PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR
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PROTOCOL TITLE
Collaborative Study: Testosterone Antidepressant Augmentation in Women

FUNDING
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5/19/2017

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

Inadequate response to antidepressant therapy in major depressive disorder (MDD) is disabling and highly prevalent, particularly among women. Although the use of antidepressant augmentation strategies is common, there are few effective therapies and few strategies that have been studied in a rigorous, controlled fashion.

Because studies in other female populations suggest that low-dose testosterone administration (in which testosterone levels are raised within the normal female range) has antidepressant effects with minimal side effects, testosterone is a candidate for a novel augmentation treatment for women with MDD. Preliminary studies from our group demonstrate antidepressant efficacy of low-dose testosterone administration in women with inadequate response to inhibitors of serotonin uptake, as well as in other female relatively androgen-deficient populations. We propose to test the efficacy and tolerability of testosterone as an augmentation treatment for depressed women with inadequate response to antidepressant therapy in a randomized, placebo-controlled trial, which, if positive, will form the basis of a future, large, randomized, controlled trial.

An additional potential benefit of testosterone therapy is that it targets two specific persistent/residual symptoms of MDD – fatigue and sexual dysfunction. Fatigue/loss of energy and reduced libido/sexual function are common symptoms associated with MDD and ones for which few effective therapies are available. Moreover, data suggest that libido and sexual function may be independently and adversely affected by antidepressant therapy administration in a substantial subset of women. Our preliminary data suggest that low-dose testosterone may be an effective therapy for these two target symptoms in depressed women with partial/nonresponse to antidepressant therapy. In addition, data suggest that low-dose testosterone therapy increases energy and improves libido and sexual function in women. However, the specific therapeutic potential of low-dose testosterone augmentation has not been tested with a randomized, placebo-controlled study in MDD women with inadequate response to antidepressant therapy.

Rigorous, randomized, placebo-controlled trials are necessary to determine whether low-dose testosterone augmentation will be an effective treatment for depression in women with inadequate response to antidepressant **therapy**. In this application, a double-blind, placebo-controlled trial of adjunctive low-dose testosterone for inadequate response to antidepressant **therapy** among MDD women is proposed.

Specific Aim 1. Low-dose testosterone augmentation improves depressive symptoms in women with MDD and partial/nonresponse to antidepressant therapy.

Aim 1A. We will investigate in female antidepressant therapy partial/nonresponders whether low-dose testosterone augmentation for 8 weeks leads to a significantly greater reduction in depressive symptoms than placebo.

Aim 1B. We will investigate whether a key mechanism underlying the effects of testosterone administration on depressive symptoms is an increase in neuroactive testosterone metabolites, including 3alpha-diol.

Specific Aim 2. Adjunctive low-dose testosterone is safe and well-tolerated in women with MDD and partial/nonresponse to antidepressant therapy. We will investigate whether low-dose testosterone augmentation therapy for 8 weeks is safe and well-tolerated in female antidepressant therapy partial/non-responders compared to placebo.

Specific Aim 3. Low-dose testosterone augmentation improves two specific RDoC construct symptoms (in the Arousal/Physiologic Processes Domain) in women: fatigue and sexual dysfunction. We will investigate in female antidepressant therapy partial/nonresponders whether low-dose testosterone augmentation for 8 weeks improves associated fatigue and sexual dysfunction compared to placebo.

Specific Aim 4. Low-dose testosterone therapy in women with treatment resistant depression will cause functional changes in the brain. We will investigate the mechanisms and functional neuroanatomy of the effects of testosterone on different regions of the brain in women with treatment resistant depression. This will serve as preliminary data for future studies and grants.

Exploratory Aim: We will investigate androgen and other hormonal correlates of functional and structural neuroanatomy in women with treatment resistant depression compared with healthy, non-depressed controls. This will serve as preliminary data for future studies.

Specific Aim 5. **Androgen and neuroactive steroid levels will be lower in postmenopausal women with treatment resistant depression than in healthy controls.**

We will investigate androgen and neuroactive steroid levels in postmenopausal women with treatment resistant depression in comparison to androgen and neuroactive steroid levels in postmenopausal healthy controls without significant psychiatric disease.

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Although a number of potential augmentation strategies for MDD patients with partial/nonresponse to antidepressant therapy have been studied, few have been shown to be effective and fewer have acceptable side-effect profiles. The development of low-dose physiologic testosterone therapy for women is an innovative and potentially important approach to the prevalent problem of partial/nonresponse to antidepressant therapy among women with MDD. This is an approach that has never been pursued previously in any double-blind, randomized fashion. Furthermore, examination of the effects of experimental manipulation of peripheral gonadal hormone concentrations on RDoC candidate constructs (anergia/fatigue and

sexual dysfunction) within the domain of Arousal/Regulatory Processes reflects an innovative approach. The RDoC perspective we propose involves focus on these constructs independent of depression diagnosis, to generate data that may contribute to a larger body of findings supporting new ways of classifying mental disorders based on observable behavior and neurobiological measures. The classic gonadal steroid dichotomous paradigm (estrogens as the female hormone/testosterone as the male hormone) has only recently been understood to be an oversimplified framework. Although testosterone levels in women (and estrogen levels in men) are much lower than that in the opposite sex, there is increasing evidence that their role in the regulation of brain function may be important. This new area of investigation is an innovative approach that may have important clinical implications and may identify new potential targets for drug development in MDD. Investigation of neuroactive, GABAergic steroid metabolites of testosterone as a possible mechanism responsible for brain effects is another innovative component.

Partial/nonresponse to antidepressant **therapy** is extremely prevalent in the U.S., disproportionally affects women, and is associated with substantial morbidity and functional impairment. An effective, well-tolerated therapy would have a significant impact on public health. Antidepressant treatments with novel mechanisms of action and benign side effect profiles are needed. Preliminary data suggest that low, physiologic doses of testosterone may be effective to improve mood among women with partial/nonresponse to antidepressant therapy, yet with few side effects. If these preliminary findings are confirmed, results from the proposed study would form the basis for a larger, multi-center trial with active comparators to definitively establish that low-dose testosterone augmentation in women with antidepressant therapy partial/non-response is effective, well tolerated and exerts durable effects on mood in antidepressant-treated women with MDD, through effects on neuroactive steroids.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

Study Design: After antidepressant therapy partial or nonresponse is established by documentation of failure to at least 8 weeks' treatment with an adequately dosed antidepressant therapy, A minimum of 100 subjects study-wide (60 from MGH) with MADRS≥12 will enter the double-blind augmentation randomized-placebo-controlled treatment study. 50 of these subjects will be on active study drug and 50 will be control subjects on placebo. Eligible subjects will undergo baseline assessment of symptoms and biological measures and then be randomized to 1 of 2 groups: low-dose physiologic testosterone augmentation by transdermal preparation (AndroFeme®, 1.0 mL daily, 10mg testosterone, Lawley Pharmaceuticals) or placebo. Antidepressant dose will be held steady during the trial. Outcome measures will examine the antidepressant efficacy of physiologic testosterone treatment (Aim 1), its tolerability (Aim 2) and its effects on 2 specific RDoC symptoms (Aim 3).

Subject Enrollment: A total of 330 women will be enrolled study-wide, of which 100 women with antidepressant-resistant MDD and 50 women with no psychiatric history will be eligible study-wide. Study subjects for the depressed population will be women, ages 21-75 with failure to remit following at least 8 weeks antidepressant trial documented by past treatment history. The patients will be recruited from the MGH Depression Clinical and Research Program (DCRP)

and Butler Hospital in Providence, Rhode Island. In addition, 20 healthy controls without depression will be recruited for the fMRI substudy healthy control population.

Up to 60 postmenopausal women who do not have current or past psychiatric disorders will also be screened to obtain 30 women who will be enrolled at the MGH Depression Clinical and Research Program as healthy controls.

Randomized Study Subject Population (n=60)

Inclusion Criteria for Randomized Study Subjects:
1. Female, age 21-75, who provide written informed consent
2. Free testosterone level no higher than the third quartile of the normal range
3. Meet DSM-IV criteria (by SCID) for current Major Depressive Disorder and have MADRS≥12
4. Currently treated with an antidepressant (1^{st}, 2^{nd} or 3^{rd} trial in current episode), that has been taken at an adequate dose[103] for at least eight weeks with sufficient source documentation to confirm high level of confidence in treatment details (using the MGH ATRQ). Current treatment may include a combination therapy as long as the subject has not exceeded three failed trials in the current episode.
5. Persistent depression symptom burden of the same dose of the currently (ineffective) antidepressant therapy, evidenced by MADRS≥12.

Exclusion Criteria for Randomized Study Subjects**:**
1. Serious suicide or homicide risk, as assessed by evaluating clinician
2. Unstable medical illness including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic
3. Substance use disorder active within last six months, or clinical suspicion of ongoing substance use disorder at the discretion of the study clinician at time of screening based on history and/or laboratory results.
4. Any history of psychotic features, bipolar disorder, or primary obsessive compulsive disorder, as assessed by SCID
5. Untreated hypothyroidism. If treated hypothyroidism, change in levothyroxine dose within the prior 3 mos
6. Use of androgens, including testosterone, DHEA and methyltestosterone, within the prior three months
7. Any investigational psychotropic drug within the last 30 days
8. In the judgment of the study clinician, unlikely to be able to participate safely throughout the study period (three or more episodes of self-harm in the past year, documented history of poor treatment adherence, or frequent missed appointments (>50%) in the past year)
9. Alanine aminotransferase (ALT) > 3x upper limit of normal or creatinine> 3x upper limit
10. History of a hormone-responsive cancer
11. History of testosterone abuse
12. Almond allergy (AndroFeme contains almond oil)
13. History of hypercalcemia or thromboembolism
14. Women who are breastfeeding
15. Pregnant women, women who desire to become pregnant, or women of child bearing potential who are not using a medically accepted means of contraception (to include condom, diaphragm, spermicide, intrauterine device, tubal ligation, or partner with vasectomy)

Additional Exclusion Criteria for Randomized study subjects in fMRI Substudy Only (n=20)

Exclusion criteria for the fMRI substudy participants include routine MRI exclusion criteria as listed below:

- Cardiac pacemaker
- Surgical aneurysm clips
- Neurostimulator
- Implanted pumps
- Metal fragments in body / eyes
- Pregnancy
- Nitroglycerin patch (if non-removable)
- Weight >250
- Severe claustrophobia

Healthy Control fMRI Population (n=20)

Inclusion criteria for fMRI Healthy Control Subjects

- Match a randomized subject based on:
    - Age (+/- 2 years)
    - BMI (+/- 2 kg/m^2)
    - Hormonal status

Exclusion criteria for fMRI Healthy Control Subjects

- Any history of psychiatric illness including major depressive disorder, bipolar disorder, psychotic features, or obsessive compulsive disorder, as assessed by SCID
- Current or prior use of any psychiatric medications
- Current or prior substance use disorder
- Unstable medical illness including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic
- Hypothyroidism
- Use of androgens, including testosterone, DHEA and methyltestosterone, within the prior three months
- Abnormal alanine aminotransferase (ALT) or creatinine
- Women who are breastfeeding
- Pregnant women, women who desire to become pregnant, or women of child bearing potential who are not using a medically accepted means of contraception (to include condom, diaphragm, spermicide, intrauterine device, tubal ligation, or partner with vasectomy)
- Additional Routine MRI exclusion criteria as listed below:
    - Cardiac pacemaker
    - Surgical aneurysm clips
    - Neurostimulator
    - Implanted pumps
    - Metal fragments in body / eyes
    - Nitroglycerin patch (if non-removable)
    - Weight >250
    - Severe claustrophobia

Inclusion Criteria for Healthy Controls:

1. Female, age 50-75, who provide written informed consent
2. Free of lifetime psychiatric medication use
3. Postmenopausal, defined as amenorrhea for greater than one year and/or elevated FSH for women with prior hysterectomy

Exclusion Criteria for Healthy Controls:

1. Unstable medical illness including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic
2. Current or past Axis I psychiatric or substance use disorder (non-prescribed medications, recreational drugs, or alcohol)
3. Use of androgens, including testosterone, DHEA and methyltestosterone, within the prior three months

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Study visits for randomized subjects

Screening Visit: A screening visit will take place to determine eligibility for enrollment. Participants will be asked to complete the MGH ATRQ and sign release of information forms to request documentation from prescribing clinicians. It is estimated that after review of detailed source documents describing past treatment details, 60 women will be confirmed to have partial/nonresponse to antidepressant therapy and be eligible for the randomized, placebo-controlled phase. Screening visit testing will include:

- complete medical history
- physical examination, including height and weight
- 0800h Blood draw for ALT, creatinine, CBC, thyroid stimulating hormone (TSH), free testosterone, FSH, urine pregnancy and toxicology
- diagnostic interview with SCID
- MDD symptom assessment with MADRS
- completion of the MGH ATRQ

If antidepressant-resistance is confirmed following screening procedures, the subject will be scheduled for a baseline visit approximately 1-2 weeks.

Baseline Visit for the Randomized, Placebo-Controlled, Augmentation Study: Eligible subjects will undergo baseline testing immediately before the augmentation phase of the protocol. Baseline visit testing will include the following. However, the blood draw will not be repeated if the Baseline visit occurs within 2 weeks of the screening visit.

- Weight, vital signs, waist-to-hip ratio, and consumptive habits (alcohol, caffeinated beverages) will be recorded
- Urine pregnancy test (for premenopausal women only). Postmenopausal is defined as at least one year since last menses and FSH in the postmenopausal range
- Baseline endocrine blood testing: free testosterone, estradiol, neuroactive steroid panel, DHEAS, blood cortisol, late night salivary cortisol and 8 a.m. salivary cortisol
- Baseline safety blood testing: ALT, creatinine, CBC
- Depression: MADRS, IDS-SR, CGI-I, S
- Cognitive Functioning: CPFQ
- Persistent/residual symptom assessment: SF-36, BFI, ESS, FSS, DISF
- Safety assessments: SAFTEE-SI, CHRT, and hirsutism and skin assessments
- We will distribute the Study Drug Compliance and Hot Flash Diary or Menopausal Symptom Diary

- We will also collect DNA to be banked for a future exploratory study into whether androgen receptor CAG repeat length moderates androgen-behavior relationships (based on data by Seidmanet *et al.*).

Follow-up Visits (Placebo-Controlled Augmentation Phase): Subjects will then be randomized to receive low-dose testosterone or placebo. Follow-up visits will take place at weeks 2, 4, 6 and 8 post-baseline. Follow up visits will include:

- Urine pregnancy test (for premenopausal women only). Postmenopausal is defined as at least one year since last menses and FSH in the postmenopausal range (weeks 2, 4, 6 and 8)
- Free testosterone levels (weeks 2, 4, 6 and 8)
- Skin evaluations to monitor for side effects (weeks 4 and 8)
- Weight and vital signs will be recorded (weeks 4 and 8)
- Waist-to-hip ratio (week 8)
- Depression: MADRS, IDS-SR, CGI-I, S (weeks 2, 4, 6 and 8)
- Cognitive Functioning: CPFQ (weeks 2, 4, 6 and 8)
- Persistent/residual symptom assessment: SF-36, BFI, ESS, FSS, DISF (weeks 2, 4, 6 and 8)
- Safety: SAFTEE-SI, CHRT (weeks 2, 4, 6 and 8)
- Estradiol levels (weeks 2, 4, 6 and 8)
- Salivary cortisol (week 8)
- Medication management sessions (weeks 2, 4, 6 and 8)
- Neuroactive steroid panel (week 8)
- We will distribute the Study Drug Compliance and Hot Flash Diary or Menopausal Symptom Diary (weeks 2, 4, 6, and 8)

fMRI Substudy Visits for Randomized Subjects

- fMRI substudy participants will be further evaluated at the screening visit for suitability to undergo MRI using the Martinos Center Patient/Volunteer Screening Form.
- Subjects will undergo urine pregnancy tests throughout the study as noted above.
- One hour fMRI sessions will be scheduled at baseline before intervention occurs and at 8 weeks (weeks 0,8)
- Subjects will be administered the IDS-SR, FSS and BFI before each scan (weeks 0, 8)
- A blood draw to measure blood hormone levels on the day of the fMRI scan will occur at both the baseline and 8 week scanning visit.

fMRI Healthy Control Substudy Visits

Screening visit: A screening visit will take place to determine eligibility for enrollment and ensure that subjects meet inclusion and exclusion criteria. Twenty women with no history of depression or other psychiatric illness will be eligible for the baseline fMRI scan. Screening visit testing will include:

- Complete medical history
- Diagnostic interview with SCID
- Physical examination, including height and weight
- Morning blood draw for ALT, creatinine, CBC, thyroid stimulating hormone (TSH),

free testosterone, FSH, and urine pregnancy test

If a subject is found to be eligible following screening procedures, the subject will be scheduled for the baseline fMRI visit in approximately 1-2 weeks.

Baseline fMRI Healthy Control Visit:

Healthy control subjects will receive one fMRI scan at this visit. Other study procedures include:

- Urine pregnancy test
- Study questionnaires: IDS-SR, FSS and BFI
- A blood draw to measure blood hormone levels on the day of the fMRI scan will occur at both the baseline and 8 week scanning visit.

Healthy control Visits

Screening Visit (n=60): A "Screening Visit" will be conducted to determine eligibility. It is estimated that 60 women will need to be screened to obtain 30 eligible study subjects.

The screening visit will include:

- Abbreviated SCID
- Medical and psychiatric history including past psychiatric medication use

Baseline Visit (n=30): The baseline visit will occur if subjects are found eligible.

Baseline visit will include:

- Psychiatric assessments: MADRS, IDS-SR, CGI-I, S, CPFQ, SF-36, BFI, ESS, FSS, DISF, SAFTEE-SI, CHRT
- Height, weight, vital signs, waist-to-hip ratio, and consumptive habits (alcohol, caffeinated beverages) will be recorded
- Salivary cortisol
- Blood for androgens including total testosterone, free testosterone, neuroactive steroid levels, including allopregnanolone, and cortisol, and other hormones

Blood collection

All randomized subjects will complete a blood draw at all study visits. This will total 526mL of blood over the approximate 10-week study period from screening to the final visit. All subjects who choose participate in the fMRI substudy will have an additional 40mL of blood drawn for a total of 566mL over this 10 week period. fMRI healthy control subjects will participate in only a screening and single fMRI visit. fMRI healthy control subjects will have a total of 106mL of blood drawn over a 2-3 week study period. Aim 5 healthy control subjects for Aim 5 will have a total of 130 mL of blood drawn.

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

Subjects being studied in this protocol on active study drug (testosterone) will still have at least moderate depression, and subjects on placebo will still have MDD. Standard care alternatives are presented in the Consent form. Subjects at MGH are offered three months of free medication management clinic services for depression treatment after completion of a clinical trial such as this one. Research staff will assist in arranging subsequent treatment by providing referrals and communications to subsequent providers.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

The procedures to protect against or minimize potential risks include the following: (1) the assignment of unique study subject numbers to patients, (2) the use of these primary identifiers throughout the study, (3) storage of information in locked file cabinets, and (4) access limited to study personnel for these file cabinets and data.
We have set up exclusion criteria to minimize risks:

- Subjects must be medically stable as defined in this protocol.
- We exclude people who may be considered particularly vulnerable in clinical trials, including those at acute risk for suicide, those with active substance abuse or dependence, and those with psychosis (or treated with antipsychotic medications).
- Because we are investigating the efficacy of a hormone, it will be important to exclude patients with other endocrine abnormalities which could contribute to treatment resistance or with a changing endocrine status.
- The potential effects of testosterone on the fetus are not known and therefore precautions against administration to pregnant patients will be instituted. In order to prevent pregnancy, we will: 1) specifically ask potential study subjects if they are seeking pregnancy, and we will not allow anyone who responds in the affirmative to participate in the study, 2) administer urine pregnancy tests at every study visit (screening, baseline and then every two weeks for the 8-week study) and require premenopausal study subjects to use contraception.
- No history of testosterone abuse by women because testosterone at the doses necessary to build muscle and that are typically abused by male athletes, are more than 10 times the dose proposed in this application, and are virilizing to women.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Suicide Prevention: Suicidal ideation will be assessed by the Beck Suicide Index at each visit. Patients who develop active suicidal ideation, or who are felt by the study clinician to be at high risk for suicide, will be discontinued from the study and referred for hospitalization and further treatment if clinically indicated. In addition, each study subject will be extensively interviewed at each study visit by a highly trained psychiatrist, who not only has extensive experience in the treatment of major depression, but also with these issues in the setting of clinical trial protocols. Patients in research protocols have much more access to psychiatrists than patients in the community, as a study psychiatrist is available by pager 24 hours per day. Moreover, the studies are performed in hospitals in which patients can be immediately admitted, if necessary to ensure safety.

Drug Safety: We will measure free testosterone levels every two weeks and decrease the dose to 5 mg (0.5 ml) in any subject with a free testosterone level of more than 2x the upper limit of normal for females at one visit or 1.5x the upper limit of normal at two visits. If the free testosterone level remains above the same limits listed above after the initial dose decrease, the dose will be decreased to 2.5mg (0.25ml). In order to ensure success at raising levels during the

trial, dose increases by 5 mg increments (0.5 ml) daily will be made in subjects in whom the free testosterone level is less than the mean for age at any visit.

Safety: Patients will be carefully monitored by clinicians for side effects and potential toxicities with hirsutism and skin assessments. The blood draws will be performed by trained staff who have certificates documenting their ability to draw blood. We are performing a physical examination and routine laboratory tests prior to allowing anyone to enter the randomized phase of the protocol to ensure that subjects are medically stable. Close monitoring of patients throughout the study will ensure that adverse effects from treatment, exacerbation of symptoms, or emergence of suicidality, mania or psychosis will be promptly recognized so that patients can be treated appropriately following study discontinuation. All patients will be instructed on how to contact study clinicians in the case of an emergency. They will also be instructed on how to contact the Acute Psychiatry Service (APS) at MGH and the Patient Assessment Services (PAS) at Butler Hospital, which provide emergent psychiatric care on a 24h/day basis.

Every effort will be made to keep patients in the study. Acceptable reasons for early discontinuation include: 1) request of patient, 2) decision of physician, 3) serious adverse event, 4) worsening of depression requiring hospitalization, 5) emergence of hypomania, mania or psychotic symptoms, 6) pregnancy.

A Data Safety Monitor will review safety data, including adverse events and free testosterone levels twice-a-year and will be available in the interim to consider any urgent matters.

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Risks of Testosterone Administration: Because free testosterone levels will be raised to within the normal range for women, we expect the risks to be minimal. Administration of low-dose testosterone using the Intrinsa patch at a comparable dose to the AndroFeme dose we are proposing, to over 2,000 women has shown no significant increase in hirsutism or acne, reflecting the safety of low-dose testosterone in terms of androgen side-effects and lipid parameters. Our own experience in administering low-dose testosterone at a comparable dose to 40 women with anorexia nervosa for three weeks, 45 women with HIV wasting for 12 weeks and, 51 women with hypopituitarism for one year, resulted in no significant side effects. Shifren *et al.* studied 75 women with bilateral oophorectomy for 12 weeks and also found no significant side effects. Four additional studies investigating the effects of low-dose testosterone in comparable doses to what we are propsing in larger groups of women confirmed Shifren *et al.*'s findings of minimal side effects of low-dose testosterone. Additional details of the studies published are as follows.

1- Our experience in administering low-dose testosterone to 40 women with anorexia nervosa for three weeks, 45 women with HIV wasting for 12 weeks and 51 women with hypopituitarism for one year resulted in no significant side effects. Our pilot data in nine women with treatment-resistant depression similarly showed no significant side effects.
2- There is now an extensive literature reporting experience with testosterone versus placebo in several thousand female patients using comparable dosing to that proposed in this application. These studies have overall reported a lack of significant side effects, including

hirsutism or any signs of virilism, alopecia or voice deepening. Overall, administration of low-dose testosterone in over 2,000 women has shown no significant increase in hirsutism or acne reflecting the safety of low-dose testosterone in terms of androgenic side-effects and lipid parameters. The exception is one published study which showed a small but statistically significant increase in body hair. The lack of significant androgenic side effects in the vast majority of studies speaks to our ability to maintain blinding of patients. Nevertheless, patients will be carefully monitored by clinicians for side effects and potential toxicities.

3- Results of a study of 508 postmenopausal women suggest that the addition of testosterone to postmenopausal estrogen replacement regimens may actually be protective against breast cancer. In that study, women who received testosterone in addition to estrogen/progestin hormone replacement therapy were shown to have a breast cancer rate that was substantially less than that for conventional estrogen/progestin hormone replacement therapy users, i.e., breast cancer rate for testosterone subjects was similar to that reported for subjects who never used hormone therapy in the "Million Women Study". This result is consistent with experimental data from animal studies, including a study by Dimitrakakis *et al.* that demonstrated that androgen receptor blockade results in a greater than twofold increase in mammary epithelial proliferation, which could be almost completely prevented by concomitant physiologic testosterone replacement. The same group also showed in ovariectomized rhesus monkeys that testosterone decreases estrogen receptor expression and inhibits estrogen-induced mammary eptithelial proliferation. Likewise, in rodent breast cancer models, androgen administration exerts apoptotic and antiproliferative mammary effects, including a decrease in the number of progressing tumors. In a recent study, testosterone inhibited proliferation and increased apoptosis in cultured human breast tissue cells and opposed estrogen-stimulated proliferation. Therefore, these data are very reassuring with respect to the safety of testosterone administration on breast cancer risk and consistent with data demonstrating that the risk of breast cancer is not increased in women with polycystic ovary syndrome and many decades of elevated testosterone levels (and may even be reduced by as much as 50%). They are also consistent with some, but not all, studies examining the association of endogenous gonadal steroid levels, including androgens, with breast cancer risk. An exception is an analysis from the Nurses' Health Study II, in which higher levels of androgens were associated with a non-statistically significant increase in overall breast cancer risk but a statistically significant increase in risk of invasive and ER+/PR+ breast cancers. Also, a report on effects of testosterone administration without concurrent estrogen therapy in 814 postmenopausal women included observations of four cases of breast cancer, though the interpretation of this finding is limited since additional review of the cases revealed historical factors suggesting a casual relationship between the study treatment and the onset of new cancers was unlikely. More work recently published by the same author reported no increased risk of breast cancer in a retrospective cohort study of 631 women treated with testosterone between January 1989 and December 2007. Overall, although these data prevent any definitive conclusions in this matter, they are overall reassuring with regard to risk of breast cancer with low-dose testosterone administration in the normal physiological range, and in aggregate they support the notion that an 8-week trial of low-dose testosterone as proposed here does not pose a risk for new onset breast cancer. There is no evidence of an increase in any other types of cancer with low-dose testosterone use in women. As a safety measure, women with histories of hormonally responsive cancers will be excluded from participation in the study.

4- Although AndroFeme has not specifically been tested in pregnancy, and there are no published cases of pregnancy while receiving other low-dose testosterone preparations, the risk during pregnancy is low because the serum testosterone levels that will be achieved with

AndroFeme during this study will be within the normal female range. In addition, it is important to note that aromatase enzymes (the enzymes that convert testosterone to estradiol) are present in abundance in the placenta and convert androgens to estrogens, protecting the fetus. In order to prevent pregnancy, we will: 1) specifically ask potential study subjects if they are seeking pregnancy, and we will not allow anyone who responds in the affirmative to participate in the study, 2) administer urine pregnancy tests at every study visit (screening, baseline and then every two weeks for the 8-week study) and require premenopausal study subjects to use contraception.

Close skin contact with the area of application within an hour of application by a partner or child should be avoided.

AndroFeme contains almond oil. Therefore, individuals with almond allergies may be allergic to this preparation.

Risks of blood sampling: Blood sampling is performed in the study, and there is always a very minor risk of infection, bruising, or syncope during a blood draw. There is also the discomfort of having one's blood drawn.

Discomforts with Questionnaires: Answering detailed questionnaires may create some inconvenience for subjects.

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

The study may provide relief of depressive symptoms for some patients who participate.
The study may benefit other people with depression, by furthering our understanding of the antidepressant efficacy of low-dose testosterone in women with antidepressant-resistant depression, and by providing information on the relationship between hormones and depression. The study will also systematically test the safety and efficacy of this drug. The study will examine the safety and efficacy of a novel intervention for partial/nonresponse to antidepressant therapy and investigate potential mechanisms responsible for such an effect. Few data exists to guide treatment for antidepressant therapy partial/nonresponse in MDD. Therefore, the identification of a safe, tolerable drug with antidepressant efficacy could help to improve treatment for these patients. Furthermore, since this drug acts by a mechanism distinct from other antidepressant agents, the study may help to suggest new targets for drug development, or new drugs which may be efficacious in treating depression.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or

ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

The investigators will make every attempt to include English-speaking persons of all race and ethnicity in the study. Non-English speakers will be excluded from participation, as not all questionnaires and scales used have been validated in languages other than English

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

Non-English speakers will be excluded from participation in the research because all questionnaires and scales used have been not been validated in languages other than English.

For guidance, refer to the following Partners policy:
Obtaining and Documenting Informed Consent of Subjects who do not Speak English
http://healthcare.partners.org/phsirb/nonengco.htm

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

All materials used to recruit subjects for this protocol will be reviewed and approved by the Partners Healthcare, Inc. IRB prior to their use. We intend to place flyers in the offices of affective illness and general psychiatric clinics, as well as primary care practices at MGH, and to recruit for the study as part of other clinic-wide recruitment efforts. We also plan to use HOPE as a recruitment method. HOPE is a service which will allow potential research subjects to search for studies that may be appropriate for them through patient gateway. Other web-based advertisements will be used as a recruitment method for this protocol such as a departmental website, researchmatch.org and clinicalconnection.com

We also plan to use Facebook advertising to reach potentially interested subjects living in the Boston area. Women ages 21-75 living within a 20 mile radius of Boston will see advertisements for this research study in the "News Feed" section of their Facebook and be directed to an IRB approved RedCap prescreen survey. These advertisements will in no way be designed to target individuals who indicate they may be depressed on Facebook. The ads are shown to individuals based on the demographic requirements but have no link to any specific personal, profile or browsing history information. Examples of these advertisements are attached to this study protocol in Insight. A general Facebook page has also been created for this study (a Facebook requirement) and includes only basic contact and study information. A copy of this page is also attached to the study protocol in Insight.

Beth Israel Deaconess Medical Center will serve as an additional site to aid in recruitment efforts. Recruitment efforts at this site will be overseen by Dr. Roscoe Brady, the site responsible investigator for BIDMC. Dr. Brady will supervise the identification of patients who may be

eligible for this research study and refer such patients to MGH study staff for eligibility screening. Subjects will be identified by BIDMC using CQ2 (i2b2) and correspondence will be sent to their clinical provider identifying them as a potentially eligible participant. With permission from a clinical provider, a letter will then be sent to the patient that includes MGH study staff contact information. All study procedures will be performed at MGH.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Compensation for each follow-up visit will be $50 to account for transportation costs and time for a total of $200 after the completion of the 8-week study. One payment will be dispersed to the study subject upon the completion of their final study visit and reflect the number of visits the subject attended.

Subjects in the fMRI substudy will be compensated an additional $100 per MRI session ($200 maximum compensation for randomized subjects, $100 for healthy controls).

For guidance, refer to the following Partners policies:

Recruitment of Research Subjects
http://healthcare.partners.org/phsirb/recruit.htm

Guidelines for Advertisements for Recruiting Subjects
http://healthcare.partners.org/phsirb/advert.htm

Remuneration for Research Subjects
http://healthcare.partners.org/phsirb/remun.htm

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

All subjects will receive the consent form for the study. This document will be read by the patients and also reviewed by the patient with a licensed physician investigator on the research staff prior to participating in the study. Any questions, concerns, or ambiguities will be clarified by Dr. Miller, Dr. Fava or another licensed physician investigator prior to the patient signing consent. Patients will sign informed consent and only then will begin participation in the study. If new information is ascertained during the study, we will modify our consent and re-consent our patients. There is an additional consent form for the substudy of 20 randomized subjects and 20 healthy control subjects who will undergo fMRI as part of Aim 4.

NOTE: When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the New Submissions page on the PHRC website:
http://healthcare.partners.org/phsirb/newapp.htm#Newapp

For guidance, refer to the following Partners policy:
Informed Consent of Research Subjects
http://healthcare.partners.org/phsirb/infcons.htm

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

Each site will conduct weekly research meetings with co-investigators and study staff to review study progress, including recruitment strategies, enrollment numbers, protocol issues, and safety issues. The PIs of the two sites will participate in monthly conference calls regarding study progress. Site visits will occur twice a year (Dr. Carpenter will visit MGH annually and the MGH PI(s) will visit Butler annually).

A Data Safety Monitoring Board (DSMB) will also be established consisting of 1) a psychiatrist with specific interest and expertise in major depressive disorder, Andrew Nierenberg, M.D., 2) an established clinical researcher in androgens, Janet Shifren, M.D, and 3) a biostatistician, Peter Forbes, MA, Clinical Research Center, Boston Children's Hospital. The Data Safety Monitoring Board will meet every 6 months after study activation. The DSMB will review safety data, including adverse events, and will be available in the interim to consider any urgent matters. Any serious or unexpected adverse events, or any other unanticipated problems involving risks to subjects or others, will be formally reported to the Partners Healthcare System Institutional Review Board (IRB) and FDA.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

Documentation of the presence of any side effect or adverse event will be completed at every visit using the SAFTEE-SI. Patients will be asked to contact an investigator or study staff member at any time concerning adverse events or worsening symptoms. Suicidal ideation and behavior will be assessed by the CHRT and MADRS at each visit. Patients who develop active suicidal ideation, or who are felt by the study clinician to be at high risk for suicide, will be discontinued from the study and referred for hospitalization and further treatment if clinically indicated. A study psychiatrist is available by pager 24 hours per day. Our research groups at the Massachusetts General Hospital and Butler Hospital have extensive experience managing suicidality in clinical trials[123]. All treatment emergent serious adverse events will be documented and reported immediately to the IRB at Partners HealthCare System, as well as to the safety board and to the FDA (as appropriate). An event that is serious must be recorded on the case record and requires expeditious handling to comply with regulatory requirements. In the event that a patient becomes ill or injured as a direct result of participation in the research study, necessary medical care will be made available. All adverse effects will be reported as per Partners Healthcare System requirements. Potentially serious adverse events (SAEs) will be followed to resolution or stabilization and reported as SAEs if they become serious.

A serious adverse event is one that meets any one of the following criteria:

a) Fatal or life threatening
b) Requires inpatient hospitalization
c) Results in persistent or significant disability or incapacity
d) Congenital anomaly
e) Important medical event that may jeopardize the patient or require intervention to prevent serious outcome
f) Cancer
g) Overdose
h) Results in development of drug dependency or drug use

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

Karen K. Miller, MD will oversee and monitor the study to ensure that it is being executed accurately and per the protocol. She will be responsible for monitoring the data collected, and the reporting of study-related adverse events to the IRB in accordance with adverse event reporting policies.

For guidance, refer to the following Partners policies:

Data and Safety Monitoring Plans and Quality Assurance
http://healthcare.partners.org/phsirb/guidance.htm#13

Reporting Unanticipated Problems (including Adverse Events)
http://healthcare.partners.org/phsirb/guidance.htm#7

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

Medical information produced by this study will be stored in the investigator's file and identified by code number only. The code key connecting a specific name to specific information will be kept in a separate, secure location. Information contained in the records will not be given to anyone unaffiliated with the Massachusetts General Hospital in a form that could identify the subject without the subjects written consent, except as described in the consent form or as required by law.

It is possible that medical and research records, including sensitive information and/or identifying information, may be inspected and/or copied by federal or state government agencies, or hospital accrediting agencies, in the course of carrying out their duties. However, they are required to maintain confidentiality in accordance with laws and policies of the Hospital.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

We will be sending samples to Dr. Graziano Pinna at the University of Illinois and Dr. Ravinder Singh at Mayo laboratories in Minnesota for research purposes. Samples will be anonymously coded prior to sending the samples. The samples will not be able to be linked to individual subjects by the outside collaborators.

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw

their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

No data or specimens will be stored outside of MGH.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

Specimens from Butler Hospital in Providence, Rhode Island will be sent to MGH for assaying. These samples will contain coded identifiers that study investigators are able to link to individual subjects.