 ppm wheat gluten, and fermented for 72 hrs. Samples were collected every 24 hours for analysis. A multiplex-competitive ELISA was used to analyze the samples, using a gluten-incurred yogurt calibrant, and six gluten-specific antibodies (G12, R5, Total gluten, 2D4, MIoBS, and Skerritt). Calibration curves were established using a four-parameter logistic (4 PL) regression, and the gluten concentrations in the samples were estimated from the curves. The average % gluten recovery and % CV (coefficient of variation) were calculated from the analysis results.
- **Results**
  - Based on the antibody reactivity profiles, average gluten concentration values of the MIoBS and the Total gluten antibodies were used as the final gluten concentration for the samples. The average gluten recovery was between 55-195% for all fermented samples. The % CV ranged between 2-31%, with most samples resulting in % CV of ≤ 20%. These values are consistent with the acceptance criteria (50-200% recovery, and ≤ 20% CV) of AOAC SMPR 2017.021(quantification of gluten in oats).
- **Implications**
  - The multiplex-competitive ELISA can provide accurate and precise quantitation of wheat gluten in fermented sourdough prepared with different starter cultures. The effects of heat treatment and several other variations in sourdough preparation on quantitation are currently

being evaluated.

93. **Reichard, Grace**
    - **Abstract title:** Determination of mycotoxins in tree nuts, confectionery and spices using LC-MS/MS – a matrix extension study
    - **Authors:** Reichard, Grace, FDA/CFSAN (Student); Zhang, Kai, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
    - **Abstract:**
        - **Synopsis**
            - Gluten, found in wheat, barley, and rye, is avoided by individuals with celiac disease due to adverse effects caused by the consumption of gluten. Regulations set in place by the FDA allow gluten-free products to be labeled properly. Accurate quantitation of gluten, especially in fermented and hydrolyzed products, is necessary to comply with the requirements. A multiplex-competitive ELISA was used to quantify gluten in fermented sourdough. The sourdough was prepared by combining different starters with rice flour and water, and then incurring with varying amounts of gluten. Sourdough was then fermented for 72 hours, and samples were taken every 24 hours for analysis. Samples were analyzed using the multiplex-competitive ELISA with a gluten-incurred yogurt calibrant, and six gluten-specific antibodies. The average % gluten recovery, and % CV (coefficient of variation) were calculated from the results. Average gluten recovery was between 55-195%, and the % CV was between 2-31%, with most samples resulting in % CV of ≤ 20%. The multiplex-competitive ELISA can provide accurate and precise quantitation of wheat gluten in fermented sourdough prepared with different starter cultures. Effects of heat treatment and several other variations in sourdough preparation on quantitation are currently being evaluated.
        - **Purpose**
            - Gluten is a protein found in wheat, barley, and rye which can cause adverse reactions in individuals with celiac disease. As a precaution, these individuals need to follow a strict gluten-free diet. There are FDA regulations in place that define gluten-free foods. Accurate quantification of gluten in different foods, including fermented and hydrolyzed foods, is necessary to comply with these regulations. In this study, we report a multiplex-competitive ELISA for the quantification of gluten in fermented sourdough.
        - **Methods**
            - Gluten-incurred sourdough was prepared using four types of sourdough starters. The starter cultures were combined with rice flour and water. Then, the mixtures were incurred with 8, 20, and 100 ppm wheat gluten, and fermented for 72 hrs. Samples were collected every 24 hours for analysis. A multiplex-competitive ELISA was used to analyze the samples, using a gluten-incurred yogurt calibrant, and six gluten-specific antibodies (G12, R5, Total gluten, 2D4, MIoBS, and Skerritt). Calibration curves were established using a four-parameter logistic (4 PL) regression, and the gluten concentrations in the samples were estimated from the curves. The average % gluten recovery and % CV (coefficient of variation) were calculated from the analysis results.

- **Results**
    - Based on the antibody reactivity profiles, average gluten concentration values of the MIoBS and the Total gluten antibodies were used as the final gluten concentration for the samples. The average gluten recovery was between 55-195% for all fermented samples. The % CV ranged between 2-31%, with most samples resulting in % CV of ≤ 20%. These values are consistent with the acceptance criteria (50-200% recovery, and ≤ 20% CV) of AOAC SMPR 2017.021(quantification of gluten in oats).
- **Implications**
    - The multiplex-competitive ELISA can provide accurate and precise quantitation of wheat gluten in fermented sourdough prepared with different starter cultures. The effects of heat treatment and several other variations in sourdough preparation on quantitation are currently being evaluated.

94. **Khuda, Sefat E. (Mentor) and Xi, Cynthia (Student)**
    - **Abstract title:** Establishment of co-culture models of the human intestinal epithelium to assess gut barrier functions after exposure to emulsifiers and live microbials
    - **Authors:** Tartera, Carmen, CFSAN; Xi, Cynthia, JIFSAN (Student); Sawyer, Marianne, CFSAN; Pereira, Marion, CFSAN; Bigley, Elmer, CFSAN; Balan, Kannan, CFSAN; Alonso-Claudio, Almaris, CFSAN; Khuda, Sefat, CFSAN (Mentor).
    - **FDA Strategic Initiative:** Empowering Patients and Consumers
    - **Abstract:**
        - **Synopsis**
            - Dietary components have the potential to regulate gut barrier integrity and functions. The interaction of food components (emulsifiers and live microbials) with the intestinal epithelium and the subsequent responses have not been thoroughly evaluated.
        - **Purpose**
            - To establish co-culture models that simulate the intestinal epithelium for better assessing the responses to mixed-food components.
        - **Methods**
            - Human intestinal epithelial cells (Caco-2: goblet HT-29-MTX) were mixed to represent small (90:10) and large (75:25) intestinal barriers. Monolayers of Caco-2, HT-29-MTX, and co-cultures were treated with emulsifier polysorbate (P)-80, and live microbials: Lactobacillus acidophilus (LA), Streptococcus thermophilus (ST), and Bacillus subtilis (BS). The trans-epithelial electrical resistance (TEER), lucifer yellow flux, cellular viability, and expression of biomarkers (tight junction, mucin, and cytokines genes) were examined.
        - **Results**
            - Upon treatment with live microbials at a multiplicity of infection (MOI) of 10:1 and 20:1, the TEER values for all monolayers markedly increased with LA but did not change with ST or BS. (P)-80 (0.001%-0.2%) alone resulted in a dose dependent decrease of TEER in treated Caco-2 and 90:10 mixed monolayers, while HT-29-MTX and 75:25 mixed monolayers were less responsive, as shown by lucifer yellow flux and cell proliferation. Following recovery experiments, the integrity of monolayers was restored with proliferating cells. Except for claudins, there were no significant differences in the expression of the tight junction genes of microbials treated co-culture monolayers. Differential

expression of mucin and cytokine genes were observed between microbial treatments in co-culture models. Noticeably proinflammatory cytokine CXCL8 was downregulated when treated with LA in both co-culture models.

- **Implications**
    - Preliminary data indicate that presence of live microbials do not adversely affect the integrity and functions of the epithelium but there are differences in their potencies. These established co-culture models allow us to further investigate the impact of live microbials combined with emulsifiers on the gut mucosal responses.

95. **Zhang, Anya**
    - **Abstract title:** Database for Skin Permeability of Chemicals and the Interface of Predictive Models
    - **Authors:** Zhang, Anya, FDA/CFSAN (Student); Vo, Elis, (Student); Li, Miao, FDA/CFSAN (Mentor); An, Nan, FDA/CFSAN (Mentor); Fairman, Kiara, FDA/NCTR (Mentor); Zang, Janet, FDA/CFSAN (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - Prediction of absorption and disposition of chemicals through skin into systemic circulation is an essential step for health risk assessment of cosmetic ingredients with dermal routes of exposure, and skin permeability is a key parameter in this exercise. Office of Cosmetics and Colors (OCAC) is investigating a New Approach Methods (NAMs) application to assess skin permeability in a risk assessment of cosmetic ingredients. This approach includes establishing a database of skin permeation data and a repository of skin permeability predictive models to support dermal exposure assessment of cosmetic ingredients and potential impurities in cosmetic products. Based on published literature, a complete repository of skin permeability data of over 260 chemicals were compiled. A database written in R was created, equipped with a graphical user interface with Rshiny. The database displays the collected data alongside an interface to predict skin permeability of a chemical using various publicly available models, including Mitragotri, Potts-Guy, Barratt, and Abraham. Currently, we are working on converting the database from R to Python to utilize Python's AI/ML tools. Such a tool would enhance the performance of OCAC's ability to dynamically select the best model available for a given cosmetic ingredient, as there is no single model universally appropriate in predicting skin permeability for all chemicals. Using NAMs to accurately predict skin permeability will be more time-sensitive and cost-efficient than in vivo testing and would align with the "sense of the Congress" stated in the Modernization of Cosmetics Regulation Act of 2022 (MoCRA) that animal testing should not be used for the purposes of safety testing on cosmetic products, except when appropriate.
        - **Purpose**
            - Prediction of absorption and disposition of chemicals through skin into systemic circulation is an essential step for health risk assessment of cosmetic ingredients with dermal routes of exposure, and skin permeability is a key parameter in this exercise. While in vivo testing is

the ideal method for determining skin permeability, FDA is strongly committed to reducing use of animal testing, and replacing it with alternative methods when they are available, further the Modernization of Cosmetics Regulation Act of 2022 (MoCRA) states that it is the sense of Congress that animal testing should not be used for safety testing of cosmetic products with some appropriate allowances. Therefore, it is critical to develop and validate New Approach Methods (NAMs) for skin permeability of chemicals. Predictive modeling of transdermal absorption has evolved since the early 1940s, leveraging computational models and various algorithms. To enhance our understanding, a well-curated database containing permeability data for chemicals relevant to risk assessment of cosmetic products would be valuable. Therefore, a database of skin permeation data and a repository of skin permeability predictive models would support dermal exposure assessment of cosmetic ingredients and potential impurities in cosmetic products, which would effectively reduce the need for animal testing.

- **Methods**
  - The objective of this study is to establish a database for skin permeability of chemicals, which includes collection of skin permeability data from published literature, curation of the data obtained, and organization of these data into a single repository. Additionally, we established a repository of various mathematical models for skin permeability prediction. Existing models have been reviewed to develop a repository with model equations in R. A user-friendly interface has been developed for the database and repository of current models. Following the establishment of the database and repository of current models, we will use several Artificial Intelligence/Machine Learning (AI/ML) models to train on skin permeability data to predict skin permeability directly or to identify the best models to predict chemical skin permeability. Appropriate AI/ML algorithms will be applied for chemical skin permeability, and the predictions will be compared with mathematical and mechanistic models.
- **Results**
  - As of now, we have successfully reviewed published literature to compile a repository with over 1700 lines of skin permeability data of various chemicals. This dataset includes a compound's octanol-water partition coefficient (LogP), molecular weight (MW), number of hydrogen bonds (Hb), molecular volume (MV), melting point (MPt), solute hydrogen bond acidity ($\Sigma\alpha 2H$), solute hydrogen bond basicity ($\Sigma\beta 2H$), solute dipolarity/polarizability ($\pi 2H$), McGowan characteristic molecular volume (Vx), and excess molar refraction (R2). With the collected data we were able to establish a database and create a graphical user interface (GUI) with Rshiny, a R library to develop GUI. Users can sort and display data by reference or by specific compounds. Additionally, the interface also has a feature to predict skin permeability of a chemical with existing mathematical models including Mitragotri, Potts-Guy, Barratt, and Abraham. Currently, we are working on converting the database from R to Python to utilize AI and ML tools. The Python database has the same basic functionality as R and utilizes TKinter, a python toolkit to develop GUI, to create a user interface.

- **Implications**
    - Compiling skin permeability data into a single repository streamlines the process of predicting skin permeability of a chemical using existing models. The reliable and robust skin permeability data will be helpful in predicting the dermal exposure to cosmetic ingredients. However, due to the variability in compound structures, there is no single model universally appropriate in predicting the skin permeability for all chemicals. This can be combatted with the introduction of AI/ML into skin permeability research. The AI/ML can provide predictions directly or it can dynamically select the best model for different chemicals. Developing and validating NAMs for skin permeability will reduce reliance on in vivo testing, aligning with the sense of what Congress stated for cosmetic ingredient safety with MoCRA.

Center for Veterinary Medicine (CVM)

96. **Garcia, Nina**
    - **Abstract title:** Developing inventory databases for analytical methods used in animal food ingredient submissions, and in medicated animal feed
    - **Authors:** Garcia, Nina, FDA/CVM (Student)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **Synopsis**
            - The CMC/Technical Additives Branch in DAFI provides scientific assessments on the CMC section of new animal food ingredients and new uses of existing ingredients in regulatory submissions. This project has two objects, 1) to develop an inventory database of analytical methods submitted as part of the regulatory submissions to DAFI, 2) to convert historic paper records of medicated assay methods to digital form and develop an inventory database of such methods.
        - **Purpose**
            - The Division of Animal Food Ingredients (DAFI) in the Center for Veterinary Medicine (CVM) is responsible for the pre-market approval of ingredients to be used in animal food. DAFI reviews different types of submissions, such as Food Additive Petitions (FAP), Generally Recognized As Safe (GRAS) notices, and AAFCO Feed Ingredient Definition (FID) requests. As part of the scientific assessments of these submissions, the Chemistry, Manufacturing and Controls (CMC)/Technical Additive Branch reviews the analytical methods used to determine the identity, specifications, quality (that includes any potential impurities and contaminants), stability, and mixability of the ingredient, and for some instances the methods used to demonstrate the utility and safety of the ingredient. The CMC/Technical Additive Branch also manages a repository of analytical methods used to measure the animal drugs in medicated feeds and provides relevant method information in response to public requests through the Freedom of Information Act (FOIA). This internship project has two objectives, 1) to develop an inventory database of analytical methods submitted as part of FAP submissions, 2) to convert historic paper records of medicated feed assay methods to digital form and develop an inventory database of these methods.

- **Methods**
  - This project requires the intern to read through all FAPs and compile the data and information relevant to the analytical methods provided in the submissions. First, using CVM's Corporate Database Portal (CDP), submissions are filtered with the criteria “FAP” and “Approved”. There are 33 approved FAPs. The analytical method information submitted as part of each FAP are collected and entered into an inventory database. The information used to generate the database include the name of the analytical method, applicable sample matrices, brief description of the method procedure, validation parameters such as linearity, range, limit of detection (LOD), limit of quantification (LOQ), and any limitations of the use. Also, a library of various compendial methods used in these FAPs is created. To achieve the second objective of the project, historical paper records of analytical methods used to measure animal drugs in medicated feeds were converted to digital format to be stored in DAFI’s Common Automated Submission Exchange and Reporting (CASPER) system.
- **Results**
  - This project will result in a completed database of analytical methods from all 33 FAPs with the “Approved” Status. The database contains analytical method information provided in FAPs for various types of food additives such as omega-3-fatty acids, minerals, chemical preservatives, feed acidifiers etc. This analytical method inventory database consists of different types of analytical methods such as inductively coupled plasma mass spectrometry (ICP-MS), high-pressure liquid chromatography (HPLC), gas chromatography (GC), Fourier transform infrared spectroscopy (FTIR), mass spectrometry (MS), and microbiological methods that were used to establish the identity, composition, specifications, and to analyze potential impurities and contaminants of various food additives in these 33 FAPs. The database also includes links to the compendial methods used in these FAPs, such as the methods published by the Association of Official Analytical Chemists (AOAC), American Oil Chemists' Society (AOCS), and the United States Pharmacopeia (USP), etc. Additionally, historical paper records are converted to digital format to be stored in DAFI’s CASPER system to develop an inventory database of official analytical methods used to measure animal drugs in medicated feeds.
- **Implications**
  - The analytical method database generated from approved FAPs will provide the DAFI scientific reviewers a quick reference when they conduct their scientific assessments of the analytical method sections for future submissions. This inventory provides a quick reference for analytical methods used to establish identity, to analyze various types of impurities and contaminants, and information on the analytical parameters used to establish specifications based on additive types, method validation parameters, and the extent of the method validation. The compendial method library also provides scientific reviewers a quick reference if such a method is used in a regulatory submission. The CMC/Technical Additive Branch's current digital depository on medicated feed assay does not contain all analytical methods used to measure animal drugs in medicated feeds. The

additional information converted from paper records provides a more comprehensive repository for the medicated feed assay methods. This database would provide quick reference to DAFI reviewers and more efficient responses to FOIA requests on medicated feed assay methods.

97. **Lawrence, Jalyn**
    - **Abstract title:** Exploring Target Animal Safety Endpoints of Drugs Across Fish Species and Water Conditions: Development of a User-Friendly Database
    - **Authors:** Lawrence, Jalyn FDA/CVM (Student); Pulver, Stacey, FDA/CVM (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
        - **<u>Synopsis</u>**
            - In the United States, many different fish species are reared and maintained for numerous purposes, including commercial food production, recreational fishing, ecological restoration, and display. There is a need for more approved drugs for use in aquaculture, but evaluating new animal drugs in all fish species and all water conditions would be impractical. This project aims to organize available information in order to assess variability in safety profiles across fish species, water conditions, and drugs. This may help CVM and the aquaculture industry make informed decisions about which fish species should be studied to ensure the safety of drugs across a variety of fish species.
        - **<u>Purpose</u>**
            - The purpose of this project is to create a database of existing data from Freedom of Information (FOI) Summaries and Public Master Files (PMFs) to allow for the comparison of target animal safety profiles across different fish species, water conditions, and drug classes.
        - **<u>Methods</u>**
            - Data from target animal safety studies of immersion aquaculture drugs reported in FOI Summaries or PMFs were transferred to a Microsoft Excel sheet. The Excel sheet included columns to classify the following information: drug's active ingredients, route of administration, fish species, life stage, dose, duration, mortality, survival, and water chemistry.
        - **<u>Results</u>**
            - Data from 340 immersion drug-fish exposures were assessed and entered into the Excel sheet. Data across five different drugs and 16 species of fish were identified. Fry, fingerlings, and juvenile fish were the life stages tested in these exposures with fingerlings representing the majority. The water temperature range across all exposures was 8 to 32 °C.
        - **<u>Implications</u>**
            - The results of this project may assist CVM in comparing target animal safety endpoints across multiple fish species and water conditions. This data will be further evaluated to help identify ideal fish species, water temperature, and life stage to include in target animal safety studies.

98. **Mascorro, Ashley**
    - **Abstract title:** Enhancing awareness and compliance of companion animal drug disposal methods: bilingual infographics and social media strategies.

- **Authors:** Mascorro, Ashley, FDA/CVM (Student); Hansen, Honorata, FDA/CVM (Mentor); Qu, Shen, FDA/CVM (Mentor)
- **FDA Strategic Initiative:** Empowering Patients and Consumers
- **Abstract:**
    - **Synopsis**
        - The current disposal methods for unused, expired, or unwanted companion animal medications are community-based drug "take-back" programs, flushing those on the FDA's flush list through the lavatory, and disposal via household trash (U.S. Food and Drug Administration, 2024). Proper storage and appropriate disposal of animal drugs is critical in keeping pets, humans, and the environment safe from harm. These practices further CVM's One Health mission of supporting the best possible public health outcome for humans, animals, and the ecosystem. Public health communications are used to increase the public's comprehension understanding and inform their decision-making for their health (Riegelman, Kirkwood, 2019). To be effective, the quality and display of public health communications must be given adequate attention. This project focused on developing promotional infographics and educational social media content as potential strategies to enhance public awareness and practice of the appropriate animal drug disposal methods for pet owners. To reach a broader audience, the infographics and social media content were developed in English and Spanish. With 62 percent of US households being Spanish-speaking, and Spanish being the second most-spoken language in the United States, it is essential to make English-based material accessible in Spanish (Census, 2022). This project is expected to increase comprehension and awareness of companion animal drug disposal methods and to increase overall compliance with safe drug disposal through the development and publication of multilanguage health communications.
    - **Purpose**
        - This project focused on developing promotional infographics and educational social media content as methods to enhance the knowledge and common practice of appropriate animal drug disposal methods for pet owners. In an effort to reach a broader audience, the infographics and social media content were developed in English and Spanish.
    - **Methods**
        - Initial research via the reading of previously published FDA CVM articles and sites was conducted to understand the current FDA CVM recommended animal drug disposal methods. These included articles and sites centered around animal drug disposal, drug approval, and One Health. Interviews with professionals including animal scientists, veterinarians, DEIA officials, and representatives from a variety of committees in FDA within the topic of interest were consulted for increased insight. After gathering the necessary information, and using previous experience in health communication design, infographics and social media content were produced to inform pet owners on how to dispose of their expired, unused, or unwanted animal drugs.
    - **Results**
        - This project is expected to increase comprehension and awareness of companion animal drug disposal methods and to increase overall

compliance with safe drug disposal among English and Spanish speaking communities through the development and publication of multilanguage health communications.

- **Implications**
  - Developing animal drug disposal educational material in multiple languages may encourage the development and publication of multilanguage content in other public health areas. Equitable and inclusive health communications could increase the awareness and practice of public health-supporting actions like appropriate animal drug disposal across communities that speak different languages.

National Center for Toxicological Research (NCTR)

99. **Berryhill, Johnna**
    - **Abstract title:** Identification of Prescription Opioid-Associated Cardiovascular Adverse Events Through Comprehensive Analysis of FAERS Data
    - **Authors:** Berryhill, Johnna, FDA/NCTR (Student); Ma, Li, FDA/NCTR (Student); Chen, Ru, FDA/CDER; Rogers, Paul, FDA/NCTR; Lyn-Cook, Beverly, FDA/NCTR; Hong, Huixiao, FDA/NCTR; Tong, Weida, FDA/NCTR; Zou, Wen, FDA/NCTR (Mentor)
    - **FDA Strategic Initiative:** Unleashing the Power of Data
    - **Abstract:**
      - **Synopsis**
        - This study retrieved 217,419 opioid-CVDAE pairs from FAERS from 2004 to 2024, and 100,678 pairs were identified as potential safety signals using EBGM, including 17 FDA-approved opioid classes. Oxycodone and hydrocodone had the highest prevalence, while remifentanil exhibited the highest proportion of CVD safety signals at 61.1%. Network and hierarchical clustering analyses revealed distinct groupings and shared CVDAEs among opioids, with notable associations between fentanyl and remifentanil, fentanyl and tramadol, and hydrocodone and oxycodone. The study clarified specific cardiovascular risks associated with different groups of prescription opioids. These findings may benefit clinicians and patients in establishing safer opioid prescribing practices and use to avoid potential cardiovascular risks.
      - **Purpose**
        - Prescription opioids are prescribed for moderate-to-severe pain management. The US is facing an unprecedented opioid epidemic, and prescription opioid use (POU) is associated with increased risks of cardiovascular disease (CVD). Utilizing the FDA Adverse Event Reporting System (FAERS), this study investigated prescription opioid-associated cardiovascular adverse events (CVDAEs) to systematically evaluate the CVD-related risk profiles of multiple prescription opioids and their interrelationships.
      - **Methods**
        - All adverse event (AE) reports in the FDA Adverse Event Reporting System (FAERS) corresponding to 17 FDA-approved opioid classes over a 20-year period (2004, Quarter 1 to 2024, Quarter 1) were downloaded. These pairs were filtered using 741 cardiovascular disease (CVD)-related Preferred Terms (PT) of MedDRA, leaving only the opioid-CVDAE pairs. Subsequently, Empirical Bayes Geometric Mean (EBGM) was utilized to identify potential safety signals. A comparative analysis of these safety

signals was pursued to provide an overview of the 17 classes of FDA-approved prescription opioid-associated CVDAEs. The top 10 most reported CVDAEs for each opioid class were presented and compared. Additionally, network and hierarchical clustering analyses were conducted to further explore the relationships between the opioids.

- **Results**
  - In an analysis of 217,419 opioid-CVDAE pairs from FAERS, 100,678 pairs were identified as potential safety signals using EBGM. This included 531 unique opioid-CVDAE pairs. Oxycodone had the highest prevalence at 37.9% (38,146 pairs), followed by hydrocodone at 11.5% (11,538 pairs). Sufentanil had no identified safety signal pairs, while levorphanol and dihydrocodeine had minimal presence (5 and 10 pairs, respectively). Remifentanil showed the highest proportion of potential safety signal pairs at 61.1%. Comparison of the top 10 CVDAEs for each opioid class revealed 47 unique CVDAEs, with significant overlap (33 CVDAEs common). Network analysis highlighted tramadol, fentanyl, remifentanil, and hydrocodone as opioids with the highest shared CVDAEs (103, 100, 86, and 81, respectively). The strongest connections were between fentanyl and remifentanil (25 shared CVDAEs), fentanyl and tramadol (18 shared CVDAEs), and hydrocodone and oxycodone (17 shared CVDAEs). Hierarchical clustering identified 4 isolated opioids (levorphanol, dihydrocodeine, oxymorphone, and butorphanol) and 2 clusters. Cluster A included codeine, buprenorphine, morphine, tapentadol, tramadol, fentanyl, and remifentanil—sharing the CVDAE 'tachycardia.' Cluster B consisted of hydrocodone, meperidine, hydromorphone, and oxycodone, which were similar in the absence of certain CVDAEs.
- **Implications**
  - Prescription opioids are associated with increasing risks for cardiovascular disease, but systematic knowledge is lacking. The findings of this study from millions of FAERS reports revealed multiple kinds of prescription opioid-associated specific cardiovascular risks and possible associations among various opioids. The results may provide information and knowledge to help FDA drug reviewers and physicians be aware of cardiovascular risks of certain prescription opioids or combinations of opioids with other prescription drugs, which could prevent or reduce risks of prescription opioid-associated CVD.

100. **Byron, Andrew**

- **Abstract title:** Model expansion from peripheral blood mononuclear cells to CD14+ monocytes to distinguish sex-based differences in the immunotoxicity of silver nanoparticles and silver ions in healthy human donors
- **Authors:** Byron, Andrew, FDA/NCTR (Student); Canup, Brandon, FDA/NCTR; McBride, Kaitlyn, FDA/NCTR; Fahmi, Tariq, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Empowering Patients and Consumers
- **Abstract:**
  - **Synopsis**
    - The increasing use of silver nanoparticles (AgNPs) in healthcare products such as bandages and implants as well as consumer products—like food packaging, clothing, and cosmetics—increases the potential for exposure to AgNPs. There are previously reported sex-

based differences in both the innate and adaptive immune systems, thus expanding upon this knowledge is important for protecting consumer health. The study objective was to expand upon our previously published model to investigate sex-based differences in inflammasome activation due to exposure to AgNPs using $CD14^+$ monocytes isolated from human peripheral blood mononuclear cells (PBMCs) of healthy donors. PBMCs from whole blood were isolated using gradient separation and $CD14^+$ monocytes were further isolated using negative selection via magnetic bead separation. Treatments consisted of 1, 10, and 30 μg/mL AgNPs and 1 and 10 μg/mL Ag^+, with or without lipopolysaccharide (LPS), for the subsequent cell viability and enzyme-linked immunosorbent assay (ELISA) investigations. The ELISAs were conducted to assess the expression markers for inflammasome activation (IL-1β), cytokines (IL-10 and TNF-α), and chemokine (IL-8). There was a concentration-dependent decrease in cell viability for the AgNPs and Ag^+ treatments. Significant upregulation of IL-1β was observed for the LPS-primed Ag^+ treatments in both males and females, while the AgNPs showed slight upregulation. IL-10 expression was downregulated by the 30 μg/mL AgNPs treatment with LPS priming for both males and females. AgNPs induced IL-8 with both males and females in a concentration-dependent manner. No significant effects were observed with TNF-α. The data obtained from this study is the preliminary step towards consolidating donor data using $CD14^+$ monocytes to identify potential sex-based differences from exposure to AgNPs and Ag^+.

- **Purpose**
  - The increasing use of silver nanoparticles (AgNPs) in healthcare products such as bandages and implants, as well as consumer products—like food packaging, clothing, and cosmetics—increases the potential for exposure to AgNPs. There are previously reported sex-based differences in the innate and the adaptive immune systems, thus expanding upon this knowledge is important for protecting consumer health. There is a lack of human models that distinguish the potential sex-based differences in the immunotoxicity of nanomaterials. The study objective was to expand upon our previously published model to investigate sex-based differences in inflammasome activation due to exposure to AgNPs using $CD14^+$ monocytes isolated from human peripheral blood mononuclear cells (PBMCs) of healthy male and female donors.
- **Methods**
  - In-house characterization of AgNPs was performed using transmission electron microscopy (TEM) and dynamic light scattering (DLS). PBMCs from whole blood were isolated using gradient separation and $CD14^+$ monocytes were further isolated using negative selection via magnetic bead separation. Treatments consisted of a 6-hour exposure to 1, 10, and 30 μg/mL AgNPs (30 nm) and 1 and 10 μg/mL Ag^+, with or without a 4-hour lipopolysaccharide (LPS) priming step prior to the treatments, for the subsequent cell viability and ELISA investigations. The inflammatory response was assessed by detecting the expression of IL-1β for inflammasome activation, in addition to the cytokines IL-10 and TNF-α. The chemokine IL-8 was measured to determine effects of AgNPs and

Ag^+ on chemotaxis. LPS priming was performed for the cytokines and chemokine experiments to determine potential effects from the inflammasome activation.

- **Results**
  - The average hydrodynamic size of the AgNPs using DLS was 32.80 nm, and the average primary size was measured to be 28.4 nm using TEM. These measurements are consistent with the manufacturer's measurements. There was a concentration-dependent decrease in cell viability for the AgNPs and Ag^+ treatments. Significant upregulation of IL-1β was observed for the LPS-primed Ag^+ treatments in both the male and female, while the AgNPs showed slight upregulation. IL-10 expression was downregulated by the 30 µg/mL AgNPs treatment with LPS priming in both the male and female. AgNPs induced IL-8 in both the male and female in a concentration-dependent manner. No significant effects were observed with TNF-α.
- **Implications**
  - The investigations in this study were performed using $CD14^+$ monocytes from one pair of male and female donors. This work is the preliminary step towards consolidating donor data using $CD14^+$ monocytes to identify potential sex-based differences from exposure to AgNPs and Ag ions.

101. **Dogra, Neil**

- **Abstract title:** Immunohistochemical Effects of Pre- and Postnatal Exposure to Purified Synthetic Cannabidiol (CBD) in Sprague-Dawley Rats
- **Authors:** Dogra, Neil (Student) NCTR, Timothy Flanigan (Mentor) and W. Drew Gill (NCTR)
- **FDA Strategic Initiative:** Empowering Patients and Consumers
- **Abstract:**
  - **Synopsis**
    - In the United States, cannabidiol (CBD) use has risen due to its perceived safety and described benefits in alleviating anxiety, pain, and insomnia—common symptoms during pregnancy. CBD is also known for its anti-inflammatory properties, but excessive anti-inflammatory effects may result in immunosuppressive or immunomodulatory outcomes. Concerns still exist regarding CBD's safety due to insufficient research, and the impact of CBD on the developing immune system is largely unknown. This study aims to identify the effects of perinatal CBD exposure on immune system development, focusing on the neuro-immune system analyzed via histology. To explore CBD's impact during pregnancy, pregnant Sprague-Dawley rats were administered CBD via oral gavage from gestational day (GD) 6 to just before birth at doses of 15 mg/kg, 100 mg/kg, or vehicle control. After birth, pups continued to receive the same doses from postnatal day (PND) 1 to PND 21. On the day of weaning—one hour after their last CBD dose—half of the rats were given an immune challenge by injecting lipopolysaccharide (LPS) to illicit an immune system response, and the other half were injected with saline. Twenty-four hours later, rats were euthanized and underwent transcardial perfusion with saline and a paraformaldehyde solution. Brains were removed, cryopreserved, and sectioned frozen at 30 µm. We then utilized immunohistochemistry methods to examine various immune functions in the brain, including assaying microglia activation.

We hypothesized that microglia activation would be decreased in CBD-exposed animals with the LPS injection, compared to the control animals with LPS injection.

- **Purpose**
  - In the United States, cannabidiol (CBD) use has risen due to its perceived safety and described benefits in alleviating anxiety, pain, and insomnia—common symptoms during pregnancy. CBD is also known for its anti-inflammatory properties, but excessive anti-inflammatory effects may result in immunosuppressive or immunomodulatory outcomes. Concerns still exist regarding CBD's safety due to insufficient research, and the impact of CBD on the developing immune system is largely unknown. This study aims to identify the effects of perinatal CBD exposure on immune system development, focusing on the neuro-immune system analyzed via histology.
- **Methods**
  - To explore CBD's impact during pregnancy, pregnant Sprague-Dawley rats were administered CBD via oral gavage from gestational day (GD) 6 to just before birth at doses of 15 mg/kg, 100 mg/kg, or vehicle control. After birth, pups continued to receive the same doses from postnatal day (PND) 1 to PND 21. On the day of weaning—one hour after their last CBD dose—half of the rats were given an immune challenge by injecting lipopolysaccharide (LPS) to illicit an immune system response, and the other half were injected with saline. Twenty-four hours later, rats were euthanized and underwent transcardial perfusion with saline and a paraformaldehyde solution. Brains were removed, cryopreserved, and sectioned frozen at 30 µm. We then utilized immunohistochemistry methods to examine various immune functions in the brain, including assaying microglia activation.
- **Results**
  - We hypothesized that microglia activation would be decreased in CBD-exposed animals with the LPS injection, compared to the control animals with LPS injection.
- **Implications**
  - By collecting this data, FDA will be able to make more informed decisions regarding CBD. It may also aid healthcare providers in making better decisions regarding the use of CBD amongst pregnant individuals.

102. **Jaikumar, Svanik**

- **Abstract title:** Potential Effects of In-Utero Antiretroviral Exposure on Key Inflammatory and Oncogenic Markers in the Rat Colon
- **Authors:** Jaikumar, Svanik, FDA/NCTR (Student); Strizencova, Michaela FDA/NCTR (Student); Gokulan, Kuppan, FDA/NCTR (Mentor); Sutherland, Vicki, DTT/NIEHS (Collaborator); Cunny, Helen, DTT/NIEHS (Collaborator); Khare, Sangeeta, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response, Empowering Patients and Consumers
- **Abstract:**
  - **Synopsis**
    - Patients diagnosed with (or exposed to) HIV are typically prescribed Antiretroviral Therapy (ART) as treatment or prophylaxis. The most common ART regimen is a "tri-combination" therapy containing one

integrase inhibitor and two nucleoside reverse transcriptase inhibitors. These drugs interfere with important enzymatic activities needed by HIV to survive and may have long-term impacts. This study sought to analyze the effects of ART on the offspring of perinatally-treated (gestational day 6 through postnatal day 21) control (zero), medium, and high-dose recipient Sprague Dawley rats. Male and female pups of the control or drug-receiving dams were allowed to mature with no direct dosing to 12 months of age, at which time colon tissue samples were collected from each of the pups (30 pups total; n=5 per sex in each treatment group). Inflammatory/oncology markers were examined by analyzing mRNA gene expression by qPCR, and cytokine/chemokine protein levels were measured using a magnetic-bead multiplex immunoassay. The mRNA expression of 14/84 genes examined were found to have clear ($|Log2FC| > 1$) and significant ($p < 0.05$) change due to ART exposure. A noticeable sex-associated difference was also observed, with female offspring having more affected genes on average, but with males having the highest single-gene fold change (downregulation by a factor of 25). The majority of affected genes belonged to the chemokine (CXCR, CCR, and CCL) gene groups. In addition, the levels of 6/23 cytokines examined were found to have clear ($|Log2FC| > 0.5$) and significant ($p < 0.05$) change due to the ART exposure. In conclusion, a significant effect on mRNA gene expression and protein levels of cytokine in the colon tissue was noted as a result of perinatal ART exposure. In the future, longitudinal follow-up/studies are needed to assess the transgenerational effects of ART.

- **Purpose**
  - Antiretroviral Therapy (ART) represents a significant milestone in the management and treatment of infection by Human Immunodeficiency Virus (HIV). ART has dramatically improved the quality of life and prognosis for individuals afflicted with HIV and has transformed this formidable illness into a manageable condition. The anti-retroviral drugs that constitute ART function in a variety of ways. Since HIV requires certain enzymes to invade a host cell, replicate its genetic material, and hijack the host cell's genome, most antiretrovirals inhibit those enzymes. The most common ART regimen is a "tri-combination" of drugs containing one integrase inhibitor and two nucleoside reverse transcriptase inhibitors. While ART has been given to pregnant women, transgenerational effects have not been extensively studied. There is strong interest in understanding if there are long-term impacts in individuals exposed to ART perinatally (in utero and via lactation), and that is the motivation behind our study.
- **Methods**
  - This study sought to analyze the effects of ART on the offspring of perinatally-treated (gestational day 6 onwards through postnatal day 21) control (zero), medium-dose, and high-dose recipient Sprague Dawley rat dams. After weaning, male and female pups of the control or drug-receiving dams were allowed to mature to 12 months of age without further exposure, and colon tissue samples were collected from each of the offspring (30 pups total; n=5 in each treatment group). The colon tissue samples were processed in one of two ways, corresponding to the analysis that would be run downstream. An optimized protocol

for RNA extraction was used to purify mRNA from the tissue and analyze gene expression. The collected RNA was converted to cDNA, and then quantitative (real-time) PCR was used to generate gene expression data. Protein extraction was conducted using Bio-Rad reagents to purify protein from the tissue, and a multiplex system was used to compare cytokine/chemokine levels across doses.

- **Results**
  - The mRNA expression of 14/84 genes examined were found to have clear ($|Log2FC| > 1$) and significant ($p < 0.05$) expression change due to ART exposure. An additional 3 genes had $Log2FC > 0.5$ and were significant. A noticeable sex-associated difference was also observed, with female offspring having more affected genes on average, but males having the highest single-gene fold change (*CCL20* downregulation by a factor of 25). The majority of affected genes belonged to the chemokine (CXCR, CCR, and CCL) gene groups. Notable genes that experienced fold change in mRNA gene expression were *TP53*, *MYC*, *IGF-1*, *TGFβ-1*, and *TLR4*. *TP53* is a noted tumor suppressant, and upregulation of the human homolog gene is associated with an increase in apoptosis. In addition, many genes whose downregulations are associated with various cancers in humans were found to be downregulated in the rats in the study. Aside from gene expression analysis, protein levels of 6/23 cytokines (GM-CSF, GRO/KC, IL-17, IL-1α, M-CSF, and VEGF) examined were found to have clear ($|Log2FC| > 0.5$) and significant ($p < 0.05$) change due to the ART exposure. While it's not possible to draw analogous comparisons from rats to humans, translational research is needed to understand if similar profiles of ART are observed across species and in diseased models.
- **Implications**
  - A significant effect on mRNA gene expression and the protein levels of key cytokines was noted as a direct result of fetal/lactational ART exposure. In the future, longitudinal studies are needed to assess the transgenerational effects of ART, as well as comparisons between animal and human data to understand impacts on children exposed to ART during gestation.

103. **Kaswer, Ashley**

- **Abstract title:** Identification of Metabolite Profiles of Montelukast in Rat Plasma Using LC/MS-Based Metabolomics Analysis
- **Authors:** Kaswer, Ashley, FDA/NCTR (Student); Sun, Jinchun, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
  - **Synopsis**
    - Montelukast (MTK) is widely used in addition to inhaled corticosteroids to manage asthma symptoms for adults and children. Recently, there have been increasing concerns of adverse neuropsychiatric drug reactions such as depression and suicidal ideation. However, a direct link of MTK medication to neuropsychiatric events remains unknown. The goal of this study was to profile MTK metabolites in plasma from rats treated with MTK for 14 days and to further investigate MTK metabolism pathways to understand the underlying mechanisms of MTK-induced neurotoxicity, if any. Liquid chromatography-high

resolution mass spectrometry-based metabolomics was conducted to profile MTK metabolites from rat plasma. Metabolomics data detected 9 MTK metabolites including glutathione-conjugated MTK metabolites and 2 downstream metabolites, which might be associated with the glutathione detoxification system. This could be evidence of the glutathione detoxification system functioning appropriately, but further studies must be conducted to confidently correlate MTK medication to neurotoxicity. While not yet verified, a new MTK metabolite (methylated-MTK) was identified for the first time in this study. The newly discovered methylated-MTK metabolite confirmed that metabolomics is a powerful tool for drug metabolite profiling.

- **Purpose**
  - Montelukast (MTK) is widely used in addition to inhaled corticosteroids to manage asthma symptoms for adults and children. It functions as a potent antagonist of cysteinyl leukotriene receptor 1 (CysLTR1) as well as CysLTR2, P2Y12, and GPR17 receptors to block the substances that cause asthma symptoms. Recently, there have been increasing concerns of adverse drug reactions largely amongst the youth, including adverse neuropsychiatric effects such as sleep deprivation, tremors and shakiness, depression, and suicidal ideation. A direct link of MTK medication to neuropsychiatric events remains unknown. However, a recent report indicates that MTK-glutathione-conjugated metabolites, hypocretin-1 and the genetic biomarker HLA DQB*0602 may be associated with increased risk for MTK-induced neurotoxicity. The goal of this study was to profile MTK metabolites in plasma from rats treated with MTK for 14 days and to further investigate MTK metabolism pathways to understand the underlying mechanisms of MTK-induced neurotoxicity, if any.
- **Methods**
  - Rats were treated daily with a vehicle or a 10mg/kg dose of MTK for 14 days. After the last dose on Day 14, blood samples from the control group were collected at 0hrs, and blood samples from the treated group were collected at 2, 4, and 24hrs (n=3/group). Plasma metabolites were extracted after protein precipitation. Liquid chromatography-high resolution mass spectrometry-based metabolomics was conducted to profile MTK metabolites. Aliquots of plasma metabolite extracts (3µL) were introduced into a reversed-phase column using a linear gradient of 0.1% formic acid in acetonitrile (B) and 0.1% formic acid in water (A) for the mobile phase. Pooled plasma extracts were run after every 10th sample for monitoring equipment variability and data filtering. The metabolomics data was collected in both positive and negative ionization modes with the Orbitrap Exploris 240 mass spectrometer. Raw data was processed using the Compound Discoverer Software.
- **Results**
  - The identification of metabolites was based on accurate mass measurements, tandem mass spectrometry spectra, and retention time in comparison to an in-house library as well as mzCloud. In total, metabolomics detected 9 MTK metabolites including glutathione-conjugated MTK metabolites and 2 downstream metabolites which might be associated with the glutathione detoxification system. While not yet verified, a new MTK metabolite (methylated-MTK) was

identified for the first time in this study. Levels of all identified metabolites were highest at 2 hours after the final dose. Further studies are warranted to determine whether the highest level occurs between 0-2 hours.

- **Implications**
    - Consistent with previous reports, this metabolomics study detected the presence of glutathione (GSH)-conjugated MTK metabolites in the plasma of rats treated with MTK for 14 days. This could be evidence of the glutathione detoxification system functioning appropriately. Since the decrease of GSH levels in neurons can lead to neurodegeneration, further studies must be conducted to confidently correlate MTK treatments to neurotoxicity. Furthermore, the newly discovered methylated-MTK metabolite confirmed that metabolomics is a powerful tool for drug metabolite profiling.

104. **Kingsley, Jessica**

- **Abstract title:** Comparison of Single-Output and Multioutput Random Forest Models for Predicting Compound-Induced Responses in Rat Serum Liver Enzymes
- **Authors:** Kingsley, Jessica FDA/NCTR (Student); Chen, Xi, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
    - **Synopsis**
        - This study evaluates the performance of single-output and multioutput random forest models in predicting seven critical liver enzymes in compound-treated rats, utilizing data from the Open TG-GATES database. By incorporating compound, dose, and duration of treatment, the study compares the predictive accuracy and computational efficiency of both modeling approaches. The results demonstrate that multioutput models generally outperform single-output models for several enzymes—particularly ALT, LDH, and DBIL—with significant improvements in predictive accuracy and computational efficiency. The findings suggest that multioutput models offer a promising approach for hepatotoxicity assessment in preclinical stages, potentially reducing the reliance on animal testing and supporting more ethical and efficient drug development processes.
    - **Purpose**
        - Accurate prediction of liver enzyme levels is crucial for assessing hepatotoxicity, a common and serious side effect in drug development. Seven critical liver-function-related enzymes—alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), gamma-glutamyl transferase (GTP), and lactate dehydrogenase (LDH)—serve as key biomarkers for liver function and injury. Precise prediction of these enzymes is essential for evaluating drug safety in preclinical stages, potentially reducing risks in clinical trials and supporting more ethical research practices. This study aims to develop and evaluate the performance of single-output and multioutput random forest models in predicting these enzyme levels in compound-treated rats. By incorporating the exposure information (i.e., dose and duration of treatment), this research seeks to determine whether predicting enzyme levels simultaneously (multioutput) offers superior performance compared to predicting them

separately (single-output). The comparative analysis aims to enhance our understanding of the interrelationships between these liver enzymes and potentially improve the efficiency and accuracy of hepatotoxicity assessments. Ultimately, this study contributes to the broader goal of refining predictive models in toxicology, supporting more effective drug development processes, and advancing efforts to reduce, refine, and replace animal testing.

- **Methods**
  - We used data from the Open Toxicogenomic Project-Genomics-Assisted Toxicology System (Open TG-GATES) database, consisting of the seven liver enzymes tested from 8,078 rats treated with 138 compounds under 1,649 treatment conditions. For our study, we randomly selected 110 compounds (~80%) corresponding to 6,442 rats under 1,317 treatment conditions as the training set. The remaining 28 compounds (~20%) involving 1,636 rats under 332 treatment conditions were used as the test set. Each treatment condition was applied to approximately five rats, and the enzyme levels were averaged to produce a single target value per treatment condition. Molecular descriptors for each compound were calculated using Mordred, resulting in 1,826 descriptors per compound, which were then reduced to 167 using cluster-based feature selection. Both single-output and multioutput random forest models were developed, with hyperparameter tuning and 5-fold cross-validation employed to optimize model performance. Model performance was evaluated using the root mean squared error (RMSE), and statistical significance was assessed with the Wilcoxon signed-rank test.
- **Results**
  - This study revealed mixed but generally favorable results for the multioutput random forest model. It outperformed single-output models for four out of the seven enzymes: LDH, GTP, DBIL, and ALT. The most significant improvement was observed in ALT prediction, with a substantial 240.47% reduction in RMSE. For LDH, DBIL, and ALT, the improvements were statistically significant with p-values less than 0.001. GTP showed a modest improvement with an 8.08% reduction in RMSE, although this was not statistically significant (p-value 0.34). On the other hand, the multioutput model did not show improvements for ALP, AST, and TBIL. AST and TBIL had slight increases in RMSE (-0.17% and -2.80% respectively), while ALP showed a 9.53% decrease in performance (p-value 0.0004). Despite these variations, the multioutput model demonstrated higher computational efficiency compared to running multiple single-output models. Additionally, the multioutput approach showed promise in capturing potential interdependencies among the enzymes, which could be valuable for understanding the complex relationships in liver function. These results suggest that while not universally superior, the multioutput model offers significant advantages for certain enzymes and in overall efficiency.
- **Implications**
  - The findings of this study have several important implications for toxicological research and drug development. Firstly, the generally superior performance of multioutput random forest models in predicting liver enzyme levels suggests a more efficient approach to

assessing hepatotoxicity in preclinical stages. This could lead to more accurate and comprehensive evaluations of drug safety, potentially reducing the risk of liver-related adverse effects in clinical trials. Secondly, the improved performance in predicting certain enzymes indicates possible associations among enzyme activities, which warrants further investigation to enhance our understanding of liver function and drug-induced injury mechanisms. From a practical standpoint, these models can support the reduction of animal testing in drug development, aligning with the toxicological research community's "3Rs" principle (Reduce, Refine, Replace). The computational efficiency of the multioutput model also suggests potential cost and time savings in drug safety assessments.

105. **Marks, Sarah**

- **Abstract Title:** Evaluation of Drug-induced Liver Injury Biomarkers in a Commercially Available Microphysiological System
- **Authors:** Marks, Sarah, FDA/NCTR (Student); Yeisley, Daniel, FDA/NCTR (Mentor); Ren, Lijun, FDA/NCTR (Mentor); Papineau, Katy, FDA/NCTR (Mentor); Schnackenberg, Laura, FDA/NCTR (Mentor); Shi, Qiang, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
- **Abstract:**
    - **<u>Synopsis</u>**
        - Microphysiological systems (MPS) are novel in vitro cell models that seek to increase the physiological relevance of cell culture and may have great potential as drug discovery/development (DDD) tools. However, MPS need to be evaluated to ensure the robustness and reproducibility of the data obtained. In the case of liver MPS, this is typically done through their ability to accurately model and predict drug-induced liver injury (DILI), which is critical for ensuring safety of novel pharmaceuticals as the liver is the primary organ for drug metabolism. This work specifically seeks to evaluate the Lena Biosciences (LB) MPS, a 3D culture model that may better maintain hepatic metabolism and function through increased oxygen availability utilizing an oxygen-saturated perfluorocarbon blood substitute (BS). Initial assessment of the LB MPS utilized acetaminophen (APAP), a model hepatotoxicant, to treat primary human hepatocytes (PHHs) for one week. Preliminary data of APAP-treated PHHs indicated discrepancies between markers of cytotoxicity and typical mechanisms of toxicity for APAP induced DILI—namely lactate dehydrogenase (LDH), cellular adenosine triphosphate (ATP), aspartate aminotransferase (AST), and reduced glutathione (GSH). Future work exploring these is necessary to qualify this system through deconvolution of potential sources of these unexpected results such as challenges with performing assays on 3D cultured cells, the inherent biological variance in PHHs from different donors, or greater resistance to APAP-driven DILI due to the increased oxygen supply.
    - **<u>Purpose</u>**
        - Microphysiological systems (MPS) are novel in vitro cell culture models that seek to better capture physiological cellular function through various strategies to improve the system’s performance and complexity. While MPSs may have great potential as drug discovery/development

(DDD) tools, they need to first be qualified and eventually validated to ensure the robustness and reproducibility of the data obtained. For liver, this is often evaluated through the ability of the MPS to accurately model and predict drug-induced liver injury (DILI), which is critical to DDD for ensuring safety since the liver is involved in the metabolism of most drugs. This work aims to evaluate the Lena Biosciences (LB) MPS, a commercial 3D culture model that is designed to better maintain hepatocytic metabolism and function through increased oxygen availability utilizing in-well perfusion and an oxygen-saturated perfluorocarbon blood substitute (BS). Initial evaluation of the LB MPS utilized acetaminophen (APAP), a known, well-characterized hepatotoxicant. DILI biomarkers such as lactate dehydrogenase (LDH) activity levels, cellular ATP, glutathione (GSH), and aspartate aminotransferase (AST) can be assessed to validate the system.

- **Methods**
  - To assess the assay performance of DILI biomarkers in the LB system, primary human hepatocytes (PHHs) were either cultured in the LB system per the manufacturer's protocol or under the standard sandwich culture conditions. Briefly, PHHs from an individual donor were either seeded encapsulated in SeedEZ fiberglass scaffolds in 4 mg/ml Matrigel, incubated at 37°C for 10 minutes, and then placed into the LB 48-well Perfusion Pal plate, or seeded onto collagen 1 coated 24-well plates and allowed to adhere for 4 hours then overlaying with 0.25 mg/mL Matrigel; for both PHH culture conditions, cells post seeding were cultured overnight prior to treatment. All cultures were maintained in a phenol- and serum-free William's E Medium. On the day of treatment, PHHs were treated with fresh medium or 0.1 to 10 mM APAP for 7 days in the LB system or 3 days under 2D conditions. LDH and AST were measured from cell culture supernatants and ATP and GSH were measured from cell lysates. LDH was also measured from PHHs lysed in 1% Triton X-100 prepared in the maintenance medium.
- **Results**
  - LDH activity in all concentrations of APAP treated PHHs exhibited no increase across 7 days compared to untreated PHHs, including the most cytotoxic, 10 mM APAP. Similarly, there was no significant decrease in GSH in the APAP treatments compared to the control by day 7. This contrasts the ATP data at day 7 where an expected and significant decrease is observed with the 10 mM APAP treatment compared to the control **(~100% reduction).** AST was also reduced **(~17% to 32%)** in all APAP concentrations except the lowest concentration of 0.1 mM APAP on day 7. To further investigate the LDH results, LDH activity was measured from PHHs lysed with 1% Triton X-100. LDH was observed in 2D PHH lysates to be increased ~370% compared to 3D lysed PHHs in the LB system at 1 h despite adjusting for equivalent cell numbers. This persisted through 48 h with a ~300% increase observed. Similarly, at 48 h there was unexpectedly no difference in LDH activity between the lysed and un-lysed PHHs in the LB system.
- **Implications**
  - The preliminary results indicate discrepancies in purported cytotoxicity between the four DILI biomarkers. Due to the LB MPS utilizing a hydrogel encapsulation and 3D architecture, assays suitable for 2D cell

culture analysis may not be robust or reproduceable in this system. Cellular ATP appears to be a robust marker within this system—unlike LDH, which may not perform well potentially due to molecular weight/diffusion kinetics or inhibition of activity. Further experimentation utilizing other donors to ensure these effects are not due to inherent biological variability are also necessary. Identification and qualification of alternative appropriate assays of cytotoxicity will also be necessary in future work characterizing the ability of the LB system to capture APAP-driven DILI.

106. **Strizencova, Michaela**

- **Abstract title:** Impact of Perinatal Exposure to HIV Antiretroviral Therapy on Intestinal Cell Permeability Genes and Cytokine Production in the Colon of Matured Rats
- **Authors:** Strizencova, Michaela (Student) NCTR, S. Jaikumar (Student), S. Khare (Mentor), Sutherland, Vicki, DTT/NIEHS (Collaborator); Cunny, Helen, DTT/NIEHS (Collaborator), and K. Gokulan
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
  - **<u>Purpose</u>**
    - Antiretroviral Therapy (ART) is a combination of drugs commonly used to treat patients with human immunodeficiency virus (HIV) and is often initiated immediately upon diagnosis. Pregnant women are typically recommended this treatment both due to the risks HIV poses to maternal health and because of the potential for transmission to the fetus. While ART is crucial in suppressing HIV replication, residual viral presence is associated with chronic low-grade inflammation that increases the risk of cardiomyopathy and other chronic inflammatory conditions. ART itself may contribute to these pro-inflammatory effects, though its full impact is unclear. Studies have shown ART to induce functional and compositional changes to the intestinal microbiome, and research from our laboratory further suggests that in-utero exposure to ART may transmit these microbiome abnormalities to offspring in a rat model. Microbiome homeostasis is essential for health and is impacted by the permeability of the gastrointestinal tract. Permeability is largely regulated by the intestinal epithelial layer, playing a critical role in maintaining structural integrity, intercellular communication, and barrier function of the gut. Dysfunctional cell junctions may disrupt these processes and lead to dysbiosis, which is associated with various disease States. Thus, investigating the role of ART and its long-term impact on individuals who are exposed during gestation and lactation is imperative in understanding its broader health implications.
  - **<u>Methods</u>**
    - This study investigates how a tri-combination ART impacts intestinal permeability, specifically focusing on potential alterations in colonic cell junctions among Sprague Dawley rat pups born to ART-treated dams during gestation (from GD 6) through lactation (postpartum 12). To examine gene expression changes in intestinal cell junctions of the offspring, pregnant and lactating dams received varying doses of tri-combination ART (control, mid-dose, high-dose). Once the pups were born, they were allowed to mature for 12 months. At this time (12-month age) animals were sacrificed, and the colon tissue was collected.

RNA was extracted from the tissue, treated with DNAse to remove any residual DNA, and converted into cDNA which then underwent quantitative RT-PCR array analysis to test the mRNA gene expression levels of cell junction genes (a total of 84 genes) across all treatment groups. To further examine the ART's inflammatory effect, protein was extracted and purified from the colon tissue, and a multiplex immunoassay was used to analyze concentrations of the multifunctional cytokine Transforming growth factor beta (TGF-β) using the Bio-Rad multiplex assay kit.

- **Results**
    - Of the 84 cell junction genes that were tested, 25 were significantly upregulated or downregulated (p-value<0.05, fold regulation>|1.414|) across the mid-dose and high-dose groups in both male and female offspring. An additional 14 genes were notably altered, however, were not found to be statistically significant. The female offspring displayed a greater number of altered genes than male offspring, especially pups born in the high dose group (DAM). Male offspring displayed larger gene alteration with mid-dose treatment. The *CLDN5* gene was significantly upregulated in both sexes. When male and female samples were considered together, an additional 20 genes were significantly altered including the upregulation of *ITGAM* and *ICAM*1, and the downregulation of *CLDN4, CLDN*15, and *GJB*3. Protein levels of TGF-B were found to be notably decreased across all groups, especially TGF-B3, but not at a significant level. Male offspring displayed a greater decrease in cytokine concentrations compared to females.
- **Implications**
    - The implications of this study emphasizes the need for long-term animal and human studies to comprehensively understand how ART affects cell junction pathways and its relationship with extraintestinal diseases. Changes observed in mRNA gene expression of cell junctions indicate potential alterations in gut permeability. This may lead to adverse effects such as the loss of essential nutrients or infiltration of pathogens due to the downregulation of tightening claudins such as Cldn4. Conversely, downregulation of pore forming claudins, such as Cldn15, could lead to overly tight junctions that impair the paracellular passage of ions. A delicate balance in permeability is crucial for optimal gut health, therefore disruptions in cell junctions caused by ART may have harmful long-term consequences. A decrease in TGF-B may indicate dysregulation in immune response pathways. Further investigation using longitudinal animal models is essential to evaluate the potential detrimental effects of ART-induced cell junction dysregulation.
- **Synopsis**
    - Antiretroviral Therapy (ART) is a combination of drugs commonly used to treat patients with human immunodeficiency virus (HIV) and is often initiated immediately upon diagnosis. Pregnant women are typically recommended this treatment due to the risks HIV poses to both maternal health and potential transmission to the fetus. While ART is crucial in suppressing HIV replication, it has been shown to induce functional and compositional changes to the intestinal microbiome. This may result from dysfunctional cell junctions that impact intestinal permeability and lead to microbiome dysbiosis, which is linked to

various disease states. Research from our laboratory suggests that in-utero exposure to ART may transmit microbiome abnormalities to offspring in a rat model. This study investigates how tri-combination ART impacts intestinal permeability, specifically focusing on potential alterations in colonic cell junctions among rat pups born to ART-treated dams during gestation and lactation (from GD 6 through postpartum 21). To examine gene expression changes in colonic cell junctions, pregnant dams received varying doses of tri-combination ART (control, mid-dose, high-dose). Pups were born and matured for 12 months, at which time they were sacrificed. RNA was extracted from their colon tissue, treated with DNase to remove any residual DNA, and converted into cDNA, which then underwent quantitative PCR array analysis. Of the 84 cell junction genes, 25 were significantly upregulated or downregulated across the mid-dose and high-dose groups in both male and female offspring. Additionally, protein was extracted and purified from the colon tissue and a Bio-Rad multiplex assay kit was used to analyze concentrations of the cytokine Transforming growth factor beta (TGF-β); however, no significant changes were found. This study emphasizes a need for longitudinal studies to better understand the impact of ART on cell junction pathways and its relationship with extraintestinal diseases.

107. **Xiang, Melissa**

- **Abstract title:** Prediction of Placental Drug Permeability Using Machine Learning
- **Authors:** Xiang, Melissa, FDA/NCTR (Student); Fairman, Kiara, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
    - **<u>Synopsis</u>**
        - With at least 80% of pregnant women taking medication, it is necessary to find an efficient way to identify what substances can cross the placental barrier to reduce risk in fetal development. However, there is a lack of data regarding the specific combination of properties necessary for a drug or toxicant to cross the placenta. Using machine learning (ML) models, it is possible to identify common patterns in the key physicochemical properties of a drug that allow it to cross or not cross. The ML models will be trained on a previously established dataset from recently published databases and studies, including data on whether a drug can cross or not cross the placenta. The PubChem predicted values of each drug's molecular weight (MW), logP, hydrogen bond donors (HD), hydrogen bond acceptors (HA), and total polar surface area (PSA) will also be added to the database. The following ML models will be used on this newly formed dataset: Support Vector Classifier (SVC), K-Nearest Neighbor (KNN), Random Forests (RF), and Logistic Regression (LR). The models were chosen due to good performance on small, complex datasets. These models will be fed the data and tested on how accurately they can predict a substance's capability of crossing or not crossing depending on patterns found in MW, logP, HD, HA, and PSA. If the ML models are proven accurate in their predictions, this will help with guiding research in placental permeability. It also helps with distinguishing approved drugs for which the ML model identified as crossing drugs but little information is available.

- **Purpose**
  - The placenta, which forms during pregnancy, is crucial to fetal development as it delivers nutrients and compounds from the mother to the fetus. Drug intake during pregnancy has been an issue due to the possible risk of certain drugs crossing the placental barrier, potentially harming the fetus. Since there is limited data on how drugs interact with the placenta, it is difficult to identify the combination of parameters that allow these drugs to cross the placenta. Consequently, there has been concern with prescribing certain medications to pregnant women. Understanding what features cause drugs to cross the placenta could significantly improve maternal and fetal health and safety. It is possible to use ML to recognize such patterns among various drugs, as ML is more efficient than relying on human assessment. It can be used consistently when validated and can be easily replicated.
- **Methods**
  - Several different ML models will be used, including SVC, KNN, RF, and LR. With the benefit of using multiple methodologies, we can determine which are more effective in predicting which drugs can cross the placental barrier or not based on a limited dataset. This dataset was derived from previous published databases and studies, which classified drugs as able to cross or not cross the placental barrier. The dataset was expanded with values implemented from PubChem to include each drug's MW, logP, HD count, HA count, and PSA values, which are suspected to be relevant to a substance's capability of crossing the placental barrier. The ML models will be trained to recognize these features to establish if they impact drug placental permeability.
- **Results**
  - The results for running tests on SVC, KNN, RF, and LR are still pending. We will report the accuracy, precision, and recall of each model. However, previous publications and internal research suggest that SVC, KNN, RF, and LR perform well with a smaller dataset, performing at over 80% accuracy. SVC will likely perform better than the other methods chosen since it can handle complex, non-linear relationships in datasets which is something that those particular methods lack in comparison. KNN and RF were chosen because they can handle complex and small datasets, which is a disadvantage of ML models like Neural Networks (NN) and Stochastic Gradient Descent (SGD). NN has difficulties with over-fitting in smaller datasets and SGD is unable to scale data points and needs extensive tuning of parameters. LR is a binary classifier that works well with binary dependent variables but may struggle with non-linear data. The range of ML models used will prove beneficial in identifying what works best with the complex and small volume of data that we have.
- **Implications**
  - Effectively predicting and identifying key parameters for placental crossing based on the physicochemical properties of a drug or toxicant with ML models have the potential to be cost-efficient as well as greatly beneficial for maternal and fetal health. ML models will be able to guide research or surveillance efforts in placental permeability. More research will lead to a better, more robust body of literature in the maternal-fetal space, with the additional possibility of identifying how new molecular

entities interact with the placental barrier. Since artificial intelligence (AI) and therefore the use of ML has been recently incorporated in biochemical research, these efforts can also be expanded upon and developed in further detail and relevance.

108. **Yu, Caleb**

- **Abstract title:** Developing a Random Forest Model for Predicting Human Sex Hormone-Binding Globulin Binding Activity
- **Authors:** Yu, Caleb, FDA/NCTR (Student); Guo, Wenjing, FDA/NCTR (Mentor); Liu, Bailang, FDA/NCTR (Mentor); Hong, Huixiao, FDA/NCTR (Mentor)
- **FDA Strategic Initiative:** Unleashing the Power of Data
- **Abstract:**
  - **Synopsis**
    - Chemicals binding to nuclear receptors such as the androgen receptor (AR) and estrogen receptor (ER) can interfere with the endocrine system, making it crucial to assess their AR and ER binding activity to evaluate endocrine toxicity. When chemicals bind to serum transport proteins—such as human sex hormone-binding globulin (hSHBG)—their entry into target cells is reduced, limiting the quantity available to bind to ER and AR. Hence, hSHBG binding data is essential for a comprehensive assessment of endocrine activity. However, experimentally measuring hSHBG binding for the vast number of chemicals is impractical due to time and cost constraints. This study aims to develop and validate a Random Forest (RF) model to predict hSHBG binding activity. We curated 125 and 164 chemicals with experimental hSHBG binding data from literature and the ChEMBL database, respectively, as training and external validation datasets. Molecular descriptors were computed using Mold2, and non-informative descriptors were removed. The RF models were validated through 100 iterations of 5-fold cross-validation, achieving average scores of 0.824 accuracy, 0.892 sensitivity, 0.666 specificity, and 0.574 MCC. External validation yielded 0.914 accuracy, 0.929 sensitivity, 0.625 specificity, and 0.402 MCC. The results suggest the RF model is a useful tool for predicting hSHBG binding activity, aiding in the assessment of chemical endocrine activity.
  - **Purpose**
    - Chemicals binding to nuclear receptors such as the androgen receptor (AR) and estrogen receptor (ER) have the potential to interfere with the endocrine system. Assessing the AR and ER binding activity of chemicals is important for evaluating their endocrine toxicity. When a chemical binds to transport proteins in serum—such as human sex hormone-binding globulin (hSHBG)—its entry into target cells is reduced, leading to a lower quantity available to bind to ER and AR. Therefore, data on hSHBG binding activity is essential for a comprehensive evaluation of the endocrine activity of chemicals. However, experimentally measuring hSHBG binding activity for the vast number of chemicals is impractical due to time and cost constraints. Consequently, efficient and cost-effective new approach methods (NAMs)—such as machine learning predictive models—are urgently needed to improve the assessment of endocrine activity. This study aims to develop and validate a model using the Random Forest (RF) machine learning algorithm to predict

hSHBG binding activity of chemicals.

- **Methods**
  - Chemicals with experimental hSHBG binding activity data were curated from literature and the ChEMBL database. This resulted in 125 chemicals with competitive binding assay data from the literature as a training dataset, and 164 chemicals from the ChEMBL database as an external validation dataset. Molecular descriptors for the curated chemicals were then computed using the Mold2 software, and low informative descriptors were removed. Next, 100 iterations of 5-fold cross-validation were conducted on the training dataset to evaluate the performance of RF binary classification models. Finally, an RF model was trained using the entire training dataset and evaluated using the external validation dataset. Model performance was measured using accuracy, sensitivity, specificity, and the Matthews correlation coefficient (MCC).
- **Results**
  - For the 100 iterations of 5-fold cross-validation, the RF models achieved average scores of 0.824 for accuracy, 0.892 for sensitivity, 0.666 for specificity, and 0.574 for MCC. External validation yielded scores of 0.914 for accuracy, 0.929 for sensitivity, 0.625 for specificity, and 0.402 MCC.
- **Implications**
  - The results suggest that the RF model could be a useful tool for predicting hSHBG binding activity, therefore aiding in the assessment of the endocrine activity of chemicals.

Office of the Commissioner (OC) / Office of Digital Transformation

109. **Valerio, Luis**
- **Abstract title:** Bridging the Gap Between Government Understanding and Public Consensus with Web Crawling and AI
- **Authors:** Valerio, Luis, FDA/ODT (Student); Pishko, Greg, FDA/ODT (Mentor); Dubbins, Michael, FDA/ODT (Mentor)
- **FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
- **Abstract:**
  - **Synopsis**
    - Infant formula holds significance as the exclusive infant feeding option for many parents who are unable to breastfeed due to physiological, economic, or medical reasons. Therefore, a large portion of parents must have infant formula security by guaranteeing stable supply chains and accessibility. The sudden shortage crisis that unfolded in 2021 proved the need for rapid response and monitoring of critical supply chain infrastructure. However, additional measures can also be taken to gauge shortages and gaps in the supply chain by observing public discussion. Reddit maintains several popular subreddits to discuss infant formula and neonatal care. The use of Reddit's API can automate the collection of post data, especially with the convenience and direct accessibility offered by the Python Reddit API Wrapper (PRAW), expediting the delivery of public sentiments from online discussion to government action. In our project, charting post frequency became the primary focus for its reproducibility and explicit indication of shortage

trends. Posts were accessed by a Reddit Client using PRAW, and three subreddits relating to infant formula ('formulafeeders', 'newparents', 'parenting') were accessed. In each subreddit, posts were collected and sorted by timestamp before being visualized in shortage post frequency by month and by quarter. From our project, we found our post-frequency data to correlate with the 2021 infant formula crisis and found two other significant peaks in formula shortage posts. The quarterly and monthly histograms also correlated closely to each other's trends, indicating that monthly trends may be decisive frequently enough to determine quarterly outcomes. The efficacy of scraping social media can be hard to predict due to the post limitations set by the Reddit API. However, the alignment of significant shortage events coinciding with post-frequency data suggests the potential social media scraping has as a supply chain warning system.

- **Purpose**
  - Infant formula holds significance as the exclusive infant feeding option for many parents. Parents who are not able to breastfeed rely on infant formula. While directly protected by the FDA's regulatory process and annual inspections at infant formula manufacturing plants, infant formula must have safeguards in place to maintain the supply chain and flow of formula to consumers. Considering the unprecedented infant formula shortage in 2022, the ability to rapidly gauge supply chains has become quintessential. Social media can be a powerful tool to accumulate live shortage data from parents. Sites such as Reddit offer ease of access to information through an API to parse through posts and comments. The purpose of this project is to determine the efficacy and speed of scraping social media discussions on Reddit and to provide additional perspective to infant formula supply chain reports.
- **Methods**
  - A Reddit client was created to access the site through Reddit's API. Using the PRAW (Python Reddit API Wrapper) Wrapper of Reddit API for simplification of post retrieval, subreddits 'formulafeeders', 'newparents', and 'parenting' were chosen for scraping. The key terms 'out of stock', 'shortage' and various similar spellings were identified as phrases identifying infant formula shortage posts. The limitation of PRAW's post-retrieval methods prevented results from reaching above 1,000 posts. Therefore, up to 1,000 posts could be found for any query. The scraper also removed any posts if an inclusive time range was inputted. All posts collected were sorted by their UTC timestamp and duplicate posts found by overlapping search results were removed. The posts were then sorted into bins in a histogram by timestamp displayed post frequency by quarter and by month. The accuracy of scraped shortage data was tested by mentors independent from development manually reading the content of each post in a sample of 1376 posts to verify its relation to shortages. The sample was created by randomly shuffling subsets of 688 posts undetermined as shortage-related and 688 shortage-related. Only the post title and body were visible to each mentor since providing a timestamp of each post could introduce bias.
- **Results**
  - Among the posts collected, three peaks were observed in both the quarterly and monthly histograms. Limited by the 1,000 post maximum

during the filtering process, the data does not extend further than July of 2018. However, at each peak in 2019, 2021, and 2023, the post frequency dramatically increases. This increase becomes even more concentrated when looking at monthly post frequency, where each month with a spike in posts more than doubled the previous month.

- **Implications**
    - The rapid spikes in shortage posts during 2021 parallel the widely reported crisis at the time. However, out of the three spikes, it has the lowest post frequency. This could be explained by the prolonged increase in post-activity following the spike. A second trailing peak is visible 3 quarters away in the quarterly histogram and 10 months away in the monthly graph. The quarterly and monthly histograms correlate closely, suggesting monthly trends in shortage posts may be able to accurately reflect quarterly outcomes. The lack of data produced by the API creates a strict limitation of 6 years of information, posing a challenge for long-term tracking.