IRB-HSR PROTOCOL

Investigator Agreement

BY SIGNING THIS DOCUMENT, THE INVESTIGATOR CONFIRMS:

1. I am not currently debarred by the US FDA from involvement in clinical research studies.
2. I am not involved in any regulatory or misconduct litigation or investigation by the FDA.
3. That if this study involves any funding or resources from an outside source, or if you will be sharing data outside of UVA prior to publication that you will contact the Dean's office regarding the need for a contract and letter of indemnification. If it is determined that either a contract or letter of indemnification is needed, subjects cannot be enrolled until these documents are complete.
4. The proposed research project will be conducted by me or under my close supervision. It will be conducted in accordance with the protocol submitted to and approved by the IRB including any modifications, amendments or addendums submitted and approved by the IRB throughout the life of the protocol.
5. That no personnel will be allowed to work on this protocol until they have completed the IRB-HSR On-line training and the IRB-HSR has been notified.
6. That all personnel working on this protocol will follow all IRB-HSR Policies and Procedures as stated on the IRB-HSR Website http://www.virginia.edu/vprgs/irb/ and on the School of Medicine Clinical Trials Office Website: http://knowledgelink.healthsystem.virginia.edu/intranet/hes/cto/sops/sop_index.cfm
7. I will ensure that all those delegated tasks relating to this study, whether explicitly or implicitly, are capable through expertise, training , experience or credentialing to undertake those tasks.
8. I confirm that the implications of the study have been discussed with all Departments that might be affected by it and have obtained their agreement for the study to take place.
9. That no subjects will be recruited or entered under the protocol until the Investigator has received the signed IRB-HSR Approval form stating the protocol is open to enrollment
10. That any materials used to recruit subjects will be approved by the IRB-HSR prior to use.
11. That all subjects will sign a copy of the most current consent form that has a non-expired IRB-HSR approval stamp.
12. That any modifications of the protocol or consent form will not be initiated without prior written approval from the IRB-HSR, except when necessary to eliminate immediate hazards to the subjects.
13. Any significant findings that become known in the course of the research that might affect the willingness of subjects to enroll or to continue to take part, will be promptly reported to the IRB.
14. I will report immediately to the IRB any unanticipated problems involving risk to subjects or to others including adverse reactions to biologics, drugs or medical devices.
15. That any serious deviation from the protocol will be reported promptly to the Board in writing.
16. That any data breach will be reported to the IRB, the UVa Corporate Compliance and Privacy Office , UVa Police as applicable.
17. That the continuation status report for this protocol will be completed and returned within the time limit stated on the form.
18. That the IRB-HSR office will be notified within 30 days of a change in the Principal Investigator or of the closure of this study.
19. That a new PI will be assigned if the current PI will not be at UVA for an extended period of time.
20. Signed consent forms and other research records will be retained in a confidential manner. Records will be kept at least 6 years after completion of the study. These are considered institutional records and may not be

transferred to another institution. A copy of the documents may be taken with the Principal investigator when transferring to another institution.

The IRB reserves the right to terminate this study at any time if, in its opinion, (1) the risks of further experimentation are prohibitive, or (2) the above agreement is breached.

Investigators Experience

The PI has substantial experience in studies of the early manifestations and abnormalities of pubertal maturation during research at UVA over the past 10 years. Much of this research has involved joint collaboration with colleagues in the Department of Pediatrics (e.g., Dr. Alan Rogol), and the PI continues to maintain close ties with investigators in the Dept. of Pediatrics (e.g., Drs. Christine Burt Solorzano, Mark DeBoer, and Alan Rogol). These individuals are specialists in pediatric endocrinology and are well versed and have expertise in dealing with children of the age ranges included.

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 13 ongoing, IRB-approved studies in the adolescent population, including 12 that involve overnight admissions with frequent blood sampling. Over 100 girls to date have enrolled in overnight studies.

Signatures

Principal Investigator

_____	_____	_____
Principal Investigator Signature	Principal Investigator Name Printed	Date

Department Chair

BY SIGNING THIS DOCUMENT THE DEPARTMENT CHAIR AGREES:

1. To work with the investigator and with the board as needed, to maintain compliance with this agreement.
2. That the Principal Investigator is qualified to perform this study.
3. That the protocol is scientifically relevant and sound.

_____	_____	_____
Department Chair or Designee Signature	Department Chair or Designee Name Printed	Date

The person signing as the Department Chair cannot be the Principal Investigator or a sub-investigator on this protocol.
The Department Chair or Designee signature is ONLY required if this is a new protocol or a modification changing the Principal Investigator

Brief Summary/Abstract

Women with PCOS have decreased GnRH pulse generator sensitivity to suppression by estradiol and progesterone. Increased androgen levels appear to mediate this decrease in sensitivity, as sensitivity can be restored with the androgen receptor blocker flutamide. Adolescent hyperandrogenemia is thought to be a precursor of PCOS, and a subset of adolescent girls with hyperandrogenemia have decreased hypothalamic sensitivity to progesterone similar to their adult counterparts with PCOS. We hypothesize that flutamide will restore hypothalamic progesterone sensitivity in these girls by blocking the effects of androgens. LH (GnRH) pulse frequency will be assessed before and after 7 days of oral estradiol and progesterone in hyperandrogenic adolescent girls who have been pretreated for 14 days with flutamide. The slope of the percentage change in LH pulse frequency as a function of Day 7 progesterone level will be used as a measure of hypothalamic progesterone sensitivity. Hypothalamic progesterone sensitivity in the girls pretreated with flutamide will be compared to hypothamic progesterone sensitivity in historic controls who did not receive flutamide.

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

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. 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 P insensitivity, as P sensitivity can be restored in women with PCOS with the use of the androgen blocker flutamide (Eagleson et al 2000). Thus, in a vicious cycle, hyperandrogenemia promotes neuroendocrine abnormalities that perpeturate hyperandrogenemia.

Hyperandrogenemia as a Precursor for PCOS

Excess androgen production during adolescence is thought to be a precursor of adult PCOS. Women with PCOS often report a history of irregular menstrual cycles during adolescence. A study of girls with menstrual irregularities showed that while some subjects normalized endocrine function as they mature, the majority maintained hyperandrogenism along with the elevated LH levels and polycystic ovaries characteristic of PCOS (Venturoli et al. 1987). Adolescent hyperandrogenemia has also been shown to be associated with higher androgen levels and lower fertility rates in adulthood (Apter and Vihko 1990).

The origin of adolescent hyperandrogenemia is unclear. Along with primary adrenal, ovarian, and neuroendocrine abnormalities, obesity has recently been suggested to play an important role. Several studies have shown a correlation between body mass index (BMI) and androgen levels in adolescent girls. Elevated BMIs are associated with increased total testosterone levels and decreased SHBG levels, leading to marked elevations in free testosterone (Wabitsch et al 1995, Reinehr et al 2005, McCartney et al 2006). Androgen levels decrease with weight loss, demonstrating the importance of obesity as a contributing factor (Wabitsch et al 1995, Reinehr et al 2005). The link between obesity and elevated total and free testosterone concentrations is especially concerning in light of the rising incidence of childhood obesity. The most recent data from the National Health and Nutrition Examination Study (NHANES) estimates that 15.1% of girls aged 6-19 years are overweight (defined as BMI >/= 95th percentile on the sex-specific BMI-for-age growth chart) (Hedley et al 2004). Given that obesity is associated with hyperandrogenemia, and adolescent hyperandrogenemia often progresses to adult PCOS, there is concern that that the epidemic of childhood obesity may be followed by a rise in the incidence of PCOS.

Similar to women with PCOS, girls with hyperandrogenemia have an increased frequency of LH pulses when compared to age matched controls (Apter et al. 1994). An ongoing study by our group is investigating whether the progesterone insensitivity of the GnRH pulse generator in adult women with PCOS is also seen in adolescent girls with hyperandrogenemia. Analysis of the data to date suggests that the hyperandrogenic adolescent girls have decreased hypothalamic progesterone sensitivity when compared to adolescent controls, with a subgroup (consisting of approximately half of the hyperandrogenic girls) having marked progesterone insensitivity similar to that seen in adult women with PCOS. These data have recently been published (Blank et al. 2009).

Given that androgens mediate hypothalamic progesterone insensitivity in adult women with PCOS, we hypothesize that androgens play a similar role in adolescent girls with hyperandrogenemia and that progesterone sensitivity can be restored with the use of the androgen receptor blocker flutamide.

Better understanding the effects of hyperandrogenemia in adolescence and its role in the development of PCOS will hopefully lead to improved prevention and treatment strategies for PCOS. This may prove increasingly important if the current epidemic in childhood obesity results in a growing number of girls with elevated androgen levels.

Hypothesis to be Tested

We hypothesize that flutamide will restore the sensitivity of the GnRH pulse generator to suppression by estradiol and progesterone in hyperandrogenic adolescent girls. We will compare hypothalamic progesterone sensitivity in hyperandrogenic girls pretreated with flutamide to hypothalamic progesterone sensitivity in historic controls (from JCM010) who did not receive flutamide. The slope of the percent reduction in LH pulses/11 hours as a function of Day 7 progesterone will be used as a measure of hypothalamic progesterone sensitivity.

Study Design: Biomedical

1. Will controls be used? Yes.

►IF YES, explain the kind of controls to be used. We will use historic controls from 8588/JCM010. 8588/JCM010 is identical to this study, except flutamide was not given. Therefore, it will allow us to compare the hypothalamic progesterone sensitivity data from hyperandrogenic adolescent girls in the presence and absence of flutamide.

2. What is the study design?

This study is not blinded.

3. Does the study involve a placebo? No.

Human Participants

Ages 13-18 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.

13

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

In a similar study (JCM010), we have historically obtained complete, usable data in approximately 65% of the subjects who enrolled in the study. However, that study did not involve taking flutamide. Because of the additional demands associated with taking flutamide and coming in for additional blood draws for LFT monitoring, as well as the possibility that some participants may develop mild LFT abnormalities and therefore be forced to stop the study, we suspect that the screen failure/dropout rate will approach 50%. Therefore, we plan to enroll 26 subjects, with a goal of obtaining complete, usable data in 13.

3. **How many subjects will be enrolled at all sites?** 26
4. **How many subjects will sign a consent form under this UVa protocol?** **26**

Inclusion/Exclusion Criteria

1. List the criteria for inclusion

- Girls ages 13 to 18
- Tanner IV or V stage of puberty
- Post-menarche
- Hyperandrogenemic (total testosterone > 0.4 ng/mL or free testosterone > 35 pmol/L) with or without hirsutism
- Normal AST/ALT (AST ≤ 35 U/L, ALT ≤ 55 U/L)
- Hemoglobin ≥ 11.0 g/dL for African American subjects; Hemoglobin ≥ 11.5 for non-African American subjects
- Normal screening labs (with exception of the expected hormonal abnormalities inherent in hyperandrogenemia)
- Sexually active subjects must agree to abstain or use double barrier contraception during the study Subjects must agree not to take any other medications during the course of the study without approval by the study investigators

2. List the criteria for exclusion

- Abnormal screening labs (with the exception of the expected hormonal abnormalities inherent in hyperandrogenemia)
- Elevated AST/ALT (AST > 35 U/L, ALT > 55 U/L)
- Hemoglobin <11.5 g/dL for non-African American subjects; Hemoglobin < 11.0 g/dL for African American subjects
- Weight < 32 kg
- History of liver disease, peanut allergy, deep venous thrombosis, breast cancer, endometrial cancer, or cervical cancer
- Pregnant or breast feeding
- On medications known to affect the reproductive axis within 3 months of the study (including oral contraceptive pills, metformin, and spironolactone)
- On medications known or likely to inhibit or induce CYP1A2 or CYP3A4 (please see "Restrictions on use of other drugs or treatments" section below for common examples of such drugs)
- Are currently participating in another study or have been in one in the last 30 days.

3. List any restrictions on use of other drugs or treatments.

Subjects must not take medications known to affect the reproductive axis (including oral contraceptives, metformin, and spironolactone) for 90 days prior to and during the study.

Flutamide is a substrate of CYP1A2 and 3A4 and it inhibits CYP1A2 (weak). Thus, CYP1A2 and 3A4 inducers/inhibitors may not be used for one month prior to and during the use of flutamide. [CYP1A2 inducers

(e.g., aminoglutethimide, carbamazepine, phenobarbital, and rifampin) may decrease the levels/effects of flutamide; CYP1A2 inhibitors (e.g., amiodarone, ciprofloxacin, fluvoxamine, ketoconazole, lomefloxacin, ofloxacin, and rofecoxib) may increase the levels/effects of flutamide; CYP3A4 inducers (e.g., aminoglutethimide, carbamazepine, nafcillin, nevirapine, phenobarbital, phenytoin, and rifamycins) may decrease the levels/effects of flutamide; CYP3A4 inhibitors (e.g., azole antifungals, ciprofloxacin, clarithromycin, diclofenac, doxycycline, erythromycin, imatinib, isoniazid, nefazodone, nicardipine, propofol, protease inhibitors, quinidine, and verapamil) may increase the levels/effects of flutamide; warfarin effects may be increased by use of flutamide.]

Bio-statistical Analysis-GCRC

1. What are your plans for the statistical analysis?

The primary outcome variable for the study is the slope of the percent change in LH pulses as a function of day 7 progesterone level. The comparison will be made between the participants in this study (all of whom receive flutamide) and historical controls from study 8588/JCM010. The historical controls will be the Tanner 4-5, hyperandrogenic girls who have completed 8588/JCM010. Study 8588/JCM010 involves an assessment of hypothalamic progesterone sensitivity identical to that in this study. The only difference in the studies is that girls in this study are treated with flutamide before and during the assessment of hypothalamic progesterone sensitivity while those in 8588/JCM010 are not. Based on a similar study in adults (Eagleson et al 2000), we hypothesize that the use of flutamide will normalize hypothalamic progesterone sensitivity in hyperandrogenic adolescent girls. The power calculations were based on data from 8588/JCM010 in which Tanner 4-5 hyperandrogenic girls have a slope of 1.8, while Tanner 4-5 normal controls have a slope of 9.5. The pooled SD for these two groups was 6.6. Using this data, we determined that we would need a sample size of 13 subjects in this study (to be compared with 13 historical controls from 8588/JCM010) in order to achieve a statistical significance with a power of 80% and Type 1 error rate of 0.05. . This power calculation was performed based on an expected slope difference of 7.7 between the participants in this study and the historic controls. Given the historical dropout/withdrawal rate for 8588/JCM010 of 65% and the additional demands of this study secondary to the addition of flutamide, we anticipate a dropout/withdrawal rate of around 50%. Therefore, we propose enrolling up to 26 girls with the goal of obtaining complete, analyzable data in 13.

Given the relatively small number of subjects to be studied, we anticipate the need to use non-parametric testing. Therefore, the primary comparison will be performed using the Wilcoxon Rank Sum test. A p-value of <0.05 will be considered significant.

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

The slope of the percent change in LH pulses as a function of Day 7 progesterone.

3. Do you have an adequate sample size, or is my sample size larger than necessary?

We calculated that we will need a sample size of 13 subjects in this study (to be compared with 13 historical controls from 8588/JCM010) in order to achieve a statistical significance with a power of 80% and Type 1 error rate of 0.05. Given the historical screen failure/dropout/withdrawal rate for 8588/JCM010 of 65% and the additional demands of this study secondary to the addition of flutamide, we anticipate a screen failure/dropout/withdrawal rate of around 50%. Therefore, we propose enrolling

up to 26 girls with the goal of obtaining complete, analyzable data in 13. We believe this sample size is adequate and not excessive.

Biomedical Research

1. 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 Consent and 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 consent and 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, Chem 17 including LFTs, prolactin, LH, FSH, E_1, E_2, P, total T, androstenedione, 17-OHP, DHEA-S, fasting insulin, Insulin-like Growth Factor 1 (IGF-1), glucose, SHBG, TSH, hCG, cholesterol, LDL, HDL, and a number of cytokines and adipokines, including adiponectin, leptin, resistin, PAI-1, IL-1b, IL-6, IL-8, TNFa, MCP-1, HGF and NGF).) In the rare event that a subject has an elevated follicular phase 17-OHP on screening, she will be referred to her physician for further evaluation, including cortrosyn stimulation test to rule out congenital adrenal hyperplasia. She will only be able to continue in the study if she has a documented normal 17-OHP response to cortrosyn stimulation. Potential subjects must fall within the normal range on all blood tests to be admitted to the study, except for hyperandrogenemic girls who will be expected to have some abnormal hormone levels.

Subjects will be administered 1 month of iron supplementation following screening, provided their screening labs show they are eligible to participate in the study. As soon as screening lab results are available, and subjects are found to be eligible for study participation, subjects weighing ≤ 38 kg will be given 1- 325 mg tablet a day and subjects weighing >38 kg will be given 2- 325 mg tablets a day. The first overnight admission may occur anytime within this 30 day period or after the 30 day period. Subjects will be given the option to pick up this supply of iron in the Clinical Research Unit or have it mailed to them.

Note: Subjects who have just completed IRB-HSR# 8588/ JCM010 will be allowed to consent for JCM021 8 weeks after completing JCM010 (IRB-HSR# 8588). In addition, screening laboratory results from JCM010 (ie. CBC, Chem 17 including LFTs, prolactin, LH, FSH, E_1, E_2, P, total T, androstenedione, 17-OHP, DHEA-S, fasting insulin, glucose, TSH, hCG, cholesterol, LDL and HDL) may be used as the baseline screening results for this study unless > 3 months have occurred between completion of JCM010 and enrollment in JCM021. If > 3 months have occurred between completion of JCM010 and enrollment in JCM021, then subjects will need to complete all of the screening labs for JCM021. Subjects enrolling in JCM021 8-12 weeks following completion of JCM010 will have blood drawn for a comprehensive metabolic panel and pregnancy test, and may begin flutamide administration immediately upon confirmation that the AST/ALT is within the normal range and the pregnancy test is negative.

Day Minus 14 to Minus 16: Outpatient blood draw

Blood will be drawn for a stat comprehensive metabolic panel and pregnancy test. The results from

these tests should be back within 1 hour. In order to continue with the study, the AST/ALT must be within the normal range and the pregnancy test must be negative. The subject can obtain her study drug as soon as the normal results are confirmed. She can either wait for the results and pick up the flutamide during the same visit, or she can return within the next 2 days to pick up the medication.

Day Minus 14: Start Flutamide

The subject will begin taking flutamide. Subjects weighing ≥ 50 kg will receive 250 mg orally twice a day, and subjects weighing < 50 kg will receive 125 mg orally twice a day.

Day Minus 7: Outpatient blood draw

Blood will be drawn to check AST and ALT levels, as well as a serum pregnancy test.

Day 0: First inpatient admission

The subject will be admitted to the CRU, alternate UVA hospital unit, or hotel at 1700 hr and an IV will be placed in a forearm vein. 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 small amount of topical lidocaine and prilocaine cream (EMLA cream) may be applied to facilitate IV line placement. Blood will initially be drawn for hemoglobin, βHCG, and AST/ALT. In order to continue with the admission, girls must have a hemoglobin of ≥ 11.5 g/dL for non-African American subjects or a hemoglobin of ≥ 11.0 g/dL for African American subjects, negative βHCG, and normal AST/ALT. If the overnight portion of the study is to be done at a hotel, the hemoglobin, βHCG, and AST/ALT check will be done at the CRU 1-5 days prior to the overnight admission. Alternatively, subjects may come to the CRU early on the day of their admission before their scheduled admission time. The study team will be responsible for scheduling this with the subject. Frequent 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 5 mL sample at 6 AM will be analyzed for fasting insulin, fasting glucose, lipids, estrone, SHBG, DHEA-S, androstenedione and a number of cytokines and adipokines, including adiponectin, leptin, resistin, PAI-1, IL-1b, IL-6, IL-8, TNFa, MCP-1, HGF and NGF. A formal "lights out" will occur at 2300 hr so that we may observe any nocturnal changes in hormonal secretion patterns. 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 the overnight admission. Q10 minute blood sampling will end at 0600 hr. There will be one final blood draw at 0700 hr. The subject will be offered meals at standard CRU meal times.

Day 1: Start Estradiol and Progesterone

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 0700, 1500, and 2300 hr for seven days. If the 1500 dose is incompatible with school schedules, alternative dosing schedules can be arranged. Generally this will entail taking the afternoon dose immediately upon returning home from school without any change in the morning and evening dosing times. Dosages will be based on weight. The target mean plasma progesterone concentration is 2-8 ng/dL. Each subject will be

instructed to eat a small snack with the progesterone syrup. It has been observed that the absorption of progesterone is influenced by the presence or absence of food. Subjects will also be given oral iron supplementation at a dose of 1-2 mg/kg. Subjects weighing ≤ 38 kg will be given 325 mg of ferrous gluconate orally once a day and subjects weighing >38 kg will be given 325 mg of ferrous gluconate orally twice a day to be taken until one month after study completion. Subjects will continue to take flutamide (250 mg PO BID for subjects ≥ 50 kg or 125 mg PO BID for subjects < 50 kg).

Days 3, 5: Outpatient blood draws

On study days 3 and 5, subjects will have blood drawn at 1700 hr (two hours after the 1500 hr progesterone dose) to check serum estrogen and peak progesterone levels.

Day 7: Second inpatient admission

The second inpatient admission will begin on day 8. The procedure will be identical to the first inpatient admission. As with the first admission, blood will initially be drawn for hemoglobin, βHCG, and AST/ALT. In order to continue with the admission, girls must have a hemoglobin of ≥ 11.5 g/dL for non-African American subjects or a hemoglobin of ≥ 11.0 g/dL for African American subjects, negative βHCG, and normal AST/ALT. Subjects will discontinue flutamide, estrace, and progesterone after the completion of the second inpatient admission. They will continue to take iron supplements for one month after study completion.

Day 21: Outpatient blood draw.

Subjects will return 14 days after hormonal study drugs have been discontinued for an outpatient blood draw for AST/ALT and βHCG.

Follow-up

There is an optional 1 year follow up period. If the subject agrees to participate, she will complete follow-up questionnaires and/or phone interviews 3, 6 and 12 months after the study. These questionnaires/interviews will ask about the onset and frequency of menses and changes in hirsutism. Whenever possible we will also see the patients on 2-3 occasions during the 12 months to obtain blood for T, DHEA-S, E_2, and P measurements (depending on cycle stage).

The PI will inform the subjects verbally of all incidental findings that are of clinical significance or are of questionable significance**.** A follow-up letter describing the finding should be provided to the subject with instructions to either show the letter to their PC or if the subject has no PCP, the subject should be instructed to make an appointment at UVa or at the Free Clinic

2. Will any of the NON-RADIOLOGIC treatments/ procedures be done for research purposes only? Yes.►**IF YES, explain:** All procedures performed in this protocol are being done solely to answer a research question and generate generalizable knowledge.

3. Will any RADIOLOGIC treatments/examinations be performed for research purposes only? No.

4. **Will you be using viable embryos?** No.

5. **Will you be using embryonic stem cells?** No.

Family History/Pedigree

1. What kind of information is being sought?

Family history (No questionnaire is being used)

2. What identifiers will be recorded with the info (e.g. names, initials, relationship such as mother, father, brother, sister, random number)?

Relationship

3. Does any of the information sought potentially expose the subject or a family member to additional risk? No.

Specimens: Not to be Used for Genetic Research or Banking

If the specimens in this protocol will only be used for Genetic Research and or Specimen Banking, you may delete the Specimen Labeling and Specimen Shipping sections below.
If specimens will be taken for other reasons, the questions below should be answered and referenced to the samples taken for things other than Genetic Research and or Specimen Banking.

Specimen Information

1. Describe the type of specimen to be used:

Blood

2. Will the specimen be obtained BEFORE a subject has signed a consent form? No.

3. Will you be using discarded specimens? No.

Specimen Labeling

1. What information/ HIPAA identifiers will be on the specimen label when it is given to the study team (from clinical labs or other source outside the study team) and/or what information will you put on the specimen?

Name, medical record #, GCRC protocol #, time (date and clock hour) drawn.

2. If the specimen is given to the study team with information on the label will you delete any of the information on the specimen label? No.

3. Will any additional data be linked to the specimen by way of a code? No.

4. Will the analysis on the specimen be done soon (within 24 hours) after it is collected?

Yes and No. See the explanation below.

Samples from the screening exam that are analyzed by UVA clinical labs will be run within 24 hrs.

►**IF NO, where will the specimen be stored until analysis is done?**

If at UVa will the specimen be stored in a refrigerator/freezer in a lab, a room-provide room number, or specific location).
To protect confidentiality, whenever possible, you should consider using a central facility/repository such as the UVa Biorepository and Tissue Research Facility.

Some samples from the screening exam (TSH, LH, FSH, P, Testosterone, E_2 and insulin) will be analyzed and stored by the Research Assay and Analysis core lab
All samples from the inpatient admissions will be analyzed and stored in the Center for Research in Reproduction Ligand Core lab.

Specimen Shipping

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

Data and Safety Monitoring Plan

If you have any questions completing this section call 982-4311, 924-8660 or 243-9847 for assistance
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?

__X___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.

1.2 How will you define serious adverse events?

__X___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.

1.3 What is the definition of an unanticipated problem?

Do not change this answer

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 studies
- 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 What is the definition of a protocol violation?

Do not change this answer

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

Additional Information: see the IRB-HSR website at http://www.virginia.edu/vpr/irb/HSR_docs/Forms/Protocol_Violations_%20Enrollment_Exceptions_Instructions.doc

1.5 If pregnancy occurs how will this information be managed?

___X__ Adverse Event- will follow adverse event recording and reporting procedures outlined in section 3.

1.6 What is the definition of a Protocol Enrollment Exception?

__X___NA- No outside sponsor

1.7 What is the definition of a data breach?

Do not change this answer

A data breach is defined in the HITECH Act (43 USC 17932) as an unauthorized acquisition, access, or use of protected health information (PHI) that compromises the security or privacy of such information.

Additional Information may be found on the IRB-HSR Website: Data Breach

2. Identified risks and plans to minimize risk

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

Expected Risks related to study participation.	**Frequency**
• Significant anemia related to frequent blood sampling (hematocrit < 30%)	____Occurs frequently ____Occurs infrequently

	____Occurs rarely __X__Frequency unknown
• Mild anemia (hematocrit < 36%) related to frequent blood sampling	____Occurs frequently ____Occurs infrequently ____Occurs rarely __X__Frequency unknown
Expected Risks related to study participation- micronized progesterone	
• GI upset (nausea, abdominal bloating, diarrhea)	____Occurs frequently ____Occurs infrequently __X_Occurs rarely ____Frequency unknown
• CNS effects (sleepiness, headache, dizziness, fatigue, emotional lability, irritability)	____Occurs frequently ____Occurs infrequently __X_Occurs rarely ____Frequency unknown
Risk associated with estradiol:	
• GI upset (nausea, abdominal bloating, diarrhea)	____Occurs frequently __X_Occurs infrequently __ _Occurs rarely ____Frequency unknown
• Breast Tenderness	____Occurs frequently __X Occurs infrequently __ _Occurs rarely ____Frequency unknown
• Deep vein thrombosis	____Occurs frequently ____Occurs infrequently __ _Occurs rarely __X_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)
Risks associated with discontinuation of estrogen and progesterone.	Some subjects may observe withdrawal menstrual bleeding after discontinuation of estrogen and progesterone.
Risk associated with flutamide:	
• In pregnant women, abnormal development of external genitalia in a male fetus	☐Occurs frequently, ☐Occurs infrequently ☐Occurs rarely ☒Frequency unknown
• Gastrointestinal side effects (nausea, vomiting, upset stomach, decreased appetite, diarrhea)	☐Occurs frequently, ☒Occurs infrequently ☐Occurs rarely ☐Frequency unknown
• Mild elevation of liver transaminases	☐Occurs frequently, ☒Occurs infrequently

	☐ Occurs rarely ☐ Frequency unknown
• Serious hepatotoxicity (significant hepatitis, hepatic failure)	☐ Occurs frequently, ☐ Occurs infrequently ☒ Occurs rarely ☐ Frequency unknown
Violation of subject's privacy and confidentiality	Minimized due to the requirements of the privacy plan in this protocol
Expected Risks related to study participation- IV placement	
• Infection at needle site	☐ Occurs frequently, ☐ Occurs infrequently ☐ Occurs rarely ☒ Frequency unknown, but likely to be very rare
• Bleeding at needle site	☐ Occurs frequently, ☐ Occurs infrequently ☒ Occurs rarely ☐ Frequency unknown
• Blood clot at needle site	☐ Occurs frequently, ☐ Occurs infrequently ☒ Occurs rarely ☐ Frequency unknown
• Pain at needle site	☒ Occurs frequently, ☐ Occurs infrequently ☐ Occurs rarely ☐ Frequency unknown
• Bruise at needle site	☒ Occurs frequently, ☐ Occurs infrequently ☐ Occurs rarely ☐ Frequency unknown
Expected Risks related to study participation- iron supplementation	
• nausea	☐ Occurs frequently, ☒ Occurs infrequently ☐ Occurs rarely ☐ Frequency unknown
• constipation	☐ Occurs frequently, ☒ Occurs infrequently ☐ Occurs rarely ☐ Frequency unknown
• dark or black stools	☒ Occurs frequently, ☐ Occurs infrequently ☐ Occurs rarely ☐ Frequency unknown
•	
Reproductive Risks *Specify potential reproductive risks*	Minimized due to the requirements of this protocol.
Violation of subject's privacy and confidentiality	Minimized due to the requirements of the privacy plan in this protocol

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: The risk of not taking hormonal medications is pregnancy.

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 studies, all participants will be required to have a normal hemoglobin (≥ 11.5 g/dl for non-African American subjects, and ≥ 11.0 for African American subjects). Hemoglobin levels will be measured prior to every admission; if the hemoglobin level is <11.5 g/dl for non-African American subjects of < 11.0 g/dl for African American subjects, the study will be discontinued.
- Blood loss will be carefully recorded and limited to a maximum of 7cc/kg (10% of estimated total blood volume) in 8 weeks. As a total of < 225 ml of blood will be drawn during the study (including estimated waste from frequent blood draws), girls weighing less than 32 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.
- In order to participate, subjects will be required to have normal transaminases (AST/ALT). Transaminase levels will be checked every week while the subject is on flutamide. If the transaminase levels rise above normal range (AST > 35 U/L or ALT > 55 U/L), the flutamide will immediately be discontinued and liver function tests will be monitored at least weekly until the levels normalize. Should the abnormalities persist or escalate, the subject will be referred to a hepatologist.
- The subjects will be advised to discontinue flutamide and immediately contact the study coordinator should any symptoms of liver dysfunction develop (right upper quadrant pain, anorexia, unexplained flu-like symptoms, jaundice, dark urine, pruritis).
- We will warn participants against becoming pregnant during the study. We will require that sexually active subjects use effective double barrier methods of birth control as needed during the study. β-hCG levels will be measured at screening, immediately prior to initiation of study drug, and weekly while the subject is on the hormonal study drugs; if the β-hCG is positive, the study will be discontinued.

2.3 Under what criteria would an INDIVIDUAL SUBJECT'S study treatment or study participation be stopped or modified

___X__At subject, PI or sponsor's request

- Transaminase levels will be monitored weekly while the subject is on flutamide. The flutamide will be stopped immediately if the subject develops elevated transaminases (AST > 35 U/L or ALT >55 U/L) or symptoms concerning for liver dysfunction.

- Hemoglobin levels will be measured prior to every admission; if the hemoglobin level is <11.5 for non-African American subjects or < 11.0 g/dl for African American subjects, the study will be discontinued.
- β-hCG levels will be measured at screening, immediately prior to initiation of study drug, and weekly while the subject is on the hormonal study drugs; if the β-hCG is positive, the study will be discontinued.

2.4 Under what criteria would THE ENTIRE STUDY need to be stopped.

__X___Per IRB, PI, DSMB, or sponsor discretion

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 What are the criteria for breaking the blind/mask?

_X____NA – Not blinded/masked

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

__X___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.

3.2 How will adverse event data be collected/recorded?

_____Paper AE forms/source documents

__X___Spreadsheet *(paper or electronic*)

_____Database *specify name/type of database(s)*:

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

_____NCI CTCAE Version 4.0

__X___Mild/Moderate/Severe

__X__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.*

__X___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?

___X__After subject signs consent

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

__X___Other: ~ 2 weeks post study drug intervention, at time of last blood draw.

3.7 How will Adverse Events, Unanticipated Problems, Protocol Violations and Data Breaches be reported? Complete the table below to answer this question

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 participation (Note: An internal event is one that occurs in a subject enrolled in a UVa protocol.)	IRB-HSR	Within 24 hours	IRB Online and phone call 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. *Timeline includes submission of signed hardcopy of AE form.*	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	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)
Protocol Violations (*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* *Go to 3rd bullet from the bottom.*
Data Breach	The UVa Corporate Compliance and Privacy Office, a ITC: if breach involves electronic data- UVa Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 **ITC:** Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Phone- (434) 924-7166

OUTSIDE SPONSOR			
All Serious adverse events	GCRC	Within 7 calendar days from the time the study team received knowledge of the event.	We will send an email, fax, or letter to the GCRC.
External, Serious and Unexpected adverse event resulting in change to the protocol or consent..	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form*	IRB Online www.irb.virginia.edu/

UVa PI HELD IND/IDE			
Life-threatening and/or fatal unexpected events related or	FDA	Within 7 calendar days of the study	Form FDA 3500A (MedWatch) or narrative

possibly related to the use of the investigational agent.		team learning of the event	
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

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

__X___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 ?

e.g. Who is looking at data in aggregate form to identify trends?
Check all that apply

___X__No additional oversight body other than PI at UVa (*skip question 5.2*)

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

5.3. What items will be included in the aggregate review conducted by the PI?

___X__All adverse events

_X____Unanticipated Problems

__X___Protocol violations

___X__Audit results

_____Application of dose finding escalation/de-escalation rules *These should be outlined under 2.4.*

___X__Application of study designed stopping/decision rules

___X__Early withdrawals

___X__Whether the study accrual pattern warrants continuation/action

___X__Endpoint data

5.4 **How often will aggregate review occur?**
For additional information on aggregate review see:
www.virginia.edu/vpr/irb/hsr/continuations.html#aggreview

__X___Annually

5.5. How often will a report, regarding the outcome of the review by the DSMB/DSMC, be sent to the UVa PI?

A copy of these reports must be sent to the IRB and GCRC if applicable as soon as they are received by the PI. Do not wait until the next continuation to submit them to the IRB.

__X___ NA- there is no DSMB/ DSMC overseeing this study

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

___X__Part of IRB-HSR continuation status form

Payment

1. Are subjects being reimbursed for travel expenses (*receipts /mileage required***)?** No.

2. Are subjects compensated for being in this study? Yes.

2a. What is the maximum TOTAL compensation to be given over the duration of the protocol?
$300

2b. Explain compensation to be given.
All participants will receive a $75 Simon Mall American Express gift card per inpatient admission. In addition, participants will receive a $150 bonus, also in the form of a gift card, for completing the medication regimen and outpatient blood draws. Therefore, a participant who completes the entire study will receive a total of $300 in gift cards. If a subject completes only one inpatient admission and withdraws from the study, she will be given a $75 gift card. If the subject is removed from the study by the investigators because of safety issues (i.e. elevated transaminases while on flutamide), she will receive $150 compensation in the form of gift cards despite not finishing the study in addition to compensation for any completed admissions.

2c. Is payment pro-rated (*e.g. some compensation is given even if subjects do not complete the entire study)?*

Yes. Please see above explanation.

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

3a. How will the researcher compensate the subjects?
__X___ Gift card

3b. Which category/ categories best describes the process of compensation?

__X___ Compensation will include an alternative method (petty cash, gift card, other) and tax information will be collected.

►If an alternate method will be used justify why you are unable to issue checks through the UVa Oracle or state system.

Compensation is being made in the form of a gift card that is accepted by most merchants. The volunteers for this study are adolescent girls and this population is most easily recruited when their compensation is tangible and available at the time of the overnight admission.

Risk/ Benefit Analysis

1. 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?

The goal of this study is to advance our knowledge of the effects of adolescent hyperandrogenemia and the mechanisms of development of polycystic ovary syndrome. Hopefully, this knowledge will lead to more effective means of preventing and treating PCOS in the future.

While treatment is not the goal of this study, it is possible that individual subjects may experience modest, transient improvement in symptoms of hyperandrogenemia as a result of androgen blockade with flutamide.

2. Analyze the risk-benefit ratio.

This study involves moderate risks and may provide valuable information about the development of polycystic ovary syndrome (PCOS). There are no long-term risks associated with blood sampling or the short term administration of physiologic doses of estradiol and progesterone. The risk of liver dysfunction with flutamide is very low, and serious complications should be avoided with close monitoring of transaminases and rapid cessation of flutamide should abnormalities develop. Although there are not direct benefits to the individual study participants, there are significant potential benefits to society as a whole and girls with hyperandrogenemia in particular. We hope that a better understanding of the effects of hyperandrogenemia in adolescent girls and the role of adolescent hyperandrogenemia in the development of PCOS will lead to improved prevention of the disorder as well as more effective treatments. Therefore, potential benefits are great while risks are small.

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APPENDIX: Sponsor

Sponsor Information

This study will be supported by NIH funds through the General Clinical Research Center Core Grant (435) and through a U54 Specialized Cooperative Centers Program for Research in Reproduction and Infertility (IRB# 14191).

APPENDIX: Legal/Regulatory

Recruitment

The following procedures will be followed:

- *Finders fees will not be paid to an individual as they are not allowed by UVa Policy*
- *All recruitment materials will be approved by the IRB-HSR prior to use. The advertisements will be submitted to the IRB after the protocol has been approved.*
- *Only those individuals listed as personnel on this protocol will recruit and or conduct the consenting process with potential subjects.*

Clinical Privileges

The following procedures will be followed:

- *Investigators who are members of the clinical staff at the University of Virginia Medical Center must have been granted clinical privileges to perform specific clinical privileges whether those procedures are experimental or standard.*
- *The IRB cannot grant clinical privileges.*
- *Performing procedures which are outside the scope of the clinical privileges that have been granted may result in denial of insurance coverage should claims of negligence or malpractice arise.*
- *Personnel on this protocol will have the appropriate clinical privileges in place before performing any procedures required by this protocol.*
- *Contact the Clinical Staff Office- 924-5871 for further information.*

Sharing of Data/Specimens

Data and specimens collected under an IRB approved protocol are the property of the University of Virginia. You must have "permission" to share data/ specimens outside of UVa other than for a grant application and or publication. This "permission" may come in the form of a contract with the sponsor or a material transfer agreement (MTA) with others. A contract/ MTA is needed to share the data outside of UVa even if the data includes no HIPAA identifiers and no code that could link the data back to a HIPAA identifier.

- *No data will be shared outside of UVa, beyond using data for a grant application and or publication, without a signed contract/MTA approved by the SOM Grants and Contracts office/ OSP or written confirmation that one is not needed.*
- *No specimens will be shared outside of UVa without a signed contract/MTA approved by the SOM Grants and Contracts office/ OSP or written confirmation that one is not needed.*

APPENDIX: Recruitment and Consenting

1. **How do you plan to identify potential subjects**

____Chart Review/ Clinic Schedule Review/ Database Review such as CDR, pharmacy lists to create a list of potential subjects and obtain contact information for the purpose of recruitment via letter, phone call, or direct email contact. **Study team requests Waiver of HIPAA Authorization for Recruitment**

____Potential subject's treating physician supplies the study team with a referral for the study and the study team then makes a contact via letter, phone or direct email. **Study team requests Waiver of HIPAA Authorization for Recruitment**

____Chart Review/ Clinic Schedule Review/ Database Review such as CDR, pharmacy lists to create a list of potential subjects.
The list will only be used to perform a review of medical records. Subjects will not be contacted in any way.

____Chart Review/ Clinic Schedule Review/Referrals from health care providers, Database Review such as CDR, pharmacy lists to create a list of potential subjects and meet with them during a clinic/hospital visit. Subjects will not be contacted prior to their UVa visit.

_X___Potential subject is approached by the treating physician/ health care provider and given information about the study and provides the potential subject the information necessary to contact the study team if they are interested.

_X___Potential subjects will not be directly identified. They will respond to an advertisement such as a flyer, brochure etc. *If this is checked #3 below should be checked INDIRECT CONTACT.*

2. **How will potential subjects be recruited?**

____Direct contact of potential subjects by the study team via letter, phone, direct e-mail.

__X__Potential subjects will be approached while at UVa Health System

__X__ Indirect contact-see below

3. How will the consenting process take place?

Hyperandrogenic subjects who have completed study 8588/JCM010 within the past year will be contacted by letter. The letter will explain that we are conducting a new study similar to the one in which they previously participated. It will also briefly explain the study and the theory behind assessing hypothalamic progesterone sensitivity after treatment with flutamide. Potential subjects will be given the research coordinator's contact information and will be asked contact us if they are interested in the study.

Consenting Process:

An outpatient consenting and screening visit is scheduled for volunteers who express interest in thestudy
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 consent and screening visit is held in an outpatient examination room in the CRU. This allows a private conversation between the screening physician and/or study coordinator, the potential participant, and at least one of her parents if the participant is < 18 years of age (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 and/or study coordinator 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, physical, screening blood tests) occurs immediately thereafter. Participants generally begin the main part of the study within 1-2 months of the screening visit.

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

Subjects will be asked to fast (nothing to eat or drink except water after midnight) prior to the screening visit. Subject will be advised that only individuals who have taken no hormonally-active medications for 3 months prior to the study will be eligible for participation. Subject will be advised to consult her personal physician prior to stopping any medications.

** Request waiver of documentation of consent for minimal risk screening procedures**

5. Do you need to perform a "dry run" of any procedure outlined in this protocol?

APPENDIX: Participation of Children

In the state of Virginia a person under the age of 18 is considered a child.

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

Initial abnormalities of hypothalamic-pituitary-ovarian function appear to manifest during puberty in adolescents with hyperandrogenemia, who are at high risk of developing adult PCOS. Thus, we believe that the pathophysiologic mechanisms leading to abnormal hypothalamic-pituitary-ovarian function in women with PCOS have their genesis during the pubertal transition. Women with PCOS have decreased GnRH pulse generator sensitivity to suppression by estradiol and progesterone. Increased androgen levels appear to mediate this decrease in sensitivity, as sensitivity can be restored with the androgen receptor blocker flutamide. Adolescent hyperandrogenemia is thought to be a precursor of PCOS, and a subset of adolescent girls with hyperandrogenemia have decreased hypothalamic sensitivity to progesterone similar to their adult counterparts with PCOS. We propose to study whether flutamide can restore hypothalamic progesterone sensitivity in these girls by blocking the effects of androgens. Therefore, the proposed studies will include children (defined as subjects < age 18 y).

The PI and co-mentors have experience conducting clinical research studies in children (all) and in peripubertal girls with and without hyperandrogenemia (Drs. Solorzano, Marshall, McCartney). The proposed research will involve close collaboration with other colleagues in the Division of Pediatric Endocrinology. These individuals are specialists in pediatric endocrinology and metabolism, and are highly experienced in the performance of clinical research in children and adolescents. All studies will be performed in the CRU at the University of Virginia or with CRU approved staff, which has facilities appropriate to children and adolescents. This facility has nursing and support staff with expertise in working with pediatric subjects, and they have facilitated prior pediatric endocrinology protocols with our group and others. In particular, the CRU is well equipped for frequent sampling protocols and sleep assessments in pediatric subjects, and alternate locations such other UVA hospital units or off-site hotel space will also be equipped for such studies. The proposed studies involving adolescents were specifically powered to make meaningful analyses of this group.

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

The knowledge being sought is not currently available in the literature or 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 children. If there is no adult data available, 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.

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 (i.e., Progesterone USP, micronized, for prescription compounding [NDC 39822-6000-3] Mfg: Spectrum Chemical Manufacturing Corporation) is FDA approved. We have used this progesterone suspension in other protocols, and we have thus far administered the progesterone suspension to 25 adolescent girls and at least 12 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. Although Estrace is not often prescribed to adolescent girls, there is significant experience with estrogens in this 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.

There is much experience with flutamide as a treatment for men with prostate cancer; it is FDA-approved for this use. It is unknown how many men have received flutamide, but based on an aforementioned report (i.e., Wysowski DK and Fourcroy JL, Flutamide hepatotoxicity. J Urol 1996; 155: 209), approximately 150,000 men received flutamide from 1989 to 1994. Many more men have received this drug since 1994. In men with prostate cancer, flutamide is generally used at a dose of 250 mg three times a day, and its safety profile at this dosage is well described. In men, it may cause gynecomastia, hot flashes, breast tenderness, galactorrhea, impotence, and decreased libido; these side-effects in men are not uncommon, and they are expected based on flutamide's mechanism of action as an androgen-receptor antagonist. Gastrointestinal side effects (nausea, vomiting) occur in approximately 10%. Mild, transient increases in AST and LDH may occur also. Less common (< 10%) associations in men with prostate cancer include hypertension, edema, drowsiness, confusion, depression, anxiety, nervousness, headache, dizziness, insomnia, pruritus, ecchymosis, photosensitivity, herpes zoster, anorexia, increased appetite, constipation, indigestion, upset stomach (4% to 6%), diarrhea, anemia (6%), leukopenia (3%), thrombocytopenia (1%), and weakness (1%). Rare side effects or associations in men with prostate cancer (limited to important or life-threatening) include hepatic failure, hepatitis, jaundice, malignant breast neoplasm (male), myocardial infarction, pulmonary embolism, sulfhemoglobinemia, thrombophlebitis, and yellow discoloration of the urine.

When flutamide is used in men with prostate cancer at a dose of 250 mg three times a day (our protocol's dose is 126-250 mg twice daily), hepatotoxicity occurs in approximately 3 per 10,000 patients, and deaths from severe hepatotoxicity have been reported in this context. The above estimate is based on a review of case series submitted to the MedWatch Spontaneous Reporting System of the FDA between February 1989 and December 1994, during which time the FDA received reports of 20 deaths and 26 hospitalizations for hepatotoxicity due to flutamide (Wysowski DK, Fourcroy JL. Flutamide hepatotoxicity. J Urol 1996; 155: 209-12).

Flutamide is not FDA-approved for use in women, but it has been studied and used (off-label) in women with hirsutism, polycystic ovary syndrome, acne, congenital adrenal hyperplasia, and various cancers, occasionally in the U.S., but more so in other countries. Published research reports describe flutamide use in over 1000 women. However, substantially more women have used flutamide in the clinical setting, although the numbers involved are unknown. However, flutamide is the only currently available androgen-receptor blocker that is relatively free of other actions (e.g., the effects of spironolactone on the kidney) and that has a short half-life (approximately 6 hours).

We are aware of 4 case reports of severe hepatotoxicity in females taking flutamide, including one fatal case. Serious hepatotoxicity has been described in a 41-year-old woman taking flutamide for 3 months (Kackar and Desai 2003); in a 14-year-old girl taking flutamide for hirsutism (Andrade et al 1999); and a 20-year-old woman taking flutamide for hirsutism (Wallace et al 1993). One fatal case of flutamide hepatotoxicity has been reported in an 18-year-old women treated with flutamide (250-375 mg per day) for 5 months for acne and hirsutism (Osculati and Castiglioni 2006). Importantly, in the one fatal case of hepatotoxicity, there was unfortunately no monitoring of LFTs either before or during the 5 months of flutamide treatment.

We are cognizant that the administration of an anti-androgen during the early stages of fetal development following conception might possibly (i.e., in theory) be associated with abnormal development of genitalia in a male fetus. Thus, in this proposed study, we are careful to exclude early pregnancy before and during flutamide use. Additionally, all women will have agreed to avoid conception either by the use of abstinence or barrier-means of contraception.

Flutamide has been given to hyperandrogenic adolescent girls in several European studies. De Leo and colleagues treated 8 hyperandrogenic girls between the ages of 16-19 years old with 250 mg flutamide twice a day for 6 months (DeLeo et al 1998). The only adverse effect that they reported was a "slight, not clinically significant increase in serum transaminases and lipid profile." The Ibanez group in Spain has more extensive experience with flutamide in hyperandrogenic girls, although they have given lower dose flutamide than proposed in this study (62.5-250 mg/day vs. 250 mg twice a day). They have treated 190 hyperandrogenic girls with low dose flutamide for 3-54 months (mean duration of flutamide 19 months), and have not seen any evidence of hepatotoxicity (Ibanez et al 2005).

We have used flutamide in a number of our earlier clinical studies at UVAHS. We have administered flutamide to at least 27 women with PCOS and at least 13 normal controls. A few subjects had minimally elevated transaminases during study; although the causes of these mild (or borderline) test abnormalities were not clearly established, the transaminase levels invariably normalized within 1-2 weeks. We have encountered no serious adverse events related to flutamide. In the 4 girls studied to date on this protocol, there have been no transaminase abnormalities after administration of flutamide.

4. **Is the potential subject population likely to include wards of the state or children who are more at risk for becoming a ward of the state? yes**

   4a. Is the research is this protocol related to the childs' status as a ward of the state? No.

   4b. Is the research to be conducted in schools, camps, hospitals, institutions, or similar settings in which the majority of children involved as subjects are not wards? Yes

   4c. Are you aware of the following requirement?
   If the consent form contains a signature line for both parents the study team will notify the IRB immediately, if at any time during the course of the research, it becomes known that a potential subject is a ward of the state or that a child already enrolled in this protocol becomes a ward of the state. Yes**.**

APPENDIX: Pharmacy-Investigational Drugs/Biologics

1. **What is the name of the investigational drug/biologic?**
   Micronized progesterone suspension #64125 (John Marshal IND holder)

2. **Where will the subjects be seen for the administration/dispensing of the drug?**
   __X___ Inpatient Unit: *specify:* Beginning on the day of discharge from the first inpatient admission, subjects will be given the prescription for oral progesterone suspension to be taken three times a day at 0700, 1500, and 2300 hours for seven days.

3. **What dose will be utilized in this study?**
   20mg/ml, 25-100 mg

4. **What will be the frequency of dosing in this study?**

Three times a day at 0700, 1500, and 2300 hours.

5. What will be the duration of dosing in this study?
7 days

6. What route of administration will be utilized?
PO

7. Will drug need to be prepared by the UVa Investigational Drug Service (IDS)?
__X___ YES
_____ NO- Drug will be prepared and/or administered per package insert

►*IF YES, complete the following information under 7a-7d.*
If you need assistance completing this section contact the Investigational Pharmacists at 982-1048

7a. Concentration
__X___ Standard
_____ Non- Standard- *specify*

7b. Diluents
__X___ Standard
_____ Non- Standard- *specify*

7c. Stability after prepared
_X____ Standard
_____ Non- Standard- *specify*

7d. Special storage requirements
__X___ Standard
_____ Non- Standard- *specify*

8. Are there any special handling instructions mandated by the study (e.g. weighing hazardous materials)? No.

9. Does the protocol provide provisions for dose titration, dose reductions, and or re-challenged (if drug is stopped), etc.? No.

10. How will missed doses be handled?
The subject should take the missed dose when she remembers it unless she is due for the next dose. If the next dose is due, it should be taken and the missed dose should be skipped.

11. Will a comparator (active or placebo) be utilized in the protocol? No.

12. Does this study involve research on a drug, biologic, supplement or food additive? No.

►IF YES, is this study investigator initiated?

If no, answer question # 13 only.

13 Are you using a drug/supplement/ food additive in a manner not approved by the FDA?
Yes.

IF YES, answer questions 13a-13f
You may reference the non-IRB protocol to answer these questions.

13a. Describe pertinent animal data that is available regarding the toxicity/safety of this drug.
Not applicable, as abundant human data is available.

13b. 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 (i.e., Progesterone USP, micronized, for prescription compounding [NDC 39822-6000-3] Mfg: Spectrum Chemical Manufacturing Corporation) is FDA approved. We have used this progesterone suspension in other protocols, and we have thus far administered the progesterone suspension to 25 adolescent girls and at least 12 adult women; no adverse events have occurred.

13c. Have there been any human deaths associated with this drug?
To our knowledge, there have been no human deaths associated with micronized progesterone.

13d. In how many humans has this drug been used previously?
We have used this progesterone suspension in other protocols, and we have thus far administered the progesterone suspension to 25 adolescent girls and at least 12 adult women; no adverse events have occurred.

13e. If this protocol will be used in children describe any previous use of this drug with children of a similar age range.
The oral micronized progesterone suspension formulated by the University of Virginia Investigational Drug Pharmacy has been given to over 25 adolescent girls as part of our protocols. There have been no adverse effects.

14. Do the following criteria apply? *Check all that apply*

_____ 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;

_____ 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;

_____ 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 Not checked- explain why you believe the risk to subjects is not increased:

The oral micronized progesterone suspension formulated by the University of Virginia Investigational Drug Pharmacy has been given to over 25 adolescent girls as part of our protocols. There have been no adverse effects.

_X___ 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 *This item must be checked.*

_X___ The investigation will be conducted in compliance with the requirements of 21CFR312.7 (Promotion and charging for investigational drugs)
This item must be checked.

15. Is this a post-marketing study? No.

►IF YES is the study required to be done by the FDA?

APPENDIX: Pharmacy-Investigational Drugs/Biologics

1. What is the name of the investigational drug/biologic?
Estrace #64125 (John Marshal IND holder)

2. Where will the subjects be seen for the administration/dispensing of the drug?
__X___ Inpatient Unit: *specify:* Beginning on the day of discharge from the first inpatient admission, subjects will be given the prescription for oral estrogen to be taken once a day for 7 days.

3. What dose will be utilized in this study?
0.5-1 mg once a day

4. What will be the frequency of dosing in this study?
Daily

5. What will be the duration of dosing in this study?
7 days

6. What route of administration will be utilized?
PO

7. Will drug need to be prepared by the UVa Investigational Drug Service (IDS)?

___X__ YES
_____ NO- Drug will be prepared and/or administered per package insert

►*IF YES, complete the following information under 7a-7d.*
If you need assistance completing this section contact the Investigational Pharmacists at 982-1048

7a. Concentration
__X___ Standard
_____ Non- Standard- *specify*

7b. Diluents
___X__ Standard
_____ Non- Standard- *specify*

7c. Stability after prepared
__X___ Standard
_____ Non- Standard- *specify*

7d. Special storage requirements
__X___ Standard
_____ Non- Standard- *specify*

8. Are there any special handling instructions mandated by the study (e.g. weighing hazardous materials)? No.

9. Does the protocol provide provisions for dose titration, dose reductions, and or re-challenged (if drug is stopped), etc.? No.

10. How will missed doses be handled?
The subject should take a dose as soon as she remembers, unless it has been a full 24 hours, at which point the missed dose should be skipped and the regularly scheduled daily dose should be taken.

11. Will a comparator (active or placebo) be utilized in the protocol? No.

12. Does this study involve research on a drug, biologic, supplement or food additive? No.

►**IF YES, is this study investigator initiated?**
If yes, answer questions # 13 and 14

13 Are you using a drug/supplement/ food additive in a manner not approved by the FDA?
Yes.

IF YES, answer questions 13a-13f
You may reference the non-IRB protocol to answer these questions.

13a. Describe pertinent animal data that is available regarding the toxicity/safety of this drug.

Not applicable, as abundant human data is available.

13b. Describe pertinent human data that is available regarding the toxicity/safety of this drug.

Estrace has used extensively in women and has a well described safety profile. It is FDA approved for use in adults.

13c. Have there been any human deaths associated with this drug?

To our knowledge, there have been no human deaths associated with estrace.

13d. In how many humans has this drug been used previously?

Oral estrogens, in various formulations, have been used to treat millions of women, although exact numbers are unknown.

13e. If this protocol will be used in children describe any previous use of this drug with children of a similar age range.

Although Estrace is not often prescribed to adolescent girls, there is significant experience with estrogens in this 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.

14. Do the following criteria apply? *Check all that apply*

_____ 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;

_____ 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;

_____ 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 Not checked- explain why you believe the risk to subjects is not increased:

Although Estrace is not often prescribed to adolescent girls, there is significant experience with estrogens in this 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.

__X__ 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 *This item must be checked.*

__**X**__ The investigation will be conducted in compliance with the requirements of 21CFR312.7 (Promotion and charging for investigational drugs)
This item must be checked.

15. Is this a post-marketing study? No.

►IF YES is the study required to be done by the FDA?

APPENDIX: Pharmacy-Investigational Drugs/Biologics

1. What is the name of the investigational drug/biologic?
Flutamide #64125 (John Marshal IND holder)

2. Where will the subjects be seen for the administration/dispensing of the drug?
_____ Inpatient Unit: *specify:*_____
__X___ Outpatient Unit: *specify:* Subjects will come in for an outpatient blood draw 14-16 days prior to first inpatient admission. Subjects will be given their prescription for flutamide at this time.

3. What dose will be utilized in this study?
Subjects weighing ≥ 50 kg will receive 250 mg orally twice a day, and subjects weighing < 50 kg will receive 126 mg orally twice a day.

4. What will be the frequency of dosing in this study?
Subjects will take medication twice daily.

5. What will be the duration of dosing in this study?
22 days

6. What route of administration will be utilized?
PO

7. Will drug need to be prepared by the UVa Investigational Drug Service (IDS)?
___X__ YES
_____ NO- Drug will be prepared and/or administered per package insert

►*IF YES, complete the following information under 7a-7d.*
If you need assistance completing this section contact the Investigational Pharmacists at 982-1048

7a. Concentration
___X__ Standard
_____ Non- Standard- *specify*

7b. Diluents
__X___ Standard
_____ Non- Standard- *specify*

7c. Stability after prepared

___X__ Standard
_____ Non- Standard- *specify*

7d. Special storage requirements
__X___ Standard
_____ Non- Standard- *specify*

8. Are there any special handling instructions mandated by the study (e.g. weighing hazardous materials)? No.

9. Does the protocol provide provisions for dose titration, dose reductions, and or re-challenged (if drug is stopped), etc.? No.

10. How will missed doses be handled?
If a subject misses a dose, that dose should be skipped and the next regularly scheduled dose should be taken.

11. Will a comparator (active or placebo) be utilized in the protocol? No.

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

►IF YES, is this study investigator initiated?

If yes, answer questions # 13 and 14

13 Are you using a drug/supplement/ food additive in a manner not approved by the FDA? Yes.

IF YES, answer questions 13a-13f
You may reference the non-IRB protocol to answer these questions.

13a. Describe pertinent animal data that is available regarding the toxicity/safety of this drug.

Not applicable, as abundant human data is available.

13b. Describe pertinent human data that is available regarding the toxicity/safety of this drug.

There is much experience with flutamide as a treatment for men with prostate cancer; it is FDA approved for this use. In men with prostate cancer, flutamide is generally used at a dose of 250 mg three times a day, and its safety profile at this dosage is well described. In men, it may cause gynecomastia, hot flashes, breast tenderness, galactorrhea, impotence, and decreased libido; these side-effects in men are not uncommon, and they are expected based on flutamide's mechanism of action as an androgen-receptor antagonist. Gastrointestinal side effects (nausea, vomiting) occur in approximately 10%. Mild, transient increases in AST and LDH may occur also. Less common (< 10%) associations in men with prostate cancer include hypertension, edema, drowsiness, confusion, depression, anxiety, nervousness, headache, dizziness, insomnia, pruritus, ecchymosis, photosensitivity, herpes zoster, anorexia, increased appetite, constipation, indigestion, upset stomach (4% to 6%), diarrhea, anemia (6%), leukopenia (3%), thrombocytopenia (1%), and weakness (1%). Rare side effects or associations in men with prostate cancer (limited to important or life-threatening) include

hepatic failure, hepatitis, jaundice, malignant breast neoplasm (male), myocardial infarction, pulmonary embolism, sulfhemoglobinemia, thrombophlebitis, and yellow discoloration of the urine.

Flutamide is not FDA approved for use in women, but it has been studied in women with hirsutism, polycystic ovary syndrome, acne, and congenital adrenal hyperplasia. Flutamide is and has been used (off-label) in clinical settings for these indications, occasionally in the U.S., but more so in other countries. However, flutamide is the only currently available androgen-receptor blocker that is relatively free of other actions (e.g., the effects of spironolactone on the kidney) and that has a short half-life (approximately 6 hours).

Serious hepatotoxicity has been described in a 41-year-old woman taking flutamide for 3 months (Kackar and Desai 2003); in a 14-year-old girl taking flutamide for hirsutism (Andrade et al 1999); and a 20-year-old woman taking flutamide for hirsutism (Wallace et al 1993). One fatal case of flutamide hepatotoxicity has been reported in an 18 year old women treated with flutamide (250-375 mg per day) for 5 months for acne and hirsutism (Osculati and Castiglioni 2006). However, liver function tests were not checked prior to initiation of treatment, nor were they monitored during treatment.

We have used flutamide in a number of our earlier clinical studies at UVAHS. We have administered flutamide to at least 27 women with PCOS and at least 13 normal controls. A few subjects had minimally elevated transaminases during study; although the causes of these mild (or borderline) test abnormalities were not clearly established, the transaminase levels invariably normalized within 1-2 weeks. We have encountered no serious adverse events related to flutamide.

We are cognizant that the administration of an anti-androgen during the early stages of fetal development following conception might possibly (i.e., in theory) be associated with abnormal development of genitalia in a male fetus. Thus, in this proposed study, we are careful to exclude early pregnancy before and during flutamide use. Additionally, all women will have agreed to avoid conception either by the use of abstinence or barrier-means of contraception.

13c. Have there been any human deaths associated with this drug?

When flutamide is used in men with prostate cancer at a dose of 250 mg three times a day, hepatotoxicity occurs in approximately 3 per 10,000 patients, and deaths from severe hepatotoxicity have been reported in this context. The above estimate is based on a review of case series submitted to the MedWatch Spontaneous Reporting System of the FDA between February 1989 and December 1994, during which time the FDA received reports of 20 deaths and 26 hospitalizations for hepatotoxicity due to flutamide (Wysowski DK, Fourcroy JL. Flutamide hepatotoxicity. J Urol 1996; 155: 209-12).

We are aware of 4 case reports of severe hepatotoxicity in females taking flutamide, including one fatal case. Importantly, in the one fatal case of hepatotoxicity, there was unfortunately no monitoring of LFTs either before or during the 5 months of flutamide treatment.

13d. In how many humans has this drug been used previously?

Since before 1990, flutamide has been used in men for prostate cancer, which is a common disease. It is unknown how many men have received flutamide, but based on an aforementioned report (i.e., Wysowski DK and Fourcroy JL, Flutamide hepatotoxicity. J Urol 1996; 155: 209), approximately 150,000 men received flutamide from 1989 to 1994. Many more men have received this drug since 1994.

Flutamide has been studied and used (off-label) in women with hirsutism, polycystic ovary syndrome, acne, congenital adrenal hyperplasia, and various cancers. Published research reports describe flutamide use in over 1000 women. However, substantially more women have used flutamide in the clinical setting, although the numbers involved are unknown.

13e. If this protocol will be used in children describe any previous use of this drug with children of a similar age range.

Flutamide has been given to hyperandrogenic adolescent girls in several European studies. De Leo and colleagues treated 8 hyperandrogenic girls between the ages of 16-19 years old with 250 mg flutamide twice a day for 6 months (DeLeo et al 1998). The only adverse effect that they reported was a "slight, not clinically significant increase in serum transaminases and lipid profile." The Ibanez group in Spain has more extensive experience with flutamide in hyperandrogenic girls, although they have given lower dose flutamide than proposed in this study (62.5-250 mg/day vs. 250 mg twice a day). They have treated 190 hyperandrogenic girls with low dose flutamide for 3-54 months (mean duration of flutamide 19 months), and have not seen any evidence of hepatotoxicity (Ibanez et al 2005).

14. Do the following criteria apply? *Check all that apply*

_____ 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;

_____ 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;

_____ 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 Not checked- explain why you believe the risk to subjects is not increased:

Flutamide has been given to hyperandrogenic adolescent girls in several European studies. De Leo and colleagues treated 8 hyperandrogenic girls between the ages of 16-19 years old with 250 mg flutamide twice a day for 6 months (DeLeo et al 1998). The only adverse effect that they reported was a "slight, not clinically significant increase in serum transaminases and lipid profile." The Ibanez group in Spain has more extensive experience with flutamide in hyperandrogenic girls, although they have given lower dose flutamide than proposed in this study (62.5-250 mg/day vs. 250 mg twice a day). They have treated 190 hyperandrogenic girls with low dose flutamide for 3-54 months (mean duration of flutamide 19 months), and have not seen any evidence of hepatotoxicity (Ibanez et al 2005).

___X_ 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
This item must be checked.

_**X**___ The investigation will be conducted in compliance with the requirements of 21CFR312.7 (Promotion and charging for investigational drugs)
This item must be checked.

15. Is this a post-marketing study? No.

►IF YES is the study required to be done by the FDA?

APPENDIX: Privacy Plan for Studies With Consent

1. Describe your plan to protect the identifiable data from improper use and disclosure.

__X__ Option # 2

Health information may be stored with HIPAA identifiers.
Specimens will be stored with or without HIPAA identifiers depending on security measures in place (see below).

See Table A for list of HIPAA identifiers which apply
See instructions in italics below for additional information to be completed.

1a. Will any of the data be stored electronically at UVa?

Yes.**►IF YES, where will it be stored?**
___X__ a Health Systems Computing Services (HS/CS) managed server that is configured to store data regulated by HIPAA,

1b. Will any of the data be stored in hard copy format at UVa *e.g.- on paper***?**

Yes.**►IF YES, where will it be stored?**

_X____ case report forms will be stored in a secure area with limited access.

1c. The following procedures will also be followed

- Only investigators for this study and clinicians caring for the patient will have access to the data. They will each use a unique log-in ID and password that will keep confidential.
- If specimens stored: The following security precautions will be implemented for specimens stored at UVa:
    - Specimens will be stored in a locked freezer/ or locked room
      __X___Access to the freezer/room will be limited to study personnel
- Each investigator will sign the University's Electronic Access Agreement available at http://www.itc.virginia.edu/policy/form/eaa.pdf and forward the signed agreement to the appropriate department as instructed on the form.
  If you currently have access to clinical data it is likely that you have already signed this form. You are not required to sign it again.
- UVa Institutional Data Protection Standards will be followed
  http://itc.virginia.edu/security/dataprotection
- If identifiable data (*data with health information and HIPAA identifiers*) is transferred to any other location such as a desktop, laptop, memory stick, CD etc. the researcher must follow the ITC Policy "Electronic Storage of Highly Sensitive Data".http://itc.virginia.edu/security/highlysensitivedata/

- If the HIPAA identifiers and health information are combined on an additional computer off UVa premises, the researcher will follow the UVa "Guideline for Safeguards When Removing PHI Off- Premises for Work" https://www.healthsystem.virginia.edu/intranet/privacyoffice/Policies/PHI_Off_Premises.doc
- The data will be securely removed from the server, additional computer(s), and electronic media according to the University's Electronic Data Removal Policy. https://etg07.itc.virginia.edu/policy/policydisplay?id=IRB-004
- The data may not be analyzed for any other study without additional IRB approval

2. Describe your/central registry's plan to destroy the HIPAA identifiers at the earliest opportunity consistent with the conduct of the research.

__X___The HIPAA identifiers (except full dates and or address information if needed) will be destroyed as soon as all publications are complete.

This wording would allow the researcher to keep HIPAA identifiers until all queries/ request for additional information from publisher are addressed

3. **Do you confirm that you will not reuse the identifiable data (HIPAA identifiers or health information) or disclose any of this information to any other person or entity except as outlined in this protocol, except as required by law, for authorized oversight of the research study, or use it for other research unless approved by the IRB-HSR? YESThis means that after the study is closed at UVa:**

- ***You cannot contact the subject by any method (you cannot call them, send a letter, talk to them in person about the study, etc) without additional IRB approval***
- ***You cannot use the data for any research that is not already described in your IRB protocol without additional IRB approval (if you change your hypothesis you must modify your protocol)***
- ***You cannot share your research data with another researcher outside of your study team without additional IRB approval***
- ***Any health information with HIPAA identifiers will be shredded or discarded by using recycling bins for confidential material found in clinic settings. For large item disposal of confidential material contact Environmental Services at 2-4976 or University Recycling at 2-5050.***

TABLE A: HIPAA Identifiers (Limited Data Set)

1. Name
2. Postal address information, other than town or city, state, and zip code
3. Telephone numbers
4 Fax numbers
5. Electronic mail addresses
6. Social Security number
7. Medical Record number
8. Health plan beneficiary numbers
9. Account numbers
10. Certificate/license numbers
11. Vehicle identifiers and serial numbers, including license plate numbers
12. Device identifiers and serial numbers
13. Web Universal Resource Locators (URLs)
14. Internet Protocol (IP) address numbers
15. Biometric identifiers, including finger and voice prints
16. Full face photographic images and any comparable images
17. Any other unique identifying number, characteristic, code that is derived from or related to information about the individual (e.g. initials, last 4 digits of Social Security #, mother's maiden name, first 3 letters of last name.)