Consent Agenda: April ICR Governing Board Meeting

Agenda Item II.a.

ICR Governing Board Meeting Minutes

April 8, 2022 12:00 – 1:00 pm

Meeting By Zoom: https://csupueblo.zoom.us/j/95780770453

Meeting Minutes:

In Attendance: Dr. Kinney, Dr. Cinnamon Bidwell, Mr. Sal Pace, Mr. Scott McWhorter, Dr. Malik Hassan, Ms. Elyse Contreras, Mr. Sherard Rogers, Dr. Sue Sisley, Dr. Joanna Zeiger, Ms. Valdez, Mr. Dieter Raemdonck and Ms. Wendy Fairchild

I. Welcome (12:00-12:03pm) Dr. Bidwell

II. Consent Agenda (12:03-12:08pm) Dr. Bidwell

a. Minutes from 03/11/2022
   - Elyse Contreras– correction to page 2 – "money from other sources" rather than other regions/states
   - Malik Hassan motions to approve, Elyse Contreras seconds, all vote yes

b. Fully Executed MOU

c. Approved Budget Scenarios

d. Preview of new one-page infographic

III. Fully Executed MOU – ICR Gov. Board and CSU Pueblo (12:08-12:13pm) Dr. Bidwell

- Dr. Cinnamon Bidwell thanks all for working on this – ethical and collaborative, all voices heard

IV. Legislative Update (See Executive Summary 12:13-12:28pm) Dr. Bidwell with Ms. Valdez and/or Mr. Raemdonck

a. Budget Process updates
   - Ms. Valdez – ICR Budget ($2.8 million) made its way through the legislature without amendment.
   - It is key to continue to educate legislators about ICR and what it does
   - Governor recommended 3.6 million for the ICR, but this was revised by the Joint Budget Committee in response to available funding in the MJ tax cash fund (MTCF) – Ms. Valdez says $1 million increase to a total of $2.8 million is a. positive outcome
   - Ms. Valdez - As a cash fund it is not restricted and used for many purposes and therefore did not as much of an increase as hoped
   - Dr. Bidwell states we need to continue to demonstrate the value of the ICR and the need for the state to invest MTCF in research.
   - Ms. Valdez and Mr. Raemdonck will help put package together and reach out to key members of the JBC and other leaders, and educate new members about the ICR as world class institution – need a great fact sheet to distribute
   - Dr. Bidwell asks for feedback regarding fact sheet

b. Other Outstanding Legislation
   - HB 1321 – Ms. Valdez states that the ICR could apply for funds for the research needs specified in the bill.
   - Dr. Kinney mentions the funds to CDOT are for the development tool to measure impairment and that the ICR would not likely apply to conduct the research but would like to support the competition to
     - Mr. Raemdonck says there is general support for the bill and the

development of the tool

- Dr. Kinney asks how HB 1321 is being perceived and Ms. Valdez replies, "well received" and thinks it will be funded
- Sal asks if feasible to be amended in Senate to collaborate with ICR? Conversations can happen and Ms. Valdez sees this as broad impairment and not just cannabis
- Dr. Bidwell - supports Sal in ICR involvement as there are unique facets of cannabis research administration and funding that the ICR has already navigated
- Dr. Kinney says indicates that the ICR could use the developed infrastructure to facility competition for this research on behalf of CDOT. The ICR would like to be helpful in such processes – if we can leverage the ICR to support CDOT this could help to raise profile of ICR
- Ms. Valdez can meet with sponsors while still in the house and meet with Ean to recommend meeting with ICR so all aligned to move funds forward
- (Ms. Valdez and Dieter leave meeting at 12:30)

V. Current Year Unobligated Funds (See Executive Summary; 12:28 – 12:45pm) Drs. Bidwell and Kinney

a. Source of unspent funds
b. Priorities (Budget Subcommittee) for use of the funds
    - Dr. Kinney – Based on most recent assessment about $100,000 unspent - Budget
    - Address contractual obligations for Journal of Cannabis Research - APC
    - CRC 2022 Registration for Board Members, Staff, and Emerging Scientist Awardees
    - Senior Scientists research program Dr. Kinney to vet research requests.
    - Motion from the Board - Dr. Hasan motions to support, Dr. Zeiger seconds, all approve
c. Potential unspent funds derived from research projects.
    - Authorized to roll forward 25% in funded monies - reinvest money next year to support research

V. In person Meeting (12:45-12:55pm) Dr. Kinney

a. May Meeting (05/13/22) Grant-Humphreys Mansion in Denver
b. Extended Meeting (roughly 9:30am – 130pm-ish) with a working lunch
c. Interruption in email system at CSU Pueblo
    - Email conversion – May 11-15, Dr. Kinney will share alternate email address to group
d. Agenda items – Seeking input
    i. RFP – Marketing and Communication
    ii. Research Presentation – Senior Scientist
    iii. Budget Request/Process for FY24

    Additional Suggestions Included:
    - Updated from the Research Subcommittee – plans for external funding
    - Mr. McWhorter – request a presentation to better understand research capabilities/capacity of the ICR
    - Dr. Zeiger suggests inviting ICR Funded PIs to give presentations researchers
    - Dr. Kinney – we can share project reports – bring researchers in to present if time allows
    - Dr. Bidwell would like discussion the CRC Conference on next agenda

e. Next in person meeting in the late summer or early fall
    - Dr. Hasan has graciously agreed host the meeting in Pueblo

VI. Public Comment (12:55-1:00pm) Dr. Bidwell

- No Public Comment

VII. Adjourn – 1:00pm

Agenda Item II.b.

There will no regularly scheduled ICR Gov. Board Meeting on June 10th.

Agenda Item II.c.

Dr. Bidwell will not be available for regular communication (email or phone) between May 14th and May 31st.

Agenda Item II.d.

Year 1 Project Summaries, ICR FY22 RFA

Included here are a portion of the progress reports (summary of progress) submitted by each of the currently funded PIs. If a Board member is interested in the full progress report for any of the projects, please contact the ICR office and this can be provided.

1. **Defining the effects of CBD consumption during pregnancy on embryonic neurodevelopment and postnatal anxiety**

   PI: Emily Bates

   AIM 1: Determine the transcriptional consequences of CBD exposure on pro-neuroinflammatory and developmental signaling. Marijuana use during pregnancy is associated anxiety and ADHD in the exposed child42, but we do not know to what extent these outcomes can be attributed to CBD and/or TRPV1 activation. TRPV1 activation induces pro-inflammatory transcriptional changes in multiple cell types30. The first step in understanding how CBD and TRPV1 activation affects brain development is to determine how CBD and TRPV1 affect expression of genes expressed in the hypothalamus, which mediates stress and anxiety. We hypothesize that chronic CBD induced activation of TRPV1 induces transcriptional changes in the developing hypothalamus. We will assess gene expression using single cell RNA sequencing (scRNA-seq) in the hypothalamus of embryonic day (E) 14.5 vehicle and CBD exposed wild type mice. By comparing gene expression in TRPV1KO/KO and WT mice exposed to CBD, and non-CBD exposed controls, we will deduce how CBD and TRPV1 activity affect gene expression. We will focus assessment on the expression of pro-neuroinflammatory genes and developmental signaling pathways.

   Progress for Aim 1: We administered 50 mg/kg CBD or vehicle (sunflower oil) by oral gavage to pregnant dams from E5.5 to birth. We dissected hypothalamus from 2 males and 2 females from each exposure group. The male samples from the same exposure group were pooled and the female samples from the same exposure group were pooled to fit into the budget. We have completed the single cell RNA sequencing of CBD exposed and vehicle exposed P2 hypothalamus at 5000 cells per sample at a depth of 100,000 reads per cell. We have processed the data and have identified clusters of cells based on gene expression. We found that fetal CBD exposure significantly changed gene expression in in several clusters of cells. For example, many genes that are important for regulating energy balance (feeding behavior and metabolism) were dysregulated in the CBD exposed hypothalamus compared to control. We are in the process of analyzing this large dataset.

   AIM 2: Investigate effects of in utero CBD-induced TRPV1 activation on anxiety behavior. CBD activates TRPV128, which is expressed in a brain structure that mediates stress and anxiety, the hypothalamus. Acute pharmacological TRPV1 activation causes anxiety behavior in adult mice15, suggesting that TRPV1 mediates anxiety behavior. However, we do not know if CBD consumed during pregnancy impacts fetal brain development to affect postnatal anxiety. We hypothesize that chronic in utero CBD exposure activates TRPV1 and predisposes animals to postnatal anxiety. Following the exposure of CBD or vehicle daily throughout pregnancy, we will quantify anxiety behaviors of wild type and TRPV1KO/KO offspring postnatally. We will conduct the same behavior experiments in age-matched mice that express constitutively active (ca)TRPV1 in the hypothalamus to determine how constant activation of TRPV1 influences anxiety behavior. These experiments will determine if, and the extent to which, CBD exposure during in utero development affects postnatal anxiety, and if these effects are dependent on TRPV1 activation.

   Progress in Aim 2: We administered 50 mg/kg CBD or vehicle (sunflower oil) by oral gavage to

pregnant dams from E5.5 to birth for wild type and TRPV1 KO/KO dams. Pups from these exposure groups have now undergone behavior tests for anxiety including the open field test, the light-dark box, and the elevated zero maze. We are in the process of analyzing this data. We also tested thermal pain sensitivity using the Hargreaves apparatus. We found that fetal CBD exposure increased sensitivity to heat in male mice (n=8 for each exposure group). We tested cognitive function using the puzzle box. We found that fetal exposure to CBD reduced cognitive ability in female mice (n=12 mice in each exposure group).

Because we found that CBD exposure changed expression of metabolism and insulin sensitivity genes in the hypothalamus single cell sequencing data, we also tested glucose tolerance in male and female mice exposed to CBD or vehicle throughout gestation. We found that CBD reduced glucose tolerance in male mice exposed to CBD (n=14 mice for each exposure group), while it had no impact on glucose tolerance in female mice (n=10 mice for each exposure group).

AIM 3: Determine if in utero CBD exposure induces neuroinflammation. TRPV1 activation induces inflammation in adult mice7. In the brain, inflammation can be detrimental to neural development, neuronal function, and survival29.TRPV1 receptors are expressed in the hypothalamus throughout development and adulthood4. Therefore, CBD activation of TRPV1 during brain development could induce neuroinflammation. We will determine if chronic CBD exposure during in utero development activates TRPV1 sufficiently to cause neuroinflammation. We will collect brains at E14.5, postnatal day 7 (P7), and postnatal week 10. We will obtain transverse sections of the hypothalamus and quantify microglia, TRPV1, apoptosis, and cellular stress markers via immunohistology. The compilation of this data will allow us to interpret if, and to what extent, the immune system is stimulated in the developing brain when exposed to CBD, as well as if neurons show altered levels or stress and survival.

Progress in Aim3: We have not started this aim yet, but are planning to start this in July of 2022.

2. **Is what you see what you get? A systematic, public health-driven analysis of cannabis product label claims vs. actual cannabinoid content - CU Boulder**
   PI: Cinnamon Bidwell

   Below I summarize our activities and progress during the first budget period of our project:

   Developed Standard Operating Procedures (SOP) for all study procedures, including:
   Product selection and procurement
   Blinding procedures
   Workflow for potency/contaminant testing
   Transfer of data (i.e., test results) from MedPharm to CUChange
   Data verification and quality control
   Created electronic data capture (EDC) system using REDCap platform

   Complete as of January 2022
   Purposes include:
   Data entry
   Data cleaning/query resolution
   Transfer of data between sites (i.e., MedPharm and CUChange Lab)
   Conducted several comprehensive test-runs of product testing/data entry procedures
   Initiated necessary certifications and regulatory changes
   ISO 17025 certification for MedPharm testing facilities (currently in-progress)

3. **Observational study of the effects of acute cannabis use on ocular activity relevant to driving**

   PI: Ashley Brooks-Russell

   The project goals are to leverage an ongoing research study to add eye-scanning technology. In the larger study, participants will drive a driving simulator before and after cannabis use, to study how cannabis impacts driving performance. This study funds the addition of eye tracking technology, from Seeing Machines, to collect real-time measures of head position and eye movements to better understand the impact of cannabis use on driving performance. The study has the following 3 aims:

   Aim 1 – Temporal pattern of impairment: What is the predictive value of eye movements against validated benchmarks of driving simulator performance over time?
   Aim 2 – Cannabis Use History: Do the relationships in Aim 1 vary by cannabis use history (daily vs. occasional)?
   Aim 3 – Cannabis product: Do the relationships in Aim 1 vary by THC concentration of the product used?

   To achieve these aims we have engaged in several activities. First, the University of Colorado executed a collaboration agreement with Seeing Machines to share data, software, and technology. Second, we developed new driving scenarios, informed by our prior study, for improved measures of driving performance. This development occurred over many months of pilot testing and included a site from Dr. Brown (University of Iowa) and a member of his team to fine tune the scenarios. We recruited over a dozen participants (sober) to pilot test drive the scenarios and ensure they will perform as expected.

   We also pilot tested our blood collection protocols with 28 participants (cannabis-using). From the pilot testing, we encountered a challenge with our capillary blood collection strategy. In working with capillary device, we identified unexpected blood cannabinoid results. Specifically, it seems that some cannabinoids (e.g. THC) had substantially different values between the venous and capillary samples. Although there might be a physiologic explanation for this, what contradicts that explanation is that some metabolites did not differ. Furthermore, there were differences at baseline when differences would have only been expected after acute use. This led our team to consider the possibility that polar compounds like THC where either adhering to the plastic tubes or other plastic components of the mechanism. We conducted lab-based test to identify the issue by looking at different plastics, different storage temperatures, and different lengths of time of storage. It appears that the storage temperature affects the rate at which THC adheres to plastic. Luckily, we now know how to store samples to prevent this and can continue working with our capillary device which has the feature of virtual no discomfort to the participant.

   Despite this progress, we have encountered delays in our recruitment timeline. To accelerate data collection, we are currently hiring at least 2 additional staff so that we can run participants more frequently (at no added cost; funded by parent study). We are also hiring a social media firm to accelerate recruitment (funded by parent study). With these steps we can complete the data collection within the study timeline.

4. **Short-term effects of cannabis use and cannabinoids in youth: A sibling-comparison study**

   PI: Jarrod Ellingson

   This project has two primary aims: 1) examine whether siblings with greater cannabinoid exposure have worse mental health functioning than their co-sibling; and 2) examine whether participants demonstrate worse mental health functioning when their recent cannabis use is elevated (indicated by higher relative THC-COOH levels, compared to each participant's typical levels). Further, we will examine, given the availability of high-potency cannabis products (80+% THC) and a lack of knowledge on precisely how these products are used by adolescents, an exploratory aim will investigate these questions. Specifically, we will investigate whether high-potency cannabis product use is associated with higher cannabinoid exposure (THC-COOH) or worse mental health functioning in adolescents.

   Our proposed recruitment was limited, specifically by COVID-related policy changes affecting the clinical settings from which we recruit. Specifically, we were unable to have a person physically in the clinical space. Additionally, clinical staff were strained. Together, these factors resulted in very few referrals. Although recruitment is improving as COVID moves into an endemic stage, we have modified our IRB protocol to broaden the scope of our recruitment to community adolescents. If we remain below our targeted recruitment by mid-FY2, we will further broaden the scope of recruitment to emerging adults (through age 21). To complete recruitment, we will need to recruit approximately 1.5 families per month in the next award period. We are on track to meet this, with two families recruited in March 2022. As a result of slow recruitment during much of FY1, we have a 27% carry forward budget.

5. Dissecting the genetic basis of sex and dioecy in Cannabis sativa
PI: Nolan Kane

Aim 1. Mendelian sex determination: the evolution of sex chromosomes in hemp

We have obtained seed material from diverse lineages of hemp, germinated seeds from them, and now have growing plants that will soon provide DNA for sequencing multiple diverse hemp sex chromosomes from males and females. We also have collaborations established with Alex Harkess, who will provide affordable sequencing for these sex chromosomes, enabling us to do more sequencing than we had thought. Below I've listed the diverse hemp lines we are growing, along with their type of sexes.

Dioecious: Carmagnola Selectionata, Otto II (Winterfox Farms)
Dioecious with frequent monoecy: A2R4 (Winterfox Farms), Wild Horse (Winterfox Farms)
Monoecious cultivars: USO-30, Bialobrzeskie and Futura 75.

We have planted enough seeds to grow 20 plants per line, which will be phenotyped to give accurate sex ratios for each lineage. DNA will be extracted for sequencing from each plant.

Aim 2. Quantitative expression of sex: the role of modifier genes in sex determination

These growing plants will be used in multi-factorial crosses to identify the inheritance and sex ratios when diverse sex chromosome types are combined. The quantification of the sex ratios will itself be highly informative about the inheritance of these traits, but the genetic mapping will be the most definitive for understanding why there is so much variation in hemp between monoecy and dioecy, and diverse intermediates.

To assist with this analysis, we have configured a new computer server system, priced at $19,600, configured with 2x Intel Xeon E5-2680 V2 2.8GHz 10 Core, 512GB DDR3, 8x 3.5 Drives (8x8Tb hard drives), which will be ordered shortly. We are also in the process of ordering supplies for DNA extraction and sequencing these plants, which will be accomplished over the summer. To prepare for this, we are optimizing DNA extractions now.

Supplemental Aim: Network analysis of genes related to sex determination and chemistry

Leonardo Orozco ($5k supplement) has made very substantial progress on his analysis of the genetics of chemistry in a genetic pathway context, as well as substantial background and preparation for his analysis of the network analysis of genes involved in genetics of sex determination. He has clearly learned a ton, as well as getting sophisticated software installed and working, and yielding interesting and meaningful results.

To facilitate all of this work, we have hired a lab tech (Zach Marcus) with substantial expertise in this area, particularly related to the hemp industry, plant growing, genetics and chemistry. He has set up our growing space, started growing the plants, started with DNA extraction and continued to improve on and optimize our DNA extraction protocols.

6. Exploring Intoxication During Acute Alcohol and Cannabis Co-Administration: A Focus on Cannabinoid Content and Order Effects

PI: Hollis Karoly

The overall project aims are below:

Aim 1: Compare the effects of cannabis containing different ratios of THC/CBD potency on intoxication when cannabis is ingested immediately after a moderate dose of alcohol. Hypothesis 1: Within the Order AC group (alcohol before cannabis), we expect to observe linear effects of THC/CBD ratio on subjective and objective intoxication outcomes such that the THC-dominant group will demonstrate greatest subjective and objective intoxication, followed by the 1:1 group, the CBD-dominant group and the placebo group. Aim 1 focuses on potency results using the ordering procedure (alcohol before cannabis) that is typically used in co-use studies to facilitate comparisons to prior findings.

Aim 2: Compare intoxication levels in individuals who consume cannabis before alcohol and those who consume alcohol before cannabis, within THC/CBD potency groups. Hypothesis 2. Individuals in the order AC condition will experience a delayed onset of peak BAC, lower peak BAC, increased subjective intoxication, increased objective intoxication, higher blood-THC levels and prolonged duration of intoxication compared to those in the CA condition, within each of the 4 potency groups.

Exploratory Aim 3: Explore the interaction of THC/CBD potency and order effects on intoxication. Exploratory Hypothesis 3. The linear relationship between potency and intoxication (where higher THC levels are associated with more intoxication) will be stronger for those in the Order AC group compared to those in the Order CA group. We lack prior data to inform directional hypotheses regarding the interactive effects of potency and order on peak BAC, time to BAC and blood-THC levels; however, examining this interaction is a natural extension of Aims 1 & 2 and may yield important details about the interplay of potency and order.

For the first year of the project, we planned to accomplish the following:

pre-award: complete the IRB submission, prepare all study protocols, RA hiring and begin training.

Year 1: Recruitment, complete 60 study visits, submit protocol manuscript and present protocol at conferences.

Since receiving the funding for the project, we have completed the IRB submission, finalized all study protocols, hired our RAs and completed their training, began recruiting for the study and enrolled our first 3 participants. We had initially reserved two months of year 1 to complete study set up (e.g., obtain final IRB approvals, finalize training of research staff, recruitment). Thus, we were expecting to enroll our first subject in approximately September, 2021. However, the set-up of the study took longer than anticipated, due to the fact that this is the first study in the PIs laboratory that is implementing the mobile laboratory procedure, and a number of logistics had to be worked out (e.g., setting up Wi-Fi connection in the mobile lab, regulating temperature inside the mobile lab, blood storage within the mobile lab). In addition, due to COVID safety concerns (specifically, research assistants being in close quarters with participants who could potentially be unmasked or unvaccinated), we took extra time working with our university IRB to put protections in place for our research staff (i.e., requiring participants to be vaccinated and wear a mask during all sessions). This delayed our progress in running our first participants as well. Finally, after we began recruitment we found that most individuals we screened did not drink enough to meet the heavy drinking criteria for the study (i.e., as of Feb 17, 2022, we screened 22 individuals for only 2 eligible participants). Thus, we changed the drinking criteria from SAMSHA's binge drinking criteria (5 binges per month) to NIAAA's heavy drinking criteria: (For men, consuming more than 4 drinks on any day or more than 14 drinks per week; For women, consuming more than 3 drinks on any day or more than 7 drinks per week). Since implementing this small change, we have had 5 eligible participants scheduled and have

run 2 participants so far. Thus, we anticipate that we will be able to get back on track with our goals, and plan to run extra participants in late spring-summer to increase our total number of participants run during year 1, so that we will come as close to our goal of n=60 as possible during the first project year. To do this over the summer, we will be able to increase the hours of our primary study RA (Emma Smith) from 10 to 20 during the summer months, and have at least 2 undergraduate volunteers that will be assisting with the study over the summer.

Another goal that we set for year 1 is to submit a manuscript detailing our study protocol. This manuscript is in preparation and we anticipate being able to submit it during year 1 (we anticipate it will be submitted to a journal by April 15, 2022 at the latest). We also planned to present the protocol at a conference. The protocol is submitted as part of a symposium to be presented at the Research Society on Marijuana (RSMJ) conference in July, 2022 in Boston, MA.

7. **Cannabinoid conversion to CBN during hemp extraction and post-extraction fluorination of CBD and CBN for increased bioavailability.**

   PI: Kenneth Olejar

Aim 1: In-extraction CBD modification to CBN.

Work on this aim is progressing. Initial studies following the methodology outlined by Pollastro, et al 2018 using reflux have been successful in the conversion of CBD to CBN, thereby demonstrating that the laboratory can perform the conversion. Following on from the conversion of pure CBD, hemp biomass was decarboxylated and underwent the same reflux procedure. The CBD in the hemp has been consumed in the reaction and purification is being performed to identify if CBN has been produced.

Upon successful completion of CBD to CBN in hemp biomass using reflux, attention will turn to the conversion in the biomass within the pressurized liquid extraction system.

Aim 2: Post-extraction fluorination of CBD and CBN.

Fluorination of CBN has been examined using the F-TEDA reagent. Optimum conditions for the complete conversion of CBN have been evaluated. Reagents, temperature and time have been established for the reaction. Currently several compounds have been generated with isolation and identification of these compounds being performed.

Aim 3: Assessment of synthesized compounds in cell lines.

No work has been completed on Aim 3. Compounds derived from Aim 2 are required prior to work beginning on Aim 3.

8. Microbiome mediated effects of Cannabis and CBD on neurotransmitter-related molecular networks and anxiety

PI: Nichole Reisdorph

Overall Research Goals:

Determine if 4 week oral consumption of Cannabis or CBD changes anxiety-like defensive behavioral responses in mice

Quantitate levels of THC/CBD in plasma and brain
Measure neurochemicals and endocannabinoids in plasma and brain
Assess changes in anxiety-related defensive behaviors
Determine changes in microbiome
Determine changes in physiology using surgically implanted telemetry devices

Determine if molecular and behavioral effects of Cannabis or CBD depend on the make-up of the gut microbiome.

Prior to beginning new studies, we finished analyzing preliminary data to ensure proposed study design would be appropriate. Importantly, we learned that the PK of THC/CBD requires collecting blood <24h following final dose. The relatively rapid turnover was also described in a recently published study that performed i.p. of THC in mice (Torrens, et al 2020, PMID 32345621) and further confirmed in studies involving rats. Based on this new data, we will perform sample collection at <24h following administration of the final dose. In addition, we will include analysis of plasma at 1 week to assess THC/CBD levels. Through our preliminary work, we also learned that THC seems to be driving changes in neurotransmitters rather than CBD. Finally, we observed that our proposed CBD dose may not be high enough to promote physiological and molecular changes. Overall, our analysis of preliminary data has resulted in some minor changes to experimental design, but all of these are within the scope of the original proposal. There are no resulting changes to our budget.

To date, IACUC approval has been granted based on a previous application. Following extensive discussion with Dr. Nicolas Busquet at the Mouse Behavioral Core, it was determined that the addition of 2-3 behavioral tests plus a series of pilot studies would ensure success and high quality data. These tests are within the scientific scope of the original proposal and budget. However, these changes require an amendment to the IACUC protocol. Following discussions with the head Veterinarian and head of the Animal Facility, Dr. Jori Leszczynski, a brand new IACUC protocol was advised. This new protocol has been submitted and is currently undergoing final revisions. Note that all studies could be conducted under the original protocol based on an amendment but a new submission was strongly recommended, which has meant a delay in conducting the studies.

The original application proposed only using an Open Field test to assess changes in anxiety. Because anxiety can be difficult to assess using a single test, Dr. Busquet recommended orthogonal tests/tasks to fully understand changes in behavior. The additional behavioral tests are as follows: Raised elevated maze, light/dark box, and object exploration. These tests will be conducted on the same mice and can be conducted with 4 mice at a time, hence no addition to the budget is required. Because Mr. Nate Anderson was awarded an Emerging Scientist award, which included assisting with these studies, no changes to salary are required.

Dr. Busquet also strongly recommended a short-term pilot project using high/no Cannabis to determine if behavioral differences could indeed be determined using the high dose. In addition, recent data strongly suggests that male and female rats respond differently to Cannabis/THC (Baglot, et al 2021). Therefore, we will be conducting the short term pilot study using both sexes; if differences due to sex are noted, we will conduct all studies in males and

females.

While progress has been made, there have been delays for two major reasons, as discussed with ICR staff. First, the PI of the project, Dr. Reisdorph became ill with COVID in November, 2021 and is still recovering. This caused delay in designing the final behavioral studies with Dr. Busquet and in submitting the new IACUC proposal. The new IACUC proposal was also delayed due to a new requirement for Biosafety protocols; Dr. Reisdorph had to have a new biosafety protocol accepted before the IACUC would be accepted. This has been approved. Second, the Mouse Behavioral Core was scheduled to move to a new building on the Anschutz campus near the end of 2021. This date has been delayed several times and is now scheduled for April, 2022. The new facilities are being upgraded to include amenities such as housing the mice in the same room in which the behavioral studies will be conducted. While we could have conceivably conducted some of the studies in the old facility and some in the new, this would have introduced a significant source of variation. To ensure high quality results and remove this variation, we are choosing to wait and conduct all Goal #1 studies in the new facility. However, due to construction delays, this means that the mouse studies will not be completed in Y1 as expected. Therefore, we will now be conducting these studies starting in May, 2022 and completing them in 2022. The overall timeline will not be affected as the mass spectrometry assays can be conducted on a shortened timeframe; their timing was based on expected completion of the Goal #1 mouse studies and not on capacity of the instruments.

9. Quantification of Endo- and Phytocannabinoids with Comparison to Pain Medication Requirements and Surgical Outcomes for Patients Undergoing Abdominal Surgery for Cancer

PI: Camille Stewart

Specific Aims:

Determine if post-operative pain medication requirements are different between daily users and non-users of cannabis products who undergo abdominal surgery for the treatment of cancer.

Compare post-operative pain medication requirements to pre-operative plasma endo- and phytocannabinoid concentrations in daily users and non-users of cannabis products who undergo abdominal surgery for the treatment of cancer.

Compare 30-day post-operative complications to pre-operative and post-operative plasma endo- and phytocannabinoid concentrations in daily users and non-users of cannabis products who undergo abdominal surgery for the treatment of cancer.

Summary of progress in achieving goals, objects, and aims:

A research team was assembled.

This team met every 2 weeks during preparation for study enrollment and during the first 3 months of study enrollment.

The team now meets monthly (second Thursday of the month from 9-9:30 AM) to review enrollment numbers, any issues related to the study, and discuss next steps.

The study team has executed the following steps towards completing the research study:

Obtained Colorado Multi-Institutional Board Review approval – COMIRB protocol #21-3544. Initial approval was obtained July 19, 2021.

The study protocol has been amended twice since initial approval –

December 6, 2021 – enrollment locations amended to include patients undergoing surgery at UCHealth Highlands Ranch Hospital. This was performed to increase the number of patients eligible for enrollment.

January 25, 2022 – enrollment criteria amended to include chronic cannabinoid users defined as weekly use for at least 3 months prior to study enrollment. This was performed due to increase enrollment speed of cannabinoid users.

Created a RedCap study database

Created a Microsoft Teams study team page for communication about study protocols, consents, pre-screened patients, and enrolled patients

Ordered supplies for biospecimen collection and preparation to measure endo- and phytocannabinoids

Research team members were trained on biospecimen collection and preparation

Established storage location for biospecimens

Began screening for enrollment of the research study on September 16, 2021. As of March 26, 2022 (~6 months of enrollment) –

123 patients have been pre-screened for enrollment
115 patients have been approached for enrollment
37 patients have been enrolled

29 patients have completed all study activities including data collection
5 patients have pending or ongoing study activities and data collection
2 patients were screen failures

Goal enrollment is 40 non-users of cannabinoids and 40 chronic cannabinoid users. Of the 35 patients who have successfully been enrolled:

24 patients are non-users of cannabinoids
11 patients are chronic cannabinoid users

At this enrollment rate, anticipating 1.8 chronic cannabinoid users enrolled per month, enrollment and data collection should be complete after 23 months (August 2023).

This enrollment rate is ahead of schedule.

Began the first batch of biospecimen endo and phytocannabinoid assays for the first 35 patients enrolled – results are currently pending.

10. Is what you see what you get? A systematic, public health-driven analysis of cannabis product label claims vs. actual cannabinoid content – MX

PI: Tyrell Towle

1. MedPharm ("MP") will purchase authentic Retail Marijuana and transport the samples to the MP laboratory

2. A MP representative will assign an internal code, remove all identifying and packaging information, and provide the blinded MP sample to a MP analyst for potency analysis.

a. The potency analysis includes 10 of the most commonly encountered cannabinoids (CBD, CBDA, d-9 THC, d-8 THC, THCA-A, CBG, CBGA, THCv, CBC, and CBN).

3. The information collected from the packaging and photos of the packaging and product will be uploaded to Dr. Bidwell's REDCAP system.

4. The potency of the samples will be determined in triplicate and the average result will be uploaded to Dr. Bidwell's REDCAP system.

5. Dr. Bidwell's laboratory will analyze the data received from MP.

Summary of Progress:
Funding has not been released from CDPHE to purchase samples, so work on live samples has not begun. We are required by the MED to achieve ISO 17025 certification prior to beginning any work as well. We have made substantial progress on achieving ISO 17025 certification and have had our initial audit. It is estimated that we will have certification on or around May 1, 2022. Once this is achieved and funding is released from CDPHE, we will be able to commence the work with live samples.

11. Investigating the effect of cannabidiol and cannabidiol-trazodone combination treatment on naturally- occurring canine cognitive dysfunction syndrome as a surrogate for Alzheimer's disease

PI: Stephanie McGrath

The objective of this study is to evaluate the effect and tolerability of CBD with and without trazodone in dogs with naturally occurring CCD as a model for AD.

Aim 1: To determine if CBD alone or in combination with trazodone is successful in improving clinical signs and delaying disease progression in dogs with CCD, as a model for AD. Dogs with CCD will be treated with either CBD alone, CBD/trazodone combination therapy, or a placebo to assess efficacy in treating neurodegenerative disease.

Progress towards Aim 1: During the last 8 months, a team of personnel have been assembled, IACUC approval has been secured, study documents have been created to support the clinical trial, and recruitment techniques have been instituted. The study officially launched in March and 4 dogs have been successfully enrolled. Since the study is double blinded, no efficacy data will be available until the end of the active enrollment.

Aim 2: To assess the tolerability and safety of co-administration of CBD and trazodone in dogs with CCD. A group of dogs with naturally occurring CCD will be treated with CBD and trazodone in combination and closely monitored for adverse events at home by pet owners and objectively via laboratory tests (CBC, biochemistry panel, bile acid test).

Progress towards Aim 2: It was decided that Aims 1 and 2 would be altered. Since neither CBD nor trazodone have been used in the past to treat canine cognitive dysfunction, we made the decision to administer each medication alone, rather than in combination. This will allow us to assess the efficacy of each prior to studying combination therapy. We are gathering data (n = 4 thus far) on safety and tolerability of each medication in older dogs suffering from cognitive decline. Again, because the study is double blinded, data are not available. However, no adverse events have been noted to date.