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## ROLES OF INVESTIGATORS AND OTHERS IN THE TESTOSTERONE TRIALS

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TITLE: THE TESTOSTERONE TRIAL

PROTOCOL

Sponsors
National Institute on Aging (NIA) and AbbVie, Inc.

NIH Grant Number
U01 AG030644

University of Pennsylvania Protocol Number
808676

Principal Investigator
Peter J. Snyder, MD

Study Drug Provider
AbbVie Inc

IND Number
104707

Clinical Trials.gov Number
NCT00799617

Date:
October 6, 2008

Version Number: 1.0

Amended:
December 22, 2008

Version Number: 1.2

Amended:
April 1, 2009

Version Number: 1.3

Amended:
June 4, 2009

Version Number: 1.4

Amended:
September 15, 2009

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Amended:
April 8, 2010

Version Number: 2.0

Amended:
June 22, 2010

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Amended:
September 13, 2010

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Amended:
November 23, 2010

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## Study Summary

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<td>Protocol Number</td>
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<tr>
<td>Study Design</td>
<td>Randomized, placebo-controlled, double-blind study of five coordinated trials</td>
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<td>Study Duration</td>
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### Objectives

The primary specific aims are to test the hypotheses that testosterone treatment of elderly men whose serum testosterone concentrations are unequivocally low – and who have symptoms and/or objectively measured abnormalities in at least one of five areas that could be due to low testosterone (physical or sexual function, vitality, cognition, and anemia) – will result in more favorable changes in those abnormalities than placebo treatment.

### Number of Subjects

800

### Diagnosis and Main Inclusion Criteria

A constellation of conditions that occur as men age will be studied: mobility disability, decreased libido, low vitality, reduced memory performance, as well as anemia, all of which could be at least partially the result of low testosterone. Primary entry criteria will be age ≥65 years, an unequivocally low testosterone concentration (average of 2 morning testosterone values, < 275 ng/dL), and symptoms and objective manifestations of mobility disability, low libido, or low vitality.

### Study Product, Dose, Route, Regimen

AndroGel®, testosterone in an alcohol-water gel, will be administered transdermally in doses from 5 to 15 grams per day, as necessary to maintain the serum testosterone concentration within the range of normal for young men.

### Duration of administration

AndroGel or placebo will be administered to each subject for 12 months.

### Reference therapy

The effects of AndroGel on the primary and secondary end points will be compared to effects of placebo on these end points.

### Statistical Methodology

The primary end points for each of the five trials (Physical and Sexual Function, Vitality, Cognitive Function and Anemia) will be analyzed separately by random effects models for each specific trial.
1. **Introduction**

This document is a protocol for a human research study to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures.

This trial is supported by the National Institute on Aging (NIA), the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute of Child Health and Human Development (NICHD), the National Heart, Lung and Blood Institute (NHLBI) and AbbVie, Inc.

1.1. **Background**

As men get older, they experience many conditions, often together, that eventually result in the inability to perform many activities of daily living, an increased propensity to fall, and decreased independence. These conditions include mobility disability and low vitality. Elderly men also experience increased anemia, metabolic syndrome, decreased sexual function and memory impairment. These conditions likely have multiple causes, but one cause that could contribute to all of them is a low serum testosterone concentration. When young hypogonadal men are treated with testosterone, they experience improvements in sexual function, muscle mass and strength, bone mineral density, sense of well being, and anemia. However, the benefits of testosterone therapy in older men with age-related decline in testosterone concentration are not known and are the subject of this investigation.

1.2. **Decrease in Testosterone as Men Age**

As men age, their serum testosterone concentration falls gradually from age 20 to over age 80, as demonstrated by both cross-sectional (1) and longitudinal studies (2-4). By the eighth decade, approximately 30% of men have concentrations of total testosterone lower than normal for young men and 70% have free testosterone concentrations lower than normal for young men (3). Age-related decline in testosterone concentrations is associated with decreases in physical function, sexual function, vitality, and, in some studies, decreases in memory and cognitive function.

1.3. **Conditions that Testosterone Treatment Might Improve**

1.3.1. **Physical Function**

As men age, they experience a decrease in muscle mass and strength and in physical function (5). Decreased muscle mass and strength leads to impairment of physical function and mobility (6, 7). Mobility disability is a highly prevalent recognized geriatric syndrome. The 6-minute walk test, which assesses walking speed and distance, is a standardized, reliable measure of mobility (8).

In population studies in elderly men (9, 10), lower testosterone concentrations are associated with decreased physical function. Testosterone treatment of young hypogonadal men significantly increases lean body mass (11, 12) and muscle strength (11). Clinical trials in which elderly men with low-normal serum testosterone concentrations were treated with testosterone have consistently demonstrated an increase in muscle mass, but less consistently demonstrated increases in muscle strength and physical function (13-15). Limited data from clinical trials suggest that testosterone therapy might improve walking speed.
1.3.2. Sexual Function
Aging in men is associated with reduced sexual activity, which may respond to testosterone. Sexual desire, erection, and ejaculation decrease linearly from 20 to 70 years (16-18). Erectile dysfunction occurs in approximately 20-30% of men in their 50s, and by age 70, most men have lost the capacity for firm erection or satisfying orgasm. One possible explanation for the decline in sexual function with age is the concomitant fall in testosterone. A meta-analysis of randomized, placebo-controlled studies concluded that testosterone improves sexual function, the more so the lower the pretreatment testosterone concentrations (19). The possibility that testosterone treatment will improve sexual function may depend on other factors, such as the availability of a willing partner, use of PDE5 inhibitors, and overall health. It is also possible that testosterone will affect some aspects of sexual function more than others, especially Hypoactive Sexual Desire Disorder (diminished libido).

1.3.3. Vitality
Several lines of evidence suggest that a decrease in testosterone contributes to age-related decreases in vitality, sense of well-being, and quality of life. Several epidemiologic studies have documented an association between serum testosterone concentrations and mood and vitality, mostly in the setting of depression (20, 21). Low testosterone in elderly men is associated more with subsyndromal depression and related symptoms than with major depression. The ability of testosterone treatment to improve vitality, mood, and well being in men who are severely hypogonadal due to known pituitary or testicular disease is accepted by endocrinologists. A few prospective studies have also documented improvements in vitality and well being during testosterone therapy (22-24).

1.3.4. Cognitive Function
The fall in testosterone levels with increasing age is accompanied by a decline in cognitive function, including reductions in verbal and visual memory, spatial ability and executive function (25-27). Several studies of elderly men suggest that age-related declines in circulating testosterone levels are associated with reduced cognitive function, and elderly men with prostate cancer made hypogonadal by androgen deprivation therapy show cognitive impairments relative to their pretreatment performance in verbal memory, visual memory, spatial ability, and executive function (28-30). In addition, a number of small randomized trials suggest that testosterone may benefit memory in elderly men. Together, these studies suggest that lower testosterone levels or androgen action are associated with poorer cognitive functioning in otherwise healthy elderly men and that testosterone treatment may improve memory functioning.

1.3.5. Anemia
Testosterone is well known to stimulate erythropoiesis (12). Low testosterone is associated with anemia in elderly men (31). Anemia in elderly men is associated with current disability (32) and predicts future morbidity and mortality after adjusting for comorbidities (33). We shall therefore determine if testosterone corrects anemia.

1.4. Conditions Testosterone Might Worsen

1.4.1. Prostate Cancer
Elderly men often harbor clinically silent prostate cancer. The testosterone-dependency of metastatic prostate cancer is illustrated by regression following surgical or medical castration
(34) and exacerbation following testosterone treatment (35). Many elderly men harbor occult prostate cancer (36). There is no direct evidence, however, that either high endogenous serum testosterone concentrations or testosterone treatment of men with low testosterone concentrations increases the risk of clinical prostate cancer. Randomized, placebo-controlled trials of testosterone in elderly men show that testosterone increases PSA but not prostate cancer, although their statistical power to detect a difference between treatment groups was very small (37).

One challenge with regard to prostate cancer in planning this trial is to protect individuals who volunteer. We shall exclude men who have a prostate nodule by manual examination or a serum PSA concentration above a defined value; and then we will monitor men who do enroll by repeating the manual examination and the PSA measurement during the trial. Unfortunately, there is no PSA value that has both high specificity and sensitivity for detecting prostate cancer (38). A Prostate Cancer Risk Calculator was devised to allow prediction of a man’s risk of both overall and high-grade prostate cancer. This Risk Calculator (http://www.compass.fhcrc.org/edrnnci/bin/calculator/main.asp) has been applied to another population of 3488 men (39). Because this Calculator does not take into account the serum testosterone concentration, and a low serum testosterone concentration results in a lower PSA, we shall adjust the serum PSA concentration to account for the low testosterone. This adjustment will be based on the regression coefficient (0.00128) derived from data from the European Male Aging Study showing a direct correlation between serum testosterone and PSA. Each man's PSA will be adjusted to what it would be if his serum testosterone were 460 ng/dL as in the following equation: Adjusted PSA = PSA + (460 - testosterone level) x 0.00128. For example, if a man's measured PSA is 1.0 ng/mL and testosterone is 200 ng/dL, the PSA will be adjusted upward by (460 - 200) x 0.00128 = 0.33. The adjusted PSA, 1.33 ng/mL, would then be used in the Prostate Cancer Risk Calculator. The serum PSA will also be increased, and specifically, doubled, before its use, when the subject is taking a 5-alpha reductase inhibitor.

In addition to selecting men at relatively low risk of developing prostate cancer by using the Prostate Risk Calculator, we have proposed criteria by which to monitor them during a testosterone trial. We chose a PSA increment criterion based on data from the placebo arm of a finasteride study. Taking into account the upward adjustment of the baseline PSA of 0.3-0.4 ng/mL, as above, we shall use an increment of 1.0 ng/mL above the adjusted baseline PSA confirmed by repeat determination, as the criterion for referral for urological evaluation and prostate biopsy.

1.4.2. Benign Prostatic Hypertrophy (BPH) and Lower Urinary Tract Symptoms (LUTS)

Despite the theoretical reasons that testosterone treatment could increase the risk of LUTS due to BPH, interventional studies have not demonstrated this risk. We shall monitor lower urinary tract symptoms during this trial.

1.4.3. Erythrocytosis

Testosterone stimulates erythropoiesis, so a potential consequence is erythrocytosis. We shall determine if a man whose hemoglobin is normal before treatment experiences an increase above normal (erythrocytosis) during treatment.

1.4.4. Sleep Apnea

Some evidence suggests that testosterone may exacerbate sleep apnea, although the evidence is weak. To be safe, we shall exclude men with diagnosed but untreated sleep apnea from these trials.
1.4.5. Cardiovascular Disease

In a recent study in men ≥65 years of age, men treated with testosterone experienced significantly more cardiac serious adverse events than men treated with placebo (unpublished). However, in another recent study (Srinivas-Shankar, J Clin Endocrinol Metab 95: 1220, 2010), no such excess occurred. In addition, Murad and Montori performed a meta-analysis of 51 randomized controlled trials of testosterone, in which nine reported serious cardiac adverse events. The quality of evidence was considered low because of few events, brief length of observation, and substantial loss of subjects to observation. Nonetheless, they concluded that “Compared with placebo, testosterone therapy was not associated with a significant increase in the risk of death, myocardial infarction (MI), revascularization procedures, cardiac arrhythmias, or a cardiac composite that included MI, revascularization procedures and cardiac arrhythmias.” Including these new trials did not change the conclusions.

1.5. Genetic Propensity to Respond to Testosterone

Variability in both beneficial and deleterious effects of testosterone may be explained by the fact that serum testosterone concentrations do not predict androgen responsiveness well, most likely due to genetic differences in 1) androgen action or metabolism due to relative conversion to active metabolites (such as estradiol or dihydrotestosterone), binding proteins (i.e. SHBG), and/or tissue-specific coactivators or corepressors; or 2) tissue-specific end-organ response to androgens due to coexistent polymorphisms of modulator genes. Our strategy is to evaluate this genetic predilection to beneficial and adverse effects of treatment by testosterone. Therefore, we shall collect peripheral blood lymphocytes for genetic analyses.

2. Study Objectives

The primary specific aims of the coordinated set of randomized, placebo-controlled clinical trials are to test the hypotheses that testosterone treatment of elderly men whose serum testosterone concentrations are unequivocally low – and who have symptoms and/or objectively measured abnormalities that could be due to low testosterone (physical or sexual function, vitality, cognition, or anemia) – will result in more favorable changes in those abnormalities than placebo treatment. The trials are highly coordinated, but each trial has its own primary, secondary, and exploratory specific aims, as follows:

2.1. Physical Function Trial

Primary specific aim: To test the hypothesis that testosterone treatment for one year, compared with placebo, of men ≥65 years who have an average serum testosterone concentration < 275 ng/dL and mobility disability, as defined by self-reported difficulty in walking 1/4 mile and objectively measured gait speed <1.2 meters/second on the six-minute walk test, will be associated with a greater proportion of men improving their six-minute walking distance by >50 m.

Secondary specific aim: To test the hypotheses that testosterone treatment of these same men for one year, compared with placebo treatment, will be associated with greater improvement in self-reported physical function by the 10-item physical function (PF10) component of the SF36.

Exploratory aims: To determine if testosterone treatment, compared with placebo, will be associated with

1. Better patient global impression of change in walking ability,
2. A greater proportion of men in all of the trials (combined) improving their six-minute walking distance >50m,
3. A lower frequency of falls in men in this Trial and in all trials.

2.2. Sexual Function Trial

Primary specific aim: To test the hypothesis that testosterone treatment for one year, compared with placebo, of men ≥65 years who have an average serum testosterone concentration < 275 ng/dL and decreased libido by self-report and by the Derogatis Interview for Sexual Functioning in Men-II (DISF-M-II) questionnaire, will be associated with greater improvement in sexual activity, as assessed by the Harbor-UCLA 7-day Sexual Function Questionnaire, question 4.

Secondary specific aims: To test the hypotheses that in these men, testosterone treatment for one year, compared with placebo treatment, will be associated with more favorable outcomes in

1. Harbor-UCLA 7-day Sexual Function Questionnaire, Questions 1–3, and 5-6,
2. Libido, as assessed by the DISF-M-II,
3. Erectile function as assessed by International Index of Erectile Function (IIEF).

Exploratory aims: To determine if testosterone treatment for one year, compared with placebo, will be associated with

1. Better patient global impression of change in sexual activity,
2. More favorable change in the UCLA 7-day Sexual Function Questionnaire among men in all trials combined.

2.3. Vitality Trial

Primary specific aim: To test the hypothesis that testosterone treatment for one year, compared with placebo treatment, of men ≥65 years who have an average serum testosterone concentration < 275 ng/dL and poor vitality, as defined by a score of <40 on the FACIT-Fatigue scale, will be associated with a greater percentage of men who have an improvement of ≥4 points in this test.

Secondary specific aims: To test the hypotheses that testosterone treatment for one year, compared with placebo, will result in more favorable outcomes in vitality/fatigue, as measured by the

1. SF-36 vitality scale,
2. Mood, as assessed by the Positive and Negative Affect Scales (PANAS)
3. PHQ-9 depression scale

Exploratory aims: Testosterone treatment for one year, compared with placebo treatment, will be associated with

1. A greater improvement in a patient global impression of vitality
2. A greater percentage of subjects who have an improvement of ≥4 units in the FACIT-Fatigue scale among men in all trials combined
2.4. Cognitive Function Trial

Primary specific aim: To test the hypothesis that testosterone treatment for one year, compared with placebo treatment, of men ≥65 years who have an average serum testosterone concentration < 275 ng/dL, who have subjective memory complaints as determined by their score on the MAC-Q questionnaire, and who demonstrate memory impairment as defined by a score on the Wechsler Memory Scale Revised Logical Memory II subscale recall (WMS-R LM II) or by Benton Visual Retention Test (BVRT) more than one SD below the performance for young men, aged 20-24 years [a criterion for age-associated memory impairment (AAMI) (40)] will result in greater improvement, or less decline, in verbal memory as assessed by the WMS-R LM II.

Secondary specific aims: To test the hypotheses that testosterone treatment for one year, compared with placebo, in the impaired subset of the study population defined above, will result in greater improvement or less decline in:

1. Visual memory assessed by the Benton Visual Retention Test (BVRT),
2. Spatial ability assessed by the Card Rotation test, and
3. Executive function/working memory assessed by the Trail Making Test (TMT).

Exploratory aims: Testosterone treatment for one year, compared with placebo treatment will result in greater improvement, or less decline in:

1. Verbal memory as assessed by the Wechsler Memory Scale Revised (WMS-R) Logical Memory II (WMS-R LM II) subtest, in all subjects, regardless of extent of memory impairment at baseline. The rationale for performing these tests in all subjects, regardless of presence or absence of impairment at baseline, is to determine if the cognitive response to testosterone depends on a demonstrated baseline impairment.
2. Patient global impression of change (PGIC) in memory,
3. Global cognitive function assessed by the Modified Mini-Mental State Examination (3MSE).

2.5. Anemia Trial

Primary Specific Aim: Testosterone treatment for a year, compared with placebo, of men who are anemic at baseline (hemoglobin concentration < 13.5 g/dL) will be associated with a greater proportion whose anemia is corrected.

2.6. Measurements Across All Trials

The close coordination of the trials will permit measurements across all trials and hypotheses testing in the entire study group.

Secondary aim:

1. Testosterone treatment for one year, compared to placebo, of men in all trials will be associated with improved mood, as assessed by the Positive and Negative Affect Scales (PANAS) (41).

Exploratory aims:

1. Testosterone treatment for a year, compared with placebo, of men in all the trials, not just those who qualify for an individual trial, will be associated with better scores in each of the five primary end points.
2. Testosterone treatment for one year, compared with placebo, of all men in the study will be associated with a decrease in falls.

3. Testosterone treatment for one year, compared to placebo, of all men will be associated with better depression scores on PHQ-9.

4. Testosterone treatment for one year, compared with placebo, of men in all trials, will be associated with better scores on:
   - a patient global impression of change (PGIC) question in each primary efficacy area,
   - the sum of the PGIC questions in all primary efficacy areas, and
   - an overall PGIC question.

5. Testosterone treatment for one year, compared to placebo, will increase the incidence of a rise in prostate specific antigen (PSA), even after correction of the baseline value for low testosterone, sufficient to trigger a prostate biopsy.

3. Study Design

3.1. General Design
This study is designed as five separate, but highly coordinated, randomized, placebo-controlled clinical trials of the effect of testosterone in men ≥65 years who have a low serum testosterone concentration and symptoms and objective manifestations of abnormalities in the areas of physical function, sexual function, vitality and/or cognition.

The study will be conducted at 12 clinical sites across the United States. The data coordinating center at the University of Pennsylvania will coordinate the activities of the trial sites, central laboratory, central pharmacy, and associated reading centers. The trials are planned to take six years.

3.2. Study Endpoints

3.2.1. Physical Function Trial Endpoints
The physical function trial endpoints will be measured at 3, 6, 9 and 12 months.

Primary Endpoint:
- Mobility, as assessed by the 6-minute walk test

Secondary Endpoints:
- Physical function, as assessed by the physical function 10-item scale (PF10) of MOS SF36

Exploratory Endpoints:
- Patient global impression of change in walking a quarter mile
- Fall frequency in men in this trial and in all men
3.2.2. Sexual Function Trial Endpoints

The sexual function trial endpoints will be measured at 3, 6, 9 and 12 months.

Primary Endpoint:
- Overall sexual activity, as assessed by question 4 of the Harbor-UCLA 7-Day Sexual Function Questionnaire

Secondary Endpoints:
- Harbor-UCLA 7-day Sexual Function Questionnaire, Questions 1–3, and 5-6
- Libido, as assessed by the DISF-M-II
- Erectile function as assessed by International Index of Erectile Function (IIEF)

Exploratory Endpoints:
- Patient global impression of change in sexual activity

3.2.3. Vitality Trial Endpoints

The vitality trial endpoints will be measured at 3, 6, 9 and 12 months.

Primary Endpoint:
- Fatigue, as assessed by the 13-item FACIT-Fatigue Scale

Secondary Endpoints:
- Well-being, as assessed by the positive and negative scale (PANAS)
- Vitality scale of the SF-36
- PHQ-9 depression score

Exploratory Endpoints:
- Patient global impression of change in fatigue/vitality

3.2.4. Cognitive Function Trial Endpoints

The Cognitive Function Trial endpoints will be measured at 6 and 12 months. All subjects in all trials will be assessed by all cognitive function end points.

Primary Endpoint (in those subjects who have memory impairment at baseline):
- Verbal memory, as assessed by score on the WMS-R LM II

Secondary Endpoints (in those subjects who have memory impairment at baseline):
- Visual memory, as assessed by the BVRT
- Spatial ability, as assessed by the Card Rotations Test
- Working memory/executive function, as assessed by the Trail Making Test (B-A score)

Exploratory Endpoints:
- Patient global impression of change in cognitive function
- WMS-R LM II, BVRT, Card Rotations, and Trail Making Test in all subjects (both subjects who have memory impairment at baseline and those who do not)
3.2.5. **Anemia Trial Endpoint**

**Primary Endpoint:**
- Correction of anemia, as assessed by hemoglobin increasing from <13.5 to ≥13.5 g/dL.

3.3. **Safety Measurements**

Although this study will not have sufficient statistical power to assess the effect of testosterone on the safety parameters below, we shall monitor subjects for the development of these conditions because of the possibility that testosterone treatment could increase the risk.

3.3.1. **Prostate Cancer**

Prostate cancer will be diagnosed by prostate biopsy. Men will be referred for urologic evaluation for consideration of biopsy when either a prostate nodule is palpated on digital rectal examination or the serum PSA concentration increases ≥ 1.0 ng/mL above the testosterone-corrected baseline value, confirmed by a repeat determination.

3.3.2. **Lower Urinary Tract Symptoms Due to Benign Prostatic Hyperplasia**

Lower urinary tract symptoms will be evaluated by the International Prostate Symptoms Score (IPSS) questionnaire. An increase of >5 points or to an absolute value of >19 will result in a review of medications that affect urine flow rates and evaluation for prostatitis. If a cause is found, it should be treated. If no cause is found, treatment with an alpha blocker should be considered. If the subject is treated and symptoms persist, or if acute urinary retention occurs, the subject should be referred for urological consultation. If the urologist treats the subject and the score does not decrease below the above thresholds, gel treatment will be discontinued.

3.3.3. **Erythrocytosis**

Erythrocytosis will be evaluated by hemoglobin. If the value increases to ≥ 17.5 g/dL, the subject will have repeat hemoglobin and testosterone measurements. If the testosterone concentration is above the target range, the number of depressions daily will be decreased and the hemoglobin repeated again. If the hemoglobin is still elevated, he will be referred for evaluation. If no treatable cause is found, the dose of testosterone will be decreased. At month 12, when treatment has stopped, men with elevated hemoglobin upon repeat will return for another hemoglobin test after 3 months time. The expectation is that after 3 months any effect the testosterone had on the hemoglobin will have dissipated.

3.3.4. **Sleep Apnea**

Men will be asked if they have been diagnosed with sleep apnea and are being treated. If they have been diagnosed but are not being treated, study medication will be discontinued. If subjects are being treated for sleep apnea, they will continue in the study.

3.3.5. **Cardiovascular Disease**

In order to determine if there is a relationship between testosterone treatment and cardiovascular events, we will administer a focused questionnaire about cardiovascular health at baseline and two others during treatment, one about incident cardiovascular events and one about incident symptoms. When a myocardial infarction, emergency revascularization, congestive heart failure, stroke or sudden death is reported, hospital records will be acquired.
and evaluated. A committee of experienced cardiologists and neurologists will be appointed to adjudicate these events. The baseline questionnaire will allow us to assess balance between the two treatment arms in cardiovascular disease. The questionnaires about incident cardiovascular events and symptoms will help determine if testosterone treatment is associated with an increase in cardiovascular events. These questionnaires will be administered at each visit during the one year of treatment and also during the year of observation after treatment.

3.3.6.Fractures
Fractures will be monitored in follow up visits during the course of the trial. If a participant reports they had a fracture at a follow up visit the sites will follow up and request all possible medical records related to the fracture. This will include, but is not limited to, X-rays, CT scans, MRIs, other imaging exams, orthopedic and or operating notes. These documents will be sent for review to confirm the fracture diagnosis.

4. Subject Selection and Withdrawal

4.1. Number of Subjects
Subjects will be evaluated for study eligibility during Screening Visit 2. The total sample size is 800 for the entire study. Each clinical site is expected to enroll approximately 67 subjects. It is projected that 85% of subjects allocated to treatment will complete the 12 months of treatment.

4.2. Common Inclusion Criteria
The inclusion criteria common to all subjects in all trials are as follows:

- Men ≥65 years old
- Total serum testosterone concentration at screening visit 1 (SV1) < 275 ng/dL, at screening visit 2 (SV2) < 300 ng/dL and an average serum testosterone concentration of < 275 ng/dL
- If the main T Trial has reached its enrollment goals, men must be eligible for either the Bone Trial or the CV Trial, if they are still open to enrollment (Please refer to the separate Bone Trial and CV Trial protocols for study details and study specific inclusion/exclusion criteria)

Blood should be collected from subjects who have been fasting (only water in the previous 8 hours). Only fasting samples are acceptable.

4.3. Common Exclusion Criteria
The exclusion criteria common to all subjects are as follows:

- Diagnosed prostate cancer or prostatic intraepithelial neoplasia (PIN) or, by the Prostate Cancer Risk Calculator, a >35% risk of having overall prostate cancer or >7% risk of having high grade prostate cancer
- Severe lower urinary tract symptoms (score of > 19) by the International Prostate Symptom Score questionnaire
- Hemoglobin < 10 g/dL or > 16.0 g/dL. Subjects who have hemoglobin level below 10 g/dL will be referred to their primary care providers for evaluation of anemia.
• Sleep apnea, diagnosed but untreated
• Alcohol or substance abuse within the past year (based on self report)
• Angina not controlled by treatment,
• NYHA class III or IV congestive heart failure
• Myocardial infarction within the previous 3 months
• Stroke within the previous 3 months
• Hypertension, defined as systolic blood pressure of >160 mm Hg or a diastolic blood pressure >100 mm Hg.
• Severe pulmonary disease that precludes physical function tests
• Serum creatinine >2.2 mg/dL; ALT 3x upper limit of normal; hemoglobin A1c >8.5%
• TSH > 7.5 mIU/L
• Kidney disease requiring dialysis
• Diagnosis or treatment for cancer within the past 3 years, with the exception of nonmelanotic skin cancers
• Body mass index (BMI) >37 kg/m²
• Average testosterone concentration > 275 ng/dL; SV1 value > 275 ng/dL or an SV2 value of > 300 ng/dL
• Mini Mental State Exam (MMSE) Score <24

• Major psychiatric disorders, including major depression (PHQ-9 score > 14), mania, hypomania, psychosis, schizophrenia or schizoaffective disorders, that are untreated, unstable, have resulted in hospitalization or medication change within the previous three months, or would result in inability to complete the trial efficacy instruments. Subjects whose disorders have been stable while being treated for more than three months are eligible.
• Skin conditions at the testosterone gel application site, such as ulcer, erosion, lichenification, inflammation, or crust, or generalized skin conditions such as psoriasis or eczema that might affect testosterone absorption or tolerability of the testosterone gel
• Known skin intolerance to alcohol or allergy to any of the ingredients of testosterone gel

Medications:

Subjects who are using the following medications will be excluded:

• Drugs that affect serum testosterone concentration, (eg, testosterone, androstenedione, DHEA, estrogens, GnRH analogs, spironolactone, and ketoconazole) for 2 months during the previous 12 months or within the previous three months.
• rhGH or megesterol acetate within the previous three months.
• Anti-depressant medication that has been introduced within the past three months. (Subjects with diagnosed depression who have been stable for more than three months while taking anti-depressant medication are eligible.)
• Prednisone (dose of greater than 5 mg daily) use daily for more than two weeks, or equivalent doses of other glucocorticoids for more than two weeks during the previous three months.
• Opiate use within the past three months. Subjects who are using opiate analgesics intermittently for relief of chronic pain at doses that do not equal or exceed the equivalent of 20 mg methadone daily will be included. The following doses of opiate analgesics are considered equivalent:
  - Methadone 20 mg
  - Hydrocodone 30 mg
4.3.1. Evaluation of T Level < 100

Men with a testosterone level < 100 ng/dL at SV1 or SV2 will be evaluated by the study physician or referred to an endocrinologist for the measures described below. Assessment of the following laboratory test results in combination will inform the physician of the need for further testing by MRI.

- serum LH > 9.3 mIU/mL
- total T4 < 4.5 μg/dL
- prolactin >30 ng/mL
- cortisol <10 μg/dL
- repeat testosterone

a. These five (5) tests will require a 10 cc venous blood draw. Blood must be drawn between 7 – 10 AM. Participants must be fasting for these tests which is defined as drinking only water after midnight of the night before the blood draw.

b. Men will be excluded who have a sellar mass >1 cm by an MRI scan of the head, in the absence of an elevated LH level.

c. Men will be excluded who have a history of mumps orchitis, castration, Klinefelter’s syndrome or chemotherapy with an elevated LH level.

d. Clinical site staff must document that the participant has been told that standard medical treatment for a serum testosterone concentration < 100 ng/dL, is testosterone replacement, yet there is a 50% chance he would receive placebo for one year if he participates in The Testosterone Trial.

4.4. Inclusion and Exclusion Criteria for Physical Function Trial

**Inclusion criteria:** symptomatic mobility disability, defined by

- Self-reported difficulty in walking one-quarter mile and/or self-reported difficulty in walking up one flight of stairs and
- Walking speed <1.2 meters/second on the 6-min walk test

**Exclusion criteria:**

- Not ambulatory
- Other conditions affecting mobility of sufficient severity that testosterone is unlikely to improve, including neurological conditions (multiple sclerosis) and severe disabling arthritis of the lower extremity, joints, or back
4.5. Inclusion and Exclusion Criteria for Sexual Function Trial

Inclusion criteria:
- Self-reported decreased libido and a sexual partner willing to have sexual intercourse ≥ twice/month
- Decreased libido, defined by a score of ≤20 on the DISF-M-II SR questionnaire

Exclusion criteria:
- Medical or nonmedical reasons that would preclude sexual activity (e.g., penile deformity, Peyronie’s disease, pelvic surgery for bladder cancer)
- Severe peripheral vascular disease associated with an absence of pedal pulses
- Autonomic neuropathy

4.6. Inclusion and Exclusion Criteria for Vitality Trial

Inclusion Criteria:
- Decreased energy, self-reported
- Low vitality, defined by a score <40 on the FACIT-Fatigue Scale

4.7. Cognition Trial

Cognitive function tests will be performed in all men in all trials, so there will be no specific inclusion or exclusion criteria for this Trial.

During the informed consent process, subjects will be asked for permission to audio-tape the testing sessions. Subjects may refuse and continue to participate in the study. This is done for quality control purposes at the Wake Forest University (WFU) Cognitive Function Reading Center. Recordings will be erased after scoring is completed.

4.8. Inclusion and Exclusion Criteria for Anemia Trial

Inclusion Criterion:
- Hemoglobin concentration <13.5 g/dL, the lower limit of normal for the central laboratory

Exclusion Criteria:
- Hemoglobin <10.0 g/dL

4.9. Subject Recruitment and Screening

The principal goals of recruitment are to identify men who have conditions that might be caused by a low testosterone concentration and who are representative of the United States population geographically, racially and ethnically. Recruitment techniques will include use of national media, local media, mass mailings by zip code, including retirement communities, retired employee groups (military, unions), graduates of local universities of appropriate graduating classes; local talks; direct recruitment at residential facilities for the elderly; focus groups to identify potential barriers to recruitment; and listing on ClinicalTrials.gov.
4.10. Early Withdrawal of Subjects

Because these trials are based on the principle of “intent-to-treat”, every attempt will be made to follow and evaluate all enrolled subjects for the duration of the trials. Therefore, even if treatment is discontinued, the subject will be asked to complete the appropriate evaluations.
5. Study Drug

5.1. Description

The study drug is AndroGel® (AbbVie, Inc., North Chicago, IL), which contains 1% testosterone in an alcohol-water gel and is FDA-approved for treatment of low testosterone in men. AbbVie will provide AndroGel in pumps, which deliver 1.25 g of gel per depression. AbbVie will also provide identical pumps with placebo gel.

5.2. Treatment Regimen

AndroGel or placebo will be applied to the abdomen, shoulders or upper arms once a day at the same time to dry, intact skin. Subjects will be instructed to wash their hands after application and to let the gel dry before dressing. It is important not to have contact with women or children while the gel is wet. They will also be asked not to bathe or get this area wet for five hours after application. Subjects will be taught how to apply the gel and they will be provided with written instructions and precautions. This information will be reviewed at each contact and visit.

The initial dose of AndroGel will be 5.0 g once a day. The serum testosterone concentration will be measured monthly for the first three months. If the testosterone concentration is not between 500 and 800 ng/dL at any time point, the dose will be either increased by increments of 1.25-2.5 g/day, up to a maximum of 15 g/day or decreased by increments of 1.25-3.75 ng/day. If the serum testosterone concentration is >800 ng/dL following two consecutive reductions in Androgel dose, treatment will be discontinued. A placebo subject will also be discontinued.

Men who stop treatment due to two consecutive reductions will have a repeat testosterone determination after two weeks. If the repeat testosterone value is <500 ng/dL (the lower limit of the target range) the participant will resume gel use. In this situation, the initial dose will be 1.25 g (one depression) a day, no matter how low the serum testosterone concentration. The matched placebo participant will also resume gel use as well.

Men who are increased to the maximum dose of 15g/day will be asked to return for a serum testosterone determination within one month of the dose change. A subject from the placebo-treated group will also be asked to return for testosterone determination.

If the serum testosterone level is >1500 ng/dL the testosterone test will be repeated by the central lab, Quest. If the level is still >1500 ng/dL, the participant will be called in for an unscheduled blood draw for safety and so appropriate dosing can be insured. Levels of testosterone which are this high are typically caused by incorrect application or contamination at the blood draw site; for this reason proper gel application instructions will be reviewed at the time of the unscheduled blood draw. In order to maintain blinding of the sites, the DCC staff will instruct a site to also bring in a placebo subject for a repeat blood draw.

Furthermore in the case of a need for a repeat value due to a problem with the blood draw or an out of range value for any lab result, participants will be asked to return for a repeat blood draw and will be matched with a placebo participant to maintain the blind. The matching will be done by the DCC.

To maintain blinding when the dose of a subject in the testosterone group needs to be changed, a designated, unblinded DCC staff member will instruct the clinical trial site personnel to change that subject’s dose, and also instruct that the dose (i.e., amount of gel) of a subject in the placebo group be changed at a randomly selected site (if possible) as well.
Reasonable efforts will be made to maintain blinding of investigators and staff members at clinical sites, provided such efforts do not impede subject safety.

5.3. Method for Assigning Subjects to Treatment Groups

Treatment assignment and balancing on prognostic factors will be done by the technique of minimization, rather than stratified randomization, because the sample size for this study (800) is not large enough to assure balance given the large number of strata that would be needed using the latter technique. Minimization will be performed by using a computer program developed at the Mayo Clinic in SAS Version 8. Factors for balancing for each of the five primary efficacy trials in which a subject may participate include study site, baseline serum testosterone concentration, age, and current use of an antidepressant. Additionally, use of a PDE-5 inhibitor will be balanced for those participating in the Sexual Function Trial.

5.4. Preparation and Administration of Study Drug

AndroGel pumps containing active and placebo gels will be supplied by AbbVie to the Investigational Drug Service (IDS) at the University of Pennsylvania, which will be the Central Pharmacy. The IDS will supply the pumps to the research pharmacies at each of the 12 trial sites. Subjects will be asked to return used pumps, which will be weighed. The weight will provide an assessment of the subject’s compliance.

5.5. Storage

Bulk supplies of study medication will be stored in the central pharmacy at controlled room temperature (20-25 Celsius). Study medication that is labeled for individual study subjects and shipped to participating study sites will be stored at controlled room temperature (20-25 Celsius) with short temperature excursions allowed within the range of 15 to 30 Celsius.

5.6. Dispensing of Study Drug, and Return or Destruction of Study Drug

Blinded, tamper-sealed treatment kits containing a 3-month supply of testosterone or placebo, will be shipped to each site and stored securely. Each kit will be labeled with a specific randomization number, which will be repeated on each individually-labeled pump bottle. The initial set of pumps will be dispensed to the study subject only after randomization has taken place. Additional blinded and tamper-sealed sets of pumps will be provided to the sites in 3-month increments as refills, labeled for the individual study subject, after randomization has taken place. At appropriate intervals, there will be reconciliation of drug shipped, drug consumed, and drug remaining.

5.7. Concomitant Medications

Concomitant medications will be recorded. Subjects will be asked specifically if they are taking PDE5 inhibitors, antidepressants, antipsychotic drugs, or androgenic drugs.

6. Study Procedures and Visits

6.1. Telephone Prescreening

Potential subjects who call the trial site in response to advertisements or respond to a trial staff member at a health fair, etc., will be asked the following questions:
Testosterone Trial Protocol

- Are you willing to answer questions about your possible participation in a testosterone research study?
- Are you 65 years of age or older?
- Do you have difficulty walking a quarter of a mile or climbing one flight of stairs?
- Has your desire for sex decreased?
- Is your energy low?

Subjects will be asked several questions about major exclusion criteria such as recent use of testosterone, use of medications that affect bone, history of spinal surgeries and spinal conditions, history of cancer, stroke, heart attack, atrial fibrillation (if the CV Trial is open to enrollment), and height and weight to calculate body mass index. If a potential subject is willing to answer questions, is ≥65 years old, and not excluded by the medical history, he will be asked to schedule Screening Visit 1, the first in-person visit.

6.1.1. Screening Visit 1

Subjects will first be asked to give written, informed consent for Screening Visits 1 and 2 and to be assessed for eligibility for the Bone and/or CV Trial, using the Screening Consent Form.

Screening Visit 1 - Assessments and Procedures

- Screening Consent
- Brief medical and medications history
- Blood draw – 30 mL (Serum T, PSA reflex and chemistry panel reflex/ eGFR )

If the serum testosterone concentration is <275 ng/dL, and the risk of overall prostate cancer is ≤35% and of high grade prostate cancer ≤7%, as determined by the National Cancer Institute Prostate Cancer Risk Calculator, the subject will be asked to schedule Screening Visit 2.

If the subject has a testosterone level <100ng/dL at either screening visit 1 or screening visit 2, he will be evaluated as described in Section 4.3.1.

6.1.2. Screening Visit 2

The following procedures and questionnaires will be completed:

- Complete medical history, including medications
- Blood draw - 30 mL (Serum testosterone, CBC, Hgb A1c, TSH)
- Urinalysis
- Height and weight (for BMI); waist, hip and blood pressure measurements
- Digital rectal examination (DRE)
- International Prostate Symptom Score (IPSS)
- 6-Minute Walk Test (Physical Function Trial screening test)
- Derogatis Inventory of Sexual Function Male (Sexual Function Trial screening test)
- PHQ-9 (Trial eligibility depression screening test)
- MMSE (for exclusion of moderate to severe dementia)
- Interactive Voice Response (IVR) System instruction
- Please refer to the Bone Trial protocol and/or CV Trial protocol, for specifics of procedures that may need to occur at SV2 for those trials
Eligibility will be determined based on the results of these screening tests. Subjects who have a second testosterone concentration <300 ng/dL, and an average testosterone concentration between screening visit 1 and screening visit 2 of <275 ng/dL, meet all the common eligibility criteria, described in 4.4, and meet all of the inclusion and exclusion criteria for at least one of the Physical Function, Sexual Function or Vitality Trials, described in 4.5 - 4.8, will be asked to schedule a baseline visit.

6.1.2.1. Data Collection and Interactive Voice Response (IVRS) System
Several methods will be used to collect data from study subjects including self-administration and interviewer-completed questionnaires. Data from a few questionnaires will be collected using the Interactive Voice Response System. IVR is a computer-based, automated touch-tone telephone system used increasingly to collect self-reported, personally sensitive data.

Clinical site personnel will train subjects in the use of the IVR system during the second screening visit in preparation for data collection by IVR, prior to randomization. Subjects will be registered in the IVR system by their T Trial identification number. Each subject will be provided a secure username and password that they will be instructed to change the first time they access the IVR system. The subject will complete the FACIT Fatigue Scale during SV2 using the IVR system. If the T level indicates that a subject is eligible, subjects will be contacted by site personnel and instructed to submit the following forms via the IVR system before the baseline visit:

- UCLA 7-day diary
- PANAS
- PHQ-9.
- Baseline status questions: general health, physical function, sexual function, vitality and cognitive function.

Clinical site personnel will communicate with subjects regarding use of the IVRS and missed responses. Data from the IVR database will be transferred to the DCC database electronically.

6.1.3. Baseline Visit
The entire study, including rationale, assessments, treatment, and potential risks, will be described to the subjects who are deemed eligible. Subjects will be given the option of participating in one or more of the Physical Function, Sexual Function, or Vitality Trials, if they qualify for them. Those who agree will sign the Trial Informed Consent. All subjects will participate in the Cognitive Function Trial, and those who are anemic will be considered as participating in the Anemia Trial. Only subjects who qualify for and agree to participate in the Sexual Function or Vitality Trials will be tested by the secondary end points in that trial.

6.1.3.1. Assessments and procedures for all subjects
All subjects will be tested for the primary efficacy endpoints for all the trials and by other common endpoints as listed below.

- Concomitant medications
- International Prostate Symptom Score
- Cardiovascular History Questionnaire
- Weight (for BMI), waist, hip and blood pressure measurements
- Blood draw - 30 mL (serum testosterone, PSA, Hct/Hgb, creatinine, FSH and LH, extra serum archived for SHBG, DHT, estradiol; pharmacogenomics)
Additional serum (60 mL), plasma (10 mL), and urine (10 mL) will be collected and stored at -80° for assay of 25 hydroxyvitamin D and unanticipated assays.

An additional 10 mL of blood will be drawn for development of lymphoblastoid lines from men who agree to sign the separate and optional Genetics Consent Form.

Primary Efficacy Endpoints for Each Trial:
  - 6-Minute Walk Test (Physical Function Trial)

Other Common Endpoints:
  - Patient Global Impression Questions
  - Falls
  - 3MSE, WMS-R LM II, BVRT, Card Rotations, and Trail Making Test
  - MAC-Q
  - SF-36 (entire form)

Please refer to the Bone Trial protocol and/or CV Trial protocol for specifics of procedures that may need to occur at the Baseline Visit for those Trials.

### 6.1.3.2. Secondary efficacy endpoints

Subjects will be tested for secondary efficacy endpoints only for those trials in which they are specifically enrolled, with the exception of the physical function trial secondary endpoints, which will be tested in all men. All endpoints in the Vitality Trial will be completed by IVR. In the Sexual Function Trial, the UCLA Questionnaire will be completed via IVR.

#### Baseline Visit – Secondary Efficacy Endpoints for Each Trial

- Subjects enrolled in the Physical Function Trial:
  - PF-10
- Subjects enrolled in the Sexual Function Trial:
  - DISF-M-II SR
  - IIEF
- Subjects enrolled in the Vitality Trial:
  - SF-36 Vitality scale (IVR)

### 6.1.3.3. Endpoint for Anemia Trial

All subjects will have blood drawn for hemoglobin and hematocrit at the baseline visit, and at 3, 6, 9 and 12 months. Subjects who are anemic at the baseline visit will considered to be enrolled in the Anemia Trial. They will require no additional tests.

### 6.1.3.4. Medication instructions

All subjects will be instructed in the use of the gel and given a three-month supply.

### 6.1.4. Months 1 and 2 Visits (± 7 days)

- Blood draw for serum testosterone (15 mL)
- Additional serum (20 mL), plasma (10 mL), and urine (10 mL) will be collected and stored at – 80°
- Concomitant medications
- Adverse Events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire
- Weigh used pumps
- Review gel application technique
- Dose adjustment, if necessary

After each of these visits, subjects will be notified by phone if an adjustment in gel dose is necessary.

6.1.5. **Month 3 Visit (± 2 weeks)**

6.1.5.1. **Common assessments and procedures**

- Concomitant medications
- Adverse events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire
- Weight, waist, hip and blood pressure measurements
- Digital rectal exam
- International Prostate Symptom Score
- Blood Draw – 30 mL (Serum T, PSA, Hct/Hgb; extra sera saved for SHBG, DHT, estradiol)
- Additional serum (60 mL), plasma (10 mL), and urine (10 mL) will be collected and stored
- Primary Efficacy Endpoints for Each Trial:
  - 6-Minute Walk Test (Physical Function Trial)
  - UCLA Sexual Function Questionnaire question 4 (Sexual Function Trial, IVR)
  - FACIT-Fatigue (Vitality Trial, IVR)
- Other Common Endpoints:
  - Patient Global Impression of Change (PGIC) Questions (IVR)
  - Falls
  - Positive And Negative Affect Scales (IVR)
  - PHQ-9 (IVR)
- Weigh used pumps
- Review gel application technique
- Dose adjustment, if necessary
- Dispense medication for three months

6.1.5.2. **Secondary and exploratory efficacy endpoints for each trial.**

Secondary endpoints will be performed only on subjects specifically enrolled in that trial, with the exception of the physical function trial secondary endpoints, which will be tested in all men enrolled in the Trial.

**Month 3 Visit – Secondary and Exploratory Efficacy Endpoints for Each Trial**

- Physical Function
  - PF-10
  - PGIC question for physical function (IVR)
- Sexual Function
  - UCLA Sexual Function Questionnaire-complete (IVR)
  - DISF-M-II SR
  - IIEF
  - PGIC question about sexual function (IVR)
- Vitality
  - SF-36 Vitality scale (IVR)
Testosterone Trial Protocol

6.1.6. **Months 4 and 5 Assessments (± 7 days)**

Subjects will be asked by telephone about adverse events, concomitant medications, and gel use.

- Concomitant medications
- Adverse events
- Review of instructions for use of testosterone gel

6.1.7. **Month 6 Visit (± 2 weeks)**

The Month 6 visit assessments will be similar to those of the Month 3 visit.

- Concomitant medications
- Adverse events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire
- Weight, waist, hip and blood pressure measurements
- Blood Draw – 30 mL (Serum T, Hct/Hgb; extra sera saved)
- Additional serum (60 mL), plasma (10 mL), and urine (10 mL) will be collected and stored at – 80°
- **Primary Efficacy Endpoints for Each Trial:**
  - 6-Minute Walk Test (Physical Function Trial)
  - UCLA Sexual Function Questionnaire - question 4 (Sexual Function Trial, IVR)
  - FACIT-Fatigue (Vitality Trial, IVR)
- **Other Common Endpoints:**
  - Patient Global Impression of Change Questions (IVR)
  - Falls
  - Positive And Negative Affect Scales (IVR)
  - WMS-R LM II, BVRT, Card Rotations, and Trail Making Test
  - MAC-Q
  - PHQ-9 (IVR)
- Weigh used pumps
- Review gel application technique
- Dose adjustment, if necessary
- Dispense medication for three months

**Month 6 Visit – Secondary and Exploratory Efficacy Endpoints for Each Trial**

- Physical Function
  - PF-10
  - PGIC question for physical function (IVR)
- Sexual Function
  - UCLA Sexual Function Questionnaire-complete (IVR)
  - IIEF
  - PGIC question about sexual function (IVR)
- Vitality
  - SF-36 Vitality scale (IVR)
  - PGIC question about vitality (IVR)
6.1.8. Months 7 and 8 Assessments (± 7 days)
Subjects will be asked by telephone about adverse events, concomitant medications and gel use.
- Concomitant medications
- Adverse events
- Review of instructions for use of testosterone gel

6.1.9. Month 9 Visit (± 2 weeks)
Assessments and procedures at the Month 9 visit will be similar to those at the Month 3 visit except that prostate evaluation will not be performed.
- Concomitant medications
- Adverse events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire
- Weight, waist, hip and blood pressure measurements
- Blood Draw – 30 mL (Serum T, Hct/Hgb; extra sera saved)
- Additional serum (60 mL), plasma (10 mL), and urine (10 mL) will be collected and stored at – 80°
- Primary Efficacy Endpoints for Each Trial:
  - 6-Minute Walk Test (Physical Function Trial)
  - UCLA Sexual Function Questionnaire question 4 (Sexual Function Trial, IVR)
  - FACIT-Fatigue (Vitality Trial, IVR)
- Other Common Endpoints:
  - Patient Global Impression of Change Questions (IVR)
  - Falls
  - Positive And Negative Affect Scales (IVR)
  - PHQ-9 (IVR)
- Weigh used pumps
- Review gel application technique
- Dose adjustment, if necessary
- Dispense medication for three months

Month 9 Visit – Secondary and Exploratory Efficacy Endpoints for Each Trial
- Physical Function
  - PF-10
  - PGIC about physical function (IVR)
- Sexual Function
  - UCLA Sexual Function Questionnaire-complete (IVR)
  - IIEF
  - PGIC about sexual function (IVR)
- Vitality
  - SF-36 Vitality scale (IVR)
  - PGIC about vitality (IVR)

6.1.10. Months 10 and 11 Assessments (± 7 days)
Subjects will be asked by telephone about adverse events, concomitant medications, and gel use.
Testosterone Trial Protocol

- Concomitant medications
- Adverse events
- Review of instructions for use of testosterone gel

6.1.11. Month 12 Visit (± 2 weeks)

Month 12 will be the end of treatment. All common and trial-specific assessments will be made at this visit for all trials.

Common Assessments and Procedures

- Concomitant medications
- Adverse events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire
- International Prostate Symptom Score
- Height, weight, waist, hip and blood pressure measurements
- Blood Draw – 30 mL (Serum T, PSA, Hct/Hgb, HgbA1c, chemistry panel; extra sera saved for SHBG, DHT, estradiol)
- Additional serum (60 mL), plasma (10 mL), and urine (10 mL) will be collected and stored
- Digital rectal exam
- Primary Efficacy Endpoints for Each Trial:
  - 6-Minute Walk Test (Physical Function Trial)
  - UCLA Sexual Function Questionnaire question 4 (Sexual Function Trial, IVR)
  - FACIT-Fatigue (Vitality Trial, IVR)
- Other Common Endpoints:
  - Patient Global Impression of Change Questions (IVR)
  - Falls
  - Positive And Negative Affect Scales (IVR)
  - 3MSE, WMS-R LM II, BVRT, Card Rotations, and Trail Making Test
  - MAC-Q
  - PHQ-9 (IVR)
- Weigh used pumps
- Please refer to the Bone Trial protocol and/or CV Trial protocol for specifics of procedures that may need to occur at the Month 12 Visit for these trials

Month 12 Visit – Secondary and Exploratory Efficacy Endpoints for Each Trial

- Physical Function
  - PF-10
  - PGIC about physical function (IVR)
- Sexual Function
  - UCLA Sexual Function Questionnaire-complete (IVR)
  - DISF-M-II SR
  - IIEF
  - PGIC about sexual function (IVR)
- Vitality
  - SF-36 Vitality scale (IVR)
  - PGIC about vitality (IVR)
6.1.12. Months 18 and 24 Assessments (± 1 month)

These are post-treatment assessments. Month 18 visit will occur at the trial site. The Month 24 visit will be conducted over the telephone.

**Months 18 and 24 Assessments**

- Blood draw – 15 mL for serum PSA – Month 18 only
- Adverse events
- Cardiovascular Event Questionnaire
- Cardiovascular Symptom Questionnaire

6.2. Subject Compensation

Subjects will be compensated during the course of the trial, based on the number of visits completed and the number of trials in which they participate. In addition, travel and parking expenses, and meal tickets will be provided for study visits.
### 6.3. Screening, Assessment, & Monitoring Schedule

<table>
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<th>Phone</th>
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<th>Screen Visit 2</th>
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<th>Post-Rx</th>
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Abbreviation: IPSS, International Prostate Symptom Score; PGIC, Patient Global Impression of Change questions; PANAS, Positive and Negative Affect Scales

Primary efficacy endpoints will be assessed in all subjects; secondary efficacy endpoints will be assessed only in those who specifically qualify for that trial, except the endpoints for the Cognition Trial, which will be assessed in all subjects.
7. **Statistical Plan**

7.1. **Analytical Methods and Sample Size Estimations: Overview**

7.1.1. **Analysis of Primary and Secondary Endpoints for the Individual Trials**

Each of the efficacy trials (Physical Function, Sexual Function, Vitality, Cognitive Function and Anemia) is considered a separate trial, so the results will be analyzed separately. The primary and secondary endpoints of each of these trials will be evaluated for those subjects who participate in each specific trial. Primary analysis of outcomes with interim measures in addition to baseline and 12 months measures will be performed with random effects models for longitudinal data. Logistic models will be used for binary variables. Outcomes with measures at baseline and 12 months only will be compared using chi-square tests (binary outcomes) or Student’s T test (continuous variables). Wilcoxon’s rank sum test will be used for continuous variables that deviate substantially from a normal distribution. Dichotomous outcomes have been selected rather than continuous ones in order to determine not only if testosterone has a statistically significant effect compared to placebo, but if it also has an effect that is of clinical significance. All analyses will be adjusted for balancing factors. We shall perform sensitivity analyses to assess the potential impact of missing data by fitting shared parameter models that relax the missing at random assumptions of the proposed random effects models. We shall employ the methods of Benjamini and Hochberg to control for the impact of multiple analyses. Individuals will be analyzed in the group to which they were randomized regardless of compliance with assigned treatment (intention-to-treat principle), but sensitivity analyses accounting for compliance will be performed.

7.1.2. **Sample Size Estimation**

Sample sizes for each efficacy area were calculated based on two-sided 0.05 level tests and 90% power. Although our primary analyses will include data from each visit, we have calculated sample sizes based on tests considering only values at baseline and 12 months, which provide conservative estimates of sample size; therefore, we apply only a modest inflation factor of 5% to help compensate for subjects who drop out early.

We do not expect that all trials will complete enrollment at the same time. Once a trial reaches its target enrollment, no further subjects will be enrolled in that trial unless they qualify for that trial and a trial that remains open. Open Trials include the Bone Trial and CV Trial in combination of one of the 3 main trials (sexual, physical or vitality).

7.1.3. **Physical Function Trial**

We estimated the expected changes in 6-minute walking distance on the basis of unpublished data from the control group in the Walking and Leg Circulation Study (WALCS), in which the subjects performed the 6-minute walk at baseline and after one year. In this study the proportion of untreated subjects with an increase of 50 m or more at 12 months was 16%. To detect an increase from 15% to 30% with 90% power will require 350 subjects, inflated to 370 to compensate for dropout before the three-month visit.

We shall compare the proportions in each treatment group who achieve an increase of \( \geq 8 \) points on the PF10 because such an improvement has been shown to be clinically meaningful.
We shall also compare the actual distributions of changes in distance on the 6-minute walk, and in PF10 scores, and differences in proportions with a 50m or greater decline in distance covered during the 6-minute walk, and in proportions experiencing one or more falls.

7.1.4. Sexual Function Trial
Published data suggest that testosterone treatment increases the mean sexual activity score (question 4 in the UCLA 7-day diary) by 0.75 units (SD of change: 1.86) (42). This difference of 0.75 units also appears to be clinically meaningful, in that hypogonadal men treated with a testosterone gel who increased their score by at least this amount had the same distribution of scores as eugonadal men. A sample size of 262 subjects (275 to compensate for missed visits) will be needed to detect this difference with high power.

7.1.5. Vitality/Fatigue
A change of 4 points on the FACIT-Fatigue Scale has been demonstrated to be a clinically meaningful difference (43, 44). We shall compare the proportions experiencing such a change in the two treatment groups. Because self-reported outcomes often show a substantial placebo response rate, we assume that 20% of those receiving placebo will show an improvement of 4 or more points on the FACIT-Fatigue Scale. A sample size of 420 will provide 90% power to detect an increase in this proportion to 35% in the testosterone arm.

7.1.6. Cognition Trial
We aim to detect an effect size of 0.3 (based on change from baseline to 12 months), which corresponds to a 3-point improvement in Paragraph Recall. On the WMS-R Logical Memory II Subscale Recall, the scaled-score that corresponds to the 50\textsuperscript{th} percentile performance for a man 70-74 years old is 17; an effect size of 0.3, or 3 point increase, would improve that score to 20, corresponding to the 50\textsuperscript{th} percentile performance for a man 45-54 years old. These data suggest that a 3-point difference will be clinically significant. The sample size required to attain 90% power for this difference is 235 per arm, 470 for both arms, or 500 to help compensate for missed visits. Based on previous studies, we expect that approximately 60% of men enrolled will have memory impairment more than one SD below the performance for young men, aged 20-24 years, a criterion for age-associated memory impairment. Therefore, of the 800 subjects enrolled in the Core Testosterone Trial, we expect that approximately 480 men, expected to be evenly distributed between treatment arms, will demonstrate age-associated cognitive impairment at baseline, as defined above.

7.1.7. Anemia Trial
We shall identify all subjects who have low hemoglobin at baseline and compare the proportions who are no longer anemic over the 12 months of treatment. We expect 10-20% of subjects to be anemic at baseline, providing 80 – 160 subjects in whom we shall evaluate the effect of testosterone on anemia. Assuming 10% of those assigned to placebo become non-anemic, we shall have 80-90% power to detect improvements ranging from 15 to 26 percentage points depending on the baseline proportion anemic.

7.2. Analytic Plans for Measures Across All Trials
7.2.1. Efficacy Endpoints from Individual Trials
The primary efficacy endpoint for each trial will be evaluated in all subjects, but the results will be considered exploratory. These analyses will take into account whether or not the subject
had actually participated in the trial associated with a given endpoint, in addition to all baseline balancing factors. Similarly, secondary endpoints from the physical function trial (falls) and the vitality trial (PANAS) will be evaluated in all subjects.

7.2.2. Patient Global Impression of Change (PGIC)

For the Physical and Sexual Function, Vitality and Cognition Trials and for global assessment overall, a seven-point Likert-scale for PGIC will be administered every three months. These data will be evaluated at each time point and over the entire 12-month observation period. In addition to a score for each trial for subjects who specifically participated in a trial and another for all subjects in all trials together, we will sum all scores to generate an overall score. There will also be a score for the overall questionnaire. In addition, we shall evaluate the extent to which the Likert scale outcomes are consistent with the changes in objective measures for subjects in each trial.

7.3. Adverse Events

We will compare proportions of men experiencing adverse events in each treatment group, with particular attention to areas that are plausibly associated with testosterone, including erythrocytosis, urinary tract symptoms, and prostate-related events.

7.3.1. Prostate Cancer

This is the safety parameter of primary interest and the focus of our interim monitoring plan described in Section 7.5. In addition to monitoring diagnosis of cancer, we will calculate rates and confidence intervals for biopsy requirement, and grade of cancer in those with cancer diagnoses.

7.4. Sample Size for the Entire Study

The total sample size for all trials is 1051, as shown in the table below. Assuming that approximately 33% of these men will qualify for, and participate in, two efficacy areas, the sample size for the entire study becomes 800 (1051 x 3/4 = 788, rounded to 800).

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<thead>
<tr>
<th>Table 7.4. Sample Sizes for Individual Trials and All Trials</th>
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<tbody>
<tr>
<td>Trial</td>
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<tr>
<td>------------------------------</td>
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<tr>
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</tbody>
</table>

The assumption that 33% of men will qualify for at least two trials comes from unpublished data in two studies. One is the Rancho Bernardo Study, showing that in response to the ADAM (Androgen Deficiency in the Aging Male) questionnaire of symptoms in three areas (energy, strength, sexual), 36% of men had symptoms in at least two areas, and some in three (unpublished). Because the men in the proposed study will all have low testosterone concentrations, the overlap may be even higher, an assumption supported by data from men in the EMAS study who were >65 years old and had testosterone concentrations <250 ng/dL. We now estimate that at least 33% of subjects will participate in two trials and 10% in three, but we
base our sample size estimates on the conservative assumption of only 33% participating in two trials.

We shall allow a variable degree of over enrollment in the main trials; Physical Function Trial, Sexual Function Trial and Vitality Trial if necessary to complete enrollment in one of the other trials, eg Bone Trial & CV Trial.

7.5. Interim monitoring

Interim monitoring in this trial will focus on safety; there is no intent to consider early stopping on the basis of any efficacy parameter. The primary safety concern related to testosterone treatment is increased risk of prostate cancer. Evaluating this risk during the study in an accurate and unbiased manner will not be possible, for several reasons. Approximately 60% of men this age harbor occult prostate cancer, and even after we select men who have reduced risk, we expect as many as 20% of the subjects will have biopsy-detectable cancer at study entry (unpublished data from the PCPT, rate for men >65 years). Thus, for any biopsy performed as a result of PSA changes or DRE finding, the probability of a positive finding will be at least 20%, yielding as many as 80 cases of prostate cancer per treatment arm. Because testosterone is known to cause PSA to rise, we might expect to perform more biopsies in the testosterone-treated group, and therefore might diagnose more cancers in that group, whether or not testosterone actually increases prostate cancer risk, i.e., we might have ascertainment bias. Further, the PSA increases in men receiving testosterone might be selectively observed in men with occult cancer, because testosterone may “unmask” such cancers, whether or not it exacerbates their growth. Therefore, even a large difference in numbers of cancer diagnoses between arms might not necessarily indicate a difference in cancer risk. On the other hand, any diagnosis of prostate cancer may lead to cancer treatment, which has its own potential risk of major adverse effects, particularly on quality of life. If testosterone truly does not increase cancer risk, but does increase risk of diagnosis of indolent tumors that are likely to remain asymptomatic during a man's lifetime, then these diagnoses in themselves represent an adverse consequence of treatment. By adjusting the PSA for serum testosterone, however, we might mitigate the possibility of ascertainment bias.

Given these considerations, which impose great difficulties on the development of a statistical monitoring plan, we propose to use an approach that balances benefits and risks. We assume a rate of cancer diagnosis in the placebo arm of 1%/year, based on unpublished data from the PCPT, and a follow-up time of 24 months for each subject. Under these assumptions, we expect a total of 8 cases per arm under the null hypothesis of no excess cancers in testosterone-treated subjects. We propose as a basis for monitoring cancer diagnosis a one-sided O'Brien-Fleming boundary with an overall alpha of 0.20 and with a Lan-DeMets spending function modification for comparing time to cancer diagnosis. This plan provides 90% power for detecting a hazard ratio of 2.4 or higher. We specify a looser criterion for early stopping than would be typical for an efficacy boundary while still maintaining the probability of error at a relatively low level. We shall perform three interim analyses, specifically, after 25%, 50% and 75% of our target sample size has completed 12 months of follow up. Use of the spending function approach will permit additional analyses or a modified schedule, should the DSMB so request.

8. Safety and Adverse Events

8.1. Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, Office on Human

8.1.1. Adverse Event
An adverse event (AE) is any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant’s involvement in the research, whether or not considered related to the subject’s participation in the research.

8.1.2. Serious Adverse Event
A serious adverse event (SAE) is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- results in congenital anomalies or birth defects
- an important medical event*

Important medical events* are those that may not be immediately life threatening, but are clearly of major clinical significance.

8.1.3. Unanticipated Problem
An Unanticipated Problem is any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the IRB-approved research protocol and informed consent document;
- related or possibly related to participation in the research; possible related means that there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research.
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

8.1.4. Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

8.1.5. Preexisting Condition
A preexisting condition is one that is present at the time of signing the consent form for the main study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.
8.1.6. **General Physical Examination Findings**

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

8.1.7. **Post-study Adverse Event**

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject’s personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study DCC of any death or adverse event occurring during the year after a subject has completed treatment.

8.1.8. **Abnormal Laboratory Values**

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management

8.1.9. **Hospitalization, Prolonged Hospitalization or Surgery**

Any adverse event that results in hospitalization should be documented and reported as a serious adverse event. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event and reported as a severe adverse event if hospitalization is required. Neither the condition, hospitalization, nor surgery is reported as an adverse event if the hospitalization was for diagnostic or elective surgical procedures for a preexisting condition.

8.2. **Recording of Adverse Events**

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document. All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs during the year after completion of treatment will similarly be recorded and reported.

8.3. **Reporting of Serious Adverse Events**

8.3.1. **Study Sponsor Notification by Investigator**

Clinical sites are required to report serious adverse events to the DCC, within 24 hours of first knowledge of the event. The DCC will facilitate the timely reporting and updates to regulatory authorities, the DSMB, NIH and the FDA according to the standard MedWatch guidelines. A
Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the DCC within 24 hours. The DCC will report all SAEs to the DSMB Chairman and DSMB safety Monitor within 48 hours of first knowledge of the event. The investigator will keep a copy of this SAE form on file at the study site. At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the investigator must provide further information on the serious adverse event in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on all ongoing serious adverse events should be provided promptly to the study sponsor.

8.3.2. IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.4. Unblinding

Treatment assignment will be blinded to all but a single designated "unblinded" physician at the trial site. Although testosterone treatment might increase the risk of certain diseases, such as prostate cancer, lower urinary tract symptoms due to benign prostatic hyperplasia, or erythrocytosis, the blind will not be broken even if a subject develops one of these conditions during the study. Instead, the following approach will be taken.

a. If a subject is diagnosed with prostate cancer during the study, treatment will be discontinued, whether the treatment is testosterone or placebo.

If the subject's score on the International Prostate Symptoms Score increases by > 5 points above the baseline value or to an absolute score of >19, suggesting worsening of lower urinary tract symptoms, the subject will be referred to a urologist for evaluation of medications that affect urine flow rate and for prostatitis. Treatment with an alpha blocker will be considered. If the subject’s score does not decrease below the above threshold in response to treatment, gel treatment will be discontinued.

b. If a subject develops a hemoglobin > or = 17.5 g/dL, he will be evaluated for causes of secondary erythrocytosis. If none are found, the dose of gel will be lowered. If the hemoglobin is still > or = 17.5 g/dL, treatment will be discontinued.

8.5. Stopping Boundaries

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Because of the considerations described in Section 7.5, Interim Monitoring, we would not want to base early stopping solely on the cancer cases, as these may be subject to substantial bias — any of the boundary scenarios outlined in Section 7.5. are possible without any true excess risk due to the likelihood of ascertainment bias. We shall ask the DSMB to consider the cancer data together with the interim efficacy data from all the trials. Should the interim data suggest no emerging benefit, the stopping boundary shown might be applied. If the interim data are consistent with potentially valuable effects of treatment, however, a somewhat greater imbalance in cancer cases might be tolerated. The proportion of cases on each arm that are of high grade (Gleason score ≥7) will also be a consideration, but the number of such cases we expect to observe in this trial will be small, perhaps a fourth of all cases. We shall also ask the DSMB to consider the extent to which ascertainment bias might affect the comparison of cancer rates in each arm, and in this regard shall present relevant data, eg the number of biopsies by arm and the proportion of cancers among those with biopsies.

### 8.6. Monitoring Subject Safety

#### 8.6.1. Potential Risks to Subjects

Several conditions to which elderly men are particularly prone are, at least partly, testosterone-dependent. These and other potential risks are described below:

**8.6.1.1. Prostate cancer.**

The basis for monitoring men in a testosterone trial for prostate cancer is that it is, to some degree, testosterone-dependent, and because elderly men often harbor clinically silent prostate cancer. There is no direct evidence, however, that either endogenous serum testosterone concentrations or testosterone treatment of men with low testosterone concentrations increases the risk of clinical prostate cancer.

**8.6.1.2. Prostate biopsy.**

Prostate biopsy will be performed if medically indicated. The two primary risks of this biopsy, which is performed by a transrectal, ultrasound guided approach, are bleeding and infection. By taking proper precautions, the risk of these complications requiring hospitalization is <1%.

**8.6.1.3. Benign prostatic hyperplasia.**

Testosterone treatment of elderly hypogonadal men might also increase the risk of lower urinary tract symptoms, because the prostate is a testosterone-dependent gland and because BPH is common in these men.

**8.6.1.4. Erythrocytosis.**

One potential consequence of testosterone treatment is the development of erythrocytosis. We shall therefore evaluate the men who participate in this study to determine if those whose hemoglobin values are normal before treatment experience an increase above normal (erythrocytosis) during treatment.
8.6.1.5. **Sleep apnea.**
Another potential risk is exacerbation of sleep apnea, although the evidence is weak.

8.6.1.6. **Physical function testing.**
There is a very small risk of injury from a fall or ankle sprain during the 6-minute walk test.

8.6.1.7. **Sexual function and vitality testing.**
The potential risks of these studies are the time the testing takes, minor distress of answering questions of a personal nature, and the fear of lack of confidentiality.

8.6.1.8. **Time burden.**
The large number of tests proposed could be tiring for an elderly man, especially one who participates in more than one protocol. In a pilot study in which 10 men, mean age 75 years, at each of three sites (30 men total) were administered all of the tests for all trials, the mean time for completion of all the tests was <100 min, and the subjects found most of the tests relatively easy. However, there was variability, and a few subjects took longer. Trial site personnel should be cognizant that some subjects could have difficulty in participating in multiple trials.

8.6.2. **Protection Against Risk**
Subject selection and monitoring procedures have been designed to minimize the risks. First, we shall select subjects who are at low risk of the potential side effects. Second, we shall employ procedures to minimize the potential risks. Third, we shall monitor enrolled subjects for the potential side effects.

8.6.2.1. **Erythrocytosis.**
A potential subject will be enrolled only if his hemoglobin is ≤16 g/dL. Men who enroll will be monitored by hemoglobin at 3, 6, and 12 months. An increase above the upper limit of normal (17.5 g/dL) in either treatment group will lead first to repeat measurements of hemoglobin and testosterone. If the serum testosterone level is above the target range, the gel dose will be decreased. If the repeat hemoglobin is still elevated, the subject will be referred for evaluation for causes of erythrocytosis and, if found, treatment. If no cause of secondary erythrocytosis is found, or, if erythrocytosis does not return to normal within one month, treatment will be discontinued. The external (unblinded) physician who evaluates subjects for erythrocytosis will consider all standard treatments including phlebotomy.

The exception to this is month 12, at which time all men stop treatment/placebo. At the month 12 visit, if a man has an elevated hemoglobin upon repeat, he will be brought back in after 3 months of being off of treatment. At which point it is expected his hemoglobin will have lowered. If the hemoglobin has not been lowered after 3 months, men will be referred.

8.6.2.2. **Prostate cancer.**
We shall exclude men with diagnosed prostate cancer or prostatic intraepithelial neoplasia (PIN). Men will also be excluded who have a >35% risk of having a prostate cancer and a >7% risk of having high grade prostate cancer by the Prostate Cancer Risk Calculator (http://www.compass.fhirc.org/edrnnci/bin/calculator/main.asp). This Risk Calculator will be used because it takes into account not only PSA, but also other known risk factors, including age, race, family
history, and previous biopsy and is therefore more conservative and exposes the subjects to
less risk than if exclusion were based only on PSA.

Risk will be reduced further by adjusting the baseline PSA concentrations upward to account for
the likelihood that those concentrations are lower than they would have been had the subjects’
testosterone concentrations been normal. Each man’s PSA will be adjusted to what would be
expected if his serum testosterone were 460 ng/dL. The adjusted PSA would then be used in
the Prostate Risk Calculator. Although adjusting the PSA for serum testosterone is not standard
clinical practice, we think this approach is preferable to using the unadjusted value because it
takes into account the physiologic relationship between testosterone and PSA and because, by
raising the PSA, it is more conservative.

The use of the Risk Calculator, instead of PSA alone, allows us to account not only for PSA, but
also other known risk factors, including age, race, family history, and previous biopsy and
therefore is more conservative and exposes subjects to less risk than if exclusion were based
only on PSA. It illustrates to a subject that every man of this age has some risk.

The rationale for choosing 35% of overall prostate cancer risk and 7% risk of high grade cancer
is two-fold: 1) It is low enough to be quite conservative. For example, for a 65 or 75 year-old
white man with no other risk factors to have a risk of ≤35%, his PSA would need to be ≤3.0
ng/mL, which would not be a cause for biopsy in routine clinical care. 2) It is high enough to
include enough subjects that recruitment will still be practical.

Subjects will be monitored during the one year of treatment by repeating the PSA measurement
at 3 and 12 months. An increment of ≥1.0 ng/mL above the corrected baseline PSA (for low
testosterone and 5-alpha reductase inhibitor usage) value will lead to referral for urologic
evaluation for consideration of prostate biopsy, confirmed by a repeat determination. Treatment
will be discontinued for any subject who is diagnosed as having prostate cancer during the trial.

8.6.2.3. **Benign prostatic hyperplasia.**
Men who have evidence of moderately severe lower urinary tract symptoms, i.e., a score of >19
on the International Prostate Symptom Score (IPSS) questionnaire, will be excluded. An
increase of >5 points or to an absolute value of >19 will result in a review of medications that
affect urine flow rates and evaluation for prostatitis. If a cause is found, it should be treated. If
no cause is found, treatment with an alpha blocker should be considered. If the subject is
treated and symptoms persist, or if acute urinary retention occurs, the subject should be
referred for urological consultation. If the urologist treats the subject and the score does not
decrease below the above thresholds, gel treatment will be discontinued.

8.6.2.4. **Cardiovascular disease**
Men will be monitored for the occurrence of cardiovascular events during the entire course of
the two-year trial. Treatment will be discontinued in men who have a myocardial infarction or
stroke. The number of subjects whose treatment is discontinued for serious adverse events will
be monitored and assessed with the DSMB.

8.6.2.5. **Sleep apnea.**
We shall exclude men who have diagnosed sleep apnea that is not being treated, and during
treatment we will question men for newly diagnosed but untreated sleep apnea.

8.6.2.6. **Physical and cognitive function testing.**
The small risk of physical and cognitive function testing will be minimized by training the
research assistants who perform the tests how to instruct the subjects how to perform the tests
properly. For the 6-minute walk, there will be a standardized protocol for warm-up and careful supervision of the subjects during the testing. The risks associated with cognitive testing are small and primarily consist of anxiety related to concerns about performance. Testers will be trained to encourage and reassure subjects that the tests are designed to be difficult for most people.

8.6.2.7. Sexual function and vitality testing.
We shall employ several means to minimize the burden of time, the minor distress of answering personal questions, and the perceived loss of confidentiality. Use of interactive voice response (IVR) for all vitality questionnaires and for the Harbor-UCLA 7-Day Questionnaire (the primary end point for sexual function) will allow the subjects to answer these questionnaires from their homes at their convenience and thereby reduce the time they spend at the trial sites. Their answers will also be anonymous this way, and not seen by trial site personnel. The subjects may refuse to answer a question that causes them discomfort or anxiety.

8.6.2.8. Time burden.
Subjects who qualify for more than one protocol will be offered the chance to participate in those for which they qualify, but they will also be told of the approximate time burden. Study staff will be taught to be mindful of a subject’s fatigue and to offer to a subject who appears fatigued the chance to resume testing on another day.

8.6.2.9. Prostate biopsy.
The standard precaution that minimizes the chance of bleeding during and after a prostate biopsy is avoiding agents that impair clotting, such as aspirin, nonsteroidal anti-inflammatory agents, and herbal supplements. The standard precaution that minimizes the risk of infection is administration of antibiotics.

8.6.2.10. Risk of using excessive testosterone gel.
No risk is expected if a subject takes a greater dose of AndroGel than prescribed, and any elevation of the serum testosterone from a single larger dose would be transient, i.e., 1-2 days. If a subject takes a larger dose than prescribed chronically, it would be detected in the serum testosterone measurements at 1, 2, 3, 6, 9 and 12 months, and the dose would be lowered.

8.6.3. Clinical Management of Participants
The T Trial Investigators recognize the obligation and importance of reporting information acquired during the research study visit to the health care provider (HCP) of participants. Participants and their HCP will be notified as soon as possible if potentially serious medical problems are identified during any of the T Trial procedures, or reported during a T Trial study visit.

8.6.3.1. Notification to Health Care Provider
Participants will be asked about several specific medical and cardiovascular events at each T Trial follow-up visit. They will be asked in the appropriate lay terms if they have experienced any of the signs and symptoms of angina and transient ischemic attack. If possible, this information will be evaluated by a T Trial physician who will determine the appropriate disposition. If the participant reports that he has not informed his HCP, the T Trial staff will notify the participant’s HCP (with the participant’s permission) by fax or email, as soon as possible. If determined necessary, the participant will be transported to the emergency department or escorted to an urgent care hospital visit for further evaluation and/or treatment.
If the participant is unable to identify a primary care physician or HCP, the site staff will identify one within the site’s medical institution.

### 8.6.4. Data and Safety Monitoring Board.

An external DSMB will be established to monitor all aspects of the study. The Board will consist of experts in geriatrics, biostatistics, clinical trials, endocrinology, and prostate disease. The DSMB members will not be affiliated with the study and will be appointed by the NIA Director in consultation with the principal investigator. The Board will meet every six months to review subjects’ safety, study progress and data integrity and completeness. After each meeting, the DSMB will provide the NIA Director with its recommendations, and the Director will decide whether or not to accept them.

### 9. Data Management

#### 9.1. Data Management System

The Data Coordinating Center (DCC) at the University of Pennsylvania will develop a data management system for the collection, storage and management of data. This system will be developed using Oracle Corporation’s suite of pharmaceutical applications. The data management systems will use a combination of tools to perform the following study functions:

- **Subject tracking** – to monitor recruitment and provide visit schedules for subjects and composite visit schedules for clinical sites.
- **Eligibility determination** - to evaluate screening data (serum testosterone, PSA, etc.) to determine eligibility for one or more efficacy areas.
- **Treatment allocation** - to allocate subjects to receive testosterone or placebo and to balance the treatment groups based on the minimization technique.
- **Dose modification** – to identify out-of-range testosterone levels.
- **Specimen tracking** - to document specimens from collection and processing to storage and retrieval.

#### 9.2. Data Entry

Electronic data entry will be used primarily to achieve accuracy and efficiency. The following methods will be utilized:

- Remote data capture will permit authorized personnel to enter data remotely via a secure Internet connection.
- Electronic data transfer methods will be developed and tested to ensure that data are completely and accurately transmitted. This will include data transferred from the central laboratory and associated reading centers, as well as data collected via the Interactive Voice Response System (IVRS).

#### 9.3. Data Quality

Oracle Clinical includes a data quality module to identify incorrect data based on a set of rules that describe the expected data. The DCC will collaborate with the investigative team to establish these parameters for primary and secondary outcomes, safety, regulatory, and descriptive values. The data management team will develop a data validation plan, rule set specifications, and programming logic to implement data validation rules. The DCC staff will interact with clinical site staff to verify queried data and track all queries to resolution.
9.3.1. Quality Control Activities

The Quality Control Committee and the DCC will develop a quality assurance and control plan that ensures that study data are as precise and reliable as possible.

**Manual of Procedures (MOP)** - The MOP will describe the sequence of study conduct and provide detailed instruction for the performance of screening, baseline, enrollment, treatment allocation and follow-up procedures. The MOP will provide instruction in case report form completion, use of the electronic data management system, and collection, documentation and transfer of specimens and tests to laboratories and reading centers.

**Training and certification procedures** - The DCC will conduct a training session before the study starts to train and certify personnel in the performance of study procedures.

**Site visits** – Findings from site visits will be used to resolve problems and develop corrective action plans.

**External data sources** - The DCC will monitor quality control of data received from study laboratories and reading centers.

**Internal quality control procedures** - A data validation plan, rule set specifications, and programming logic to implement data validation rules will be implemented.

9.3.2. Routine reports

The DCC will develop a set of standard enrollment, tracking, quality review, and safety monitoring reports. Adverse event reports, DSMB reports and reports for statistical analysis will be developed and produced on an appropriate schedule.

9.4. Data Security

The data management system will be designed to prevent unauthorized access to trial data and to prevent data loss due to equipment failure or catastrophic events. The procedures to do so encompass user account management, user privilege assignment, data loss prevention (database backup), computer systems validation, performance monitoring, and DMS change management. User access will be controlled by assignment of confidential usernames, passwords and role assignment. The system will meet the applicable Federal regulatory requirements and those described in the E6 Good Clinical Practice Guidelines to ensure the confidentiality of trial subjects.

Study data collected at the clinical sites will be entered into a web based data management system. This data management system uses a secure connection between the client browser at the clinical site and the web server at the DCC. Data transmitted over this connection is authenticated by the use of digital certificates and is encrypted as it travels the Internet to the DCC.

Electronic files containing data from hand held devices, the central laboratory, or the central reading center will be transferred to the DCC using secure FTP technology. The DCC team will maintain a secure FTP server. The files transmitted using this method will be encrypted during the exchange.

The DCC project team will collaborate with the Investigational Drug Service (IDS) and the biostatistics team to protect the blinding of treatment assignments and electronic access to information that could indirectly or directly lead to unblinding treatment assignment or codes. Internal access to such information is stored in password-protected files. Documentation is
stored in the locked files of the IDS at the University of Pennsylvania. Within the DCC this information is locked in files to which only department managers have access.

9.4.1. Maintaining Anonymity of Submitted Medical Records
Clinical site personnel will de-identify all medical records before sending them to the DCC by obliterating any Protected Health Information (PHI). Upon receipt, DCC personnel will review the records to ensure that no PHI is visible.

9.4.2. Confidentiality
Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Subjects will be asked to provide their Social Security Number (SSN) for the purpose of tracking their status in the National Death Index in the event they become lost to follow-up. This information will be locked in a secure location with access limited to the TTrial staff only. It will not be entered or stored in the electronic system and will be used for this purpose only. Subjects may refuse to provide this information without consequence to their study participation.
References

26. Lamar M, Resnick SM, Zonderman AB 2003 Longitudinal changes in verbal memory in older adults: distinguishing the effects of age from repeat testing. Neurology 60:82-86


### Appendix A – Questionnaire and Procedure Schedule

#### T Trial Questionnaires & Procedures  [Revised 8/27/10]

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<td>Weight, waist, hip, blood pressure measures, [Ht.(SV 2 and M12 only)]</td>
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#### All Subjects – Primary End Points

|6 Minute Walk Test| X | X | X | X | X | X | | | | | | |
|UCLA Sex Diary – Question 4** (+ entire diary after SV2 only)| X+ | X | X | X | X | | | | | | |
|FACIT Fatigue**| X | X | X | X | | | | | | | |
|WMS-R-LM-II (verbal memory - paragraph recall)| X | X | | | | | | | | | | |
|PANAS*| X | X | X | | | | | | | | | |
|Falls| X | X | X | X | | | | | | | |
|PHQ-9** (on paper at SV2 followed by IVR)| X | X | | X | X | X | | | | | | |
|DISF-M-II| X | | | | | | | | | | | |
|3MSE| X | | | | | | | | | | | |
|MMSE| X | | | | | | | | | | | |
|MAC-Q| X | X | | | | | | | | | | |
|SF-36| X | | | | | | | | | | | |
|Trial specific - Global Impression questions**| X | X | X | | X | X | | | | | | |
|General - Global Impression question**| X | X | X | | | | | | | | | |

#### Tests in Subjects in Specific Trials: Secondary End Points (except PF-10-all men)

|Physical: PF-10| X | X | X | X | | | | | | | | |
|Sexual: UCLA Sex Diary** (complete), IIEF, DISF-M-II| X | X | X | | | | | | | | | |
|Vitality: SF-36-Vitality**| X | X | X | | | | | | | | | |
|Cognitive: BVRT, Card rotation, TMT| X | X | | | | | | | | | | |

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V7.3 20130716 43
Partnership for Anemia: Clinical and Translational Trials in the Elderly

Protocol

Examining the Effects of Testosterone Treatment on Anemic and Non-Anemic Elderly, Hypogonadal Men

An Adjunctive Analysis of Data Derived From the T-Trial

Compiled by:
The PACTTE Consortium

Version 1.0: January 13, 2014

Distributed by the PACTTE Data Coordinating Center:
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Core Lab A
University of Utah
## Protocol Version and Amendment Tracking

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Protocol Approval Page

Examining the Effects of Testosterone Treatment on Anemic and Non-Anemic Elderly, Hypogonadal Men; An Adjunctive Analysis of Data Derived From the T-Trial

Phase of Study:
Protocol: PACTTE_04
Version: XXXXX
Date of Issue: XX XXXXXX 2014

Study Sponsor: PACTTE Consortium for the National Institute on the Aging (NIA)

We, the undersigned, have read and approve this protocol and agree on its content.

William Ershler, MD

Don Jurivich, DO

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Investigator Statement

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will personally oversee the conduct of this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the PACTTE consortium. I will discuss this material with them to ensure that they are fully informed about the conduct of the study. I am aware that, before beginning this study, the institutional review board responsible for such matters must approve this protocol in the clinical facility where it will be conducted. I agree to make all reasonable efforts to adhere to the attached protocol.

I agree to provide all subjects with informed consent forms, as required by government and International Conference on Harmonization regulations. I further agree to report to the sponsor any adverse experiences in accordance with the terms of this protocol and Food and Drug Administration regulation 21 CFR 312.64.

Principal Investigator Name ___________________________ Signature ___________________________

Date ___________________________
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1 OBJECTIVES

1.1 Hypothesis:
Elderly men with low testosterone levels and unexplained anemia who are treated for one year with testosterone will have higher proportion of $\geq 1.0$ g/dL increase of hemoglobin level from baseline to one year than elderly men with unexplained anemia in the placebo-treated control group.

1.2 Primary Objective:
- To test whether the proportion of elderly hypogonadal men with preexisting Unexplained Anemia of the Elderly (UAE) with an increase in hemoglobin of $\geq 1.0$ g/dL is greater for men treated with testosterone than for men treated with placebo.

Secondary Objective:
- To test whether the continuous change in hemoglobin in elderly hypogonadal men with preexisting UAE is greater for men treated with testosterone than for men treated with placebo.

1.3 Functional Exploratory Objectives:
- To test whether the benefit (i.e. improvement of hemoglobin) of 12 months of testosterone treatment in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on hemoglobin for elderly hypogonadal men without UAE.
- To test whether the benefit (i.e. improvement of the 6MWT) of 12 months of testosterone treatment in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on 6MWT for elderly hypogonadal men without UAE.
- To determine whether the benefit (i.e. improvement of the 6MWT) of 12 months of testosterone treatment in elderly hypogonadal men is mediated by an improvement in hemoglobin concentration for men with UAE and men without UAE.
- To test whether the benefit on verbal memory (i.e. improvement of WMS-R LM II) of 12 months of testosterone treatment in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on WMS-R LM II for elderly hypogonadal men without UAE.
- To test whether the benefit (i.e. improvement of WMS-R LM II) of 12 months of testosterone treatment in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on fatigue for elderly hypogonadal men with UAE.
- To test whether the benefit of 12 months of testosterone treatment on fatigue (i.e. improvement of 13 item FACIT-Fatigue Scale) in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on fatigue for elderly hypogonadal men without UAE.
- To test whether the benefit of 12 months of testosterone treatment on fatigue (i.e. improvement of 13 item FACIT-Fatigue Scale) is mediated by an improvement in hemoglobin for men with UAE and men without UAE.
- To test whether testosterone-induced erythrocytosis, as defined by a rise in hemoglobin level to $\geq 16$ g/dL, has detrimental effects on 6MWT performance.
1.4 Mechanistic Exploratory Objectives:

- To characterize changes in proteins regulating iron homeostasis including hepcidin, serum ferritin, serum iron, and soluble hemojuvelin in response to testosterone treatment in elderly hypogonadal men with UAE.
- To characterize changes in markers of erythropoiesis (erythropoietin and soluble transferrin receptor) in response to testosterone treatment in elderly hypogonadal men with UAE.
- To characterize changes in inflammatory cytokines and to determine whether they modify the erythropoietic response to testosterone replacement in elderly hypogonadal men with UAE.

2 BACKGROUND AND RATIONALE

Anemia Prevalence in the Elderly
Anemia is a common problem in the elderly. The prevalence of anemia is approximately 10% in community-dwelling men and women aged 65 and older, rising to 20-30% in those aged 85 and above (1). The United States population as a whole is both expanding in size and aging. In 2006, those aged 65 and older were estimated to comprise approximately 12% of the United States population (that is, 37 of 299 million total population (2)). By 2030, this proportion is estimated to rise to approximately 20% of the U.S. population (that is, 71 of 363 million). Thus, in the year 2030, we can reasonably anticipate that approximately 7.1 million adults over the age of 65 will be anemic.

Mechanisms to Account for Anemia in the Elderly
In younger patients with anemia, a causative explanation is most often readily apparent (e.g., iron deficiency, overt inflammatory disease, renal failure, etc.). However, with advancing age an increasing percentage of anemic patients lack a clearly definable pathogenesis. Among those residing in the community, of the 11% who met criteria for anemia, approximately 1/3 fell into this category of ‘undefined’ anemia (1). Although that data was derived from an observational cohort in which additional hematological parameters (e.g., reticulocyte count, lactate dehydrogenase [LDH], etc.) were not available, several additional studies have reported similar findings (3-12). In fact, with advancing age beyond 65 years, and with increasing features of frailty, the prevalence of all anemia, and the proportion of ‘unexplained’ anemia in particular, increases (4, 11).

UAE is characteristically mild to moderate (hemoglobin levels 9 g/dL to12.7 g/dL in men) with normocytic red cell indices. For the interventional trials conducted by the PACTTE consortium (see below) we have adapted a comprehensive clinical evaluation to exclude known causes of anemia (Appendix A). It is our current hypothesis that UAE is an amalgam of several age-associated processes; the sum total of which results in hypoproliferative or dysregulated erythropoiesis. Included would be a reduced erythropoietin response, reduced stem or progenitor cell proliferative capacity, the inhibitory effect of chronically up-regulated inflammation and diminished bioavailable androgens.

Consequences of Anemia in the Elderly
Although hemoglobin levels are often only mildly reduced, particularly in those elderly patients with UAE, the geriatric literature is now rife with associated adverse consequences including impaired quality of life (13), mobility and other functional impairments (14-16), falls (17) and even mortality (18, 19). Yet, interventional studies demonstrating reversal of adverse consequences with correction of anemia remain to be reported.
PACTTE
The Partnership for Anemia: Clinical and Translational Trials in the Elderly (PACTTE) is a consortium of eight clinical sites focusing upon the pathogenesis and treatment of unexplained anemia in the elderly. The consortium intends to conduct several carefully conducted clinical trials over a five year period, each addressing one or another of the possible mechanisms thought to contribute to UAE with particular focus on clinically important functional parameters.

Rationale for Current Research
As mentioned above, age-associated androgen deficiency has been considered one of those mechanisms contributing to the pathogenesis of UAE (20). It has been known for several decades that androgens influence erythropoiesis (21), and that low levels of circulating testosterone are associated with anemia in older people (22) and particularly in those with UAE (23).

3 BACKGROUND CONCERNING TESTOSTERONE TRIALS (TTrial) GOALS AND STUDY DESIGN

As men get older, they experience many conditions, often together, that eventually result in the inability to perform many activities of daily living, an increased propensity to fall, and decreased independence. These conditions include mobility disability and low vitality. Elderly men also experience increased anemia, metabolic syndrome, decreased sexual function and memory impairment. These conditions likely have multiple causes, but one cause that could contribute to all of them is a low serum testosterone concentration. When young hypogonadal men are treated with testosterone, they experience improvements in sexual function, muscle mass and strength, bone mineral density, sense of well-being, and anemia. However, the benefits of testosterone therapy in older men with age-related decline in testosterone concentration are not known and are the subject of this investigation.

The primary specific aims of the coordinated set of randomized, placebo-controlled clinical trials are to test the hypotheses that testosterone treatment of elderly men whose serum testosterone concentrations are unequivocally low – and who have symptoms and/or objectively measured abnormalities that could be due to low testosterone (physical or sexual function, vitality, cognition, or anemia) – will result in more favorable changes in those abnormalities than placebo treatment. Thus, the overall trials are highly coordinated, but each trial has its own primary, and exploratory specific aims.

The TTrial was designed as seven separate, but highly coordinated, randomized, placebo-controlled clinical trials of the effect of testosterone in men ≥65 years who had a low serum testosterone concentration and symptoms and objective manifestations of abnormalities in the areas of physical function, sexual function, vitality and/or cognition. Enrolled subjects received either testosterone (Androgel®, AbbVie, No. Chicago, IL) or control gel for one year, and were followed for an additional year. The study was conducted at 12 clinical sites across the United States. The data-coordinating center was at the University of Pennsylvania and they managed the trial activity, laboratory, pharmacy and other activities of this complex program.

Enrolled subjects were evaluated at 3 month intervals by a variety of outcome measures, some of which are common for all studies, and others are unique for each study. Blood was drawn at 3 month intervals and serum/plasma samples were frozen in 0.5 cc aliquots and stored at -80°C at the central laboratory facility (Quest Diagnostics).

4 STUDY DESIGN AND PROCEDURES
The proposed research is a collaborative effort between TTrial and PACTTE investigators aimed primarily to examine the erythropoietic effects of testosterone treatment administered to hypogonadal anemic and non-anemic subjects. Although the mechanisms regarding the effect of administered testosterone on erythroid kinetics are likely to be similar, the groups (anemic and non-anemic) will be studied separately.

4.1 T-Trial Anemia Cohort

TTrial-enrolled subjects who meet PACTTE criteria for anemia (hemoglobin of <12.7 g/dL) and an equal number of randomly-selected non-anemic TTrial subjects will be examined in this protocol. Data from anemic subjects will be examined in two phases. First, additional tests will be conducted to define the cause of anemia (Section 4.1.1), and second, exploratory studies will be undertaken to help understand the mechanisms whereby testosterone treatment influences erythropoiesis (Section 4.1.4). Non-anemic subjects selected for study will also be examined by the studies described in Section 4.1.4

4.1.1 Defining Anemia

Pretreatment laboratory results, including red blood cell indices and other components of the complete blood count (CBC) will be made available from Quest laboratories. Additionally, stored serum will be used to perform the following analyses:

<table>
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<td>Complete blood count and red blood cell indices</td>
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<tr>
<td>Serum iron[8]</td>
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<tr>
<td>Total iron binding capacity[8]</td>
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<td>Serum ferritin[8]</td>
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<td>Vitamin B12[1]</td>
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<td>Serum folate</td>
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<td>Serum creatinine[1] and estimated glomerular filtration rate (MDRD[3])</td>
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<td>SPEP/UPEP as indicated</td>
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<tr>
<td>CRPhs, inflammatory cytokines[1]</td>
</tr>
</tbody>
</table>

[8] If B12 is less than 200 pg/mL, a methylmalonic acid serum level will be measured.
[1] For subjects with borderline measures of iron deficiency (e.g., transferrin saturation of 15-25% or serum ferritin level 20-100 ng/mL) a soluble transferrin receptor assay will be obtained.
[3] MDRD: Four variable Modification of Diet and Renal Disease, 186.3 X Serum Creatinine–1.15 X age–0.203 X (0.742 if female) X (1.212 if black) (online calculator: http://www.kidney.org/professionals/KDOQI/gfr_calculator.cfm)

From the above data, including RBC indices, white blood cell number and differential and platelet count as well as the above laboratory measures enrolled subjects will be classified (see Appendices A and B):
Of these, we expect approximately one half will meet criteria for UAE, and the majority of the others will either have evidence for iron deficiency, inflammation or mixed ACI and IDA. These latter three categories will be examined in parallel in the following studies.

Three PACTTE hematologists, using criteria as outlined in Appendix A, will determine the anemia classification independently. All efforts will be made to arrive at a consensus diagnosis for each case. If consensus is not reached, a fourth PACTTE hematologist will review the case and the subject will be classified as UAE if three of the four consultants agree.

4.1.2 Testing the Primary Hypothesis

- To test whether the proportion of elderly hypogonadal men with preexisting Unexplained Anemia of the Elderly (UAE) with an increase in hemoglobin of ≥ 1.0 g/dL is greater for men treated with testosterone than for men treated with placebo.

4.1.3 Exploratory Objectives

These analyses will assess whether UAE status modifies the testosterone effect on the response of hemoglobin and functional outcomes (effect modification). These analyses will also assess whether the
effects of testosterone on functional outcomes are **mediated** by hemoglobin. Because we expect there may be varying responses of hemoglobin to testosterone that result from underlying differences in the pathogenesis of anemia, these analyses will be performed on the following groups:

- Non-anemic
- Anemic of known causes
- Unexplained Anemia of the Elderly (UAE)

### 4.1.3.1 UAE status as an Effect Modifier of Erythroid Response

- To test whether the benefit (i.e., increase in hemoglobin concentration) of 12 months of testosterone treatment, in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment for elderly hypogonadal men without anemia, or with anemia of known causes (listed in Appendix A).
  - For this, the effect of testosterone treatment versus placebo treatment in UAE subjects will be compared to testosterone treatment versus placebo treatment in all non-UAE groups (i.e. non-anemic and all non-UAE anemic groups combined) after one year of treatment.

### 4.1.3.2 6 minute walk test

- To test whether the benefit (i.e. improvement of 6MWT) of 12 months of testosterone treatment in elderly hypogonadal men with UAE is different than the benefit of testosterone treatment on 6MWT for elderly hypogonadal men without anemia or with anemia of known causes (listed in Appendix A).
  - For this, the effect of testosterone treatment versus placebo treatment in UAE subjects will be compared to testosterone treatment versus placebo treatment in each of the other groups (i.e. non-anemic and all non-UAE anemic groups combined) after one year of treatment.

- To determine whether the benefit (i.e. improvement of 6MWT) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by an improvement in hemoglobin concentration for non-anemic, UAE, and non-UAE (i.e. non-UAE anemic groups listed in Appendix A) groups combined.
  - If we do not find an effect of testosterone on hemoglobin concentration in any group, then we will not perform this objective for that particular group.

### 4.1.3.3 Verbal Memory

- To test whether the benefit (i.e. improvement of WMS-R LM II) of 12 months of testosterone treatment on verbal memory in elderly hypogonadal men with UAE is different than the effect of testosterone treatment on WMS-R LM II for elderly hypogonadal men without anemia or with anemia of other causes.
  - For this, the effect of testosterone treatment versus placebo treatment in UAE subjects will be compared to testosterone treatment versus placebo treatment in each of the other groups (i.e. non-anemic and all non-UAE anemic groups combined listed in Appendix A) after one year of treatment.

- To determine whether the benefit (i.e. improvement of WMS-R LM II) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by an improvement in hemoglobin concentration for non-anemic, UAE, and non-UAE (i.e. all non-UAE anemic groups listed in Appendix A) groups combined.
  - If we do not find an effect of testosterone on hemoglobin concentration in any group, then we will not perform this objective for that particular group.
4.1.3.4 Fatigue

- To test whether the benefit (i.e. improvement of 13 item FACIT-Fatigue Scale) of 12 months of testosterone treatment on fatigue in elderly hypogonadal men with UAE is different that the effect of testosterone treatment on fatigue for elderly hypogonadal men without anemia or with anemia of other causes.
  
  o For this, the effect of testosterone treatment versus placebo treatment in UAE subjects will be compared to testosterone treatment versus placebo treatment in each of the other groups (i.e. non-anemic and all non-UAE anemic groups combined listed in Appendix A) after one year of treatment.

- To determine whether the benefit (i.e. improvement of 13 item FACIT-Fatigue Scale) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by an improvement in hemoglobin concentration for non-anemic, UAE, and non-UAE (i.e. all non-UAE anemic groups listed in Appendix A) groups combined.
  
  o If we do not find an effect of testosterone on hemoglobin concentration in any group, then we will not perform this objective for that particular group.

4.1.3.5 Erythrocytosis

- To test whether testosterone-induced erythrocytosis, as defined by a rise in hemoglobin level to ≥16 g/dL, has detrimental effects on 6MWT performance.
  
  o For this, the 6MWT performance for testosterone-treated subjects with a rise in hemoglobin level to ≥ 16 g/dL will be compared to 6MWT performance for testosterone-treated subjects with a rise in hemoglobin that stayed below 16 g/dL after one year of treatment, assuming there are at least 10 subjects in each group.

4.1.4 Mechanistic Exploratory Objectives to Define Erythroid Kinetics and Responses

If we find an effect of testosterone on hemoglobin in elderly hypogonadal men with UAE, we will perform the following objectives. They will be limited to UAE subjects, only, because of the cost associated with additional assays to quantify key biomarkers. We will not consider these objectives for non-anemic subjects or for non-UAE anemic subjects.

4.1.4.1 Iron metabolism

Erythropoiesis is tightly linked to iron metabolism since iron is required for the synthesis of heme for hemoglobin. Though the molecular mechanisms that drive expansion of the erythroid compartment with testosterone treatment are not well-defined, we expect that absorption and utilization of iron must increase to accommodate the expansion.

Hepcidin is a peptide hormone produced by the liver that negatively regulates the amount of iron available to the erythron. It binds ferroportin, the only transporter known to facilitate cellular iron egress, and induces its internalization and degradation. Thus, through its interaction with ferroportin, hepcidin acts to reduce serum iron and increase macrophage iron stores. Serum hepcidin must decrease to allow for an increase in iron available for erythropoiesis.

Serum ferritin is a marker of iron stores which is often positively correlated with hepcidin expression (24). Serum iron is a marker of iron available for erythropoiesis. It is often inversely correlated with hepcidin expression in disease states.
HJV is a positive regulator of hepcidin expression. It is primarily expressed in the liver, skeletal muscle, and heart (25). A soluble form, sHJV, can be detected in human serum (26). The source of sHJV is unknown, but sHJV has been shown to negatively regulate hepcidin expression. Considering the relatively large requirement of skeletal muscle for iron in oxidative phosphorylation enzymes and in myoglobin, sHJV may be an additional systemic regulator of iron homeostasis that communicates the iron needs of the muscle (27). Initial testing of this hypothesis in mice has not demonstrated that muscle HJV is required for iron homeostasis (28, 29), though a role for sHJV under conditions of changing muscle physiology (e.g. exercise, hypertrophy, or sarcopenia) has not been investigated.

In a study comparing testosterone treatment of young men to older men, older men were characterized by decreased testosterone and increased hepcidin at baseline. Consistent with increased hepcidin, these men also had increased ferritin at baseline. The same study demonstrated that serum hepcidin decreased within one week of testosterone administration and that serum ferritin was positively correlated with hepcidin change after 16 weeks of testosterone treatment (30). We will test serum hepcidin concentration in men with UAE treated with testosterone at baseline and after 1 month and 12 months of testosterone treatment. Bachman and colleagues demonstrated hepcidin concentrations were lower than baseline at 4 and 8 weeks after the initiation of testosterone administration. We expect the three month time point will not allow us to appropriately capture the nadir of hepcidin expression. Additionally, this study demonstrated that the change in lean muscle mass was significantly correlated with the change in hepcidin after 16 weeks of treatment in both young and older men.

Our hypothesis concerning the effect of testosterone on iron regulation is that low testosterone results in increased hepcidin expression and iron-restricted erythropoiesis in hypogonadal men with pre-existing UAE. We further hypothesize that increasing testosterone will result in decreased hepcidin and improved iron utilization and erythropoiesis in men with UAE. We will explore this hypothesis in four separate steps. First, we intend to examine the relationship among testosterone, iron regulatory markers (log hepcidin, hemoglobin, log serum ferritin, serum iron, sHJV), and sites of iron utilization (lean muscle mass). This is accomplished by exploring the correlations among these variables:

- at baseline and
- at month 12 as well as
- the correlations among the changes of these variables from baseline to month 12

in men with UAE. We will determine these correlations separately for testosterone treated and placebo groups. In particular, for each treated group, we aim to test if:

- Testosterone at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12)
- Log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with hemoglobin at baseline (or at month 12 or change from baseline to month 12)
- Log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12) is positively correlated with log transformed serum ferritin at baseline (or at month 12 or change from baseline to month 12)
- Log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with serum iron at baseline (or at month 12 or change from baseline to month 12)
- Log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with sHJV at baseline (or at month 12 or change from baseline to month 12)
- sHJV at baseline (or at month 12 or change from baseline to month 12) is positively correlated with lean muscle mass in men with UAE at baseline (or at month 12 or change from baseline to month 12)
Log transformed hepcidin at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with lean muscle mass in men with UAE at baseline (or at month 12 or change from baseline to month 12).

For the 7 correlations above, we will test whether there is a significant difference between the testosterone-treated and placebo group for each of the individual correlations.

In the second step, we will investigate whether iron regulatory markers (log hepcidin, log serum ferritin, serum iron, sHJV) are immediately regulated in response to testosterone in men with UAE. We expect a decline of log hepcidin in the testosterone treated group in the first month based on a previous report (31) where hepcidin after 4-8 weeks of testosterone treatment in older men was approximately 47.6% of baseline (95% CI, 33.3–67.9), i.e., approximately $\log(0.476) = -0.74$ decline in log hepcidin. Similarly we expect to see a decline in serum ferritin, an increase in serum iron and an increase in sHJV. We will compare these changes from baseline to month 1 between the testosterone treated and placebo groups. Specifically, we aim:

- To test whether the decline in log transformed hepcidin between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.
- To test whether the decline in log transformed serum ferritin between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.
- To test whether the increase in serum iron between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.
- To test whether the increase in sHJV between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.

In a third step we will assess whether the 12 month effect of testosterone on hemoglobin concentration is mediated by changes in iron regulatory markers. We aim to determine if the benefit (improvement of hemoglobin) in elderly hypogonadal men is mediated by

- A decline in hepcidin between baseline and one month.
- A decline in serum ferritin between baseline and one month.
- An increase in serum iron between baseline and one month.
- An increase in sHJV between baseline and one month.

In the fourth step, we expect that the immediate change (from baseline to month 1) in iron regulatory markers will predict eventual erythropoiesis in men with UAE at month 12, but with stronger association for the testosterone treated group than the placebo group. Four linear regression models, one for each of the four iron regulatory markers (log hepcidin, log serum ferritin, serum iron and sHJV), will be used for exploring these relationships. In each regression model for a given iron regulatory marker, the dependent variable is the change in hemoglobin between baseline and month 12 and the independent variables are treatment group indicator, change of the marker from baseline to month 1, the interaction of treatment group and change of the marker, baseline hemoglobin, and baseline iron regulatory marker. With these regression models, we aim:

- To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in log transformed hepcidin concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.
- To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in log transformed serum ferritin concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.
To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in serum iron concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in sHJV concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

**4.1.4.2 Erythropoietin (Epo) levels and responses**

Epo is the primary survival factor for erythroid progenitors, but testosterone has not been shown to reproducibly change serum Epo concentrations (31). Instead, low testosterone may impair the ability of erythroid progenitors to respond appropriately to available Epo. sTfR can be a useful marker for erythroid mass or erythropoietic activity, though sTfR will also increase in response to iron deficiency (32). Our hypothesis concerning the effect of testosterone on erythropoiesis is that low testosterone correlates with high Epo expression and impaired erythropoiesis in hypogonadal men with pre-existing UAE. We further hypothesize that increasing testosterone will result in decreased Epo and improved erythropoiesis in men with UAE. We will explore this hypothesis in three separate steps. First, we intend to examine the relationship among testosterone and markers of erythropoiesis (hemoglobin, absolute reticulocytes, Epo, and sTfR). This is accomplished by exploring the correlations among these variables:

- **at baseline**
- **at month 12** as well as
- the correlations among the **changes of these variables from baseline to month 12**

in men with UAE. We will determine these correlations separately for testosterone treated and placebo groups. In particular, for each treated group, we aim:

- To test whether log transformed Epo at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with testosterone at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed Epo is negatively correlated with hemoglobin at baseline (or change from baseline to month 12) but positively correlated with hemoglobin at month 12.
- To test whether log transformed Epo is negatively correlated with absolute reticulocytes at baseline (or change from baseline to month 12) but positively correlated with absolute reticulocytes at month 12.
- To test whether sTfR at baseline (or at month 12 or change from baseline to month 12) is positively correlated with testosterone at baseline (or at month 12 or change from baseline to month 12)
- To test whether sTfR at baseline (or at month 12 or change from baseline to month 12) is positively correlated with hemoglobin at baseline (or at month 12 or change from baseline to month 12)
- To test whether sTfR at baseline (or at month 12 or change from baseline to month 12) is positively correlated with absolute reticulocytes at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed Epo is negatively correlated with sTfR at baseline (or change from baseline to month 12) but positively correlated with sTfR at month 12

For the 7 correlations above, we will test whether there is a significant difference between the testosterone-treated and placebo group for each of the individual correlations.

In the second step, we will investigate whether markers which signal erythropoietic activity (absolute reticulocytes, Epo and sTfR) are **immediately** regulated in response to testosterone in men with UAE. We expect to observe a decrease in Epo and an increase in sTfR. We aim:
To test whether the increase in absolute reticulocytes between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.

To test whether the decrease in log transformed Epo between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.

To test whether the increase in sTfR between baseline and month 1 of treatment will be greater in the testosterone treated group of men with UAE than the placebo treated group of men with UAE.

In a third step we will assess whether the 12 month effect of testosterone on hemoglobin concentration is mediated by changes in erythropoietic activity. We aim to determine if the benefit (i.e. improvement of hemoglobin) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by:

- An increase in absolute reticulocytes between baseline and one month.
- A decline in Epo between baseline and one month.
- An increase in sTfR between baseline and one month.

In the fourth step, we expect that the immediate change (from baseline to month 1) in markers of erythropoietic activity will predict eventual erythropoiesis in men with UAE at month 12, but with stronger association for the testosterone treated group than the placebo group. Three linear regression models, one for absolute reticulocytes, one for log Epo and one for sTfR, will be used for exploring these relationships. In each regression model for a given marker of erythropoiesis, the dependent variable is the change in hemoglobin between baseline and month 12 and the independent variables are treatment group indicator, change of the marker from baseline to month 1, the interaction of treatment group and change of the marker, baseline hemoglobin, and baseline marker of erythropoiesis (absolute reticulocytes, Epo or sTfR). With these regression models, we aim to estimate changes in hemoglobin between baseline and month 12 that correspond to:

- 1 unit change in absolute reticulocyte number between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

- 1 unit change in log transformed Epo concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

- 1 unit change in sTfR concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

### 4.2 Inflammatory mechanisms

Anemia occurs in the context of infections and chronic disease (33). The cytokine Interleukin-6 (IL-6) is most closely linked to the development of anemia in a number of disease states (34-36). IL-6 has also been shown to increase with age (37-39), consistent with the hypothesis that IL-6 may promote anemia in older adults (40). C-reactive protein (CRP) is induced by IL-6 and has been widely associated with aging and disease (41). Testosterone has been shown to decrease IL-6 (42-45). Our hypothesis concerning the effect of testosterone on inflammation is that low testosterone results in increased inflammation and impaired erythropoiesis in hypogonadal men with pre-existing UAE. We further hypothesize that increasing testosterone will result in decreased inflammation and improved erythropoiesis in men with UAE. We will explore this hypothesis in three separate steps. First, we intend to examine the relationship among testosterone, hemoglobin, and markers of inflammation (IL-6 and CRP). This is accomplished by exploring the correlations among these variables:

- at baseline and
at month 12 as well as
the correlations among the changes of these variables from baseline to month 12

in men with UAE. We will determine these correlations separately for testosterone treated and placebo groups. In particular, for each treated group, we aim:

- To test whether log transformed IL-6 at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with testosterone at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed IL-6 at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with hemoglobin at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed CRP at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with testosterone at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed CRP at baseline (or at month 12 or change from baseline to month 12) is negatively correlated with hemoglobin at baseline (or at month 12 or change from baseline to month 12)
- To test whether log transformed IL-6 at baseline (or at month 12 or change from baseline to month 12) is positively correlated with log transformed CRP at baseline (or at month 12 or change from baseline to month 12)

For the 5 correlations above, we will test whether there is a significant difference between the testosterone-treated and placebo group for each of the individual correlations.

In the second step, we will investigate whether markers of inflammation (IL-6 and CRP) are immediately regulated in response to testosterone in men with UAE. We expect to observe a decline in IL-6 and CRP. We aim:

- To test whether the decline in log transformed IL-6 between baseline and month 1 of treatment will be greater in the testosterone treated group of UAE men than the placebo treated group of men with UAE
- To test whether the decline in log transformed CRP between baseline and month 1 of treatment will be greater in the testosterone treated group of UAE men than the placebo treated group of men with UAE

In a third step we will assess whether the 12 month effect of testosterone on hemoglobin concentration is mediated by changes in inflammation. We aim:

- To determine whether the benefit (i.e. improvement of hemoglobin) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by a decline in IL-6 between baseline and one month.
- To determine whether the benefit (i.e. improvement of hemoglobin) of 12 months of testosterone treatment, in elderly hypogonadal men is mediated by a decline in CRP between baseline and one month.

In the fourth step, we expect that the immediate change (from baseline to month 1) in markers of inflammation will predict eventual erythropoiesis in men with UAE at month 12, but with stronger association for the testosterone treated group than the placebo group. Two linear regression models, one for log IL-6 and one for log CRP, will be used for exploring these relationships. In each regression model for a given marker of inflammation, the dependent variable is the change in hemoglobin between baseline and month 12 and the independent variables are treatment group indicator, change of the marker from baseline to month 1, the interaction of treatment group and change of the marker, baseline hemoglobin, and baseline marker of inflammation (IL-6 or CRP). With these regression models, we aim:
• To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in log transformed IL-6 concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.

• To estimate changes in hemoglobin between baseline and month 12 that correspond to 1 unit change in log transformed CRP concentration between baseline and month 1 for testosterone treated and placebo groups, respectively, and to test whether these changes are different between the two groups.
5. REFERENCES


APPENDICES

6.1 Appendix A: Criteria for Common Anemia

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency* IDA</td>
<td>Serum ferritin &lt; 50 ng/dL, or % transferrin saturation &lt; 20%</td>
</tr>
<tr>
<td>Chronic inflammation ACI</td>
<td>Serum iron &lt; 60 ug/dL without evidence for iron deficiency</td>
</tr>
<tr>
<td>Mixed IDA/ACI</td>
<td>Soluble transferrin receptor (sTFr/log ferritin &gt; 2)</td>
</tr>
<tr>
<td>Chronic Kidney Disease (CKD)</td>
<td>eGFR &lt; 30 mL/min</td>
</tr>
<tr>
<td>Myelodysplastic Syndromes (MDS)</td>
<td>MCV ≥ 100 fl, platelet count &lt; 120 K/uL, or neutrophil count &lt; 1200 K/uL not attributable to another cause.</td>
</tr>
<tr>
<td>Miscellaneous Others (MO)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 Deficiency</td>
<td>Vitamin B12 &lt; 200 pg/mL</td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>Folate &lt; lower limit of normal</td>
</tr>
<tr>
<td>Plasma Cell Dyscrasia</td>
<td>Monoclonal gammopathy ≥ 1 g/dL.</td>
</tr>
<tr>
<td>Hemolytic Anemia</td>
<td>Normocytic or macrocytic anemia associated with elevated LDH and low haptoglobin level</td>
</tr>
<tr>
<td>Thalassemia trait</td>
<td>MCV &lt; 80 fl and red blood cell count within the normal reference range without iron deficiency</td>
</tr>
<tr>
<td>Unexplained Anemia (UAE)</td>
<td>Does not meet criteria for IDA, ACI, Mixed IDA/ACI, CKD, MDS, or MO.</td>
</tr>
</tbody>
</table>
6.2 Appendix B: Unexplained Anemia of the Elderly Diagnosis Algorithm

Anemia Cohort (Hb < 12.7 g/dL) (n = 115)

Exclusions per CBC/creatinine (est. n = 20 exclusions):
- Suspected MDS:
  - MCV is ≥ 100 fL
  - platelet count < 120 K/uL
  - neutrophil count < 1200 K/uL
- Renal insufficiency (eGFR < 30 ml/min)

Exclusions per MCV < 90 (est. n = 63) (est. n = 14 exclusions)
- IDA
  - sFt < 50 ng/dL
- ACD
  - sFt > 50 ng/dL
  - Serum Fe < 60 mcg/dL
- Mixed
  - ACD w/ sTfR/log ferritin) > 2

Exclusions per MCV > 90 (est. n = 32) (est. n = 4 exclusions)
- B12 deficiency < 200 pg/mL
- Folate deficiency
- Hemolytic anemia
  - High LDH
  - Low Haptoglobin
- Plasma cell dyscrasia
  - TSP > 7.9 g/dL
  - High SPEP

Remainder UAE (est n = 77)
### Appendix C: T-Trial Blood Draws

<table>
<thead>
<tr>
<th>Dated 1/7/2011</th>
<th>Testosterone Trial - Lab test schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Subjects</strong></td>
<td><strong>SV 1</strong></td>
</tr>
<tr>
<td><strong>Real time testing</strong></td>
<td></td>
</tr>
<tr>
<td>CBC without differential (2)</td>
<td>X</td>
</tr>
<tr>
<td>Chemistry panel (1)</td>
<td>X (reflex)</td>
</tr>
<tr>
<td>Urinalysis (3)</td>
<td>X</td>
</tr>
<tr>
<td>FSH &amp; LH</td>
<td>X (4)</td>
</tr>
<tr>
<td>PSA</td>
<td>X (reflex)</td>
</tr>
<tr>
<td>TSH</td>
<td>X</td>
</tr>
<tr>
<td>Serum T Total</td>
<td>X</td>
</tr>
<tr>
<td>HgbA1C</td>
<td>X</td>
</tr>
<tr>
<td>Hgb/Hct</td>
<td>X</td>
</tr>
<tr>
<td>DHT</td>
<td>X</td>
</tr>
<tr>
<td><strong>Batch testing</strong></td>
<td></td>
</tr>
<tr>
<td>Storage 1 - SHBG</td>
<td>X</td>
</tr>
<tr>
<td>Storage 1 - DHT</td>
<td>X</td>
</tr>
<tr>
<td>Storage 1 - estradiol</td>
<td>X</td>
</tr>
<tr>
<td>Storage 1 – T</td>
<td>X</td>
</tr>
<tr>
<td><strong>Storage for non-specified testing at a later date</strong></td>
<td></td>
</tr>
<tr>
<td>Volume [subtotal from tests &amp; Storage 1 above]</td>
<td>30 ml</td>
</tr>
<tr>
<td>Storage 2 - whole blood for serum (mL)</td>
<td>0</td>
</tr>
<tr>
<td>Storage 2 - whole blood for plasma (mL)</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>30 ml</td>
</tr>
<tr>
<td><strong>OPTIONAL-Blood for Genetics Study</strong></td>
<td>20 ml</td>
</tr>
<tr>
<td>Storage 2 – urine (mL)</td>
<td>10</td>
</tr>
</tbody>
</table>

**Notes:**
- **Yellow shading** indicates blinded results.
- 1. Chemistry panel includes: Na, K, Cl, CO2, Glu, BUN, Cr, Ca, TP, Alb, TBili, AP, AST, ALT
- 2. CBC without diff includes: H/H, WBC, PLT (Excludes rbc and indices.)
- 3. Urinalysis includes: Color, Reaction PH, glucose, protein, occult blood, nitrite, leukocytes, RBC/WBC.
- 4. FSH & LH – change to blinding result in Jan. 2011
VIII. Anemia Trial

VIII.A Primary Endpoint

VIII.A.1 Primary Analysis. The primary efficacy analysis will consist of an intent-to-treat comparison of dichotomized change in hemoglobin in all randomized subjects who exhibited unexplained anemia of the elderly (UAE) at baseline and for whom at least one post-baseline hemoglobin measurement is recorded. Unexplained Anemia of the Elderly (UAE) is defined as a hemoglobin level of <12.7 g/dL that cannot be explained by deficiencies of iron, B12, or folate, or inflammation or hemolytic reasons. It is expected that 2/3 of men with hemoglobin level of <12.7 g/dL will satisfy these criteria. At each follow-up visit change in hemoglobin will be dichotomized to ‘1’ for change from baseline of ≥ 1.0 g/dL and ‘0’ for change < 1.0 g/dL. Random effects logistic regression adjusting for balancing factors (study site, indicators of participation in each primary efficacy trial, baseline testosterone concentration (≤ 200), age (≤ 75), use of anti-depressants, and use of PDE-inhibitors) and baseline hemoglobin will be used to test for differences in anemia for Androgel versus placebo patients. All measured outcomes for each subject will be included in the primary analysis. Time will be included in the model categorically to flexibly account for time effects. The two-sided Wald p-value and confidence interval for the Androgel coefficient will be reported to evaluate significance. Imbalance of baseline smoking status, BMI, and eGFR between treatment arms will be evaluated by univariate logistic regression, and variables significantly associated with treatment arm will be added to the primary outcome model.

VIII.A.2 Sensitivity Analysis for Missing Data. The primary analysis will be repeated using methods for non-ignorable missingness to assess the robustness of results to the missing-at-random (MAR) assumption. Details of such methods are described in section II.A.2.

VIII.B. Secondary Endpoints

VIII.B.1 Continuous Change in Hemoglobin. Change in continuous hemoglobin will be compared among UAE patients randomized to treatment vs. control using a linear random effects model adjusting for balancing factors and baseline hemoglobin. As with the primary analysis all measured outcomes for each subject will be included in the analysis.

VIII.C. Functional Exploratory Endpoints / Analyses. As in the primary analysis, time will be modeled categorically with an indicator for each follow-up time > 3 months.

Men with UAE

Analyses VIII.C.1-VIII.C.4 will be performed only if there is a significant effect of testosterone on hemoglobin in men with UAE.

VIII.C.1 Six Minute Walk Test (6MWT). Continuous change from baseline 6MWT to follow-up 6MWT will be compared in subjected between subjects with UAE randomized to treatment versus
control using linear random effects models adjusted for balancing factors (study site, participation in each primary efficacy trial, baseline testosterone concentration (<200), age (≤ 75), use of anti-depressants and use of PDE-inhibitors) and the baseline 6MWT.

VIII.C.2 WMS-R LM. An intent-to-treat comparison of the change in responses to the Wechsler Memory Scale Revised (WMS-R) Logical Memory II from baseline among UAE subjects will be compared for the treatment versus the placebo arm using linear random effects models adjusting for study site, indicator variables of participation in each primary efficacy trial, baseline testosterone concentration (<200), age (≤ 75), use of anti-depressants, use of PDE-inhibitors, baseline WMSR, categorical education, and version of the WMSR, as described in VI.A.1.

VIII.C.3 FACIT-Fatigue Scale. Differences in the change in FACIT-Fatigue scores from baseline to the observed follow-up visit will be compared between UAE patients randomized to treatment versus placebo using linear random effects models adjusting for study site, indicator variables of participation in each primary efficacy trial, baseline testosterone concentration (<200), age (≤ 75), use of anti-depressants, use of PDE-inhibitors, and baseline FACIT-Fatigue score.

VIII.C.4 Erythrocytosis on 6MWT and verbal memory (WMS-R LM) in men with UAE. For each time point, an erythrocytosis indicator will be created by dichotomizing hemoglobin levels to ‘1’ for hemoglobin ≥ 16 g/dL. The dichotomous hemoglobin rise indicator will be entered into a linear random effects model predicting the 6MWT corresponding to the same visit. This analysis will assume that the rise in hemoglobin at a given follow-up visit precedes the 6MWT. The analysis will adjust for treatment and balancing factors as well as baseline walk score. The analysis of the WMS-R LM will adjust for treatment, balancing factors, categorical education, test version, and baseline WMSR.

Men with explained anemia (known causes)

Analyses VIII.C.6-VIII.C.9 will be performed only if there is a significant effect of testosterone on hemoglobin in men with anemia of known causes.

VIII.C.5 Erythroid Response in Men with Anemia of Known Causes. Men with anemia of known causes will be defined as men with hemoglobin <12.7 g/dl that can be explained by conditions such as iron deficiency, B12 deficiency, folate deficiency, inflammation or hemolytics. Continuous change in hemoglobin from baseline will be compared between men with anemia of known causes (non-UAE) randomized to treatment versus placebo using linear random effects models adjusting for baseline hemoglobin and balancing factors (study site, participation in each primary efficacy trial, baseline testosterone concentration (<200), age (≤ 75), use of anti-depressants and use of PDE-inhibitors).

VIII.C.6 Six Minute Walk Test (6MWT). Analysis VIII.C.1 will be repeated in men with anemia of known causes.
VIII. Anemia Trial

VIII.C.7 WMSR. Analysis VIII.C.2 will be repeated in men with anemia of known causes.

VIII.C.8 FACIT-Fatigue Scale. Analysis VIII.C.3 will be repeated in men with anemia of known causes.

VIII.C.9 Erythrocytosis on 6MWT and verbal memory (WMS-R LM) in men with anemia of known causes. Analysis VIII.C.4 will be repeated in men with anemia of known causes.

All anemic men

Analyses VIII.C.11-VIII.C.14 will be performed only if there is a significant effect of testosterone on hemoglobin in all anemic men.

VIII.C.10 Erythroid response in all anemic men (explained and unexplained anemia). The primary analysis of the dichotomous change in response of ≥ 1.0 g/dl or < 1.0 g/dl will be repeated among all men with hemoglobin <12.7 g/dl regardless of known or unknown causes. The analysis described in VIII.B.1 and VIII.C.4 of continuous change in hemoglobin will also be repeated on all anemic men.

VIII.C.11 Six Minute Walk Test (6MWT). Analysis VIII.C.1 will be repeated in all anemic men (explained and unexplained anemia).

VIII.C.12 WMSR. Analysis VIII.C.2 will be repeated in all anemic men (explained and unexplained anemia).

VIII.C.13 FACIT-Fatigue Scale. Analysis VIII.C.3 will be repeated in all anemic men (explained and unexplained anemia).

VIII.C.14 Erythrocytosis on 6MWT and verbal memory (WMS-R LM) all anemic men. Analysis VIII.C.4 will be repeated in all anemic men.

Non-anemic men

Analyses VIII.C.15-VIII.C.18 will be performed only if there is a significant effect of testosterone on hemoglobin in men with anemia of known causes. In contrast to the anemic groups, analysis C.19 will be performed in this group regardless of testosterone response since it is possible for non-anemic men to achieve high levels of hemoglobin.

VIII.C.15 Erythroid response in non-anemic men. The analysis in VIII.B.1 and VIII.C.4 will be repeated among non-Anemic T-Trial participants.

VIII.C.16 Six Minute Walk Test (6MWT). Analysis VIII.C.1 will be repeated in non-anemic men.

VIII.C.17 WMSR. Analysis VIII.C.2 will be repeated in non-anemic men.

VIII.C.18 FACIT-Fatigue Scale. Analysis VIII.C.3 will be repeated in non-anemic men.
VIII. Anemia Trial

VIII.C.19 Erythrocytosis on 6MWT and verbal memory (WMS-R LM) in non-anemic men. Analysis VIII.C.4 will be repeated in all non-anemic men.

VIII.D Mechanistic Exploratory Objectives

The following mechanistic exploratory analyses will be performed only in UAE subjects only if an effect of testosterone on hemoglobin is found.

VIII.D.1 Correlations in change from baseline to month 12 in iron regulatory variables, erythropoietin (epo) responses, and inflammatory markers. Correlations of the following variables will be evaluated by linear regression with interactions of treatment group and the independent variable to allow for testing of separate effects in testosterone and placebo subjects. Models will adjust for smoking status, BMI, and eGFR pending the primary outcome analyses.

Correlations among iron regulatory markers in month 12 and change from baseline to month 12.

VIII.D.1.a.i Log hepcidin and total testosterone
VIII.D.1.a.ii Log hepcidin and hemoglobin
VIII.D.1.a.iii Log hepcidin and serum ferritin
VIII.D.1.a.iv Log hepcidin and serum iron
VIII.D.1.a.v Log hepcidin and sHJV
VIII.D.1.a.vi sHJV and lean muscle mass
VIII.D.1.a.vii Log hepcidin lean muscle mass

Correlations among erythropoietin variables in change from baseline to month 12.

VIII.D.1.b.i Log epo and total testosterone
VIII.D.1.b.ii sTFR and total testosterone
VIII.D.1.b.iii Log epo and continuous hemoglobin
VIII.D.1.b.iv Log epo and absolute reticulocytes
VIII.D.1.b.v sTFR and continuous hemoglobin
VIII.D.1.b.vi sTFR and absolute reticulocytes
VIII.D.1.b.vii log transformed epo and sTFR

Correlations among inflammatory markers in change from baseline to month 12.

VIII.D.1.c.i IL-6 and total testosterone
VIII.D.1.c.ii IL-6 and total testosterone
VIII.D.1.c.iii IL-6 and hemoglobin
VIII.D.1.c.iv CRP and hemoglobin
VIII.D.1.c.v IL-6 and CRP
VIII.D.2-3. Evaluating mediation of testosterone effect on hemoglobin by 1 month change in iron regulatory markers, erythropoietic activity, and inflammatory markers.

VIII.D.2 Immediate response of iron regulatory markers, erythropoietic activity, and inflammatory markers to testosterone. For each iron regulatory, erythropoietic, or inflammatory marker below (VIII.D.2.a- VIII.D.2.i), the difference in change from baseline to 1 month for UAE patients randomized to treatment vs. control will be evaluated by linear regression of 1-month iron change onto treatment assignment adjusted for balancing factors (and possibly smoking, BMI, and/or eGFR depending on primary outcome analysis).

Iron regulatory markers
- VIII.D.2.a. Log hepcidin
- VIII.D.2.b Log serum ferritin
- VIII.D.2.c Serum iron
- VIII.D.2.d sHJV

Erythropoietic activity
- VIII.D.2.e Absolute reticulocytes
- VIII.D.2.f Log epo
- VIII.D.2.g sTFR
- VIII.D.2.h-i Inflammatory markers
  - VIII. D.2.h IL-6
  - VIII. D.2.i CRP

VIII.D.3 Immediate iron regulatory response, erythropoietic activity, and inflammatory markers as a predictor of month 12 hemoglobin. Separate linear regression will estimate the month 12 change in hemoglobin from baseline associated with 1 month change in each of the variables listed in VIII.D.2. Models will include an interaction between treatment group and markers to allow for different effects in patients assigned to treatment vs. placebo. Models will adjust for balancing factors (and possibly smoking, BMI, and/or eGFR depending on primary outcome analysis).

Iron regulatory markers
- VIII.D.3.a. Log hepcidin
- VIII.D.3.b Log serum ferritin
- VIII.D.3.c Serum iron
- VIII.D.3.d sHJV

Erythropoietic activity
- VIII.D.3.e Absolute reticulocytes
- VIII.D.3.f Log epo
- VIII.D.3.g sTFR
Inflammatory markers

VIII.D.3.h IL-6
VIII.D.3.i CRP

VIII.D.4 Effect modification of inflammatory cytokines on erythropoietic response. Modification of the erythropoietic response by baseline inflammatory markers in UAE subjects will be evaluated by a linear random effects model with continuous change in hemoglobin from baseline as the longitudinal outcome and treatment, categorical time, balancing factors, baseline hemoglobin, baseline inflammatory markers and the interaction of treatment and baseline markers as predictors. For IL-6, a cutoff will be chosen so that the interaction with treatment will include an indicator of having an IL-6 level greater than that designated level. For CRP, since no such cutoff is known, a plot of the treatment effect as a function of CRP cutoff will be generated by repeating the treatment-CRP interaction analysis with binary indicators of increasing CRP levels.