MicroRNA Activation of LOX-1 Mechanisms in Endometriosis

NCT05331053

04/18/2018

Study Protocol and Statistical Analysis Plan

PennState

HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:

Provide the full title of the study as listed in item 1 on the "Basic Information" page in CATS IRB (http://irb.psu.edu).

Role of microRNA activation of lectin-like oxidized LDL receptor (LOX-1) mechanisms in microvascular dysfunction in women with endometriosis (IRB# 9584)

Principal Investigator:

Name: Lacy M. Alexander, Ph.D.
Department: Kinesiology
Telephone: 814-867-1781
E-mail Address: lma191@psu.edu

Version Date:

Provide the date of this submission. This date must be updated each time the submission is provided to the IRB office with revisions.

4/18/18

Clinicaltrials.gov Registration #:

Provide the registration number for this study, if applicable.

NA

Important Instructions for Using This Protocol Template:

1. Add this completed protocol template to your study in CATS IRB (http://irb.psu.edu) on the "Basic Information" page, item 7.
2. This template is provided to help investigators prepare a protocol that includes the necessary information needed by the IRB to determine whether a study meets all applicable criteria for approval.
3. **Type your protocol responses below the gray instructional boxes of guidance language. If the section or item is not applicable, indicate not applicable.**
4. **For research being conducted at Penn State Hershey or by Penn State Hershey researchers only, delete the instructional boxes from the final version of the protocol prior to upload to CATS IRB (http://irb.psu.edu). For all other research, do not delete the instructional boxes from the final version of the protocol.**
5. When making revisions to this protocol as requested by the IRB, please follow the instructions outlined in the Study Submission Guide available in the Help Center in CATS IRB (http://irb.psu.edu) for using track changes.

If you need help…

University Park and other campuses:	College of Medicine and Hershey Medical Center:
Office for Research Protections Human Research Protection Program The 330 Building, Suite 205 University Park, PA 16802-7014 Phone: 814-865-1775 Fax: 814-863-8699 Email: irb-orp@psu.edu	Human Subjects Protection Office 90 Hope Drive, Mail Code A115, P.O. Box 855 Hershey, PA 17033 (Physical Office Location: Academic Support Building Room 1140) Phone: 717-531-5687 Fax number: 717-531-3937 Email: irb-hspo@psu.edu

Table of Contents

1.0 Objectives

1.1 Study Objectives

Describe the purpose, specific aims or objectives. State the hypotheses to be tested. Describe the treatment that is considered standard of care (i.e., indicate how patients would be treated in a non-investigational setting). Indicate if the study test article(s) is available to patients without taking part in the study.

Epidemiologic data demonstrate a clear association between endometriosis, reproductive risk factors, inflammation and cardiovascular (CV) risk [5, 8-11]. Circulating factors, Low-density lipoprotein (LDL) and oxidized LDL (oxLDL), are two of many biomarkers of cardiovascular and inflammatory disease of endometriosis [9, 10]. An important signaling mechanism through which circulating LDL and oxLDL act is the lectin-like oxidized LDL receptor (LOX-1). LOX-1 signal transduction functionally results in pronounced endothelial dysfunction, a hallmark of CV. We hypothesis that one factor mediating the elevated risk of cardiovascular disease in endometriosis is microRNA (miRNA) activation of LOX-1 receptor mechanisms.

Specific Aim 1. To test the hypothesis that LOX-1 receptor activation is increased leading to endothelial dysfunction in endometriosis.
Hypothesis 1A. In women with endometriosis, LOX-1 receptor activation is increased resulting in endothelial dysfunction mediated through oxidant stress, endothelial NO-synthase uncoupling, and disruptions of intracellular trafficking.

Specific Aim 2. To test the hypothesis that decreased microRNAs (i.e. let7-a, let7-b, let7-g, MiR98, Mi590-p) are driving increased LOX-1 receptor expression and function in endometriosis.
In women with endometriosis:
Hypothesis 2A. MicroRNAs will be negatively related to LOX-1 receptor expression;
Hypothesis 2B. MicroRNAs will be negatively related to endothelial function.

1.2 Primary Study Endpoints

State the primary endpoints to be measured in the study. Clinical trials typically have a primary objective or endpoint. Additional objectives and endpoints are secondary. The endpoints (or outcomes), determined for each study subject, are the quantitative measurements required by the objectives. Measuring the selected endpoints is the goal of a trial (examples: response rate and survival).

Skin blood flow (cutaneous vascular conductance)

1.3 Secondary Study Endpoints

State the secondary endpoints to be measured in the study.

LOX-1 receptor activation (soluble LOX receptor), microRNAs (i.e. let7-a, let7-b, let7-g, MiR98, Mi590-p), OxLDL, LDL, blood pressure, heart rate

2.0 Background

2.1 Scientific Background and Gaps

Describe the scientific background and gaps in current knowledge.

Endometriosis is an estrogen-dependent gynecological disorder associated with considerable chronic pelvic pain, pain during intercourse, and is a major cause of infertility [1, 2]. This disorder affects 6% - 10% of reproductive age women and can be as high as 35-50% in women experiencing pain or infertility [3-6] Endometriosis derives from the presence of endometrium-like tissue in sites outside the uterine cavity. While endometriosis is a local inflammatory syndrome, the inflammatory process is systemic [7].

Endometriosis is associated with higher risk of hypercholesterolemia and hypertension [8]. Epidemiologic data demonstrate a clear association between endometriosis, reproductive risk factors, inflammation and cardiovascular (CV) risk [5, 8-11].

Endometriosis and Endothelial Dysfunction: Circulating factors including LDL and oxidized LDL are two of many biomarkers of cardiovascular and inflammatory disease of endometriosis [9, 10]. An important signaling mechanism through which circulating LDL and oxLDL act is the lectin-like oxidized LDL receptor (LOX-1). LOX-1 is a ubiquitously expressed scavenger receptor, stimulated by oxLDL, Ang II, and other inflammatory cytokines, and inhibited by estrogen[12]. LOX-1 is the upstream signaling initiator of mechanisms including increased oxidant production, reduced nitric oxide (NO) metabolism, and impaired intracellular trafficking [13]. Thus, LOX-1 signal transduction functionally results in pronounced endothelial dysfunction.

2.2 Previous Data

Describe any relevant preliminary data.

Cell culture and rodent data implicate miRNAs from the let7 family in regulating LOX-1 expression and activation, and these LOX-1-specific miRNAs are similarly dysregulated in endometriosis. We propose to interrogate a direct association between endometriosis-specific miRNAs and elevated LOX-1-mediate endothelial dysfunction.

2.3 Study Rationale

Provide the scientific rationale for the research.

Cardiovascular disease (CVD) is the leading cause of death in women. Endometriosis is associated with higher risk of hypercholesterolemia and hypertension [8]. Epidemiologic data demonstrate a clear association between endometriosis, reproductive risk factors, inflammation and cardiovascular (CV) risk [5, 8-11]. Our scientific premise is that in women with endometriosis, elevated cardiovascular disease risk is the result of endothelial dysfunction and chronic systemic inflammation through LOX-1 receptor activation. We will explore microRNA (miRNA) activation of LOX-1 receptor mechanisms as one factor mediating the elevated risk of CVD in endometriosis.

Lipitor is a commonly used statin that has pleotropic effects. One of those is to inhibit the LOX receptor and decrease the microRNAs that stimulate the LOX receptor. We will test this on an acute (receptor activation level) with microdialysis and on a systemic level. Additionally, statins have been proposed and tested in animals to decrease the endometriosis associated inflammatory and microRNAs we hypothesize to be driving the accelerated CVD risk. The short term oral statin will help us delineate this putative effect/mechanism."

The current application comprises pilot experiments designed to yield data for the development of the final protocol. Modifications (e.g. doses of some of the investigational substances, number of subjects needed to complete study, etc.) to this protocol will be made in the future based on the results of the pilot experiments.

3.0 Inclusion and Exclusion Criteria

Create a numbered list below in sections 3.1 and 3.2 of criteria subjects must meet to be eligible for study enrollment (e.g., age, gender, diagnosis, etc.). Indicate specifically whether you will include any of the following vulnerable populations: (You may not include members of these populations as subjects in your research unless you indicate this in your inclusion criteria.) Review the corresponding checklists to ensure that you have provided the necessary information.

- **Adults unable to consent**
  - Review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information. HRP-417 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).
- **Individuals who are not yet adults (infants, children, teenagers)**
  - If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children"), review the "CHECKLIST: Children (HRP-416)" to ensure that you have provided sufficient information. HRP-416 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).
- **Pregnant women**
  - Review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information. HRP-412 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

- **Prisoners**
  - Review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information. HRP-415 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).
- **Neonates of uncertain viability or non-viable neonates**
  - Review "CHECKLIST: Neonates (HRP-413)" or "CHECKLIST: Neonates of Uncertain Viability (HRP-414)" to ensure that you have provide sufficient information. HRP-413 and HRP-414 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

3.1 Inclusion Criteria

List the criteria that define who will be included in your study.

- Healthy women between the ages of 18 and 45 years (Controls), taking oral contraceptive or with regular menses every 26-34 days
- Women between the ages of 18 and 45 years with endometriosis (diagnosis by prior laproscopy by subject's own physician, and reported by the subject to the researchers)
- Tylenol if the subject has acute pain is allowed
- Contraceptive use is allowed

3.2 Exclusion Criteria

List the criteria that define who will be excluded in your study.

- Use of nicotine-containing products (e.g. smoking, chewing tobacco, etc.)
- Diabetes (HbA1C .6.5%)
- BP>140/90
- Taking pharmacotherapy that could alter peripheral vascular control (e.g. insulin sensitizing, cardiovascular medications)
- Pregnancy
- Breastfeeding
- Taking illicit and/or recreational drugs
- Abnormal liver function
- Abnormal creatine phosphokinase (CPK)
- Rash, skin disease, disorders of pigmentation, known skin allergies
- Diagnosed or suspected metabolic or cardiovascular disease
- Persistent unexplained elevations of serum transaminases
- Known allergy to latex or investigative substances (including Lipitor)
- Previous adverse reaction to Lipitor or statin

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Insert subject withdrawal criteria (e.g., safety reasons, failure of subject to adhere to protocol requirements, subject consent withdrawal, disease progression, etc.).

Participants may withdraw at any time. We may end the participant's role if we determine that her/his health or behavior adversely affects the study or increases the risks beyond those approved by the Institutional Review Board and agreed upon by the subject in the informed consent.

3.3.2 Follow-up for withdrawn subjects

Describe when and how to withdraw subjects from the investigational product or study; the type and timing of the data to be collected for withdrawal of subjects; whether and how subjects are to be replaced; the follow-up for subjects withdrawn from investigational treatment.

The study team will not contact subjects who voluntarily withdraw. The study team contacts subjects who withdraw due to an untoward or adverse event within 5 days of the withdrawal for follow-up and feedback.

4.0 Recruitment Methods

4.1 Identification of subjects

Describe the methods that will be used to identify potential subjects or the source of the subjects. If not recruiting subjects directly (e.g., database query for eligible records or samples) state what will be queried, how and by whom.

StudyFinder: If you intend to use StudyFinder (http://studyfinder.psu.edu) for recruitment purposes, please indicate this in section 4.1 along with any other methods for identifying subjects. Note that information provided in this protocol should be consistent with information provided on the StudyFinder page in your CATS IRB study.

For Penn State Hershey submissions using Enterprise Information Management (EIM) for recruitment, attach your EIM Design Specification form on the Basic Information page in CATS IRB (irb.psu.edu). See HRP-103 Investigator Manual, "What is appropriate for study recruitment?" for additional information.

We recruit subjects from Centre County, PA and surrounding regions utilizing CTSI resources at University Park. Also, we advertise for subjects (see below). Interested persons contact us. We also avail ourselves of lists of potential subjects maintained by the CRC and our lab. People on these lists have screened for other studies and indicated that they wished to be maintained on a list of potential subjects to be contacted in the event that they may qualify for additional studies. We will use StudyFinder.

4.2 Recruitment process

Describe how, where and when potential subjects will be recruited (e.g., approaching or providing information to potential subjects for participation in this research study).

We advertise for subjects. Interested persons contact us. We discuss the study's purpose and protocol as well as the qualifications for the study with the potential subject. We discuss the informed consent with them. We invite potential subjects to tour the lab, and ask questions.

4.3 Recruitment materials

List the materials that will be used to recruit subjects. Add recruitment documents to your study in CATS IRB (http://irb.psu.edu) on the "Consent Forms and Recruitment Materials" page. For advertisements, upload the final copy of printed advertisements. When advertisements are taped for broadcast, attach the final audio/video tape. You may submit the wording of the advertisement prior to taping to preclude re-taping because of inappropriate wording, provided the IRB reviews the final audio/video tape.

StudyFinder: If you intend to use StudyFinder (http://studyfinder.psu.edu) for recruitment purposes, you do not need to upload a separate recruitment document for information placed on the STUDYfinder site to your study in CATS IRB. Necessary information will be captured on the StudyFinder page in your CATS IRB study.

Study Finder
Newspaper/magazine ads
Letters/Emails to potential participants
Flyers/posters
Web Sites
Listserv
Script – Verbal (i.e., telephone, face-to-face, classroom)

Note: We advertise through various listservs on and off campus that target under-represented groups (e.g. FOBA) as well as listservs belonging groups (e.g. endometriosis support/info groups) and others as we become aware of them.
Note: We use the information from the uploaded ad and/or the phone script in other forms of recruitment (e.g. emails, listservs, websites).

4.4 Eligibility/screening of subjects

If potential subjects will be asked eligibility questions before obtaining informed consent, describe the process. Add the script documents and a list of the eligibility questions that will be used to your study in CATS IRB (http://irb.psu.edu) on the "Consent Forms and Recruitment Materials" page.

StudyFinder: If you intend to use StudyFinder (http://studyfinder.psu.edu) for recruitment purposes, any scripts (phone, email, or other) used when contacting StudyFinder participants as well as any eligibility screening questions must be added to your study in CATS IRB (http://irb.psu.edu) on the "Consent Forms and Recruitment Materials" page.

An interview using the phone questionnaire assesses likelihood of eligibility before obtaining informed consent. Subjects are informed that the information they provide is kept confidential. The data from individuals who qualify or do not qualify are kept and stored until the end of the study, then destroyed. Permission to maintain the information for subjects who qualify and contact them for future studies is requested. See Screening under "Study Design and Procedures" for screening information.

5.0 Consent Process and Documentation

Refer to "SOP: Informed Consent Process for Research (HRP-090)", for information about the process of obtaining informed consent from subjects. HRP-090 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

5.1 Consent Process

5.1.1 Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

Describe where and when the consent process will take place.

We obtain a consent via telephone for the subject to fast before coming to the lab for the screening.
The subject reports to Noll lab for the consenting process. The subject signs an informed consent before any screening procedures are performed.

5.1.1.2 Coercion or Undue Influence during Consent

Describe the steps that will be taken to minimize the possibility of coercion or undue influence in the consent process.

Subjects are informed throughout the study that their participation is voluntary, and they may discontinue their participation at any time. It is possible that a person enrolled in the study could be a student or employee. They are advised that participation in the study is voluntary and no aspect of their participation or non-participation has an effect on their class, grade, employment, or salary. Subjects are informed that participation or withdrawal from the study does not affect the quality of their medical care, payment or enrollment in health plans, or affect eligibility for benefits.

5.1.2 Waiver or alteration of the informed consent requirement

If you are requesting a waiver or alteration of consent (consent will not be obtained, required information will not be disclosed, or the research involves deception), describe the rationale for the request in this section. If the alteration is because of deception or incomplete disclosure, explain whether and how subjects will be debriefed. Add any debriefing materials or document(s) to your study in CATS IRB (http://irb.psu.edu) on the "Supporting Documents" page. NOTE: Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to

ensure you have provided sufficient information for the IRB to make these determinations. HRP-410 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Subject may arrived to consenting-session fasted if subject wishes to give informed consent and have the medical screening on the same day.

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

Refer to "SOP: Written Documentation of Consent (HRP-091)" for information about the process to document the informed consent process in writing. HRP-091 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

If you will document consent in writing, describe how consent of the subject will be documented in writing. Add the consent document(s) to your study in CATS IRB (http://irb.psu.edu) on the "Consent Forms and Recruitment Materials" page. Links to Penn State's consent templates are available in the same location where they are uploaded and their use is required.

The potential subject signs the informed consent. A copy of the signed consent is given to the subject.

5.2.2 Waiver of Documentation of Consent (Implied consent, Verbal consent, etc.)

If you will obtain consent (verbal or implied), but not document consent in writing, describe how consent will be obtained. Add the consent script(s) and/or information sheet(s) to your study in CATS IRB (http://irb.psu.edu) on the "Consent Forms and Recruitment Materials" page. Links to Penn State's consent templates are available in the same location where they are uploaded and their use is required. Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information. HRP-411 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

If your research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, the IRB will generally waive the requirement to obtain written documentation of consent.

i. The written script of the information will be provided orally (if verbal consent is to be obtained) and will include all required elements of consent disclosure.
ii. The research presents no more than minimal risk of harm to subjects (this is for the screening fasting procedure only)
iii. The research involves no procedures for which written consent is normally required outside of the research context.
iv. Written information describing the research is to be provided to the subject (option to print the consent form for their records).

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

Indicate what language(s) other than English are understood by prospective subjects or representatives.

If subjects who do not speak English will be enrolled, describe the process to ensure that the oral and written information provided to those subjects will be in that language. Indicate the language that will be used by those obtaining consent.

Indicate whether the consent process will be documented in writing with the long form of the consent documentation or with the short form of the consent documentation. Review the "SOP: Written Documentation of Consent (HRP-091)" and the "Investigator Manual (HRP-103)"

to ensure that you have provided sufficient information. HRP-091 and HRP-103 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Not Applicable

5.3.2 Cognitively Impaired Adults

Refer to "CHECKLIST: Cognitively Impaired Adults (HRP-417)" for information about research involving cognitively impaired adults as subjects. HRP-417 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

5.3.2.1 Capability of Providing Consent

Describe the process to determine whether an individual is capable of consent.

Not Applicable

5.3.2.2 Adults Unable To Consent

Describe whether and how informed consent will be obtained from the legally authorized representative. Describe who will be allowed to provide informed consent. Describe the process used to determine these individual's authority to consent to research.

For research conducted in the state, review "SOP: Legally Authorized Representatives, Children and Guardians (HRP-013)" to be aware of which individuals in the state meet the definition of "legally authorized representative". HRP-013 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

For research conducted outside of the state, provide information that describes which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the procedure(s) involved in this research. One method of obtaining this information is to have a legal counsel or authority review your protocol along with the definition of "children" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)." HRP-013 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Not Applicable

5.3.2.3 Assent of Adults Unable to Consent

Describe the process for assent of the subjects. Indicate whether assent will be required of all, some or none of the subjects. If some, indicate which subjects will be required to assent and which will not.

If assent will not be obtained from some or all subjects, provide an explanation of why not.

Describe whether assent of the subjects will be documented and the process to document assent. The IRB allows the person obtaining assent to document assent on the consent document and does not routinely require assent documents and does not routinely require subjects to sign assent documents.

Not Applicable

5.3.3 Subjects who are not yet adults (infants, children, teenagers)

5.3.3.1 Parental Permission

Describe whether and how parental permission will be obtained. If permission will be obtained from individuals other than parents, describe who will be allowed to provide permission. Describe the process used to determine these individual's authority to consent to each child's general medical care.

For research conducted in the state, review "SOP: Legally Authorized Representatives, Children and Guardians (HRP-013)" to be aware of which

individuals in the state meet the definition of "children". HRP-013 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

For research conducted outside of the state, provide information that describes which persons have not attained the legal age for consent to treatments or procedures involved in the research, under the applicable law of the jurisdiction in which research will be conducted. One method of obtaining this information is to have a legal counsel or authority review your protocol along with the definition of "children" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)." HRP-013 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Not Applicable

5.3.3.2 Assent of subjects who are not yet adults

Indicate whether assent will be obtained from all, some, or none of the children. If assent will be obtained from some children, indicate which children will be required to assent. When assent of children is obtained describe whether and how it will be documented.

Not Applicable

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

This section is about the access, use or disclosure of Protected Health Information (PHI). PHI is individually identifiable health information (i.e., health information containing one or more 18 identifiers) that is transmitted or maintained in any form or medium by a Covered Entity or its Business Associate. A Covered Entity is a health plan, a health care clearinghouse or health care provider who transmits health information in electronic form. See the "Investigator Manual (HRP-103)" for a list of the 18 identifiers. HRP-103 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

If requesting a waiver/alteration of HIPAA authorization, complete sections 6.2 and 6.3 in addition to section 6.1. The Privacy Rule permits waivers (or alterations) of authorization if the research meets certain conditions. Include only information that will be accessed with the waiver/alteration.

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

X **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☐ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☐ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Include the following statement as written – DO NOT ALTER OR DELETE unless this section is not applicable because the research does not involve a waiver of authorization. If the section is not applicable, remove the statement and indicate as not applicable.

Not Applicable

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

Describe the plan to destroy the identifiers at the earliest opportunity consistent with the conduct of the research. Include when and how identifiers will be destroyed. If identifiers will be retained, provide the legal, health or research justification for retaining the identifiers.

Not Applicable

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

Provide an explanation for why the research could not practicably be conducted without access to and use of PHI.

Not Applicable

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

Provide an explanation for why the research could not practicably be conducted without the waiver or alternation of authorization.

Not Applicable

6.3 Waiver or alteration of authorization statements of agreement

By submitting this study for review with a waiver of authorization, you agree to the following statement – DO NOT ALTER OR DELETE unless this section is not applicable because the research does not involve a waiver or alteration of authorization. If the section is not applicable, remove the statement and indicate as not applicable.

Not Applicable

7.0 Study Design and Procedures

7.1 Study Design

Describe the type/design of trial to be conducted (e.g., double-blind, placebo controlled, parallel design, etc.). Provide a schematic diagram of study design, procedures and stages, if appropriate.

Two groups of women complete this study: 1) healthy women between the ages of 18 and 45 years (Controls); 2) women between the ages of 18 and 45 years with endometriosis. Each subject participates in a cutaneous microdialysis (MD) experiment before and after 6-9 days of oral atorvastatin (Lipitor) therapy (10mg/day). Each MD experiment includes a blood draw (e.g. miRNA, soluble LOX receptor, OxLDL, IL6, IL17, TNFα). The microdialysis experiments combine an acetylcholine dose response, perfusion of investigational agents, and local heating of the skin to explore the role of microRNA (miRNA) activation of LOX-1 receptor mechanisms in endothelial dysfunction associated with endometriosis.

7.2 Study Procedures

Provide a description of all research procedures being performed and when they are being performed (broken out by visit, if applicable), including procedures being performed to monitor subjects for safety or minimize risks. Include any long-term follow-up procedures and data collection, if applicable.

Describe where or how you will be obtaining information about subjects (e.g., medical records, school records, surveys, interview questions, focus group topics, audio or video recordings, data collection forms, and collection of specimens through invasive or non-invasive procedures to include the amount to be collected and how often). Add any data collection instruments that will be seen by subjects to your study in CATS IRB (http://irb.psu.edu) in the "Supporting Documents" page.

7.2.1 EXAMPLE: Visit 1 or Day 1 or Pre-test, etc. (format accordingly)

Provide a description as defined above and format accordingly.

Screening

1. The participants drink only water and do not eat for 12 hours before the screening.
2. The research nurse conducts the screening that includes pregnancy test for women who are not post-menopausal, 12-lead resting ECG, heart rate (HR), blood pressure (BP), height, waist circumference, weight, health history, creatine phosphokinase (CPK), and standard venipuncture to obtain blood (30 ml, 2 Tbsp) for complete blood count (CBC), chemistry analysis, lipid profile, protein kinase, and other substances of interest.
3. If participant takes thyroid medication, the nurse obtains thyroid stimulating hormone (TSH) level from participant. If TSH level is not available and/or has not been measured within 6 months, TSH is also measured from the blood sample.
4. We do not perform genetic analyses on the blood nor look for presence of disease (e.g. HIV).
5. We give subjects a note that informs her/his physician that the subject takes Lipitor as part of this study.

If the research nurse is unavailable for an extended period, the Clinical Research Center CRC staff performs her screening tasks. The CRC uses their admission form to admit potential participants for screening at the CRC.

7.2.2 EXAMPLE: Visit 2 or Day 2 or Post-test, etc. (format accordingly)

Provide a description as defined above and format accordingly.

Note: Subjects perform a pre- and post-statin therapy experiment.

1. Preparation for experiments
   a. We give printed and verbal instructions outlining what the subjects need to do before they come to the lab.
   b. Subjects continue with their normal diet except:
      i. They refrain from consuming alcohol and caffeine (ex. coffee, tea, Coca Cola, chocolate) for 12 hours before the experiment.
   c. On the day of the experiment,
      i. Subjects refrain from activity that causes them to exert themselves more than a leisurely walk.
      ii. We measure HR, BP, and oral temperature. Women who are not postmenopausal undergo a urine pregnancy test if they have not been tested within 2 weeks.
      iii. The nurse draws 10 ml blood for analysis of relevant substances of interest such as miRNA, soluble LOX receptor, OxLDL, IL6, IL17, and TNFα.
      iv. Microdialysis probe insertion: The subject washes the ventral forearm with antimicrobial soap. We place a tight band around the arm so we can visualize veins. For each MD site, we make pairs of pen-marks on the arm 2.5 cm (1 inch) apart and away from veins. We remove the tight band. The MD tubing enters and exits the skin at the marks. We clean the arm with povidone iodine and alcohol. We place an ice bag on the arm for 5 minutes to numb the skin. Then we insert a thin needle into the skin at each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch) and leaves the skin at the matching exit mark. We thread the MD tubing through the needle. Next, we withdraw the needle leaving the tubing in the skin. Any redness of the skin subsides in about 60 – 120 minutes.
      v. We initiate lactated Ringer's perfusion in all MD probes at 2 µl/min.
      vi. We attach 3 ECG leads to measure heart rate, and place a cuff on the upper arm sans the MD probes to measure blood pressure. We place a local skin heater and laser Doppler probe over each MD site. We measure heart rate, local temperature, and skin blood flow (SkBF) throughout the MD experiments. We also measure blood pressure every 5-7 minutes (brachial osculation and/or critical care monitor).

2. MD Experiment – Pre-Statin Therapy

a. Acetylcholine (ACh) Dose Response
We prepare 5 MD sites: Probe 1. Lactated Ringer's only (control)
Probe 2. Lactated Ringer's + LNAME
Probe 3. Lactated Ringer's + Poly I:C
Probe 4. Lactated Ringer's + atorvastatin
Probe 5. Lactated Ringer's + (LNAME + Poly I:C) or (LNAME + atorvastatin)*
*****Note: Pilot experiments lead to development of the final protocol. Two treatments are explored at MD Probe 5 during pilot experiments. Pilot experiments also determine final doses of investigational agents.

We clamp the temperatures of the local heaters at 34°C (93°F). We add Poly I:C or atorvastatin to probes 3, 4, and 5 as indicated above. After 30 minutes, we add LNAME to Probes 2 and 5. Twenty minutes later, we record a 10-minute baseline. After the baseline, we add the first concentration of ACh to the perfusate at each probe. Each site receives 6 increasing concentrations of ACh (10^-10M to 10^-1M) each concentration is perfused for 5 minutes. As SkBF stabilizes with the addition of each concentration of ACh, we proceed to the next concentration. After the last concentration of ACh, we switch perfusates at all sites to Ringer's only for about 30 minutes while increasing the local temperature to 43°C. We add SNP to lactated Ringer's for 10 minutes at each site. Heating and SNP perfusion causes maximum vasodilation.

b. Then the experiment is over, and we remove the MD tubing from the skin and place sterile bandages over the sites. If the subject desires, we can also place a bag of ice on the site for 10 minutes to reduce any bruising that may occur. We measure blood pressure and heart rate before the subject departs.

3. Subjects take 10 mg/day atorvastatin for 6-9 days. Women who are not postmenopausal undergo a urine pregnancy test before beginning the atorvastatin.

4. Venous blood may be drawn for Chem 24 analysis if a subject experiences side effects from the statin therapy (e.g. dark urine, muscle myalgia).

5. MD Experiment – Post-Statin Therapy
   a. The subjects return to the lab to repeat the MD experiment within days 6-9 of atorvastatin therapy.
   b. On the morning of the experiment, the subjects take their final dose of atorvastatin.
   c. All other preparation and procedures are the same as described for the Pre-statin Therapy MD Experiment.

Equipment:

Laser Doppler Flowmetry: The Laser Doppler Flowmeter (Moor Instruments, Inc.) non-invasively provides a qualitative measure of skin blood flow to a depth of about 1 mm in the skin using a weak laser light. This measure is a dimensionless value called "flux" that reflects the speed and number of blood cells moving through the microvasculature in an area of skin. The flowmeter continuously measures skin blood flow using a fiber optic probe that fits into holders taped to the skin. Approved by the FDA

Blood Pressure, ECG (Heart rate): The critical care monitor measures blood pressure via a cuff inflated every 5-7 minutes on the upper arm and heart rate via 3-lead ECG probes taped to the skin of the chest. Approved by the FDA

12-lead ECG: The critical care monitor measures standard 12-lead ECG during screening via electrodes taped to the skin. Approved by the FDA

7.3 Duration of Participation

Describe the duration of an individual subject's participation in the study.	
Screening (1 Visit)	less than 1.5 hour
MD Experiments (2 Visits)	5 hours
Total: ~11.5 Hours	

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4.1 Description

Provide a brief description of all test articles (drugs (including any foods and dietary supplements), devices and/or biologics used in the research including the purpose of their use and their approval status with the Food and Drug Administration (FDA). Include information about the form of the drug product (e.g., tablets, capsules, liquid).

Investigative Substances: Microdialysis

Acetylcholine ACh Form: solid
Role in Current Study: The local perfusion of endothelial agonist, acetylcholine, specifically examines the attenuated endothelium-dependent vasodilation associated with endometriosis.

Atorvastatin Form: solid
Role in Current Study: Atorvastatin is a LOX inhibitor.

Lactated Ringer's Ringer's Form: Liquid
Role in current study: Lactated Ringer's acts as the vehicle for the other research substances and as a flush.

LNAME NG-nitro-L-arginine methyl ester Form: solid
Role in current study: A nitric oxide synthase inhibitor. L-NAME is an analog to the amino acid, L-arginine. L-NAME is a non-specific inhibitor for nitric oxide synthases, thereby inhibiting the production of nitric oxide that causes vasodilation.

Polyinsinoic Acid Poly I:C Form: solid
Role in Current Study: Polyinosinic Acid is a LOX-1 receptor antagonist.

Sodium Nitroprusside SNP Form: solid
Role in Current Study: SNP, acting as an NO donor, dilates blood vessels maximally thereby, combined with local heating, achieving maximal skin blood flow (SkBF). Maximal SkBF is a reference point for other measures of skin blood flow.

Investigative Substances: Oral

Atorvastatin Brand name: Lipitor Form: tablet
Role in Current Study: Atorvastatin acts as a systemic LOX inhibitor.

7.4.2 Treatment Regimen

Describe dose, route of administration and treatment duration. Include information about dose adjustments.

Investigative Substances: Microdialysis

The following is a table of the research agents used with intradermal microdialysis. Based upon research literature, we use 14% for calculating maximum delivery of the research agents for all IND applications for our MD studies.

Research Agent	14% delivery (mg)
ACh	0.025
Atorovastatin	0.00061
L-NAME	0.091
Poly I:C	0.1
SNP	0.023

Investigative Substances: Oral

Atorovastatin 10 mg/day for 6-9 days

7.4.3 Method for Assigning Subject to Treatment Groups

Describe the randomization process and how the associated treatment assignment will be made.

Not Applicable

7.4.4 Subject Compliance Monitoring

Insert the procedures for monitoring subject compliance.

We note remaining atorvastatin tablets when subject returns for post-treatment experiment.

7.4.5 Blinding of the Test Article

Describe how the test article is blinded.

Not Applicable

7.4.6 Receiving, Storage, Dispensing and Return

7.4.6.1 Receipt of Test Article

Describe how the test article will be obtained and from what source. Describe how the study test article will be packaged along with amounts (e.g., number of tablets/capsules or volume of liquid) and labeling. If drug kits are used, describe all the contents of the kit and associated labeling.

See uploaded Physician Oversight SOP.

Investigative Substances: Microdialysis

Detailed records are kept in laboratory log books. The containers of the substances are dated upon receipt and when opened.

Investigative Substance	Source(s)
ACh	USP
Atorvastatin	USP
L-NAME	EMD, Tocris
Lactated Ringer's	McKesson, Moore Medical
Poly I:C	Invivogen
SNP	USP

From uploaded Physician Oversight SOP:
The investigative agents are purchased by the lab members from reputable sources as indicated in the IRB and the FDA IND applications and in accordance with the standards and guidelines imposed by the FDA.
A license from the CRC physician, the nurse manager, or the overseeing physician is filed with vendors (e.g. VWR, Owens and Minor) requiring the documentation for the purchase of some investigative agents (e.g. Lactated Ringer's).
The investigative agents are shipped to the Noll Lab or picked up from the pharmacy by lab personnel.
Copies of the prescriptions and orders are maintained in the laboratory's files.

Investigative Substances: Oral

A prescription, written by a study physician, for the Atorvastatin (10mg/tablet, 9 tablets) is filled by the Boalsburg Apothecary for each study participant. Detailed records are kept in laboratory log books and in individual subjects' charts. All medications will be double-checked by two people to ensure correctness.

Atorvastatin (Lipitor)	Boalsburg Apothecary

From uploaded Physician Oversight SOP:

Some of the investigative agents are prescription drugs... The lab personnel obtain prescriptions from a CRC physician, the research nurse manager, or the overseeing physician for the investigative agents that are prescription drugs and forward the prescriptions to the pharmacy…. The investigative agents are shipped to the Noll Lab or picked up by lab personnel.

A license from the CRC physician, the research nurse manager, or the overseeing physician is filed with reputable vendors requiring the documentation for the purchase of some investigative agents.
Copies of the prescriptions and orders are maintained in the laboratory's files.

7.4.6.2 Storage

Describe the plans to store, handle the test article so they will be used only on subjects and only by authorized investigators. Describe storage temperature requirements and how temperature will be monitored and recorded.

Investigative Substances: Microdialysis

The investigational substances are stored under environmental conditions according to manufacturers' instructions in cabinets, refrigerators, or freezers located in Room 229 or 224A Noll Laboratory. The room is locked when unoccupied. Each location has a thermometer. The temperatures are recorded daily during weekdays. The temperature of the room is also monitored by the Central Control System of the Environmental Systems at PSU.

Investigative Substances: Oral

We store the drugs in a locked cabinet in Room 229 under environmental conditions according to manufacturers' instructions. The room is locked when unoccupied. The location has a thermometer. The temperatures are recorded daily during weekdays. The temperature of the room is also monitored by the Central Control System of the Environmental Systems at PSU.

7.4.6.3 Preparation and Dispensing

Describe how the test article will be assigned to each subject and dispensed. Describe the steps necessary to prepare the test article. Include where the test article preparation will be done and by whom. Fully describe how the study treatment is to be administered and by whom.

Investigative Substances: Microdialysis

None of the microdialysis drugs are dispensed to the participant; rather we use them in the experiment, as outlined in the Standard Operating Procedures (Phys Ovrsght SOP). The physician providing oversight approves the laboratory standard operating procedure for the obtainment, preparation, and administration of all investigational agents for MD and other procedures in experiments.

The following procedures have been examined and approved by the FDA.
Trained lab personnel prepare the perfusates in accordance with the procedure described in the IRB and FDA IND applications. When mixing the perfusates, the experienced technician washes the hands, wears protection (e.g. gloves, lab coat), and uses glassware that has been washed with cleaner (designed for use with healthcare instruments, pharmaceutical process equipment , tissue culture apparatus , etc.), and rinsed multiple times in tap and then doubly- distilled water and air-dried. Most of the investigational agents are obtained in ultra-pure solid form. The solid investigational agents are weighed on a microbalance and then mixed with sterile pharmaceutical-grade Lactated Ringer's solution to the desired concentration. The solution is drawn into the syringe through a 0.2 µm filter. Prior to injecting a solution into or withdrawing solution from a sterile container through a sterile hypodermic needle, the stopper of the sterile container is cleaned thoroughly with alcohol. Most perfusates are used within minutes or hours after preparation. Stock solutions are made for some investigational agents. The stock solutions are drawn into sterile 1-cc syringes through 0.2 µm filters for storage. The sterile stock solutions are labeled accordingly (e.g. content, date), and protected from light. Stock solutions are not used beyond 1 week (refrigerated) and 6 months (frozen) after mixing. Stock solutions are diluted with

sterile Lactated Ringer's when preparing the perfusate. The final dilutions of the perfusates are drawn into sterile syringes through 0.2 µm filters within minutes or hours of their use in the experiment.
The protocol that includes the administration of the investigative substances has been examined and approved by the FDA.
The administration of the investigative substances is performed by the trained and approved lab personnel as described in the protocol included in the IRB and FDA IND applications. The overseeing physician has observed and approved the microdialysis technique as performed by Dr. Lacy Alexander. The lab personnel performing microdialysis have been trained and approved to perform the technique by Dr. Lacy Alexander, approved by the overseeing physician, and approved by the IRB. Letters of approval from the overseeing physician for each member of the lab who performs microdialysis in the research project have been submitted to the IRB.

Investigative Substances: Oral
The Boalsburg Apothecary places the pills into bottles labeled with the subject ID number, strength (mg), dosing instructions, date, etc. Each bottle contains the number of pills as prescribed by a study physician. All medications will be double-checked by two people to ensure correctness. The research nurse gives the bottle of pills to the subject along with handouts that contain detailed information and dosing instructions pertinent to their assigned drug. Detailed records are kept in laboratory log books and in individual subjects ' charts.

7.4.6.4 Return or Destruction of the Test Article

Describe the procedures for final reconciliation of the test article supply at the end of the study and whether the test article is to be shipped back to a source or destroyed on site.

Subjects are required to return unused pills to investigators. The pills are disposed of in accordance with policies of the Penn State's Environmental Health and Safety Department at University Park.

7.4.6.5 Prior and Concomitant Therapy

Describe what prior and/or concomitant medical therapy will be collected. Describe which concomitant medicines/therapies are permitted during the study. Describe which concomitant medicines are not permitted during the study.

Subjects are excluded if they are currently taking chronic medications that may affect vascular function or are contraindicated for the atorvastatin pharmacotherapy. Medications that are not specifically mentioned in exclusion criteria may be excluded on a case by case basis upon review by the research clinicians or PI.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

Indicate the total number of subjects to be accrued.

If applicable, distinguish between the number of subjects who are expected to be enrolled and screened, and the number of subjects needed to complete the research procedures (i.e., numbers of subjects excluding screen failures.)

Anticipated number of subjects to be screened: 10
Needed to complete: 6

The pilot experiments will enable the determination of the number of subjects needed for completion of the study. A future modification to this study will update number of subjects.

8.2 Sample size determination

If applicable, provide a justification of the sample size outlined in section 8.1 – to include reflections on, or calculations of, the power of the study.

The pilot experiments will enable the determination the number of subjects needed for completion of the study. A future modification to this study will add that number of subjects.

8.3 Statistical methods

Describe the statistical methods (or non-statistical methods of analysis) that will be employed.

Descriptive statistics will be calculated and the variance will be noted for a full power analysis. If appropriate, three-way repeated measures ANOVA (ANCOVAs) will be performed to examine group differences (healthy control vs. endometriosis) or systemic treatment differences (pre-treatment vs. post-treatment), and local microdialysis treatment differences across the doses of pharmacological stimuli with the primary outcome variable of cutaneous vascular conductance. Appropriate post-hoc analyses with corrections for multiple comparisons will be performed when main effects are identified, in all cases including potential confounding variables as covariates.

9.0 Confidentiality, Privacy and Data Management

For research being conducted at Penn State Hershey or by Penn State Hershey researchers only, the research data security and integrity plan is submitted using "HRP-598 – Research Data Plan Review Form Application Supplement", which is available in the Library in CATS IRB (http://irb.psu.edu). Refer to Penn State College of Medicine IRB's "Standard Operating Procedure Addendum: Security and Integrity of Human Research Data", which is available on the IRB's website. **In order to avoid redundancy, for this section state "See the Research Data Plan Review Form" in section 9.0 if you are conducting Penn State Hershey research and move on to section 10.**

For all other research, in the sections below, describe the steps that will be taken to secure the data during storage, use and transmission.

9.1 Confidentiality

9.1.1 Identifiers associated with data and/or specimens

List the identifiers that will be included or associated with the data and/or specimens in any way (e.g., names, addresses, telephone/fax numbers, email addresses, dates (date of birth, admission/discharge dates, etc.), medical record numbers, social security numbers, health plan beneficiary numbers, etc.).

If no identifiers will be included or associated with the data in any way, whether directly or indirectly, please indicate this instead.

Most of the data are coded and do not contain personal identifying information. Some data will temporarily contain the subject's name, address, and/or telephone number (Phone Interview Form). Documents allowing identification of participants do not leave our labs and are only available to authorized persons. Only authorized personnel may access the lab computer. Data forms containing identifiable information are shredded when no longer needed (within 5 years after publication of results).

9.1.1.1 Use of Codes, Master List

If identifiers will be associated with the data and/or specimens (as indicated in section 9.1.1 above), describe whether a master record or list containing a code (i.e., code number, pseudonyms) will be used to separate the data collected from identifiable information, where that master code list will be stored, who will have access to the master code list, and when it will be destroyed.

If identifiers are included or associated with the data as described in section 9.1.1 above, but no master record or list containing a code will be used, it will be assumed by the IRB that the investigator plans to directly link the identifiers with the data.

We keep data in the laboratory in locked cabinets, the password-protected folder on the secured PSU server, and on password-protected computers maintained in a locked room. Only authorized personnel have access. Coded data shared with unauthorized persons cannot be traced to individuals. The list linking code numbers to participants is not shared with unauthorized persons and destroyed when project is completed and published. The code is destroyed within 5 years of publication of the data.

9.1.2 Storage of Data and/or Specimens

Describe where, how and for how long the data (hardcopy (paper) and/or electronic data) and/or specimens will be stored. NOTE: Data can include paper files, data on the internet or websites, computer files, audio/video files, photographs, etc. and should be considered in the responses. Refer to the "Investigator Manual (HRP-103)" for information about how long research records must be stored following the completion of the research prior to completing this section. HRP-103 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Please review Penn State's Data Categorization Project for detailed information regarding the appropriate and allowable storage of research data collected according to Penn State Policy AD71. Although the IRB can impose greater confidentiality/security requirements (particularly for sensitive data), the IRB cannot approve storage of research data in any way or using any service that is not permissible by Penn State Policy AD71.

We maintain data in the laboratory in locked cabinets and on password-protected computers located in rooms that are locked when unoccupied. Most of the data are coded and do not contain personal identifying information. Documents allowing identification of participants do not leave the investigator's labs and are only available to authorized persons. Coded data shared with unauthorized persons cannot be traced to individuals. We store any list linking the code to participants' identity in locked cabinets and on password-protected computers located in rooms that are locked when unoccupied. Hard copy data forms containing identifiable information are shredded when no longer needed (within 5 years after publication of results). Screening data from subjects who are not accepted into the study are shredded when the project ends. Subjects may give permission to have their contact information retained in the investigator's secured files if they wish to be considered for participation in future studies. After we complete the study, we remove all identifiers from the study's data and store the data indefinitely. Individual data may be used without identifying the subject to illustrate representative responses.
Biological specimens are stored at University Park and Quest Labs, (Chantilly, VA). At University Park, the specimens are stored in a -80°C freezer in Noll first floor hallway. All specimens not exhausted upon analysis are maintained no longer than 5 years after publication.

9.1.3 Access to Data and/or Specimens

Identify who will have access to the data and/or specimens. This information should not conflict with information provided in section 9.1.1.1 regarding who has access to identifiable information, if applicable.

Only authorized personnel have access.

9.1.4 Transferring Data and/or Specimens

If the data and/or specimens will be transferred to and/or from outside collaborators, identify the collaborator to whom the data and/or specimens will be transferred and how the data and/or specimens will be transferred. This information should not conflict with information provided in section 9.1.1.1 regarding who has access to identifiable information, if applicable.

Some specimens are mailed or transported by lab members or couriers outside labs for analysis. The outside labs are the Biomarker Core Lab (PSU, University Park) and Quest Diagnostics

(Chantilly, VA). All specimens are coded and do not contain identifiers. We do not share the code with unauthorized persons.

9.2 Subject Privacy

This section must address subject privacy and NOT data confidentiality.

Indicate how the research team is permitted to access any sources of information about the subjects.

Describe the steps that will be taken to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on whom they interact with or to whom they provide personal information.

Describe what steps you will take to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures.

Only authorized personnel have access (e.g. computer passwords, keys to locked cabinets/labs) to sources of information about subjects. We tell participants that they may decline to answer questions and decline to participate in the study. Only authorized personnel are present during screening and experiments. On occasion (e.g. educational visit, visiting colleague, site visit) participants may give permission for visitors to observe a procedure or experiment.

10.0 Data and Safety Monitoring Plan

This section is required when research involves more than Minimal Risk to subjects. As defined in "SOP: Definitions (HRP-001)", available in the Library in CATS IRB (http://irb.psu.edu), Minimal Risk is defined as the probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests. For research involving prisoners, Minimal Risk is the probability and magnitude of physical or psychological harm that is normally encountered in the daily lives, or in the routine medical, dental, or psychological examination of healthy persons. **Please complete the sections below if the research involves more than minimal risk to subjects OR indicate as not applicable.**

10.1 Periodic evaluation of data

Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe.

NA

10.2 Data that are reviewed

Describe the data that are reviewed, including safety data, untoward events, and efficacy data.

NA

10.3 Method of collection of safety information

Describe the method by which the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls and with subjects).

NA

10.4 Frequency of data collection

Describe the frequency of data collection, including when safety data collection starts.

NA

10.5 Individuals reviewing the data

Identify the individuals who will review the data. The plan might include establishing a data and safety monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.

NA

10.6 Frequency of review of cumulative data

Describe the frequency or periodicity of review of cumulative data.

NA

10.7 Statistical tests

Describe the statistical tests for analyzing the safety data to determine whether harms are occurring.

NA

10.8 Suspension of research

Describe any conditions that trigger an immediate suspension of research.

NA

11.0 Risks

List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. For each potential risk, describe the probability, magnitude, duration, and reversibility. Consider all types of risk including physical, psychological, social, legal, and economic risks. If applicable, indicate which procedures may have risks to the subjects that are currently unforeseeable. If applicable, indicate which procedures may have risks to an embryo or fetus should the subject be or become pregnant. If applicable, describe risks to others who are not subjects.

Please keep in mind that loss of confidentiality is a potential risk when conducting human subject research and should be addressed as such.

General note: The research group's members are trained and competent in their duties. The group, led by Dr. Alexander, evaluates the effectiveness and safety of protocols and procedures in an ongoing fashion. They discuss the protocol with candidates, invite questions, and offer tours of the laboratory. Prior to medical screening, candidates read and sign informed consent forms detailing protocols, procedures, risks, sensations, compensation, etc. We give candidates witnessed copies of the signed consent forms. After accepting participants into the study, we discuss and review the procedures and protocols with them generally and at each step throughout the project. They frequently remind participants of the option to withdraw from the study at any time. Restricting access to experiments, data, and coding to authorized personnel maintains confidentiality. The Noll Lab's electronics technician certifies the electrical devices for human use. Lists of emergency numbers remain by lab telephones. At least one cell phone is present at each experiment. A hospital and emergency medical services are within 1-2 miles of the lab. All lab members who are graduate students, post docs, or staff have current American Heart Association (AHA) Basic Life Support (BLS) with automated external defibrillator (AED) training. A cardiopulmonary resuscitation (CPR) mask hangs in each lab room. An AED hangs nearby in the hallway.

Microdialysis: We insert a 23 g needle horizontally into and then out of the layers of the skin of the ventral forearm. The needle's entry and exit are about 2.5 cm apart. They thread the microdialysis "probe," comprising a tube of membrane (320 um OD) with tubing attached at both ends (650 um OD), through the needle. Then they withdraw the needle leaving the membrane under the skin. They perfuse the probe with sterile saline using a syringe pump.

Cutaneous microdialysis commonly causes some pain and bruising similar to that experienced during venipuncture. There is usually no pain after the probe is in place. The participant may experience mild pain while we remove the probe. Minor bleeding may occur. As with routine venipuncture, a participant who is nervous about needles could have increased heart rate and blood pressure, become lightheaded or nauseated, or could faint.

As with venipuncture or any event that breaks the skin, infection is possible. However, no participants in any of the researchers' experiments have reported infection. We place a sterile bandage on the site after the experiment.

Although rare, if the membrane should break in half during removal, they remove the remaining half by gently pulling the attached tubing. This presents no additional risk to the participant. In the unlikely event in which the membrane breaks during removal leaving an isolated piece of membrane under the skin, they treat the piece of membrane in a manner similar to that for a splinter in the skin. In this case, they have to make a superficial

incision for removal. Such an event has not occurred in projects that have used MD in this lab (over 1,000 MD probes have been placed by Dr. Alexander, alone).

The persons performing MD have been trained in proper technique and procedure by Dr. Alexander and had their technique witnessed / approved by Robert Mooney, D.O. and/or Sarah Ferguson, M.D. (see uploads under respective "CV"s). Ice numbs the skin and the small needle reduces pain during insertion. We stop any bleeding by applying mild pressure to the site with sterile gauze. In the unlikely event that the individual has an allergic reaction, we stop microdialysis immediately. If the reaction becomes severe, we seek emergency medical assistance. Infection is possible, but proper aseptic technique and sterile solutions / supplies keep the risk minimal. They place a sterile bandage on the site after the experiment.

Perfusate: Lactated Ringer's solution flows through the microdialysis probes. An allergic reaction to this physiological saline solution is highly unlikely.

All substances (ACh, Atorvastatin, L-NAME, Poly I:C, SNP) added to the lactated Ringer's perfusing the microdialysis probes have been used previously clinically and/or during research in humans:

Microdialysis delivers small amounts of the substances to a nickel-sized area of the skin. The small quantities used and the extremely localized administration during microdialysis is unlikely to produce systemic effects. To our knowledge, there are no reports of long side effects of these substances administered through microdialysis. The chance of adverse reactions to these substances is extremely small given the minute amount delivered to the a very small area of skin, the lack of adverse reactions to similar amounts delivered via MD in many other studies, and lack of adverse effects in human cell cultures. There is a slight chance of a sensitivity or allergic reaction to these substances that could produce redness, itching, rash, and/or swelling. A severe reaction (anaphylactic shock) could also cause fever, difficulty in breathing, changes in pulse, convulsions, and/or loss of consciousness. Although rare, it is possible that a subject could experience lightheadedness, flushing, nausea, "pounding heart" sensation, and/or vomiting. We end the perfusion in the event of these signs and symptoms.

The perfusate is sterile. We prepare the perfusate in the laboratory using sterile techniques and supplies commonly used for this purpose in research laboratories. The Ringer's solution is sterile as purchased. We add other solutions aseptically to the sterile bag of Ringer's solution through 0.2 micron Gelman Sterile Acrodisc syringe filters. We mix the perfusate for same-day use and discard excess perfusate after the experiment. During the IND application process, the FDA accepted our technique for preparing perfusates. Although unlikely, in the case of a severe reaction to the perfusate, we call 911.

Laser Doppler Flowmetry: The probe attaches to the skin with double-sided tape and measures skin blood flow in a 1mm3 volume of skin. Weak lasers can hurt the eye if one should stare into the light for a long time. The red light seen on the surface of the skin is harmless. We do not turn on the laser until the probes are taped to a surface. We remove the tape afterward carefully. We have used this technique in the lab with IRB approval for many years without incident.

Blood pressure (manual and critical care monitor (e.g. CardioCap5): The manual method and critical care monitor use a cuff that inflates on the upper arm. The cuff slowly deflates while we listen to pulse-sounds at the inside of the elbow with a stethoscope, or the critical care monitor takes a measurement. The inflated cuff may make the arm feel tingly and numb, and the cuff may temporarily bruise the arm. Efficient and competent measurement technique minimizes the duration of cuff inflation.

Povidone Iodine: Hospitals and researchers use povidone iodine to clean and sterilize the skin. Participants could be allergic to iodine. An allergic reaction could cause redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse and/or blood pressure, convulsions, shock, and/or loss of consciousness. Staff use only alcohol on participants with iodine allergy as identified during screening.

Blood draw: Blood draws can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection. A competent nurse performs blood draws using standard venipuncture-procedure and techniques that minimize the chance of infection. Participants may recline for the procedure.

Tape and adhesive disks: Participants could be sensitive to the adhesive of the tape and double-sided adhesive disks used in the study causing redness, rash, tenderness, and/or itching. We remove the tape and adhesive disks carefully. Ointment is available, if needed.

Screening: The screening includes blood sample, height, waist circumference, weight, heart rate, blood pressure, pregnancy test, and history performed by the competent research staff. Participants may be uncomfortable giving medical information or being measured. The participants may decline to answer questions or participate in measurements. We conduct screenings professionally and privately.

Initial Screening Form: We use the form to initially determine a candidate's suitability for the study. The initial interview gathers minimally invasive personal data that is kept confidential. They used similar interviews in the past without problem. Candidates may complete the form at the lab group's web site via the Qualtrics commercial survey website. The participant may decline to answer questions. We conduct phone interviews professionally and privately. Only authorized personnel may access completed forms.

Local Heating: The local heating control unit (Moor Instruments) precisely controls and monitors the temperature of the heated probe holders used with the Laser Doppler Flowmeter. To determine the maximal SkBF, we increase the temperature of the heating units slowly (about 0.1°C every 1 second). The skin feels very warm but not painful. Local heating causes temporary redness of the skin that subsides within several hours. This technique is very unlikely to produce long-term ill effects. The local heating controllers (Moor Instruments) precisely control and monitor the temperature of the heated probe holders. The system has programmed maximum temperature limits. This technique is unlikely to cause long-term ill effects.

ECG: We attach three to ten electrodes to the participant's chest and then attach the electrode wires to an ECG machine. The machine records the electrical activity of the heart. There are no adverse effects from this measure. A participant may be shy about electrodes applied to the chest. The staff carefully remove the tape afterward. They conduct the test professionally and privately.

Atorvastatin Therapy: Atorvastatin is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin is generally well-tolerated. Adverse reactions have usually been mild and transient. In controlled clinical studies of 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to atorvastatin. The most frequent adverse events thought to be related to atorvastatin were muscle pain, nausea, constipation, flatulence, dyspepsia, and abdominal pain. Statins have been associated with abnormalities in liver function. Persistent elevations in serum transaminases occurred in 0.7% of patients who received atorvastatin in clinical trials. The incidence of these abnormalities was 0.2%, 0.2%, 0.6%, and 2.3% for 10, 20, 40, and 80 mg doses, respectively. The low dose and short duration of the therapy used in this study reduces the likelihood of adverse event even more. The therapy is under the direction of the GCRC clinician with Urs Leuenberger, M.D., Professor of Medicine, Milton S. Hershey Medical Center (see attached letter) consulting. Subjects receive verbal and written information concerning the therapy. Creatine phosphokinase (CPK) is measured during screening and before each experiment. If the subject experiences symptoms of possible adverse response to atorvastatin, blood is drawn for CPK and/or Chem 24 analysis when the subject reports symptoms to the study team or study nurse. If increased transaminase develops, we monitor levels until increase resolves. If subjects develop increased CPK, ALT (alanine aminotransferase) or AST (aspartate aminotransferase), they discontinue atorvastatin and their participation in the study. We exclude subjects having contraindications to statin therapy including active liver disease or persistent unexplained elevations of serum transaminases.

12.0 Potential Benefits to Subjects and Others

12.1 Potential Benefits to Subjects

Describe the potential benefits that individual subjects may experience from taking part in the research. If there is no direct benefit to subjects, indicate as such. Compensation is not considered a benefit. Compensation should be addressed in section 14.0.

Subjects receive a medical screening and the results of their medical tests that could inform them about their health. In particular, they learn their blood pressure and blood cholesterol levels. This is important knowledge. High blood pressure and blood cholesterol contribute to many serious health problems. We advise those with high blood pressure or blood cholesterol to follow-up with a health care provider. Benefits also include the satisfaction derived from participating in a research program exploring a mechanism underlying the increased risk for CVD in women with endometriosis.

12.2 Potential Benefits to Others

Include benefits to society or others.

Cardiovascular disease (CVD) is the leading cause of death in women. Epidemiologic data demonstrate a clear association between endometriosis, reproductive risk factors, inflammation and cardiovascular (CV) risk. Endometriosis impacts 5-10% of women in the US, and its cardiovascular effects are usually left untreated. We hope to gain an improved understanding of one of mechanisms underlying the increased risk for CVD in women with endometriosis. The causes and effects of endometriosis are understudied. We also hope that this study will become part of a body of work that increases interest in the examination of endometriosis and its effects on the health of women. Also, the project provides valuable experience, education and partial fulfillment of degree-work for graduate and undergraduate students of The Pennsylvania State University.

13.0 Sharing Results with Subjects

Describe whether results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) will be shared with subjects or others (e.g., the subject's primary care physicians) and if so, describe how it will be shared.

We give participants copies of their individual lab results.

14.0 Subject Stipend (Compensation) and/or Travel Reimbursements

Describe the amount and timing of any subject stipend/payment or travel reimbursement here. If there is no subject stipend/payment or travel reimbursement, indicate as not applicable.

If course credit or extra credit is offered to subjects, describe the amount of credit and the available alternatives. Alternatives should be equal in time and effort to the amount of course or extra credit offered.

If an existing, approved student subject pool will be used to enroll subjects, please indicate as such and indicate that course credit will be given and alternatives will be offered as per the approved subject pool procedures.

MD Experiments: ($ 15.00 / MD probe inserted + $20.00 completing MD experiment.)

Experiment	$ 95.00 each	(5 MD probes; 2 experiments: 1 pre-treatment, 1 post-treatment)
Total	$ 190.00	

Subjects can receive payment for experiments not completed. We pay an amount of money equal to the part completed. For instance, if a subject completes half of Experiment 1, the subject receives $15.00 for each probe inserted plus $10.00. ($10.0 is one-half of $20.00). We may ask subjects to repeat a trial. If subjects agree to repeat a trial, they receive payment for the repeated trial as stated above. They are reimbursed for gasoline if they live more than 20 miles from Noll Lab.

15.0 Economic Burden to Subjects

15.1 Costs

Describe any costs that subjects may be responsible for because of participation in the research.

None

15.2 Compensation for research-related injury

If the research involves more than Minimal Risk to subjects, describe the available compensation in the event of research related injury.

If there is no sponsor agreement that addresses compensation for medical care for research subjects with a research-related injury, include the following text as written - DO NOT ALTER OR DELETE:
It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

For sponsored research studies with a research agreement with the sponsor that addresses compensation for medical care for research-related injuries, include the following text as written - DO NOT ALTER OR DELETE:
It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Such charges may be paid by the study sponsor as outlined in the research agreement and explained in the consent form.

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

16.0 Resources Available

16.1 Facilities and locations

Identify and describe the facilities, sites and locations where recruitment and study procedures will be performed.

If research will be conducted outside the United States, describe site-specific regulations or customs affecting the research, and describe the process for obtaining local ethical review. Also, describe the principal investigator's experience conducting research at these locations and familiarity with local culture.

The Noll laboratory is a 4-floor (35,000 sq. ft.) free-standing building devoted to basic, clinical, and applied physiological research. Biochemistry laboratories are available for sample processing and analyses. Available equipment includes centrifuges, critical care monitors, osmometer, assay plate reader, shaker, washer, NA/K analyzer, spectrophotometer, i-Stat analyzer, refractometer, hemocue, hematocrit system, fraction collector, hydrogen sulphide analyzer, sonicator, microbalances, laser doppler flowmeters, full-field laser perfusion imager, mini-syringe pumps/ controllers, sweat rate monitoring systems, hospital beds, medical infusion chair, ECG analysis system, finipres, plethysmograph, refrigerators, pharmaceutical refrigerator, -20 C and -80 C freezers, heated/refrigerated circulators, water-perfused suits, local skin cooling/ heating systems, critical care monitors, blood pressure monitors, metabolic cart, gas sterilization equipment, and autoclave facilities. In addition to ample office and laboratory laptop or desktop computers for research and student use, Noll Laboratory has two available servers and a trunk line connection to the University's mainframe computer system. Internal, office and laboratory computers are linked. The laboratory has four PC-based data acquisition systems equipped with Dataq hardware driven by Windaq software, two of which are located in computer

controlled environmental chambers equipped with treadmills and bikes. A Clinical Research Center is contiguous to the Noll Laboratory in the 14,000 sq. ft. Elmore wing, and available for the proposed studies. Up to 12 subject beds for overnight (or longer) stays, a metabolic kitchen, medical examination rooms, and several specialized procedure rooms are available. Because the CRC is contiguous with existing laboratory space, it is readily available for research support, e.g., pre-test subject screening. Noll Laboratory has exceptional in-house support facilities including machine and electronics shops.

16.2 Feasibility of recruiting the required number of subjects

Indicate the number of potential subjects to which the study team has access. Indicate the percentage of those potential subjects needed for recruitment.

We are using recruiting methods that will reach a large percent of the general population. We have employed these methods successfully for earlier projects that targeted specialized groups that have similar or rarer incidence in the US population. 1 in 10 women in the US have endometriosis. Utilizing targeted recruitment we believe we can meet our subject number goals.

16.3 PI Time devoted to conducting the research

Describe how the PI will ensure that a sufficient amount of time will be devoted to conducting and completing the research. Please consider outside responsibilities as well as other on-going research for which the PI is responsible.

We have been conducting similar experiments for many years and are familiar with the duration of the procedures/protocols/projects and the amount of staffing required to complete the project while maintaining their other obligations.

16.4 Availability of medical or psychological resources

Describe the availability of medical or psychological resources that subject might need as a result of their participation in the study, if applicable.

The CRC is located within the same building as the Noll Lab. A hospital and emergency medical services are within 1-2 miles of the lab. An AED hangs near the labs in the hallway. A CPR mask hangs in each lab. The faculty, staff, grad students, and post docs in the lab group have current AHA BLS with CPR training.

16.5 Process for informing Study Team

Describe the training plans to ensure members of the research team are informed about the protocol and their duties, if applicable.

The PI and senior lab members train new members in the necessary procedures. We conduct weekly lab meetings. They review any applicable changes or issues.

17.0 Other Approvals

17.1 Other Approvals from External Entities

Describe any approvals that will be obtained prior to commencing the research (e.g., from cooperating institutions, community leaders, schools, external sites, funding agencies).

We are modifying IND# 78954 with the FDA.

17.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – Hershey only – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of HRP-902 - Human Tissue For Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ Animal Care and Use – All campuses – Human research involves animals and humans or the use of human tissues in animals

X Biosafety – All campuses – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☐ Clinical Laboratories – Hershey only – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes, but are no longer needed for clinical use. Upload a copy of HRP-901 - Human Body Fluids for Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

X Clinical Research Center (CRC) Advisory Committee– All campuses – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – All campuses – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – Hershey only – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of HRP-903 - Radiation Review Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

X IND/IDE Audit – All campuses – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☐ Scientific Review – Hershey only – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Hershey Cancer Institute Scientific Review Committee is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website at: http://www.pennstatehershey.org/web/irb/home/resources/investigator

18.0 Multi-Site Research

If this is a multi-site study (i.e., the study will be conducted at other institutions each with its own principal investigator) and you are the lead investigator, describe the processes to ensure communication among sites in the sections below.

18.1 Communication Plans

Describe the plan for regular communication between the overall study director and the other sites to ensure that all sites have the most current version of the protocol, consent document, etc. Describe the process to ensure all modifications have been communicated to sites. Describe the process to ensure that all required approvals have been obtained at each site (including approval by the site's IRB of record). Describe the process for communication of problems with the research, interim results and closure of the study.

NA

18.2 Data Submission and Security Plan

Describe the process and schedule for data submission and provide the data security plan for data collected from other sites. Describe the process to ensure all engaged participating sites will safeguard data as required by local information security policies.

NA

18.3 Subject Enrollment

Describe the procedures for coordination of subject enrollment and randomization for the overall project.

NA

18.4 Reporting of Adverse Events and New Information

Describe how adverse events and other information will be reported from the clinical sites to the overall study director. Provide the timeframe for this reporting.

NA

18.5 Audit and Monitoring Plans

Describe the process to ensure all local site investigators conduct the study appropriately. Describe any on-site auditing and monitoring plans for the study.

NA

19.0 Adverse Event Reporting

19.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility*. For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

19.2 Recording of Adverse Events

Address the frequency and process for eliciting adverse event information from research subject, e.g., "Research subjects will be routinely questioned about adverse events at study visits."

In the response, incorporate the following as written:
All adverse events (serious or non-serious) and abnormal test findings observed or reported to study team believed to be associated with the study drug(s) or device(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy
  NOTE: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the investigator.

In the case of untoward events, those lab members present during the event are included in a debriefing and notes of the meeting are taken. The participant involved in an untoward event is interviewed in person or via telephone concerning the event and the responses are included into the notes for that event.

All adverse events (serious or non-serious) and abnormal test findings observed or reported to study team believed to be associated with the study drug(s) or device(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy
  NOTE: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the investigator.

The CRC clinicians will be notified of any reported adverse reactions.

19.3 Causality and Severity Assessments

By submitting this study for review, you agree to the following statement – DO NOT ALTER OR DELETE:

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

19.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

19.4.1 Written IND/IDE Safety Reports

For a drug study under an IND, incorporate the following from 21 CFR 312.32 as written – DO NOT ALTER OR DELETE:
The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a serious and unexpected, suspected adverse reaction. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

For a device study under an IDE, incorporate the following from 21 CFR 812.150 as written – DO NOT ALTER OR DELETE:
The Sponsor-Investigator will submit a completed FDA Form 3500Ato the FDA's Center for Devices and Radiological Health for any observed or volunteered adverse effect that is determined to be an unanticipated adverse device effect. A copy of this completed form will be provided to all participating sub-investigators.

The completed FDA Form 3500Awill be submitted to the FDA as soon as possible and, in no event, later than 10 working days after the Sponsor-Investigator first receives notice of the adverse effect.

If the results of the Sponsor-Investigator's follow-up evaluation show that an adverse effect that was initially determined to not constitute an unanticipated adverse device effect does, in fact,

meet the requirements for reporting; the Sponsor-Investigator will submit a completed FDA Form 3500Aas soon as possible, but in no event later than 10 working days, after the determination was made.

For each submitted FDA Form 3500A, the Sponsor-Investigator will identify all previously submitted reports that that addressed a similar adverse effect experience and will provide an analysis of the significance of newly reported adverse effect in light of the previous, similar report(s).

Subsequent to the initial submission of a completed FDA Form 3500A, the Sponsor-Investigator will submit additional information concerning the reported adverse effect as requested by the FDA.

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a serious and unexpected, suspected adverse reaction. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

19.4.2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

For a drug study under an IND, incorporate the following from 21 CFR 312.32 into the response, as written:
In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any unexpected, fatal or life-threatening suspected adverse reaction.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any unexpected, fatal or life-threatening suspected adverse reaction.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

19.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

By submitting this study for review, you agree to the following statement – DO NOT ALTER OR DELETE:

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

19.6 Unblinding Procedures

Describe the procedures for unblinding study therapy on a subject, including documentation of this in the subject's source document. Include example(s) here why someone might unblind a study. In most cases, the unblinding will be part of managing a serious adverse reaction and will be reported with the serious adverse event. However, in cases where unblinding was not associated with a serious adverse event, such actions should be reported in a timely manner.

NA

19.7 Stopping Rules

In studies with a primary safety endpoint or studies with high risk to study subjects, provide the rules that define the circumstances and procedures for interrupting or stopping the study. If an independent Data and Safety Monitoring (DSMB) or Committee (DSMC) is set up for the study, the same stopping rules should be incorporated into the safety analysis plan as well.

Although such events are extremely unlikely to occur, we are prepared to immediately stop experiments and seek medical assistance if the subjects should experience the more serious reactions described in the informed consents, and IRB applications such as signs and symptoms of an allergic reaction, anaphylactic shock, etc. As always, our subjects are reminded throughout the protocol that they may stop the experiment at any time. Also, we exercise the discretion to end a subject's participation if the subject should engage in behavior that could jeopardize his/her own health and well-being or that of others.
We end the experiments if the subject experiences:

- Systolic or diastolic blood pressures exceed 220 or 110 mmHg, respectively, or
- Systolic or diastolic blood pressures are less than 90 or 50 mmHg, respectively or
- Heartrate <40 bpm or > 120 bpm.

20.0 Study Monitoring, Auditing and Inspecting

20.1 Study Monitoring Plan

20.1.1 Quality Assurance and Quality Control

Include this section if FDA regulations apply to this study (see "WORKSHEET: Drugs (HRP-306)" and "WORKSHEET: Devices (HRP-307)". HRP-306 and HRP-307 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Describe how you will ensure that this study is conducted and that the data are generated, documented (recorded) and reported in compliance with this protocol, with institutional and IRB policies, with Good Clinical Practice guidelines and any other applicable regulatory requirements.

Indicate who is responsible for monitoring the conduct of the study and specify how often the study will be monitored.

For single-site studies with low risk, it may be appropriate for the principal investigator to monitor the study.

For multi-center studies or single site studies involving significant risk, an independent monitor may be required (e.g., monitoring by the staff of the PSU quality assurance program office(s) or by a clinical research organization).

Dr. Lacy M. Alexander is responsible for the conduct and monitoring of the study for its compliance with this protocol, with institutional and IRB policies, with Good Clinical Practice guidelines and any other applicable regulatory requirements. She is assisted by members of her lab group. The monitoring is ongoing.

20.1.2 Safety Monitoring

Include this section if FDA regulations apply to this study (see "WORKSHEET: Drugs (HRP-306)" and "WORKSHEET: Devices (HRP-307)". HRP-306 and HRP-307 can be accessed by clicking the Library link in CATS IRB (http://irb.psu.edu).

Indicate the process for identifying, recording and reporting adverse events.

Specify roles for adverse event recording and monitoring. Indicate each staff member's role in the adverse event reporting process. Include the following if applicable:

The **Principal Investigator** will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, sponsor and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.

The **Research Coordinator** will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA and/or DSMB of all Unanticipated Problems/SAE's.

The **Monitor** will confirm that the AEs are correctly entered into the case report forms. The Monitor will also confirm that the adverse events are consistent with the source documents and are reported to the appropriate regulatory bodies as required.

The **Principal Investigator** will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, sponsor and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.

The **Research Coordinator** will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA and/or DSMB of all Unanticipated Problems/SAE's.

21.0 Future Undetermined Research: Data and Specimen Banking

If this study is collecting identifiable data and/or specimens that will be banked for future undetermined research, please describe this process in the sections below. This information should not conflict with information provided in section 9.1.1 regarding whether or not data and/or specimens will be associated with identifiers (directly or indirectly).

21.1 Data and/or specimens being stored

Identify what data and/or specimens will be stored and the data associated with each specimen.

NA

21.2 Location of storage

Identify the location where the data and/or specimens will be stored.

NA

21.3 Duration of storage

Identify how long the data and/or specimens will be stored.

NA

21.4 Access to data and/or specimens

Identify who will have access to the data and/or specimens.

NA

21.5 Procedures to release data or specimens

Describe the procedures to release the data and/or specimens, including: the process to request a release, approvals required for release, who can obtain data and/or specimens, and the data to be provided with the specimens.

NA

21.6 Process for returning results

Describe the process for returning results about the use of the data and/or specimens.

NA

22.0 References

List relevant references in the literature which highlight methods, controversies, and study outcomes.

Objectives and Background

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Pyrogens

1. Brown, S., and A.C. Fuller, “Depyrogenation of Pharmaceutical Solutions Using Submicron and Ultrafilters,” J. Parenteral Sci. & Tech., Vol. 47, No. 6, 1993, pp. 285-288.

The effect of varying the pH and ionic strength on endotoxin removal (depyrogenation) from Water for Injections (WFI) was investigated. Studies using submicron filters showed that endotoxin aggregation and filter retention increased with increasing molarity and decreasing pH. Using a Sartorius 0.01 micron filter, greater than 98% endotoxin retention could be achieved with 10 endotoxin units (EU)/ml bulk solution, and greater than 97% endotoxin retention with the 500 EU/ml bulk solution. Depyrogenation of active and placebo solutions of the radiopaque, Iohexol (350 mgI/ml), using ultrafilters of varying nominal molecular weight limit (NMWL 10,000-300,000) and a Pall Posidyne 0.2 micron filter was also investigated. Results with the ultrafilters showed that it was possible to increase the molecular weight cut-off of an ultrafilter from 10,000 to 100,000, without affecting the efficiency of endotoxin removal, thereby increasing flow rate and reducing filtration time. The Posidyne filter was able to depyrogenate Iohexol active and placebo product. The use of submicron filtration in place of ultrafiltration would provide significant cost benefits in terms of filtration time and equipment costs, and they have been shown to be capable of efficient depyrogenation of these pharmaceutical products.

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Staphylococcus aureus is an important human pathogen, causing a variety of diseases. Major virulence factors of this organism include staphylococcal enterotoxins (SEs) that cause food poisoning and toxic shock syndrome. Our study identified a novel enterotoxin-like protein that is a member of the new subfamily (group V) of pyrogenic toxin superantigens (PTSAgs) and examined its biochemical and immunobiological properties. The gene encoding the SE-like protein is directly 5‘ of another recently identified PTSAg, SEK. The SE-like protein had a molecular weight of 26000 and an experimentally determined isoelectric point between 7.5 and 8.0. We demonstrated that the PTSAg had many of the biological activities associated with SEs, including superantigenicity, pyrogenicity, and ability to enhance endotoxin shock, but lacked both lethality in rabbits when administered in subcutaneous miniosmotic pumps and emetic activity in monkeys.

Recombinant protein stimulated human CD4 and CD8 T cells in a T cell receptor variable region, β chain (TCRVβ) specific manner. T cells bearing TCRVβ2, 5.1, and 21.3 were significantly stimulated.

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