

Assessment of the sensitivity of the hypothalamic GnRH pulse generator to estradiol and progesterone inhibition in early pubertal girls (JCM026)

Background and Brief Summary:

Background:

Etiology of PCOS

Polycystic ovarian syndrome (PCOS) is a common clinical disorder affecting 6-7% of reproductive aged women. PCOS is associated with hyperandrogenism, multiple ovarian cysts, and oligo- or amenorrhea (Stein and Leventhal 1935). It is also a leading cause of infertility. The etiology for PCOS has not yet been elucidated. It has been proposed that hyperinsulinemia, altered ovarian steroidogenesis, and neuroendocrine abnormalities may play key roles either alone or in combination.

Neuroendocrine Abnormalities in PCOS and Adolescent Hyperandrogenism

Neuroendocrine abnormalities, whether primary or secondary, play an important role in PCOS. A group of neurons collectively known as the GnRH pulse generator control the pulsatile secretion of GnRH (gonadotropin releasing hormone) from the hypothalamus. GnRH, in turn, controls the synthesis and secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland. LH and FSH are both made by the same gonadotrope cell, and which hormone is preferentially synthesized and secreted depends in part on the GnRH pulse frequency. In primates, a GnRH pulse frequency of 1 pulse per hour favors secretion of LH, whereas slower pulses, on the order of 1 pulse every three hours, favor release of FSH (Wildt 1981). In normally cycling women, the GnRH pulse frequency in the follicular phase is relatively fast, favoring LH secretion. Following the rise in estrogen and progesterone after ovulation, there is a slowing of GnRH pulse frequency, resulting in a decrease in LH and increase in FSH synthesis, which is important for subsequent follicular development. Physiologic doses of exogenous progesterone have been shown to slow LH, and by inference GnRH, pulsatility when given during the follicular phase (Soules et al 1984). Therefore, progesterone plays an important role in regulating the GnRH pulse generator.

PCOS is characterized by persistently rapid LH (GnRH) pulse frequency without the cyclic luteal phase slowing seen in ovulatory women. Our group has shown that women with PCOS have reduced hypothalamic sensitivity to progesterone mediated suppression of LH (GnRH) pulsatility compared to ovulatory controls (Pastor et al 1998). Thus, they require higher plasma progesterone concentrations to achieve the same degree of GnRH suppression seen in controls. Especially when coupled to the fact that women with PCOS generally have low levels of progesterone secondary to infrequent ovulation, this relative insensitivity contributes to the persistently rapid GnRH pulsatility characteristic of PCOS. The resultant increase in LH leads to augmented ovarian androgen production, while the resultant decrease in FSH leads to impaired follicular development and anovulation. Androgens play an important role in mediating hypothalamic progesterone insensitivity, as progesterone sensitivity can be restored in women with PCOS with the use of the androgen blocker flutamide (Eagleson et al 2000).

Adolescent hyperandrogenism is thought to be a precursor to adult PCOS. Hyperandrogenic girls have increased LH concentrations and pulse frequency as well as accelerated maturation of their GnRH pulse patterns during pubertal development (Venturoli et al, 1992; Apter et al, 1994). A recent study (IRB-HSR# 8588/ CRU# JCM010) by our group found that, like adult women with PCOS, hyperandrogenic girls are less sensitive to the suppressive effects of progesterone on the GnRH pulse generator than normal controls (Chhabra et al, 2005).

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In IRB-HSR# 8588, the normal girls in early puberty (Tanner stages 1-3, n=4) had increased hypothalamic progesterone sensitivity compared to their older counterparts (Tanner stages 4-5, n=16) (percent reduction in LH pulses after 7 days of estradiol and progesterone 86.5% in Tanner 1-3 vs. 54.1% in Tanner 4-5). Although only a small number of Tanner 1-3 subjects were studied and the results do not achieve statistical significance, the finding is quite intriguing. Free testosterone concentrations rise 4- to 8-fold during the course of female puberty (McCartney et al, 2007). As androgens mediate reduced hypothalamic progesterone sensitivity in adult women with PCOS, we hypothesize that the gradual increase in free testosterone across the course of female puberty may lead to a gradual reduction in hypothalamic progesterone sensitivity.

In this study, we propose to investigate this hypothesis by assessing the relationship between hypothalamic progesterone sensitivity and androgen levels in normal puberty. We will measure hypothalamic progesterone sensitivity and androgen levels in normal girls in early to mid puberty (Tanner 1-3). These results will be compared to historic data in normal controls in late puberty (Tanner 4-5) that were collected as part of IRB-HSR# 8588.

A better understanding of neuroendocrine function during pubertal maturation may lead to insights regarding abnormal pubertal maturation (e.g. precocious puberty) or disorders with pubertal onset (e.g. PCOS). Hopefully this research will translate into improved prevention and treatment for these disorders in the future.

In addition to the main portion of the study, we will be measuring a variety of cytokines and adipokines in these subjects. This is part of a larger project to try to understand the origins of adolescent hyperandrogenemia and the extent to which cytokines and adipokines may play a role in increasing testosterone production in preadolescent and adolescent girls. These girls will be a source of normal control data for this exploratory project.

Brief Summary: Please see the abstract on page 5.

Additional Information

A. Investigators Experience

The PI and co-investigators have extensive experience in studies of the early manifestations and abnormalities of pubertal maturation during research over the past 25 years. Much of this research has involved joint collaboration with colleagues in the Department of Pediatrics, and this continues in the present application with co-investigators in the Dept. of Pediatrics. These individuals are specialists in pediatric endocrinology and are well versed and have expertise in dealing with children of the age ranges included. The group currently has 8 ongoing, IRB-approved studies in the adolescent population, including 6 that involve overnight admissions with frequent blood sampling. Over 100 girls to date have enrolled in overnight studies.

B. Study Support

This study will be supported by NIH funds through the General Clinical Research Center Core Grant and through a U54 Specialized Cooperative Centers Program for Research in Reproduction and Infertility (IRB# 9112)

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C. International Research

Will this study be done outside the U.S. under the oversight of a UVA PI? NO

D. Has another IRB ever not given approval to this protocol? No

Drug Information

- Micronized progesterone powder (Spectrum Chemical Manufacturing Corporation, Irving, CA), IND# 64,126
- Estrace (Warner-Chilcott, Rockaway, NJ)

Please list the phase or stage of this study. Not applicable.

Does this study involve research on a drug, biologic, supplement or food additive? Yes

If yes, is this study investigator initiated? Yes

If yes, answer questions # 1 and 2

If no, answer question # 2 only.

1. Do the following criteria apply?

No The investigation is intended to be reported to FDA as a well-controlled study in support of a new indication for use or intended to be used to support any other significant change in the labeling for the drug;

No If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is intended to support a significant change in the advertising for the product;

No The investigation does involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

If No- please explain why you believe the risk to subjects is not increased: Estrogen and progesterone are frequently prescribed to older adolescent girls (Tanner 4-5 pubertal stage) in the form of oral contraceptive pills. In that setting, they are felt to be safe and well tolerated. Although the proposed population here is younger than the girls typically treated with oral contraceptive pills (Tanner 1-3 pubertal stage), we will be administering physiologic doses of estradiol and progesterone for a very short time (7 days) and do not anticipate any adverse effects. The dosage of progesterone is adjusted based on the subject's weight. We have studied 4 Tanner 1-3 girls on a protocol with identical study procedures and no adverse effects were seen.

YES The investigation will be conducted in compliance with the requirements for institutional review set part in part 21CFR56 and with the requirements for informed consent set forth in part 21CFR50 ; and

YES The investigation will be conducted in compliance with the requirements of 21CFR312.7 (Promotion and charging for investigational drugs)

2. Please answer the following questions if you are using a drug/ supplement / food additive in a manner not approved by the FDA.

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- **Describe pertinent animal data that is available regarding the toxicity/safety of this drug.**
Not applicable, as abundant human data is available.
- **Describe pertinent human data that is available regarding the toxicity/safety of this drug.**
We are using a micronized progesterone suspension which is formulated/constituted by our investigational pharmacy. There are no specific data regarding human toxicity/safety of the UVAHS's progesterone suspension, but the progesterone used to formulate the suspension is FDA approved for use in adults. We have used this progesterone suspension in other protocols, and we have thus far administered the progesterone suspension to 37 adolescent girls and at least 11 adult women; no adverse events have occurred.
Estrace has used extensively in women and has a well described safety profile. It is FDA approved for use in adults.
- **Have there been any human deaths associated with this drug?**
To our knowledge, there have been no human deaths associated with micronized progesterone or Estrace.
- **In how many humans has this drug been used previously?**
The oral micronized progesterone suspension formulated by the University of Virginia Investigational Drug Pharmacy has been used in several other IRB-HSR approved protocols. We have administered the progesterone suspension to at least 37 adolescent girls and at least 11 adult women.
Oral estrogens, in various formulations, have been used to treat millions of women, although exact numbers are unknown.
- **If this protocol will be used in minors please describe any previous use of this drug with minors of a similar age range.**
The oral micronized progesterone suspension formulated by the University of Virginia Investigational Drug Pharmacy has been given to 37 adolescent girls as part of our protocols, 4 of whom were Tanner 1-3 pubertal stage. All of these subjects were also given estrace. There have been no adverse effects.
Although Estrace is not often prescribed to adolescent girls, there is significant experience with estrogens in the older adolescent population, generally in the form of oral contraceptive pills. Oral contraceptive pills have been shown safe and effective in adolescent girls, and are commonly used for a number of medical conditions, including hyperandrogenism, as well as for contraception. However, oral contraceptive pills are generally not prescribed to the girls in early adolescence (Tanner 1-3 pubertal stage) that we will be studying in this protocol.

Hypothesis to be Tested:

We hypothesize that the sensitivity of the GnRH pulse generator to suppression by estradiol and progesterone decreases during the course of normal female puberty coincident with a rise in androgen levels. Specifically, we hypothesize that the Tanner 1-3 normal subjects in this study will have a 30% greater reduction in LH(GnRH) pulse frequency after 7 days of estradiol and progesterone than historic Tanner 4-5 normal controls.

ABSTRACT

Women with PCOS have decreased GnRH pulse generator sensitivity to suppression by estradiol and progesterone, as do hyperandrogenic adolescent girls. In adult women, hypothalamic progesterone insensitivity appears to be androgen mediated as sensitivity can be restored with the use of the androgen receptor blocker

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flutamide. Free testosterone concentrations increase 4- to 8-fold during normal female puberty. The pubertal rise in T, as well as E2 and P, is accompanied by a rise in gonadotropin levels. This concurrent rise in gonadotropins and ovarian steroids suggests that the sensitivity of the hypothalamus to sex steroid feedback, which is exquisite during childhood, gradually diminishes during pubertal development. The etiology of this decreased hypothalamic sensitivity is not known. Given that androgens mediate reduced hypothalamic progesterone sensitivity in adult women with PCOS, we hypothesize that the gradual increase in free testosterone across the course of female puberty may lead to a gradual reduction in hypothalamic progesterone sensitivity. LH (GnRH) pulse frequency will be assessed before and after 7 days of oral estradiol and progesterone in normal girls in early to mid puberty. Progesterone adjusted change in 11-hour LH pulse frequency will be used as the measure of hypothalamic progesterone sensitivity. Hypothalamic progesterone sensitivity and androgen levels in these Tanner 1-3 subjects will be compared to results from previously studied Tanner 4-5 normal girls.

Human Participants:

Ages 8-14 years

Sex Female

Race All races will be recruited and enrolled.

Subjects- see below

1. Provide target # of subjects (at all sites) needed to complete protocol to obtain statistically significant results. 10

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites.

The historic dropout/withdrawal rate in JMC010/IRB#8588 which has identical study procedures is 35%. We expect a similar drop-out/withdrawal rate with this protocol. Therefore, we plan on enrolling up to 16 subjects in order to obtain complete, usable data on 10.

3. How many subjects will be enrolled at all sites? 16

4. How many subjects will sign a consent form under this UVa protocol? 16

Will you be obtaining data from the UVa Clinical Data Repository (CDR)? No

Participation of Minors

1. Explain why this research topic is relevant to minors.

This is a study of the effects of progesterone administration on gonadotropin (LH) dynamics during early puberty. It will advance our knowledge of the mechanisms of normal puberty. Moreover, it will provide important foundational data pertinent to our hypotheses regarding the development of polycystic ovary syndrome during adolescence. If our hypotheses are correct, this study could provide critical data implicating a specific mechanism by which hyperandrogenemia can cause abnormal gonadotropin secretion in those who may go on to develop PCOS. This in turn may provide a sound rationale for treatment of hyperandrogenemic girls in early adolescence. If hyperandrogenemic girls could be treated effectively before or during pubertal maturation, development of clinical PCOS as a later adolescent and adult could be potentially avoided.

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2. Is the knowledge being sought in this study already available for children or is it currently being acquired through another ongoing study?

There is very limited data on the sensitivity of the GnRH pulse generator to suppression by estradiol and progesterone during the early pubertal period. A previous study (IRB#8588/JCM010) assessed hypothalamic progesterone sensitivity in girls ages 8-18, but the bulk of these subjects were in late puberty (Tanner 4-5). Four Tanner 1-3 normal control subjects were studied on that protocol, and the finding that they were particularly sensitive to progesterone-mediated GnRH pulse generator slowing led to the hypothesis that is the basis for this study. The knowledge being sought is not currently being acquired through another ongoing study.

3. Provide data that is available in adults in order that the IRB may judge the potential risk in minors. If there is no adult data available, please provide reasons why not. If this information is available in a sponsor's protocol, you may reference the section # here and not duplicate the information.

Given that the data sought in this protocol is specific to the peripubertal period of development, there are no similar data in adults. However, very similar studies (frequent blood sampling for LH pulse analysis before and after 7 d of estradiol and progesterone) have been performed by our group in adults (REF) and adolescents (REF) without significant adverse effects.

Inclusion/Exclusion Criteria

Criteria for inclusion:

- Girls ages 8 to 14
- Tanner 1-3 pubertal stage
- Pre-menarchal
- Normal screening labs

Criteria for exclusion:

- Abnormal screening labs
- congenital adrenal hyperplasia.
- Hyperandrogenism (e.g., hirsutism, elevated free testosterone level)
- Hemoglobin <11.5 g/dL for non-African American subjects; Hemoglobin < 11.0 g/dL for African American subjects
- Weight < 31 kg
- History of peanut allergy, deep venous thrombosis, breast cancer, endometrial cancer, or cervical cancer
- On hormonal medications (including oral contraceptive pills) or on medications known to affect the reproductive axis within 3 months of the study
- Pregnant or breast feeding
- Participation in a research study within the past 30 days that involved taking a study drug.
- Participation in a research study that involved taking up to or greater than 473 ml's of blood within the past 60 days.
- Cigarette smoking
- History of surgery that required bedrest within the past 30 days
- Family history of hypercoagulability or unexplained thromboembolic disease (not in setting of bedrest, surgery, or malignancy)

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- In order to ensure an adequate number of younger girls, no more than 4 enrolled subjects will be Tanner stage 3

Restrictions (if any) on use of other drugs or treatments:

Subjects must discontinue all hormonally-active medications at least 3 months prior to the frequent sampling study (and 2 months prior to screening visit) and must not take any other medications known to affect the reproductive axis during the study. There are no dietary requirements for this study.

Recruitment

How will participants be recruited?

- Posters/Flyers
- Television
- Radio
- Newspaper Ads
- Internet
- Medical Record/ Database Review. NOTE- potential subjects will be contacted after obtaining the approval of their attending physician and IRB-HSR approval of method of contacting potential subjects (i.e. letter)
- Medical Record/ Database Review. NOTE- subjects will not be contacted
- Referrals from other health care professionals
- Discuss protocol with patients of PI or sub-investigators during a standard clinical visit

If subjects will be contacted, explain in detail how this will be done.

We will not initiate direct contact with potential subjects. Subjects responding to advertisements or referred from other health care professionals will be given the contact information for the Center for Research in Reproduction.

Do you plan to ask the subjects to do anything for the study prior to signing a consent?

YES We will request that subjects come to the screening visit while fasting for at least 8 hours. We will tell potential subjects that only subjects who have taken no hormonally-active medications for 2 months prior to screening are eligible for screening study, although we will not advise anyone to stop prescription medications without first consulting their personal physician.

Explain the consenting process:

An outpatient screening visit is scheduled for volunteers who preliminarily qualify for the study. Copies of the approved consent and assent forms are sent to potential subjects beforehand, and we request that the volunteer and her parents review and discuss the forms prior to the screening visit. The screening visit is held in an outpatient screening room in the CRU or alternate UVA clinical unit. This allows a private conversation between the study physician, the potential participant, and at least one of her parents (other individuals such as family members are allowed in the room if desired by the potential participant). The screening visit usually occurs in the morning, although rarely it will occur in the afternoon. The aims, procedures, and potential risks of the study are first explained by the study physician. Importantly, the potential participant and her parents are given an opportunity to ask any questions, and concerns are addressed. In cases where the potential participant wants to begin the study and her parents concur, the participant, parents, and physician sign the consent form. In cases where only one parent is able to come to the screening visit, we allow the second parent to sign the form in advance of the visit. This is done in conjunction with a conversation during which we offer that parent an opportunity to ask any questions and confirm that they understand the study and are willing for their daughter to participate. We routinely inform potential participants verbally that signing the consent form does not compel them to continue participation in the study. The remainder of the outpatient screening visit (i.e., history,

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physical, screening blood tests) occurs immediately thereafter. Participants generally begin the main part of the study within 1-2 months of the screening visit.

Do you plan to enroll your own students, patients, staff, employees? Yes.

Do you plan to enroll students, patients, staff, employees of any of the other personnel listed on this protocol? Yes

Do you plan to enroll yourself or any other personnel listed on this protocol? No

If yes, which groups? Subjects may be recruited from Dr. C. McCartney's, Dr. J. Marshall's, Dr. M. DeBoer's, Dr. C. Burt-Solorzano's, and Dr. A. Rogol's clinical practices. However, Drs. McCartney, Marshall, rarely if ever see early pubertal girls in their clinics. It is also possible that the children of our clinic patients, students, or employees may be enrolled. We will not enroll children of study investigators.

If yes, explain how these potential subjects will be recruited to avoid the appearance of coercion.

Patients who appear to be appropriate candidates for this study will be made aware that they may qualify for participation in the study. If they express interest, we will offer a very brief explanation of what is involved. If the patient continues to express interest, we will give them our research coordinator's contact information. In keeping with our mandate as physicians, we will discuss the study only in instances where potential participation would not be harmful to the patient (e.g. in cases where temporarily withholding customary treatments is expected to have no harmful effects). Subjects will be informed that their participation in the study is entirely voluntary and their decision whether or not to participate will have no effect on their current or future medical care.

Biomedical Research

List what will be done in this protocol:

All procedures performed in this protocol are being done solely to answer a research question and generate generalizable knowledge.

Outpatient Screening

After a potential subject is identified, we will arrange for her to come to the CRU or alternate UVA clinical unit for an outpatient screening exam. The goals and procedures of the study will be explained to the potential subject and her parents, and they will be given the opportunity to ask any questions. The potential subject and her parents will be asked to sign the assent and consent forms. A physician will record a family and personal medical history and perform a physical exam. Subjects will need to fast for a minimum of 8 hours prior to screening blood draw. Blood will be drawn for screening tests (CBC, Comprehensive metabolic panel, prolactin, LH, FSH, E₁, E₂, P, total T, androstenedione, 17-OHP, DHEA-S, fasting insulin, glucose, TSH, hCG, cholesterol, LDL, HDL, and SHBG). Potential subjects must fall within the normal range on all blood tests to be admitted to the study. Patients with elevated 17-OHP on screening will be referred to their pediatricians for further testing to rule out congenital adrenal hyperplasia. They will only be able to continue with the study if they have documented normal 17-OHP levels following cotrosyn stimulation. If the subject's hemoglobin is low at the time of screening (< 11.0 g/dL for African American subjects or < 11.5 g/dL for non-African American subjects), we will offer the option of taking oral iron supplementation at a dose of 1-2 mg/kg for 60 days. Subjects weighing ≤ 36 kg will be given 300-325 mg oral ferrous gluconate daily (containing 36 mg of elemental iron); subjects weighing >36 kg will be given 300-325 mg oral ferrous gluconate twice daily.

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Following the course of iron supplementation, the subject will then return to the CRU or clinical unit for a repeat hemoglobin, and will only be able to proceed with the study if her hemoglobin is greater than or equal to 11.5 g/dL for non-African American subjects and greater than or equal to 11.0 g/dL for African American subjects.

If the screening tests show that the subject is eligible for the study, she will be given oral iron supplementation at a dose of 1-2 mg/kg for 30 days to help prevent anemia during and after the study. Subjects weighing ≤ 36 kg will be given 300-325 mg oral ferrous gluconate daily (containing 36 mg of elemental iron); subjects weighing >36 kg will be given 300-325 mg oral ferrous gluconate twice daily. The admission can take place any time during or after the iron supplementation.

If overnight admission is scheduled to occur greater than one month after the most recent hemoglobin (such as that obtained during screening), a hemoglobin will be repeated 2-5 days before the overnight admission. Documentation of hemoglobin ≥ 11.5 g/dL for non-African American subjects or ≥ 11.0 g/dL for African American subjects in the previous month is required for the frequent sampling protocol. If the overnight admission is scheduled to occur greater than 3 months after screening, then the safety labs (complete blood count, comprehensive metabolic panel) will be repeated at this time.

Day 0: First inpatient admission

The subject will be admitted to the CRU, alternate UVA hospital unit, or off-site hotel at ~1700 hr. In general, parents are welcome to stay with their child at the off-site hotel if they wish. If the overnight portion of the study is to be done at an off-site hotel, the subject may stay without a parent or legal guardian, as long as two CRU staff are present. Whether or not a parent needs to remain during the overnight admission will be discussed when the visit is scheduled. A urine b-HCG will be performed to rule out pregnancy and must be negative for the study to continue. A small amount of topical lidocaine/prilocaine cream (EMLA cream) may be applied to facilitate IV line placement. An IV line will be placed in a forearm or hand vein and blood draws will begin at 1900 hr. Samples will be taken every 10 minutes. Most samples will be 0.75 mL, used to analyze levels of FSH and LH. 2.5 mL samples will be taken every 2 hours to analyze levels of estradiol, progesterone, testosterone, cortisol and DHEA. An additional 3.0 mL sample will be analyzed for fasting insulin, Insulin-like Growth Factor 1 (IGF-1), fasting glucose, estrone, sex hormone binding globulin, DHEA-S, androstenedione and a number of cytokines and adipokines, including adiponectin, leptin, resistin, PAI-1, IL-1b, IL-6, IL-8, TNF α , MCP-1, HGF and NGF. (Due to new technologies adipokines and cytokines can be measured in a total of 25 ul of serum, thereby allowing us to measure these without substantially increasing the necessary blood volume).

A formal "lights out" will occur at 2300 hr so that we may observe any nocturnal changes in LH release patterns. Q10 minute blood sampling will end at 0600 hr. There will be one final blood draw at 0700 hr. During blood sampling, activity (e.g., awake, sleeping) will be recorded by the nurse every 10 minutes. Additionally, periods of sleep will be estimated using wrist actigraphy (Motionlogger Basic-L; Ambulatory Monitoring, Inc.). The Motionlogger Basic-L is a watch-like device (that includes an accelerometer) that will be worn on the wrist by the research participant during overnight admission. The subject will be offered meals at standard CRU meal times.

Day 1: Study medications

Starting the day of discharge from the first inpatient admission, subjects will be given oral estrogen (estrace, 0.5-1 mg once a day) and oral progesterone suspension (20 mg/ml, 25-100 mg) three times a day at

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0700, 1500, and 2300 hr to achieve mean plasma concentrations over the range of 2-8 ng/ml for seven days. Each subject will be instructed to eat a small snack with the medication. It has been observed that the absorption of progesterone is influenced by the presence or absence of food. Subjects will continue their iron supplementation.

Days 3, 5: Outpatient blood draws

On study day 3, subjects will have blood drawn at 0900 or 1700 hr (two hours after the 0700 or 1500 hr progesterone dose) to check serum estrogen and peak progesterone levels. On study day 5, subjects will have blood drawn at 0900 or 1700 hr (two hours after the 0700 or 1500 hr progesterone dose) to check serum estrogen, peak progesterone levels, and hematocrit/hemoglobin. For the second admission to continue, subject must have a hemoglobin ≥ 11.0 g/dL for African American subjects or ≥ 11.5 g/dL for non-African American subjects.

Day 7: Second inpatient admission

The second inpatient admission will begin on day 8. The procedure will be identical to the first inpatient admission.

Follow-up

Subjects will discontinue estrace, progesterone, and iron after the completion of the second inpatient admission. Follow-up questionnaires and/or phone interviews will be completed by the subjects 3, 6 and 12 months after the study asking about the onset and frequency of menses and changes in hirsutism. During these interviews, we will specifically address whether pre-menarchal girls had a withdrawal bleed following the study, and if so, whether or not they subsequently continued menstruating. Whenever possible we will also see the patients on 2-3 occasions during the 12 months to obtain blood for T, DHEA-S, E₂, and P measurements (depending on cycle stage).

If a potential subject does not meet the inclusion/ exclusion criteria will you repeat any of the screening procedures/ tests? Yes

If yes, please explain. Patients with elevated 17-OHP on screening will be referred to their pediatricians for further testing to rule out congenital adrenal hyperplasia. They will only be able to continue with the study if they have documented normal 17-OHP levels following cotrosyn stimulation. Additionally if safety labs are abnormal during screening (e.g., abnormal liver tests, abnormal TSH), subjects will be asked to return once for repeat (confirmatory) labs to exclude lab error. Repeat testing will generally occur within one month of the original screening lab draw. If exclusionary lab values are confirmed on such repeat testing, subjects will be excluded from participation. The exact amount of blood drawn will depend on the labs being repeated, but the total amount of blood drawn will not exceed 5 ml.

If clinically meaningful results are obtained during the course of the study, explain how you will notify the participant.

We will contact the custodial parent(s) via phone and make general recommendations (e.g., follow-up with the subject's primary care physician). We will generate a letter to the primary care physician (with a copy to the subject and her parent[s]) detailing the clinically meaningful results and associated findings of relevance.

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What treatments, normally used, will be omitted for the study?

Not applicable as these are normal, healthy subjects.

Will any of the treatments/ procedures be done for research purposes only? Yes

If yes, describe: All procedures are being done for research purposes only.

What details of the study are best kept secret from the participants?

None of the details of the study will be kept from the participants. Study participants and their parents will be advised of any and all aspects of the study. They will have ready access to their individual results after the data has been analyzed, and they may contact us to find out the results of the entire study once it has been completed.

Taping/Photography-N/A

Payment

All participants will receive a \$75 Charlottesville Fashion Square Mall gift card per inpatient admission. In addition, participants will receive a \$50 bonus for study completion, also in the form of a gift card. Therefore, a participant who completes the entire study will receive a total of \$200 in gift cards. If a subject only completes one inpatient admission, she will be given a \$75 gift card.

Is money paid from UVa or State funds (including grant funds)? **Yes**

If Yes, continue to check all that apply below

Will researcher use any of the following to compensate participants (check **all** that apply)?

Check issued to participant via UVA Oracle or State system

Petty cash account

Gift card

Other type of compensation: specify : _____

Check **all** categories that apply based on each participant group in the study:

< \$50 TOTAL possible compensation for participating in the research study over one year

>= \$50 TOTAL possible compensation for participating in the research study over one year **and** check issued to participant via UVA Oracle or State system

>= \$50 TOTAL possible compensation for participating in the research study over one year **and** researcher unable to issue checks through UVA or State system, but able to gather IRS information

No payments of >= \$50 will be approved if IRS information cannot be obtained.

Justify why you are unable to issue checks through the UVa Oracle or state system.

Additional Comments/ Explanation:

This study involves preadolescent and adolescent girls. In past and on-going studies involving the same patient population, we have found that the girls prefer being given gift cards directly at the end of the study to receiving

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a check in the mail 4-6 weeks later, and this preference is reflected in our ability to recruit subjects. Therefore, we prefer to compensate these subjects with gift cards. We obtain social security numbers for IRS purposes.

Specimens:

Type of specimen to be used: Blood

Please list the information and identifiers that will be with the specimen when it is given to you. Name, medical record #, GCRC protocol #, time (date and clock hour) drawn.

If you are using discarded tissue do you confirm that it will be obtained either from pathology, a clinical lab or the UVa Tissue Procurement Facility? Not applicable.

Will you be using viable embryos? No

Will you be using embryonic stem cells? No

Do you plan to ship any specimens outside of UVA? No

Will the specimen be obtained before a subject has signed a consent form? No

Study Design: Biomedical

What kind of controls will be used? Only normal control subjects will be studied.

Single-blind, double-blind, other: This study is not blinded.

If randomized, how? This study is not randomized.

Does the study involve a placebo? No

Bio-statistical Analysis

Explain bio-statistical analysis to be done:

Sample Size Determination. If a total of 10 normal tanner-stage 1-3 girls complete both CRU admissions, and the true group to group difference in the percentage change in LH pulse frequency following estradiol and progesterone supplementation is greater than or equal to 30%, we anticipate that we will have at least an 80% chance of rejecting the null hypothesis that estradiol and progesterone supplementation alters LH pulse frequency to the same degree in both normal tanner-stage 1-3 and normal tanner-stage 4-5 girls. This effect size was determine based on the following set of assumptions: (a) the percentage change in LH pulses frequency following estradiol and progesterone supplementation is normally distributed in both study populations, (b) the within-subject measurement variability (standard deviation) in the percentage change LH pulse frequency will not exceed 26.6% in either group (standard deviation based on the data from IRB-HSR# 8588), (c) the two-sided type I error rate of the statistical test will not exceed 0.05, and (d) the power of the statistical test will be at least 0.80.

Base on our previous protocol (IRB-HSR# 8588), we anticipated that the subject dropout rate will not exceed 35% in this study population. Therefore, we intend to enroll 16 normal tanner-stage 1-3 girls into this study.

Statistical Analyses: We will analyze the LH pulse frequency data from the tanner stage 1-3 normal girls and the LH pulse frequency data from the tanner stage 4-5 normal girls, by way of a linear-mixed effects ANCOVA model. The response variable will identify the change in the LH pulse frequency between the LH pulse frequency measured at the first overnight admission (baseline) and that measured at the second overnight admission (after progesterone supplementation). The ANCOVA model will include one independent factor and two independent continuous covariates. The independent factor will identify the girl's tanner stage (1-3, or

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4-5), while the two independent continuous covariates will identify the girl's progesterone levels at baseline and following estradiol and progesterone supplementation. Based on the parameter estimates from the ANCOVA model, we will be able to compare the magnitude of the change in LH pulse frequency after estradiol and progesterone supplementation between the two groups of girls after adjusting for the girls' progesterone concentration at baseline (overnight admission 1) and after adjusting for the girls' progesterone concentration following estradiol and progesterone supplementation (overnight admission 2). A generalized-F-test will be utilized to formally test the null hypothesis that estradiol and progesterone supplementation alters LH pulse frequency in tanner-stage 1-3 girls to the same degree as in normal tanner-stage 4-5 girls. The decision rule to reject the null hypothesis, will be based on a $P \leq 0.05$ criterion. Confidence interval construction, for estimating the true group to group difference in the percentage change in LH pulse frequency after estradiol and progesterone supplementation, will be based on the Students t-distribution multiplier. The PROC MIXED procedure of SAS version 9.1.3 EAS (SAS Institute Inc, Cary NC) will be used to conduct the aforementioned statistical analyses.

In order to ensure an adequate sampling of younger girls, no more than 4 enrolled subjects will be Tanner stage 3. The number of subjects in each Tanner stage will be reported with the final results.

What is the primary outcome variable upon which a sample size estimate is based?

Do I have an adequate sample size, or is my sample size larger than necessary? *The calculation of sample size also allows the CRU to properly budget for adequate resources to help you complete your proposal.*

Risk/ Benefit Analysis:

What are the potential benefits for the participant as well as benefits which may accrue to society in general, as a result of this study?

Although individual subjects will not benefit from participating in this study, the results will advance our knowledge of normal pubertal maturation. A better understanding of normal pubertal maturation may lead to insights regarding abnormal pubertal maturation (e.g. precocious puberty) or disorders with pubertal onset (e.g. PCOS). Hopefully this research will translate into improved prevention and treatment for these disorders in the future.

Analyze the risk-benefit ratio:

This study involves minimal risks, but may provide valuable information about normal pubertal maturation and the origins of PCOS. PCOS is the most prevalent reproductive disorder in young women and typically manifests at puberty. Enhanced understanding of normal neuroendocrine function during puberty and how these normal processes go awry in PCOS will be essential to design rational prevention and treatment strategies for PCOS. Therefore, we feel the potential benefits to society are substantial while the risks to the participants are quite small.

Data and Safety Monitoring Plan

Note: A Sponsor-is defined as entity that will receive data prior to publication.

1. Definition:

1.1 How will you define **adverse events (AE)** for this study?

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An adverse event will be considered any undesirable sign, symptom or medical or psychological condition **even if the event is not considered to be related** to the investigational drug/device/intervention. Medical condition/diseases present before starting the investigational drug/intervention will be considered adverse events only if they worsen after starting study treatment/intervention. An adverse event is also any undesirable and unintended effect of research occurring in human subjects as a result of the collection of identifiable private information under the research. Adverse events also include any problems associated with the use of an investigational device that adversely affects the rights, safety or welfare of subjects.

Will use definitions provided in the non IRB Protocol (Sponsor's, Investigator-Initiated, CTEP etc.) located in Section (insert section #).

Other

1.2 How will you define **serious adverse events**?

A serious adverse event will be considered any undesirable sign, symptom, or medical condition which is fatal, is life-threatening, requires or prolongs inpatient hospitalization, results in persistent or significant disability/incapacity, constitutes a congenital anomaly or birth defect, is medically significant and which the investigator regards as serious based on appropriate medical judgment. An important medical event is any AE that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions of SAEs.

OR

Any serious psychological and emotional distress resulting from study participation (suggesting need for professional counseling or intervention).

1.3 This is the definition of an **unanticipated problem**:

- An unanticipated problem is any event, experience that meets ALL 3 criteria below:
- Is unexpected in terms of nature, severity or frequency given the research procedures that are described in the protocol-related documents AND in the characteristics of the subject population being studied
 - Related or possibly related to participation in research. This means that there is a reasonable possibility that the incident may have been caused by the procedures involved in the research study.
 - The incident suggests that the research placed the subject or others at greater risk of harm than was previously known or recognized OR results in actual harm to the subject or others

1.4 This is the definition of a **protocol violation**:

A protocol violation is defined as any change, deviation, or departure from the study design or procedures of research project that is NOT approved by the IRB-HSR prior to its initiation or

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implementation, OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Protocol violations may or may not be under the control of the study team or UVa staff. These protocol violations may be major or minor violations.

Major Protocol Violation:

All major protocol violations must be reported to the IRB-HSR immediately upon discovering them, and no later than seven (7) calendar days from the time the study team receives knowledge of the event.

A major violation is a protocol violation that meets the following criteria:

- Represent a serious or continuing failure on the part of the study team to comply with the protocol, standard operating procedures, GCPS, federal, state or local regulations
- Impacts subject safety or substantially alter risks to subjects. May or may NOT result in actual harm (clinical, emotional, social, financial, etc)
- Significantly damages the completeness, accuracy and reliability of the data collected for the study
- Is under control of the investigator/study team/ UVa staff

Examples of **MAJOR** violations may include, but are not limited to:

- Evidence of willful or knowing misconduct on the part of the investigator(s), or study team
- Failure to obtain informed consent, i.e., there is no documentation of informed consent, or informed consent is obtained after initiation of study procedures. (Note: Data for subjects where consent is not documented may not be used in data analysis.)
- Enrollment of a subject who did not meet all inclusion/exclusion criteria which would affect subject safety or would negatively impact data integrity
- Performing study procedure not approved by the IRB-HSR
- Failure to report serious unanticipated problems/adverse events to the IRB-HSR and (if applicable), the sponsor
- Failure to perform study procedures outlined in the protocol where subject safety or data integrity may be negatively impacted. (Ex. Safety labs not done per protocol)
- Drug/study medication dispensing or dosing error under control of Investigators, study team or UVa staff.
- Study visit or procedure conducted outside of required time frame that may negatively affect subject safety
- Failure to follow Data Safety Monitoring Plan

Minor Protocol Violations:

Reporting of minor violations to IRB is not required by IRB-HSR.

2. Identified risks and plans to minimize risk

2.1 What risks are **expected** due to the intervention in this protocol?

Expected: is identified in nature, severity or frequency in the study documentation (protocol, consent, Investigator Brochure, package insert etc) is considered an expected.

Risk associated with frequent blood sampling:

Expected Risks related to study participation- volume of blood drawn	Frequency <i>Please pick one frequency from each box below</i>
<ul style="list-style-type: none"> • Significant anemia related to frequent blood sampling (hematocrit < 30%) 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown, but likely to be very rare
<ul style="list-style-type: none"> • Mild anemia (hematocrit < 36%) related to frequent blood sampling 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently

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	<input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
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Risk associated with micronized progesterone suspension:

Expected Risks related to study participation-micronized progesterone	Frequency
<ul style="list-style-type: none"> GI upset (nausea, abdominal bloating, diarrhea) 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown, but likely to be very rare
<ul style="list-style-type: none"> CNS effects (sleepiness, headache, dizziness, fatigue, emotional lability, irritability) 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
<ul style="list-style-type: none"> Mild, short-lived vaginal bleeding shortly after ingestion 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown

Risk associated with estradiol:

Expected Risks related to study participation.	Frequency
<ul style="list-style-type: none"> GI upset (nausea, abdominal bloating, diarrhea) 	<input type="checkbox"/> Occurs frequently, <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
<ul style="list-style-type: none"> Breast Tenderness 	<input type="checkbox"/> Occurs frequently, <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
<ul style="list-style-type: none"> Deep vein thrombosis 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown, however occurs very rarely with long-term estrogen use (as with oral contraceptive pills), so would expect to be exceedingly rare with the short term administration in this protocol

Risk associated with IV needle placement:

Expected Risks related to study participation-IV placement	Frequency
<ul style="list-style-type: none"> Infection at needle site 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input checked="" type="checkbox"/> Frequency unknown, but likely to be very rare
<ul style="list-style-type: none"> Bleeding at needle site 	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely

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	<input type="checkbox"/> Frequency unknown
• Blood clot at needle site	<input type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input checked="" type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
• Pain at needle site	<input checked="" type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
• Bruise at needle site	<input checked="" type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown

Risk associated with iron supplementation

Expected Risks related to study participation-iron supplementation	Frequency <i>Please pick one frequency from each box below</i>
• nausea	<input type="checkbox"/> Occurs frequently, <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
• constipation	<input type="checkbox"/> Occurs frequently, <input checked="" type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown
• dark or black stools	<input checked="" type="checkbox"/> Occurs frequently, <input type="checkbox"/> Occurs infrequently <input type="checkbox"/> Occurs rarely <input type="checkbox"/> Frequency unknown

Risk associated with EMLA cream (topical lidocaine and prilocaine, used to alleviate pain): The risks of lidocaine and prilocaine in general may include (frequency not defined) hypotension, angioedema, shock, hyperpigmentation, erythema, itching, rash, burning, urticaria, burning, stinging, edema bronchospasm, and hypersensitivity reactions. However, in the case of topical lidocaine/prilocaine use, the non-dermatologic adverse events mentioned above would be extremely unlikely unless large amounts of topical lidocaine/prilocaine were used (allowing significant systemic absorption).

Risk regarding wrist actigraphy: There are no known risks associated with the use of wrist actigraphy.

Risk of not being able to take hormonal medications: Fertility in premenarcheal girls is exceedingly unlikely. Thus, the risk of pregnancy related to inability to take hormonal birth control medication is virtually absent.

2.2 List by bullet format a summary of safety tests/procedures/observations to be performed.

- Sterile technique will be used.
- Before participation in the study, all participants will be required to have a normal hemoglobin (≥ 11.0 g/dl for African American subjects or ≥ 11.5 g/dL for non-African American subjects). Hemoglobin levels will be measured prior to every admission, and the admission will only continue if the

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hemoglobin level is ≥ 11.0 g/dl for African American subjects or ≥ 11.5 g/dL for non-African American subjects.

- Blood loss will be carefully recorded and limited to a maximum of 7cc/kg (10% of estimated total blood volume) in 8 weeks. A total of 216.5 ml of blood will be drawn during the study (including estimated waste from frequent blood draws). Therefore, girls weighing less than 31 kg will not be able to participate. Iron supplementation (325 mg once or twice a day dependent on weight) will be prescribed to all participants.
- β -hCG levels will be measured at screening and prior to each admission; if the β -hCG is positive, the study will be discontinued.
- Topical lidocaine and prilocaine (EMLA) cream may be used to lessen pain associated with IV needle insertion.

2.3 Check the criteria below under which an INDIVIDUAL SUBJECT'S study treatment or study participation would be stopped or modified

- At subject, PI or sponsor's request
- Treatment would be stopped if the subject had a Serious adverse event deemed related to study
- The study would be stopped in the patient has a positive pregnancy test or a hematocrit <36 and hemoglobin <12 prior to any of the 4 admissions.

2.4 Check the criteria under which THE ENTIRE STUDY would need to be stopped. These are called stopping rules for early termination of the entire study.

- Per IRB, PI, DSMB, or sponsor discretion
- Other: If there are an excessive number of unexpected adverse events that significantly alter the risk/benefit ratio for the study, the study will be terminated. All adverse events will be evaluated both individually and cumulatively by the study team and principal investigator as they arise, allowing for timely decisions regarding study continuation/termination.

2.5 Describe the criteria for breaking the blind/mask NA – Not blinded/masked

2.6 How will subject withdrawals/dropouts be reported to the IRB prior to study completion?

- IRB-HSR continuation status form

3. Adverse Event / Unanticipated Problem Recording and Reporting

3.1 Will all adverse events, as defined in section 1.1, be collected/recorded?

- Yes

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3.2 How will adverse event data be collected/recorded?

- Paper AE forms/source documents (please attach a copy of this form if possible)
- Spreadsheet - electronic

3.3. How will AEs be classified/graded?

- World Health Organization Criteria (WHO)
- NCI Common Toxicity Criteria, Version 2.0/ NCI Common Terminology Criteria, Version 3.0
- Mild/Moderate/Severe
- Serious/Not serious (required for all protocols)

3.4 What scale will the PI use when evaluating the relatedness of adverse events to the study participation?
(Check **all** that apply.)

- The PI will determine the relationship of adverse events to the study using the following scale:

Related: AE is clearly related to the intervention
 Possibly related: AE may be related to the intervention
 Unrelated: AE is clearly not related to intervention

3.5 When will recording/reporting of adverse events/unanticipated problems begin?

- After subject begins study drug/ device placement/intervention /study-related procedure/specimen collection

3.6 When will the recording/reporting of adverse events/unanticipated problems end?

- End of study drug/device/intervention/participation
- 30 days post study drug/device/intervention
- Subject completes intervention and follow up period of protocol

3.7 Please complete the following table to describe details of Adverse event, Unanticipated Problem, and Protocol Violation reporting

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study	IRB-HSR	Within 24 hours	IRB Online and phone call

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participation (Note: An internal event is one that occurs in a subject enrolled in a UVa protocol.)			www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. <i>Timeline includes submission of signed hardcopy of AE form.</i>	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations	IRB-HSR GCRC	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc) copy of report to IRB
Protocol Violations (Note the IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.) Or Enrollment Exceptions	IRB-HSR GCRC	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form http://www.virginia.edu/vprgs/irb/hsr_forms.html copy of report to IRB
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

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4. How will the endpoint data be collected/recorded.

Database: The endpoint data will be collected and maintained in a database kept on the CRU server.

5. Data and Safety Oversight Responsibility

5.1. Who is responsible for overseeing safety data for this study

- No additional oversight body other than PI (skip question 5.2)
- The UVa Cancer Center Data and Safety Monitoring Committee (*If your study involves cancer patients, see Question # 6 to help you decide if you should check this box*)
- Industry Sponsored-designated DSMB/DSMC/Medical Monitor
- Non-Industry Sponsored (other academic center, NIH) designated DSMB/DSMC/Medical Monitor (*If your study is NIH funded, please check with the center to determine if they require a DSMB for this study*)

5.2. What is the composition of the reviewing body and how is it affiliated with the sponsor? N/A

5.3. Please check aggregate reviews that will occur by the PI

- All adverse events
- Unanticipated Problems
- Protocol violations
- Audit results
- Application of dose finding escalation/de-escalation rules
- Application of study designed stopping/decision rules
- Early withdrawals
- Whether the study accrual pattern warrants continuation/action
- Endpoint data

5.4. How often will aggregate review occur?

- Annually

5.5. How often will a report, regarding the outcome of the reviews, be sent to the PI from the oversight body listed in 5.1. Not applicable.

5.6. How will a report of the information discussed in question 5.4 OR 5.5 be submitted to the IRB?

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Part of IRB-HSR continuation status form

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**Principal Investigator
(Print)**

**Principal Investigator
(Signature)**

Date

IRBHSR# 14100

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