Statistical Analysis Plan: Evaluating the Comparative Pharmacokinetics of Nicotine after administration via JUUL or Tobacco Cigarettes

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Summary of Protocol

Description and Outcome Measures

This is an observational, crossover design that will examine the pharmacokinetics and pharmacodynamics of smoking tobacco cigarettes compared to vaping the JUUL electronic cigarette. Participants will be recruited in two groups: primary e-cigarette vapers and primary cigarette smokers. The order of assignment to either tobacco cigarettes or the JUUL electronic cigarette will be counter-balanced. We will seek to enroll 30 participants total (15 in each group).

The following schedule of events describes the study procedures:

Study Day – Tobacco Cigarette Condition	**Study Day – JUUL E-Cigarette Condition**
Day 1	**Day 2**
←---------- **HOSPITAL** ----------→	

• **Baseline Blood Draws**
  • 1 nicotine 6ml blood draw
  • 1 catecholamine 6ml blood draw
• **Baseline Urine Nicotine and VOCs**
• **Baseline Heart Rate**
• **Baseline Blood Pressure**
• **1st Standardized Session**
• **2-hr abstinence and biological samples and measurements**
  • 9 nicotine 6ml blood draws
  • 12 heart rate measurements
  • 10 blood pressure measurements
  • 1 urine sample
  • 1 catecholamine 6ml blood draw
• **4-hr recorded ad libitum use and biological samples and measurements**
  • 8 nicotine 6ml blood draws
  • 1 urine sample
	• **Baseline Blood Draws**
	  • 1 nicotine 6ml blood draw
	  • 1 catecholamine 6ml blood draw
	• **Baseline Urine Nicotine and VOCs**
	• **Baseline Heart Rate**
	• **Baseline Blood Pressure**
	• **1st Standardized Session**
	• **2-hr abstinence and biological samples and measurements**
	  • 9 nicotine 6ml blood draws
	  • 12 heart rate measurements
	  • 10 blood pressure measurements
	  • 1 urine sample
	  • 1 catecholamine 6ml blood draw
	• **4-hr recorded ad libitum use and biological samples and measurements**
	  • 8 nicotine 6ml blood draws
	  • 1 urine sample

Power Calculation & Sample Size

Our power analysis based on a 2-condition within-subject repeated measures comparison. To estimate the required sample size the minimal power level was set at 80% and Type I error at 0.05, with use of a 2-sided test. The primary outcome on which we have powered the study is the difference in nicotine exposure for JUUL vs tobacco cigarette use. We used prior within-subject variability data on plasma cotinine (mean 200 ng/ml, coefficient of variation, COV 25%) in TC smokers whose cotinine had been measured on multiple occasions for estimation. For 15 participants, we have 80% power to detect an effect size ratio of 1.25 for daily nicotine exposure.

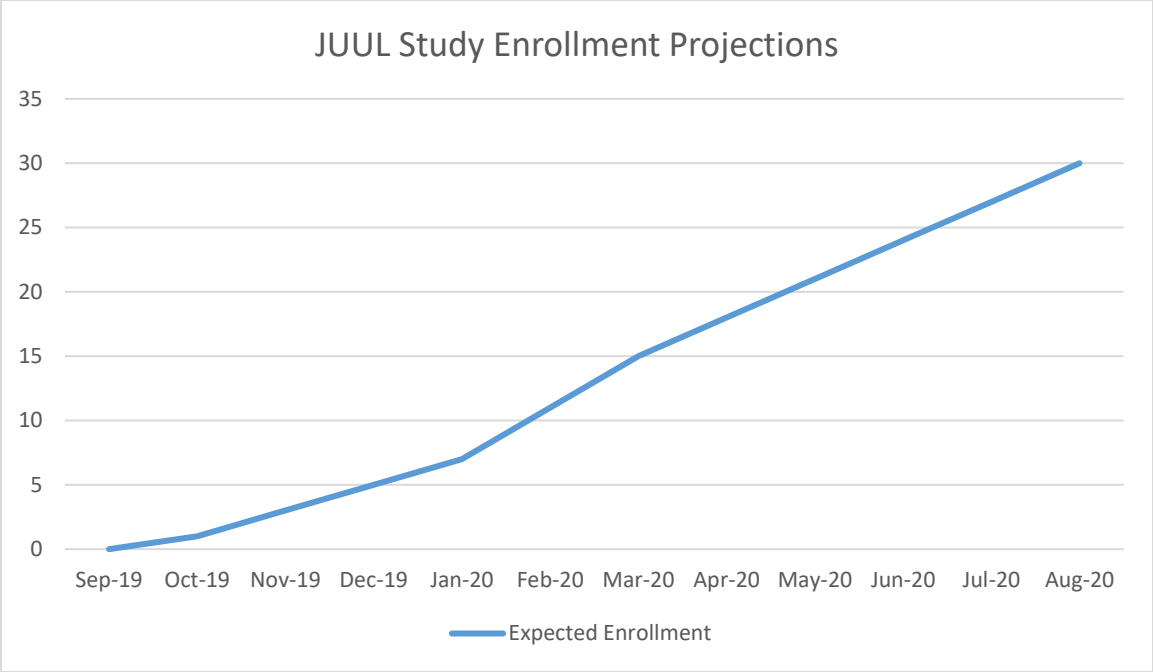
Trial Management

Study Site & Timetable

Participant visits will be conducted at Zuckerberg San Francisco General Hospital (ZSFG) at both the Tobacco Research Center and the Clinical Research Services Research Ward.

The projected study timetable is as follows:

Timeline	Specific Aim
Preparation (Months 1-2)	• Study startup (supply purchasing, case report form and database completion) • Clinical Research Coordinator & CTSI staff training • Participant Recruitment
Clinical Study (Months 3-12)	• Recruit, enroll and complete study procedures on approximately 2-4 participants per month • Conduct online analytical chemistry assays • Conduct ongoing data entry and quality assurance
Analysis (Months 9-12)	• Complete data entry, analysis and manuscript preparation • Complete analytical chemistry assays



Targeted Population

We will seek to complete a total of N=30 randomized participants. We will seek to enroll equal numbers of men and women, and all minority groups will be invited to participate. At a minimum we will enroll 25% women in the sample (about N=5). We will not enroll minors or those under tobacco purchasing product age (21 years) in the state of California.

Data Acquisition and Transmission

Data capture will occur in several ways. Identifying information will be stored locally at UCSF in a secure database and/or locked secure files. General recruitment and enrollment information, such as dates of screening and enrollment will be stored in a password protected Excel database supported by Information and Technology (IT) at UCSF.

Data will be acquired via participant self-report and in a combination of paper source documentation transcribed into electronic data capture (EDC) by clinical research staff, and by direct entry into EDC by participants. Medical history data will be collected as paper source and will remain paper source and not entered. Demographics, nicotine & tobacco product use, drug use and nicotine dependence recorded at baseline will be entered by the participant directly into EDC, and there will be no paper source files. Physiological measures such as blood pressure, urine tox and pregnancy test results will

be recorded on a paper CRF and entered into the EDC. Adverse events will be recorded on an AE log. This paper source log will be entered into the EDC. Questionnaires completed during the study on craving, satisfaction and emotions will be entered by the participant directly into EDC. Cardiovascular data will be downloaded from the ambulatory blood pressure monitor into a PDF, which will be transcribed into Excel and uploaded directly into EDC. Video files from the recorded adlib sessions will be transferred from the camcorder to an encrypted hard drive that will be stored in a secured locked cabinet accessible by study staff only. All source documents will be stored in participant binders. Data management activities will be performed using REDCap.

REDCap is a suite of software tools, supported by UCSF, which enables the collection, cleaning and viewing of clinical trial data. The database will be designed by the study team and once approved by the Investigator, Statistician, and Clinical Research Coordinator (CRC), the database will be put into production and data entry will begin. Data can be entered and changed only by those with the rights to do so. Varying interfaces allow for the CRC to view and enter data in a database format, and for the research participant to respond to questionnaires directly through electronic data capture.

REDCap has all capability to be compliant with 21CFR Part 11 and all relevant technical aspects of relevant GCP guidelines and allows for the following:

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.
- The system allows for automated field calculations, basic validation checks and double data entry comparisons.

When data entry is complete, all data will be reviewed for any missing, incomplete, or invalid data points for resolution by the Clinical Research Staff and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

Research data will be protected and maintained by:

  - Data files (and data link files) will be stored in a password-protected, secure network folder. All data files that are created and shared with the study

investigators will be encrypted and password-protected.

- Access to the data by all necessary research personnel will be through a password-protected network computer. At no time will data be downloaded on a thumb drive or other portable media or laptop. Subject identity will not be disclosed in the event of publication or sharing of data.
- Any paper files containing identifiers (if applicable to the research) will not be taken off UCSF premises; they will be kept in a locked filing cabinet in a secure building.
- Video files will not be transferred or stored on any non-encrypted devices and the hard drive containing the video files will not be taken off UCFS premises; the drive will be stored in a locked cabinet in a secure building.

- Subject identity will not be disclosed in the event of publication or sharing of data.
- PHI will be not re-used or disclosed for purposes other than research use.
- There are no outside entities to which PHI will be disclosed.

To ensure adherence to ethical practices, it will be made clear to participants that all information obtained during assessments is confidential and that no information will be shared with the participants' clinicians or others unless the participant requests this in writing and signs a HIPPA form. All investigators and staff associated with this study have been trained, and new hires will be trained, on human research ethics and Good Clinical Practice in accordance with the requirements of UCSF during initial study approval.

To ensure the integrity of the study design and procedures, standard operating procedures are in place to ensure that data is collected in a consistent manner across all Clinical Research Coordinators. The Co-Investigator (NN) will train and closely monitor the CRCs on the procedures to be used in this study. Such monitoring will consist of in-person discussion of study visits and review of case report forms. NN and CRCs will meet at least weekly to discuss any study issues and their resolutions and will meet more frequently if necessary.

To ensure minimal missing data, questionnaires will be reviewed for completeness while the participant is present. Several biochemical measures (such as expired breath CO, urine pregnancy test, urine drugs of abuse test) will be analyzed immediately, while the participant is present and retested if needed. If the volume of these samples is insufficient, a repeat sample will be requested and submitted as close to the clinic visit as possible. Oversight for quality control and adherence to protocol procedures will be conducted by both Co-Investigators, NN and GSH.

Data Entry Methods

Data entry into the REDCap database is to be completed in a timely fashion as follows:

- Entry within 48 hours of the participants' visit

Clinical research coordinators will review all entered data. Data entered directly into EDC, like questionnaires, etc. will be reviewed while the participant is present. Any issues will be clarified.

For data entered by the CRCs into the EDC (physiological measures & adverse events), queries will be performed on a sample of data entered to ensure validity. Once all queries have been resolved, the data can be released to the statistician (NAO) for analysis.

Data will remain in the REDCap system until it is downloaded and integrated into a secure, HIPAA compliant study server. Other individuals (Co-Is, post-doctoral fellows, graduate students, etc.) can submit a data request form to the PI to access relevant data. The request will require approval from the Steering Committee composed of the PI and all Co-Is.

Essential documents will be retained per UCSF's policy for 3 years from the study termination date. All files containing PHI will be de-identified by redacting any identifiers before boxing and storage. Files may be stored at UCSF's off-site storage facility before destruction.

Data Analysis Plan

The first step in the analysis will be examining the collected data using graphical analysis and descriptive summaries to ensure that all are within expected ranges, to check for the presence of outliers and abnormal values, and to ascertain that the distribution of all measures meets the assumptions of the statistical tests to be used. Means of the outcome measures for will be tested for equality using a repeated measures model. Tests will include, in addition to treatment condition, a test for order to determine possible carry-over effects. Estimation will be done using full-information maximum likelihood methods so that we can use all data in estimation even if some are missing. Analyses will primarily be conducted using SAS 9.4. More specifically, the analyses for each sub-aim are described below:

Specific Aim 1: We will determine from the standardized sessions the plasma nicotine peak concentration, time to peak concentration and area under plasma nicotine concentration-time curve (AUC) and compare JUUL vs. tobacco cigarette conditions using a mixed-effect

model for cross-over. We will also compute ratios of AUC values for the two conditions, as is commonly done in comparing the pharmacokinetics of different pharmaceutical products. The ratio of AUCs will give us information on relative nicotine doses obtained from JUUL vs. tobacco cigarettes.

Specific Aim 2: We will perform the same analysis as Specific Aim 1, but with plasma nicotine concentrations from the ad libitum periods.

Specific Aim 3: With standardized sessions, we will use a paired t test to compare peak changes in heart rate, and changes in plasma norepinephrine and epinephrine concentration, and pulmonary function tests (FEV1, FVC, FEV1/FVC).

Specific Aim 4: Satisfaction, psychological reward, unpleasant effects and withdrawal symptoms will be compared using a mixed effects model for cross-over data average measures from the mCEQ, the QSU and the MNWS for the JUUL e-cigarette and tobacco cigarette conditions.

Quality Assurance

Procedures in place to ensure the validity and integrity of the data

The Principal Investigator (NB) will be responsible for monitoring the overall safety and integrity of the study, executing the Data Safety Monitoring (DSM) plan, and complying with the regulatory and reporting requirements. The PI (NB) will provide an annual summary of the Data Safety and Monitoring Board (DSMB) report to NIH and FDA/CTP along with the progress report. The Institutional Review Board (IRB) at UCSF will approve study protocol at UCSF.

There will be a DSMB that will meet annually by telephone conference or in person, as deemed appropriate. The DSMB will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study. In addition, the Principal Investigator (NB) and Medical Monitor will be responsible for continuous data and safety monitoring of all participants enrolled in this study.

Monitoring of the study procedures and progress of the trial will be overseen by the PI and Co-Is (NN & GSH). The rate of recruitment, whether a potential participant met eligibility criteria, conduct of the study and compliance with study procedures, drop-outs and reason for drop-out will be reviewed regularly by the Co-Is (NN & GSH) and overseen by the PI. Issues pertaining to data validity and integrity will be addressed formally each week by all study staff members.

Procedures to guarantee the accuracy and completeness of the data, during data collection, entry, transmission, and analysis

All staff involved in the conduct and/or monitoring of this study will have completed the UCSF Human Subjects Protection Training and the HIPAA Research Training. Documentation of training will be in the study regulatory binder. Standard operating procedures (SOPs) are in place and all staff have been trained and certified in conducting these procedures. All assessments will be administered by these trained Clinical Research Coordinators (CRCs) under the supervision of the Co-I (NN).

All subject data and records will be maintained in binders and/or secure filing cabinets. All data and subject binders will be stored in a safe place to protect confidential subject information. Safe places will include locked filing cabinets or locked rooms accessible only by study personnel. Full subject names will not be listed on the outside of binders to protect confidentiality of study participants. A sample of entered source data will be checked against the raw data source, and the correct data entry will be used. All data entered into spreadsheets and databases will be coded by subject ID number and not by subject name (i.e., first and last name). Additionally, all entered data will be backed up on a secure study server. Computers used in the study will be password protected, accessible only by study personnel.

Protocol compliance and data integrity will be monitored by the Co-I on a weekly basis during staff meetings and periodically by reviewing the data collection and entry information.

Regulatory Issues

Reporting Adverse Events

Oversight for quality control and adherence to protocol procedures will be conducted internally by the PI (NB) and the Co-Is (NN & GSH). Data accuracy and protocol compliance will be ensured by providing the DSMP to the DSMB for their feedback; enforcing standard operating procedures; training, overseeing and monitoring the protocol quality and compliance; establishing methods to minimize drop outs and missing data and double entry of data that needs to be transferred from source documents to the database.

Safety assessments will consist of recording and monitoring all adverse events and severity, including serious adverse events. The site Medical Monitor and/or PI will review all AEs. A study participant may be discontinued from the study if the Medical Monitor or Dr. Benowitz determine it is the best decision in order to protect the safety of

a participant.

Monitoring for safety will be carried out by review of all clinical complications, assessment of severity and relatedness. Reporting of adverse events will follow requirements mandated by the IRB at UCSF. If reportable, the event will be followed by a detailed written report within five (5) working days of the PI's awareness. In addition, any reportable incidents or problems involving the conduct of the study or participant participation, including problems with the recruitment and/or consent processes will be **reported within five (5) working days** of the PI's awareness. The PI will provide a discussion of any problems noticed during each year in the course of the study to the IRB on an annual basis.

Adverse events: An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after initiating study procedures and starting the study product (e.g., ECs) even if the event is not considered to be related to the product. Medical conditions/diseases present before starting the study product are only considered adverse events if they worsen after beginning study procedures.

The standard adverse event grading scale will be used to report any potential adverse events:

- **Mild AE**: did not require treatment;
- **Moderate AE**: resolved with treatment;
- **Severe AE**: resulted in inability to carry on normal activities and required professional medical attention;
- **Life-threatening or disabling AE**
- **Fatal AE**

The relationship of event to study product and/or procedures will be documented by the Investigator as follows:

- **Unrelated:** The cause of the AE is known and the event is in no way related to any aspect of study participation. If there is any uncertainty regarding AE causality then the event must be assessed as possibly related to research participation and reported to the CHR as indicated. Often, the cause of an unrelated AE is disease progression.
- **Possibly Related:** An AE is possibly related when there is a reasonable possibility that the event might have been caused by study participation. A possibly related event may follow no known pattern of response and an alternative cause seems more likely. In other circumstances there may be significant uncertainty about the cause of the event, or a possible relationship to study participation cannot reasonably be ruled out.

- **Probably Related:** An AE is probably related when there is a reasonable possibility that the event is likely to have been caused by study participation. The AE has a timely relationship to the study procedure(s) and follows a known pattern of response, but a potential alternative cause may be present.
- **Definitely Related:** An AE is definitely related to study participation if it is clear that the event was caused by study participation. A definitely related event has a strong temporal relationship and an alternative cause is unlikely.

All routine AEs occurring between the date the participant signs the consent until the completion of study procedures will be reviewed by the supervising Medical Monitor and/or PI. Dr. Benowitz will also be notified of any severe, life- threatening/disabling or fatal AEs regardless of relatedness. Such AEs will be reported to NIH and FDA/CTP regardless of relatedness. AEs will not be reported to the IRB unless they meet criteria of relatedness. Mild and moderate AEs will not be reported unless they are also unexpected.

Expected and Unexpected AEs:

- **Expected AE:** an AE that may be reasonably anticipated to occur as a result of the study procedures or study participation and should thus be described in the research proposal, the informed consent document and Investigator's Brochure (when applicable), or is part of the normal disease process or progression.
- **Unexpected AE:** An adverse event is defined as being unexpected if the event exceeds the nature, severity, or frequency described in the current CHR application including the protocol, consent form and investigator brochure (when applicable). An unexpected AE also includes any AE that meets any of the following criteria:
    - Results in subject withdrawal from study participation,
    - Due to an overdose of study medication, or
    - Due to a deviation from the IRB approved study protocol

Serious Adverse Event (SAE)

A SAE is any AE that results in any of the following outcomes:

- Death,
- Life-threatening adverse experience – any adverse event that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does NOT include a reaction that, had it occurred in a more severe form, might have caused death;
- Inpatient hospitalization or prolongation of existing hospitalization (for >24 hours);
- Persistent or significant disability/incapacity;

- Congenital anomaly/birth defect, or cancer; or
- Any other experience that suggests a significant hazard, contraindication, side effect or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above;
- Event that changes the risk/benefit ratio of the study.

As per UCSF IRB requirements, AEs which are determined to be **definitely, probably or possibly related** AND are **serious or unexpected** will be reported to the IRB within 5 days of PI's awareness and to our NIH project officer within **72 hours** of the PI's awareness. Serious AEs, unrelated to research, as determined by the PI or medical professional, will not be reported to the UCSF IRB (this includes on site deaths).

- All related SAEs will be followed, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable, irreversible, or resolved. New information will be reported when it is received.

- In the event that a participant experiences a serious adverse event related to the study product or an unanticipated problem during the course of a study, the guidelines in the UCSF IRB Policies and Procedures Manual for adverse event and serious adverse event reporting will be followed. If appropriate, the PI will discontinue the subject's participation in the study and coordinate a referral for appropriate care for the participant elsewhere. These same actions will be taken if a participant exhibits emergence of unstable psychopathology or any significant medical disorder.

- The PI/Project Manager will report all AEs/SAEs and actions related to AEs/SAEs to the UCSF IRB if they meet reporting requirements as described above. Reports will be sent through the iRIS system.

- The PI/Project Manager will report all related AEs/SAEs and actions related to AEs/SAEs to NIDA as detailed below.

- The DSMB will receive a monthly report of all adverse events and be notified within 24 hours if an event is reported to the UCSF IRB.

- The PI will provide a discussion of any problems noticed during each year in the course of the study to the IRB and DSMB on an annual basis.

Safety evaluation: Safety analyses will be performed on all accrued subjects who completed any or all of study procedures. The assessment of safety will be based on the frequency of adverse events (AE), severity grade of AEs (mild, moderate, severe), and grade

of expectedness (unexpected, expected). Other safety data (e.g. carbon monoxide, nicotine blood levels, cotinine urine concentration, vital signs, and special tests) will be considered as appropriate.

Adverse events will be summarized by presenting the number and percentage of participants who experienced any adverse event, the number and percent reporting adverse events in each body system and the number and percent of adverse events by type. Any other information collected (e.g., severity, expectedness and relatedness to study product) will be listed, as appropriate.

A summary of clinically relevant toxic events, such as adverse events leading to death or rated as SAEs, those with a suspected relationship to study product, or adverse events requiring further medication or non-drug therapies will be provided.

Reporting Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
    - The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious as defined above.

Unanticipated problem reporting will begin at the time of participant consent. The Incident Report Form will be submitted to the UCSF IRB **within 10 working days of the PI's awareness** if the UP is determined to be related to study product or procedures.

Reporting Actions to the NIDA

Serious and related or unexpected and related adverse events as defined will be reported to the Principal Investigator (NB), site Medical Monitor, all co-investigators, the UCSF IRB, the NIDA Project Officer (Kevin Walton, PhD), and the DSMB. We will report these events to NIDA **within 72 hours of PI's awareness**.

Additionally, any severe, life-threatening/disabling or fatal AEs regardless of relatedness will be reported to NIDA **within 72 hours of PI's awareness**. Actions taken by the UCSF IRBs in response to AEs/SAEs will be reported to NIDA in the annual noncompetitive continuation application, as will reports of changes or amendments to the protocol as a result of an AE/SAE.

Any change that requires review by the IRB would be considered substantial and will be reported to NIDA for approval prior to implementation. Recommendation for trial study discontinuation, for significant changes or amendments to the protocol, or other significant findings as a result of a study related SAE will be reported immediately to the NIDA Study Officer (Kevin Walton) by the PI (NB).

Updates encompassing enrollment and completion rates as well as **all adverse events that are possibly, probably or definitely related** (including those pertaining to the symptoms within the current case definition of vaping-associated lung injury) will be **reported to NIDA on a monthly basis**.

Reporting Changes or Amendments to the Protocol

Any changes or amendments to the protocol made in response to adverse events/SAEs (or independent of AEs/SAEs) will be requested through a modification to the UCSF IRB, which will then grant or deny permission to make the requested change in protocol. The DSMB will be notified about any significant changes to the protocol. Any change that requires review by the UCSF IRB would be considered substantial and will be reported to NIH for approval prior to implementation. Changes that significantly alter the scope of the research or the ability of the research to achieve its specific aims will be submitted to the PI (GSH) and Medical Monitor, the DSMB, and NIH for approval prior to implementation.

Trial Stopping Rules

The trial will be stopped if the investigators or local IRB recommends trial discontinuation due to SAEs, if the trial is not progressing due to lack of participant recruitment, or if advised by the Data Safety Monitoring Board (DSMB).

Any questions regarding financial conflict issues can be directed to PI (Phone no.: 415-206-8324).

Conflict of Interest

The UCSF IRB requires financial disclosures to be on file for the study Investigators. For this project, Principal Investigator Neal Benowitz, MD will indicate to the IRB at the time of study submission that he does not have financial interests in the study. The UCSF IRB retains a current copy of Dr. Benowitz's financial disclosures.

The Investigators will complete the conflict of interest disclosure form and provide it to the DSMB, prior study initiation and after one year. Each member of DSMB will complete a conflict of interest disclosure form prior to each meeting.