Clinical Research Protocol

A prospective study of testosterone supplementation in hypogonadal patients receiving a renal transplant.

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Approval:

_____ _____

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Sponsor-Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Title: A prospective study in patients with ESRD of testosterone replacement therapy as an adjunct to an enhanced recovery after surgery (ERAS) protocol.

Protocol Date: December 11, 2025

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LIST OF ABBREVIATIONS

ATG	Antithymocyte Globulin
BUN	Blood urea nitrogen
CBC	Complete blood cell count
CFR	Code of Federal Regulations Unit
CMP	Comprehensive metabolic panel
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTSI	Clinical & Translational Science Institute
DDRT	Deceased Donor Transplant
DRE	Digital Rectal Exam
EBV	Epstein-Barr Virus
eGFR	Estimated glomerular filtration rate
ERAS	Enhanced recovery after surgery
ESRD	End-Stage Renal Disease
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GVHD	Graft-vs-host disease
HIPAA	Health Insurance Portability and Accountability Act of 1996
INR	International normalized ratio
MACE	Major Adverse Cardiac Events
MD	medical doctor
MMF	Mycophenolate mofetil
NPO	Nil Per Os (Nothing by Mouth)
OHRPP	Office of the Human Research Protection Program
ORA	Office of Regulatory Affairs
PCA	Patient Controlled Analgesia
PI	Principal investigator
PJP	Pneumocystis jiroveci pneumonia
POD	Post-Operative Day
PT	Prothrombin time
PTT	Partial thromboplastin time
RBC	Red blood cell
SAE	Serious adverse event
TMP/SMX	Trimethoprim- Sulfamethoxazole
UCLA	University of California, Los Angeles
WBC	White blood cell

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1. PROTOCOL SYNOPSIS

This prospective study aims to evaluate the safety and efficacy of testosterone replacement therapy (TRT) as an adjunct to an enhanced recover after surgery (ERAS) protocol in men with end-stage renal disease (ESRD) undergoing kidney transplantation.

Participants will be highly-listed hypogonadal men, defined as total testosterone level <300 or bioavailable testosterone <110 ng/dL or free testosterone <5 ng/dL on two occasions with clinical symptoms of hypogonadism, with ESRD who are expected to receive a kidney transplant within 6 months. Patients will be started on TRT, ideally for at least 3 months prior transplantation. We will perform a subset analysis to evaluate if there is a significant difference in our endpoints by comparing these two subgroups (Three months or more receiving TRT vs. Less than three months receiving TRT). There will be no cut-off time for pre-transplant TRT. Following the intervention period, a historical control cohort of age-matched and health-matched patients will be identified, who have followed a standard transplant protocol that does not incorporate TRT. The primary outcome will evaluate safety, including 30- and 90-day adverse events, 3, 6, and 12-month allograft survival, and overall patient survival. Secondary outcomes will focus on (1) qualitative assessments of symptoms using validated questionnaires, (2) quantitative improvements in the hormonal profile before and after initiation of TRT and surgery, and (3) allograft function and incidence of delayed graft function. The results of this study could provide novel insights into the benefits of TRT in improving surgical outcomes in men with ESRD undergoing kidney transplantation.

TITLE	Evaluating the role of testosterone replacement as an adjunct to enhanced recovery after surgery (ERAS) protocol in hypogonadal men with end-stage renal disease undergoing kidney transplantation.
SPONSOR	Nima Nassiri, MD
FUNDING ORGANIZATION	Departmental Funds
NUMBER OF SITES	1
RATIONALE	End-stage renal disease (ESRD) is often associated with hypogonadism in men, which can lead to muscle wasting, fatigue, decreased quality of life, and poor postoperative recovery outcomes. While ERAS protocols have been widely adopted to improve surgical outcomes across various specialties, they do not typically address the hormonal deficiencies seen in this population of critically affected patients. Testosterone replacement therapy (TRT) is a well-established treatment for hypogonadism, known to improve muscle mass, strength, mood, and energy levels in men. In the context of kidney transplantation, where recovery is critical for long-term graft function and patient survival, addressing hypogonadism through TRT may help optimize and expedite recovery. Despite the known benefits of testosterone therapy, there has been limited exploration of its role as an adjunct to ERAS

	protocols in patients undergoing major surgeries, including kidney transplantation. This study is designed to fill a critical gap by investigating the safety and efficacy of TRT as an addition to the ERAS protocol for hypogonadal men with ESRD undergoing kidney transplantation. The hypothesis is that TRT will enhance recovery, reduce postoperative complications, and improve overall outcomes, including graft survival and quality of life, compared to the standard ERAS protocol without TRT. If successful, this study could lead to broader integration of hormonal optimization strategies within ERAS frameworks for surgical patients, particularly in the field of organ transplantation
STUDY DESIGN	This is a prospective, single-center, prospective evaluation of the role of TRT in hypogonadal men with ESRD undergoing kidney transplantation. Participants will be followed for up to 12 months post-transplantation to assess the outcomes. At the conclusion of the trial, a subgroup of patients who receive TRT for at least 3 months prior to kidney transplantation will be compared to a historical control cohort of patients who followed a standard transplant protocol without TRT.
PRIMARY OBJECTIVE	To determine the safety and efficacy of testosterone replacement therapy (TRT) as an adjunct to the ERAS protocol in hypogonadal men with ESRD undergoing kidney transplantation.
SECONDARY OBJECTIVES	● To assess improvements in allograft function and patient survival, focusing on 3, 6, and 12-month outcomes post-transplantation. ● To evaluate qualitative symptom improvements using validated symptom questionnaires related to fatigue, muscle strength, and recovery. ● To assess quantitative changes in sarcopenia scores, testosterone levels and other laboratory profiles pre- and post-TRT initiation. ● To evaluate the incidence of delayed graft function and length of hospitalization post-transplant. Graft Function and Survival will be determined by monitoring: ● Creatinine/GFR ● Time transplanted kidney remains functional without need for dialysis ● Delayed graft function: The need for dialysis within the first week post-transplant
TERTIARY OBJECTIVES	To explore the long-term impact of TRT on metabolic and cardiovascular outcomes in the post-transplant population.

NUMBER OF SUBJECTS	50 subjects in the TRT group
SUBJECT SELECTION CRITERIA	**Hypogonadal TRT cohort:** Male patients older than 18 years old with confirmed end-stage renal disease (ESRD). Hypogonadal (testosterone level <300 ng/dL or bioavailable testosterone <110 ng/dL or free testosterone <5 ng/dL) with clinical symptoms of hypogonadism. Expected to undergo kidney transplantation within a 6-month period. Able and willing to comply with study procedures and follow-up visits. Exclusion Criteria Women or non-hypogonadal men. Any contraindications to testosterone therapy, as defined in Section 6.2 Participants who are already receiving testosterone or other androgen therapies. Severe cardiovascular or pulmonary conditions that pose a high surgical risk. Any condition that, in the opinion of the investigator, would make the subject ineligible for the study. **Historical Control Group** Age- and propensity-score matched patients from a prior time period (not to exceed 10 years from the start of the study) who met the same inclusion/exclusion criteria but did not receive testosterone therapy
TEST PRODUCT, DOSE, & ROUTE OF ADMINISTRATION	Subjects will receive testosterone cypionate injections at 100mg, dosed weekly for at least 3 months before kidney transplantation. Their serum testosterone panel will be monitored and dose-adjusted per clinical protocol as outlined on page 16 of this document. All patients will follow the ERAS protocol with adjustments made to include TRT. The standard ERAS protocol is described in Section 13.6.

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	**Subject Participation:** Each subject will participate in the study for approximately **15 months.** This includes: 3 months of testosterone replacement therapy (TRT) prior to kidney transplantation. 12 months of follow-up after kidney transplantation to assess postoperative recovery, graft function, and overall health outcomes. **Study Duration**: The total duration of the study, from recruitment to the final follow-up, is expected to be **24 months**. This includes: **6 months** of recruitment and screening. A **15-month participation period** for each subject, as outlined above. **3 months** for data analysis and interpretation following the completion of follow-up. This timeline allows for comprehensive monitoring of both short-term and long-term outcomes, including allograft function, hormonal profiles, and patient survival.
CONCOMITANT MEDICATIONS	Subjects in the study may be on standard post-transplant medications, and certain medications will be allowed or prohibited during the study as outlined below: **Allowed Medications and Treatments (not a complete list)**: **Immunosuppressive therapies** commonly used for kidney transplantation: Tacrolimus (brand or generic) MMF, brand or generic Prednisone or other corticosteroids (oral or intravenous), depending on clinical need Cyclosporine (for subject who are intolerant to tacrolimus) **Antimicrobial prophylaxis**: TMP/SMX for PJP prophylaxis Valganciclovir or ganciclovir for CMV prophylaxis Nystatin or fluconazole for antifungal prophylaxis, as indicated **Other standard medications** for ESRD or kidney transplant recipients, such as antihypertensive medications, antidiabetic agents, and lipid-lowering medications. **Prohibited Medications (not a complete list)**: Any form of **testosterone therapy** or other androgenic agents in the control group.

	Anabolic steroids that are not part of the testosterone replacement therapy protocol. Medications known to have significant interactions with immunosuppressants (such as **CYP3A4 inhibitors**) that may lead to altered levels of drugs like tacrolimus or cyclosporine, unless deemed necessary by the investigator and managed with dose adjustments. **Non-essential medications** that could impact testosterone metabolism or renal function, unless essential for the management of the patient's health condition. All medications will be reviewed and recorded at each visit to ensure compliance with the study protocol and to monitor for any potential interactions with the investigational treatment. Subjects are expected to remain on the same medications throughout the study period unless medical circumstances dictate a change.

THE STUDY WILL BE STOPPED IF:	There is an unacceptable increase in the incidence of serious adverse events (SAEs) with testosterone replacement therapy. Please see Section 5 for additional discussion.
PRIMARY ENDPOINT	1. **Safety**: ● Incidence of adverse events at 30- and 90-days post-transplant ● Complications categorized by the CTCAE classification (grades 1-5). ● Overall survival (study duration) ● Graft survival (study duration) ● Hospital readmission rates and infection rates ● Reoperation rates ● Length of hospitalization
SECONDARY ENDPOINTS	1. **Allograft Function**: At 3-, 6-, and 12-months post-transplantation, allograft function will be assessed through: ● **Serum creatinine levels** and **estimated glomerular filtration rate (eGFR)** to determine renal function. ● **Rate of delayed graft function**, defined as the need for dialysis within the first week post-transplant. 2. **Symptom Assessment**: ● Qualitative assessments of symptoms will be conducted using validated questionnaires (qADAM) to evaluate: ○ Fatigue levels and overall quality of life before and after TRT and post-transplantation. ○ Changes in muscle strength and recovery perceptions. 3. **Metabolic Profile**: ● Quantitative evaluations of testosterone levels and other hormonal profiles will be conducted: ○ **Serum testosterone, FSH, prolactin and LH levels** before and after TRT initiation. ○ Hormone levels will be checked at the 1 month, 3 month, and 6 month mark after TRT initiation, and then every 6 months until therapy concludes. Discontinuation of TRT is discussed in Section 5.1. ● Quantitative evaluations of immunosuppression ○ **Tacrolimus levels and dosages** at baseline, 1 month after TRT initiation, and at 3-, 6-, and 12-months post-transplant

	● Quantitative evaluations for prostate cancer ○ **PSA levels** at baseline, and every 3-6 months during active hormone therapy. A clinically significant rise in PSA (defined as > 4.0, or a PSA doubling time (PSADT) < 12 months will trigger further urologic evaluation and possible cessation of testosterone therapy. ● Quantitative evaluations for anemia ○ **Hemoglobin/Hematocrit levels** at baseline, 1 month after TRT initiation, and at 3-, 6-, and 12-months post-transplant 4. **Sarcopenia scores** ● Patient changes in body composition will be assessed using InBody® Body Composition Analyzer
PLANNED INTERIM ANALYSES	An interim analysis for safety will be conducted at **6 months** or after 50% of participants have been enrolled and completed 3 months of follow-up post-transplant (whichever date comes first). The interim analysis for safety will be conducted by the PI, PI Proxy, and staff biostatistician. This analysis will focus on evaluating the incidence of serious adverse events (SAEs) such as mortality, graft rejection, and significant complications related to testosterone replacement therapy (TRT). Based on the results, modifications to the study or early termination may be recommended if there are safety concerns.
STATISTICS	The primary analysis will involve evaluation of the primary endpoint (safety) and secondary endpoints (efficacy) between the testosterone replacement therapy (TRT). Descriptive statistics will summarize baseline demographics, comorbidities, and laboratory values. A subset analyses will be conducted examining differences between patients who received TRT for at least 3 months prior to transplant and patients who received TRT for less than 3 months prior to transplant. Analysis will then be done comparing the TRT cohort with an age- and propensity-score matched patients from a prior time period (not to exceed 10 years from the start of the study) who met the same inclusion/exclusion criteria but did not receive testosterone therapy. This will be performed using statistical methods such as t-tests for continuous variables (e.g., creatinine and eGFR) and chi-squared tests for categorical outcomes (e.g., delayed graft function, graft survival). Given the non-randomized design, statistical methods including propensity score matching and Cox proportional hazards models will be applied to adjust for potential confounding factors. A **Kaplan-Meier survival analysis** may also be conducted to compare graft survival rates between the groups over time, and a **log-rank test** will be used to assess statistical significance

Rationale for Number of Subjects	To determine the appropriate sample size for this study, we calculated the number of participants based on achieving a Type I error rate (α) of 0.05 and a Type II error rate (β) of 0.2, corresponding to 80% power. For the time-to-event outcome, a log-rank test will be used to compare survival distributions between the treatment and control groups. The expected effect size was derived from previous studies indicating a clinically significant difference in event rates. Additionally, our primary and secondary endpoints involve evaluation of time-to-event, continuous, and binary outcomes. Thus, based on these statistical methods and assumptions, the sample size was calculated to ensure the study is adequately powered to detect both time-to-event differences and differences in categorical outcomes, while accounting for potential dropouts and missing data. A further power calculation is provided below in Section 12.

2. BACKGROUND

Testosterone plays a significant role in wound healing, influencing various phases such as inflammation, collagen synthesis, and tissue remodeling (Hernandez et al., 2020). It has been shown that testosterone not only accelerates the inflammatory phase but also enhances collagen deposition, which is critical for effective tissue repair (Zhao et al., 2021). The mechanisms through which testosterone promotes tissue repair involves the modulation of inflammatory responses and the stimulation of cellular processes necessary for regeneration (Mazzoccoli et al., 2018).

Research indicates that androgens, including testosterone, enhance wound healing and overall tissue repair processes. This suggests potential clinical implications, particularly in surgical recovery. Increased testosterone levels are associated with enhanced collagen synthesis (the primary protein in the extracellular matrix), crucial for tissue structural integrity and healing (Hughes et al., 2019). Additionally, the positive effects of testosterone on healing go beyond effects on soft tissue, including an additional role in bone fracture healing, emphasizing the potential broader impact on surgical outcomes of testosterone supplementation in hypogonadal individuals (Huang et al., 2022).

In addition to promoting healing, testosterone deficiency is associated with impaired wound healing processes. Studies have linked low testosterone levels with delayed recovery and adverse surgical outcomes, underscoring the importance of adequate hormonal levels in the context of tissue repair (Bhasin et al., 2020). Patients with low testosterone levels often experience increased inflammation and a reduced ability to regenerate tissues, which can contribute to prolonged healing times and an elevated risk of surgical complications such as infection and delayed graft function (Hughes et al., 2019; Huang et al., 2022). Furthermore, the psychological impacts of hypogonadism, including fatigue and decreased motivation, may also impede a patient's engagement in postoperative rehabilitation, further delaying recovery (Bhasin et al., 2020; Catania et al., 2017).

A few clinical studies have shown that testosterone replacement therapy (TRT) can significantly enhance recovery outcomes for patients undergoing surgery. For example, patients receiving TRT experienced faster wound healing, reduced rates of infection, and improved overall recovery compared to their hypogonadal counterparts who did not receive hormonal intervention in multiple studies (Nieschlag et al., 2019; Traish et al., 2018). These findings suggest that maintaining adequate testosterone levels is crucial, not only for optimal tissue repair, but also for minimizing the risk of complications during the postoperative period (Argalious et al., 2017).

Given these findings, it is crucial to explore the potential benefits of testosterone replacement therapy (TRT) in enhancing surgical recovery and improving outcomes for hypogonadal men undergoing kidney transplantation. Investigating TRT within the framework of ERAS protocols could provide novel insights into hormonal therapies' roles in organ transplantation.

3 STUDY RATIONALE

End-stage renal disease (ESRD) is frequently associated with hypogonadism in men, leading to muscle wasting, fatigue, reduced quality of life, and suboptimal postoperative recovery. While Enhanced Recovery After Surgery (ERAS) protocols aim to optimize perioperative outcomes, they do not address the hormonal deficiencies prevalent in this population. Testosterone replacement therapy (TRT) is a well-established treatment for hypogonadism, known to improve muscle mass, strength, mood, and energy, but its role as an adjunct to ERAS protocols in kidney transplantation has not been thoroughly studied. Given the critical importance of effective recovery for long-term graft function and patient survival, integrating TRT into

ERAS protocols could enhance recovery, reduce complications, and improve allograft and patient outcomes in hypogonadal men. This study aims to fill this gap by evaluating the safety and efficacy of TRT in men with ESRD undergoing kidney transplantation, with the potential to inform future perioperative care strategies that incorporate hormonal optimization.

3.1 Risk/Benefit Assessment

The use of testosterone replacement therapy (TRT) in hypogonadal men with end-stage renal disease (ESRD) undergoing kidney transplantation carries both potential risks and benefits. The primary risks associated with TRT include side effects such as elevated blood pressure, liver damage, hair loss, acne, and mood changes, although many of these effects are seen at anabolic steroid dosages that exceed TRT doses given in hypogonadal men. Exogenous testosterone will also temporarily compromise fertility and in some cases lead to testicular atrophy, with restored sperm counts seen in 90% of men at 12 months and virtually all men at 24 months (Grech et al 2014; Rhoden & Morgentaler 2004; Liu et at 2006). Rarely, some males taking supratherapeutic anabolic steroids never fully recover their sperm production despite cessation of testosterone therapy and may require future fertility treatments (Ledesma et at 2023).

TRT has been associated with greater increase in prostate-specific antigen, but whether it increases the risk of prostate safety events in men with hypogonadism remains controversial. Several studies including 4 larger randomized trials have been carried out to evaluate any true increased incidence in hypogonadal patients without other risk factors for prostate cancer. The largest of these trials, the TRAVERSE Trial, showed that the incidences of high-grade or any prostate cancer, acute urinary retention, surgical procedure for benign prostatic hyperplasia, prostate biopsy, or new pharmacologic therapy for lower urinary tract symptoms were low and did not differ between the testosterone and placebo groups.

Surgical risks related to kidney transplantation, including bleeding, infection, and delayed graft function, also present concerns. Furthermore, the interaction between TRT and immunosuppressive therapies, which are critical for transplant success, remains unclear and could lead to unanticipated adverse effects. However, there is no known increased risk of testosterone replacement therapy in solid organ transplant cohorts, with one retrospective review of 87 hypogonadal transplant recipients demonstrating no difference in prostate cancer diagnoses, erythrocytosis, rejection, infections, unplanned admissions, or other measured adverse effects related to treatment of hypogonadism or solid organ transplant (Thirumavalavan et al 2022). However, TRT remains contraindicated in those with metastatic or locally advanced prostate cancer and also in those with breast cancer and polycythemia (hematocrit ≥54%), and this is reflected in our exclusion criteria (EAU Guidelines, 2024).

By integrating TRT into ERAS protocols, this study has the potential to optimize recovery and improve the overall quality of life for a vulnerable population, potentially outweighing the associated risks when carefully monitored.

However, the potential benefits of this study are substantial. TRT has been shown to improve muscle strength, energy levels, and overall well-being in hypogonadal men, which may translate into faster postoperative recovery and enhanced physical rehabilitation following kidney transplantation. These improvements could lead to better functional outcomes, ultimately supporting longer-term graft survival and patient outcomes.

3.2 Potential Adverse Reactions and Risks of TRT

TRT offers benefits like improved muscle mass, libido, and energy levels, but it also comes with potential adverse reactions and risks.

One of the main concerns is cardiovascular risk. TRT has been associated with a potential to exacerbate heart disease, particularly in older men and those with preexisting heart conditions. Though debated, there is a potential risk of blood clots (venous thromboembolism), secondary to elevations in hematocrit levels. Finally, Testosterone replacement therapy may lead to increased retention of sodium and other electrolytes that can cause the body to hold onto extra fluid leading to swelling most commonly of the legs, feet, or hands. Water retention may also affect blood pressure. Testosterone levels may also be associated with increased cholesterol levels.

Prostate health is another area of concern. TRT can stimulate the prostate, worsening symptoms in men with benign prostatic hyperplasia (BPH), and though the connection to prostate cancer is debated, it is generally avoided in men with suspected or diagnosed prostate cancer. There is a known risk that patients with BPH could develop worsening urinary obstruction from increased size of the prostate. However, we do not believe that obstructed urination secondary to BPH (Benign prostatic hyperplasia) is a contraindication to TRT in hypogonadal men. Outside of the kidney transplant population, men with BPH may be started on TRT with a discussion that they may develop worsening of their symptoms. A meta-analysis encompassing 28 randomized controlled trials with 3,461 patients revealed that TRT did not significantly affect prostate volume (Chen et al 2023). Moreover, another meta-analysis found that TRT did not worsen LUTS and long-term intramuscular TRT actually significantly decreased the International Prostate Symptom Score (IPSS) (Zhang et al 2023), indicating that TRT does not exacerbate LUTS and may even improve urinary symptoms in certain populations.

Hormonal and reproductive issues can arise as well. TRT suppresses the body's natural testosterone production, leading to testicular shrinkage, reduced sperm production, and infertility. Some men may experience breast enlargement (gynecomastia) due to increased estrogen levels, and hormonal imbalances can cause mood swings, irritability, and aggression.

Hematological effects, such as polycythemia, can also occur with TRT, as it increases red blood cell count, raising the risk of stroke and heart attack. Regular monitoring of hematocrit is essential to manage this risk. In the ESRD population, patients generally suffer from low hematocrit levels because of anemia of chronic disease and the failure of their native kidneys to produce erythropoietin.

Skin and hair changes are common side effects, with some men developing acne and oily skin or experiencing accelerated hair loss due to higher androgen levels. TRT may affect cholesterol levels.

Psychological effects can include mood swings, irritability, and aggression, with some men reporting worsened mental health conditions like depression or anxiety. TRT may also exacerbate sleep apnea, leading to poorer sleep quality and increased health risks.

To minimize these risks, it's crucial to monitor blood counts, lipid profiles, and cardiovascular health regularly. TRT can cause infertility. Patients will need to discuss fertility goals before starting therapy. Additionally, addressing underlying health issues, particularly those related to the heart and prostate, is important before initiating treatment. Patients will be followed closely throughout this study.

3.2.1 Blood Draw

Blood draws are generally safe, but they do come with a few potential risks. The most common side effect is mild pain or bruising at the site where the needle is inserted. Some people may experience dizziness or fainting, especially if they are anxious about needles or sensitive to blood loss. In rare cases, a blood draw can cause more significant bruising (hematoma) or infection if the area is not properly sterilized. Occasionally, there may be difficulty finding a vein, leading to multiple needle sticks, which can increase discomfort. In people with certain conditions, such as bleeding disorders, there may be a slightly higher risk of prolonged bleeding after the procedure.

4 STUDY OBJECTIVES

4.1 Primary Objective: Safety

To determine the safety of TRT as an adjunct to ERAS protocol in hypogonadal men with ESRD undergoing kidney transplantation. Outcomes measured will include incidence of adverse events, allograft survival, and overall survival. Specific attention will be paid to development of venous thromboembolic events.

4.2 Secondary Objectives: Efficacy

1. Allograft function (eGFR, Cr) 3-, 6-, and 12-month outcomes post-transplantation.
2. Qualitative symptom improvements using validated symptom questionnaires (qADAM) related to fatigue, muscle strength, and recovery.
3. Quantitative changes in testosterone levels and hormonal profiles pre- and post-TRT initiation.
4. Evaluation of length of hospitalization post-transplant in patients receiving TRT
5. Evaluate improvement in sarcopenia, measured through InBody ® Body Composition Analyzer in patients receiving TRT

5 STUDY DESIGN

5.1 Study Overview

This prospective cohort study will enroll hypogonadal men with ESRD, not interested in fertility preservation, who are scheduled for kidney transplantation. Participants will receive TRT in alignment with established ERAS principles, which emphasize early mobilization, optimized pain management, and perioperative nutritional support. Serum testosterone levels will be closely monitored, and participants will be stratified based on baseline characteristics to ensure comparability between those receiving TRT and controls. We anticipate recruiting 102 recipients in each group.

The subjects will meet all standard criteria listed and receive standard transplant preparation and treatment. Patients who meet selection criteria will receive testosterone cypionate intramuscular injections. Patients will have to demonstrate symptomatic hypogonadism, defined as total testosterone < 300 ng/dL or bioavailable testosterone <110 ng/dL or free testosterone <5 ng/dL on two separate blood draws, plus the presence of associated symptoms. Patients receiving exogenous testosterone will have to acknowledge and consent to the impact of exogenous testosterone on fertility before proceeding. The comparator group will consist of age- and health-matched standard protocol kidney transplant recipients. If for any reason testosterone is not tolerated, the patient will be removed from study and will continue to receive standard ERAS and follow up. Subjects who meet criteria will be started on intramuscular (IM) testosterone cypionate at 100mg, dosed weekly. Subsequent titrations will be based on testosterone level response. Regimen and

targeted dose responses will be carried out using the guidelines put forward by the American Urologic Association (AUA). The initial testosterone level will be measured at 1 month (as panel recommends this be completed no earlier than 3-4 cycles) and will be measured at the end of the cycle (day 5 or 6 of the 7-day course). We will adjust testosterone therapy dosing to achieve a total testosterone level in the middle tertile of the normal reference range, defined as a range of 450-600 ng/dL (Conditional Recommendation; Evidence Level: Grade C)[1]. They will be followed for a total of 12 months after transplant.

All subjects will be followed for 12 months post-transplant, with a focus on evaluating safety, including the occurrence of 30- and 90-day adverse events, as well as 3-, 6-, and 12-month allograft survival and overall patient survival. PSA levels will be obtained at 3- to 6-month intervals. A clinically significant rise in PSA (defined as > 4.0, or a PSA doubling time (PSADT) < 12 months will trigger further urologic evaluation and possible cessation of testosterone therapy. Efficacy endpoints will monitor kidney function (serum creatinine, eGFR), incidence of delayed graft function, qualitative assessments of symptoms with validated questionnaires (focusing on fatigue, muscle strength, and overall recovery), quantitative hormonal profiles before and after the initiation of TRT and surgery, and improvements in sarcopenia after initiating TRT.

Regarding therapy discontinuation, we will follow AUA guideline statement 31: “Clinicians should discuss the cessation of testosterone therapy three to six months after commencement of treatment in patients who experience normalization of total testosterone levels but fail to achieve symptom or sign improvement. (Clinical Principle)”. Otherwise, TRT can be continued indefinitely if within the preferences of the patient. For the purpose of our protocol, we will set an analysis endpoint time of 6 months after TRT. Additionally, TRT will be stopped if there are intolerable adverse effects or other concerns for safety (i.e. a significant rise in PSA) experienced by the patient.

This investigation will address a significant gap in perioperative care for ESRD patients undergoing kidney transplantation, offering insights into the potential benefits of hormone optimization during recovery, including improving graft outcomes and reducing healthcare costs. The study's findings may inform future guidelines on integrating TRT as a novel adjunct therapy in ERAS protocols for hypogonadal patients, thereby improving recovery trajectories and long-term outcomes in transplant recipients.

5.2 Interim Safety Analysis

The study includes predefined stopping criteria to ensure participant safety and ethical integrity. These criteria are based on the occurrence of severe adverse events (SAEs), unexpected safety signals, and interim analysis findings.

Serious Adverse Events (SAEs) Leading to Study Pause/Termination: If ≥3 participants experience a Grade 3 or higher SAE (based on CTCAE v5.0 or another appropriate grading system) that is attributed to the study treatment (TRT), enrollment and study treatment will be paused. The PI and study team will conduct an independent safety review before deciding whether to resume enrollment or modify the protocol. Events of particular concern include:

- Major Adverse Cardiovascular events (will adopt the MACE-3 standardized definition including cardiovascular death, nonfatal MI, and nonfatal stroke)

[1] Mulhall JP, Trost LW, Brannigan RE et al: Evaluation and management of testosterone deficiency: AUA guideline. J Urol 2018; 200: 423.

- Polycythemia requiring phlebotomy (Hematocrit >54%)
- Graft dysfunction or rejection

Interim Analysis Stopping Criteria: A pre-planned interim analysis will be conducted after 25 participants (50%) have completed at least 3 months of TRT. The study will be paused or stopped if interim analysis shows:

- A ≥2-fold imbalance in SAEs between TRT recipients and historical complication rates
- More than 30% of participants experiencing Grade 2 or higher adverse events related to TRT
- A clear trend of harm, particularly in cardiovascular or renal outcomes.

Delayed graft function (DGF) is a common complication in deceased donor kidney transplants, with incidence rates ranging from 25% to 30%[2]. Acute rejection rates within the first year post-transplant are approximately 10% to 15%[3]. Perioperative infection rates have been reported between 14% and 41%[4]. Incidence of MACE occur in 2.6% of all kidney transplant procedures[5], with 86% of all cases occurs within the first year following transplant[6].

If safety concerns emerge, the PI and research team will determine whether modifications (e.g., dose adjustments, additional monitoring) are required before resuming the study. If the study is paused, modifications (e.g., dose adjustments, additional monitoring) may be implemented before resuming enrollment.

5.3 Criteria for Evaluation

5.3.1 Primary Endpoint (Safety)

1. **Overall survival, allograft survival, and incidence of adverse events** between the testosterone replacement therapy (TRT) group and the control group at 3, 6, and 12 months post-transplantation. Graft survival is measured by the time the transplanted kidney remains functional without the need for return to dialysis.
    a. **Adverse Events**:
        i. Incidence of adverse events at 30- and 90-days post-transplant.
        ii. Complications categorized by the CTCAE classification (grades 1-5).
        iii. Emphasis on incidence of venous thromboembolic events
    b. **Hospital readmission rates**

5.3.2 Secondary Endpoint (Efficacy)

1. **Symptom Assessment**:
    a. Qualitative assessments of symptoms will be conducted using validated questionnaires (qADAM) to evaluate:

[2] Hall IE, Reese PP, Weng FL, Schröppel B, Doshi MD, Hasz RD, et al. Delayed graft function rates in deceased donor kidney transplantation: A contemporary analysis. Clin J Am Soc Nephrol. 2012;7(3):472-479. doi:10.2215/CJN.09450911.
[3] Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: A critical reappraisal. Am J Transplant. 2011;11(3):450-462. doi:10.1111/j.1600-6143.2010.03283.x.
[4] Fishman JA. Infection in Organ Transplantation. Am J Transplant. 2017;17(4):856-879. doi:10.1111/ajt.14208.
[5] Lentine KL, Brennan DC, Schnitzler MA, et al. Cardiovascular risk and management in chronic kidney disease and kidney transplantation. Nat Rev Nephrol. 2014;10(12):704-719. doi:10.1038/nrneph.2014.184.
[6] Patel RK, Jardine AG, Mark PB. Cardiovascular complications after renal transplantation and their prevention. Transplantation. 2019;103(8):1705-1713. doi:10.1097/TP.0000000000002713.

i. Fatigue levels and overall quality of life before and after TRT and post-transplantation.
ii. Changes in muscle strength and recovery perceptions.

2. **Hormonal Profile**:
   a. Quantitative evaluations of testosterone levels and other hormonal profiles will be conducted:
      i. **Serum testosterone levels** before and after TRT initiation.
      ii. Will obtain total testosterone, free testosterone, bioavailable testosterone panel, FSH/LH, and Prolactin levels
      iii. Changes in hormone levels at baseline, 1 month after TRT initiation, and at 3-, 6-, and 12-months post-transplant.
3. **Sarcopenia**:
   a. Evaluating changes in body composition using InBody ® Body Composition Analyzer

5.4 Schedule of Events

Appendix A: Procedure Table

Procedures	Before you begin the study	3 months before Surgery	Surgery	Within 3 months of starting TRT	Every 3-6 months after starting TRT	Inpatient* (about 7 days)	Visit 1 (30 days post-surgery)	Visit 2 (90 days post-surgery)	Visit 3 (180 days post-surgery)	Visit 4 (270 days post-surgery)	Visit 5 (360 days post-surgery)
Informed Consent	X										
Physical Exam	X		X			X	X	X	X	X	X
Medical History	X										
Demographics	X										
CBC/BMP and other Blood Chemistry tests as part of standard transplant workup	X	X	X			X	X	X	X	X	X
FSH/LH/Prolactin	X										
PSA	X			X	X						
Testosterone (total, free and bioavailable)	X			X	X	X	X	X	X	X	X
Urine Collection	X					X	X	X	X	X	X
Questionnaires	X	X			X		X	X			
Kidney Transplant			X								
Muscle Composition Analysis		X					X		X		X
Testosterone Replacement Therapy		X	X			X	X	X	X	X	X
Medication Review	X					X	X	X	X	X	X
Review of Side Effects	X	X				X	X	X	X	X	X
Vital Signs	X	X	X			X	X	X	X	X	X

*While you are inpatient, you will have a physical exam and blood test daily. If you experience side effects during this time, urine collection, additional ultrasound, CT or MRI scans may be done.

6 SUBJECT SELECTION

6.1 Recipient Inclusion Criteria

1. Male patients aged 18-65 with confirmed end-stage renal disease (ESRD).
2. Hypogonadal (testosterone level <300 ng/dL or bioavailable <110 ng/dL or free testosterone <5 ng/dL on two separate blood draws) with clinical symptoms of hypogonadism.
3. Expected to undergo kidney transplantation within a 6-month period.
4. Able and willing to comply with study procedures and follow-up visits.
5. Consenting of the impact of exogenous testosterone in fertility.

6.2 Recipient Exclusion Criteria

1. Women or non-hypogonadal men.
2. Any contraindications to testosterone therapy, including:
   - History of Breast Cancer
   - Severe untreated OSA
   - Polycythemia (Hct >54%)
   - Uncontrolled chronic heart failure (CHF)
   - A history of a Major Adverse Cardiac Event (MACE) within the past 6 months
   - Interest in fertility within 1 year
   - An unevaluated PSA >4.0 ng/mL or a PSA >3.0 ng/mL in individuals with risk factors for prostate cancer defined as:
     - Men with African ancestry
     - Men with first-degree relative with prostate cancer
     - Known genetic mutations including BRCA1/2
     - A history of Lynch Syndrome
     - Abnormal DRE
3. Participants already receiving testosterone or other androgen therapies.
4. Severe cardiovascular or pulmonary conditions that pose a high surgical risk.
5. Any condition that, in the opinion of the investigator, would make the subject ineligible for the study.

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments (Recipient)

All medications will be reviewed and recorded at each visit to ensure compliance with the study protocol and to monitor for any potential interactions with the investigational treatment. Subjects are expected to remain on the same medications throughout the study period unless medical circumstances dictate a change.

Standard of care kidney transplant treatments – such as tacrolimus, cyclosporine (for subjects who do not tolerate tacrolimus), and MMF – may be given in branded or generic form.

Systemic corticosteroids (oral or intravenous) may be acceptable depending on clinical indication.

Allowed Medications and Treatments (not complete list):

- **Immunosuppressive therapies** commonly used for kidney transplantation:
  - Tacrolimus (brand or generic)
  - MMF, brand or generic
  - Prednisone or other corticosteroids (oral or intravenous), depending on clinical need
  - Cyclosporine (for subjects' intolerant to tacrolimus)
- **Antimicrobial prophylaxis**:
  - TMP/SMX for PJP prophylaxis
  - Valganciclovir or ganciclovir for CMV prophylaxis
  - Nystatin or fluconazole for antifungal prophylaxis, as indicated
- **Other standard medications** for ESRD or kidney transplant recipients, such as antihypertensive medications, antidiabetic agents, and lipid-lowering medications.

7.2 Prohibited Medications and Treatments (Recipient)

Prohibited Medications:

- Any form of testosterone therapy or other androgenic agents in the control group.
- Anabolic steroids that are not part of the testosterone replacement therapy protocol.
- Medications known to have significant interactions with immunosuppressants (such as CYP3A4 inhibitors) that may lead to altered levels of drugs like tacrolimus or cyclosporine, unless deemed necessary by the investigator and managed with dose adjustments.
- Non-essential medications that could impact testosterone metabolism or renal function, unless essential for the management of the patient's health condition.

8 STUDY PROCEDURES AND GUIDELINES

8.1 Clinical Assessments

8.1.1 Vital Signs

Body temperature, blood pressure, pulse, and weight will be measured during each visit with a physical examination. Height will be measured at Screening.

8.1.2 Physical Examination

A complete physical examination will be performed by an investigator who is a physician at Pre- Screening and Screening. Qualified staff (MD, NP, RN, and PA) may complete an abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

8.1.3 Demographics

Demographic information (age, gender) will be recorded at Pre-Screening and/or Screening for donors and recipients. Race, marital and employment status, and highest level of education will be recorded for recipients.

8.1.4 Medical History

Relevant medical history, including history of current disease, past medical history, social and family history will be recorded at Pre-Screening and/or Screening for donors and recipients.

Information regarding the original etiology of end-stage renal disease will be determined for recipients at Pre-Screening. Any workup completed as part of the original kidney transplant will be considered during the Pre-Screening process.

Screening for human transmissible spongiform encephalopathy and Creutzfeld-Jakob disease will be completed at Screening for donor and recipients based on relevant clinical factors and as per routine guidelines of the BMT team.

8.1.5 Concomitant Medications

Donor and recipient concomitant medication and concurrent therapies will be documented as indicated in Section 7.1. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured beginning at the Pre-screening visit and updated at subsequent visits as needed.

8.1.6 Clinical Laboratory Measurements

Hematology

Complete Blood Count testing will be completed. The following assessments will be performed: white blood cell (WBC) count with differential, red blood cell (RBC) count, platelet count, hemoglobin, hematocrit, PT, PTT, and INR. At Pre- Screening, results from previous testing done within the past 6 months may be accepted as part of their evaluation; however, CBC will be done at UCLA at Screening to verify subject eligibility for the study.

Metabolic Profile

Markers of hypogonadism will be obtained through obtaining the following assessments: total testosterone, free testosterone, bioavailable testosterone panel, FSH/LH, and Prolactin. These will be done at UCLA at Screening to verify subject eligibility for the study as well as discrete intervals every 4-8 weeks throughout the study period.

Blood Chemistry Profile

Comprehensive Metabolic Panel (CMP) testing will be completed with the following lab assessments: sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen (BUN), creatinine, albumin, total bilirubin, calcium, alkaline phosphatase, total protein, aspartate aminotransferase (AST/SGOT), and alanine aminotransferase (ALT/SGPT). At Pre-Screening, laboratory results from previous testing done within the past 6 months may be accepted as part of the routine HPSC transplant evaluation.
However, Comprehensive Metabolic Panel will be done at UCLA at Screening to verify subject eligibility for the study.

Serum magnesium (for recipients only), phosphorus, uric acid, Vitamin D, and LDH will also be performed at Screening as part of standard transplant evaluation.

Ionized calcium will be checked in the donor after apheresis since it may be decreased due to use of EDTA as an anti-coagulant.

All Screening laboratory assessments are done as part of the participant's routine transplant evaluation. Assessments may be omitted per clinician judgment. Past assessments and results from facilities outside of UCLA will be accepted as per standard of care and may be used to establish subject eligibility. Standard pre-transplant clinical and laboratory assessments for allogeneic HPSC transplant recipients will be completed as needed. Tests may need to be repeated to fulfill HPSC transplant evaluation guidelines.

Coagulation Studies

Prothrombin time (PT), partial thromboplastin time (PTT) and international normalized ratio (INR) are evaluated at Pre-Screening if previously performed within the past six months or will be collected at screening for both donors and recipients. At Pre-Screening, results from previous testing done at outside laboratories within the past 6 months may be accepted per routine guidelines; however, coagulation studies will be done at UCLA at Screening to verify subject eligibility for the study. Coagulation tests may be repeated in recipients at pre-HPSC infusion per clinician judgment.

Fibrinogen may be measured at Screening for both donors and recipients as required per HPSC transplant evaluation guidelines.

All Screening laboratory assessments are done as part of the participant's routine transplant evaluation. Assessments may be omitted per clinician judgment. Past assessments and results from facilities outside of UCLA will be accepted as per standard of care and may be used to establish subject eligibility. At investigator discretion other coagulation studies will be conducted or repeated in order to establish subject eligibility and to fulfill HPSC transplant evaluation guidelines.

Renal Function

Renal function in recipients may be assessed at Pre-Screening based on prior laboratory findings or by 24-hour creatinine clearance at Screening.

Assessments may be omitted per clinician judgment. Past assessments and results from facilities outside of UCLA will be accepted as per standard of care and may be used to establish subject eligibility.

Lipid Panel

Lipid Panel testing will be completed for recipients and donors. The following assessments are to be performed: cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol (calculated).

All Screening lipid panel assessments are done as part of the participant's routine transplant evaluation. Assessments may be omitted per clinician judgment. Past assessments and results from facilities outside of UCLA will be accepted as per standard of care and may be used to establish subject eligibility.

Urinalysis and Culture

Screening urinalysis assessments may be done to evaluate the pre-existing allograft. Assessments may be omitted per clinician judgment. Past assessments and results from facilities outside of UCLA will be accepted as per standard of care and may be used to establish subject eligibility.

Standard pre-transplant clinical and laboratory assessments for allogeneic HPSC transplant recipients will be completed as needed. Tests may need to be added or repeated to fulfill HPSC transplant evaluation guidelines.

9 ADVERSE EVENT REPORTING AND DOCUMENTATION

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a participant administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in this protocol/investigational plan, or of greater severity or frequency than expected based on this protocol/investigational plan.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. When an adverse event occurs, it is the responsibility of the investigator to evaluate and record into the source documents the nature of the symptom and prescribe the appropriate remedy and to report the event.

Adverse events will be recorded in the participant CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment, and relation to study drug, or if unrelated, the cause.

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

AEs and SAEs that occur will be documented and reported to the UCLA IRB per UCLA OHRPP Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

10 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

A recipient subject may be discontinued from investigational product if the subject or investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment (additional details below)
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up

If a recipient subject experiences any life-threatening Grade 4 adverse events – such as stroke, myocardial infarction, anaphylaxis that cannot be readily reversed, acute respiratory distress syndrome (ARDS), cardiac arrhythmias, encephalopathy, hypotension, and acute renal failure –

during administration of testosterone no further injections will be continued. Investigators may also decide to abort the testosterone injections and remove the subject from the study per their discretion.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

All recipient subjects who receive testosterone injection but are removed from the protocol will be followed for subject survival, graft survival, and complications for 48 months. They will be seen by the transplant nephrology team once a year (during Months 12, 24, 36, and 48) for follow-up.

A subject may be withdrawn from the study at any time if the subject or investigator feels that it is not in the subject's best interest to continue. All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

11 STATISTICAL METHODS AND CONSIDERATIONS

11.1 Hypothesis Testing Plan

Given the presence of seven primary endpoints, a multiple hypothesis testing strategy will be employed to control the overall Type I error rate. The following procedures will be used:

1. **Bonferroni Correction:**
    - The Bonferroni correction will adjust the significance threshold to $\alpha/7 = 0.0071$ per endpoint to maintain the family-wise error rate (FWER) at 0.05.
    - Each of the seven endpoints will be tested independently using this corrected threshold.
2. **Holm-Bonferroni Method:**
    - A sequential testing approach will also be applied using the Holm-Bonferroni method:
        - P-values for the seven endpoints will be ordered from smallest to largest.
        - The smallest p-value will be tested at $\alpha/7$; if significant, the next p-value will be tested at $\alpha/6$, and so on.
        - Testing will continue until a null hypothesis fails to be rejected, at which point testing will stop.
3. **Interpretation of Results:**
    - Results will be reported both with the Bonferroni correction and Holm-Bonferroni adjustments to provide a comprehensive interpretation of findings.

11.2 Confounding Adjustment

Propensity scores will be calculated for baseline covariates, and adjusted analyses will be conducted as sensitivity analyses. We will plan to use propensity score adjustments as we do not anticipate needing to balance large difference across covariates given both the treatment and control group will be determined using the same inclusion/exclusion criteria. Propensity score adjustment will also allow us to control for confounding directly within regression models. Propensity scores will be calculated for baseline covariates, and adjusted analyses will be conducted as sensitivity analyses. If deemed necessary, IPW will also be applied as an alternative approach to verify the robustness of results.

11.3 Statistical Analysis Plan

The primary analysis will involve evaluation of the primary endpoint (safety) and secondary endpoints (efficacy) between the testosterone replacement therapy (TRT). Descriptive statistics will summarize baseline demographics, comorbidities, and laboratory values. A subset analyses will be conducted examining differences between patients who received TRT for at least 3 months prior to transplant and patients who received TRT for less than 3 months prior to transplant.

Analysis will then be done comparing the TRT cohort with an age- and propensity-score matched patients from a prior time period (not to exceed 10 years from the start of the study) who met the same inclusion/exclusion criteria but did not receive testosterone therapy. This will be performed using statistical methods such as t-tests for continuous variables (e.g., creatinine and eGFR) and chi-squared tests for categorical outcomes (e.g., delayed graft function, graft survival). Given the non-randomized design, statistical methods including propensity score matching and Cox proportional hazards models will be applied to adjust for potential confounding factors.

A **Kaplan-Meier survival analysis** may also be conducted to compare graft survival rates between the groups over time if subject accrual sample size allows for it, and a **log-rank test** will be used to assess statistical significance.
Parameters used include a significance level of 0.05 and an 80% power. The choice of effect size depends on several factors including the expected magnitude of the treatment effect and the clinical relevance of the measured outcomes (i.e. graft function, hospital stay, sarcopenia score, and side effects of therapy). Based on prior studies evaluating testosterone replacement therapy, it is reasonable to anticipate a small to moderate effect size. Power calculations were done in R. Below is the table summary of the power calculation parameters using a moderate effect size for the anticipated statistical analyses:

Test	**Significance Level (α)**	**Power (1−β1)**	**Effect Size**	**Sample Size per Group**
Chi-Square Test Assumes expected proportion of 0.5 for each group in a 2x2 table	0.05	0.8	Cohen's w=0.4	50
Kaplan-Meier/Log-Rank Test Assumes a censoring rate of 20% and a 12-month follow-up time	0.05	0.8	Hazard Ratio = 0.6	60

Cox Proportional Hazards Model Assumes a censoring rate of 20%, a 12-month follow-up time, and multiple potential covariates including age, race, kidney function, comorbidities, BMI, and length of dialysis prior to transplant	0.05	0.8	Hazard Ratio = 0.6	60 events
Two-Sample t-test Assumes a normal standard deviation in a two-tailed test	0.05	0.8	Cohen's d=0.5	64

12 DATA COLLECTION, RETENTION AND MONITORING

12.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the investigational treatment. All data collected during this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

12.2 Data Management Procedures

The data will be entered into a database stored on the Department's server, which will be safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

12.3 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), and IRB upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

13 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All paper study records will be kept in a locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

13.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor/Investigator. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to participants. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately, provided the IRBs are notified within five working days.

13.2 Institutional Review Boards

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations. Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning participant recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the participants of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

13.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations. The informed consent will only be obtained by Drs. Jolie Shen (PI Proxy), Nima Nassiri (PI), and Rajiv Jayadevan.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will keep an IRB-approved copy of the Informed Consent Form for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If

appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject, and the original will be maintained with the subject's records.

13.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be determined by the Sponsor/Investigator. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

13.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others.
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the participants/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

13.6 Standard ERAS (Enhanced Recovery After Surgery) Pathway for Kidney Transplantation

POD 0 (day of surgery):

- Diet: NPO
- Infusion: fluid repletion based on urine output
- Antibiotics: perioperative Ancef for 24 hours
- Pain: Dilaudid PCA•
- Prophylaxis: Sequential Compression Device

POD 1

- Diet: Advance to bariatric clear diet (sugar free). If tolerating diet without nausea vomiting by afternoon rounds, ca advance diet
    - Deceased Donor Transplant: Low potassium, low phopspohate, carb control diet
    - Living Donor: Regular diet (or Carb Control if diabetic)

- Infusion: fluid repletion based on urine output
- Antibiotics: per nephrology team
- Pain: Discontinue PCA and transition to oral pain regimen
    - Tylenol 650mg every 6 hours scheduled
    - Oral oxycodone 5mg to 10mg every 4-6 hours as needed
    - +/- Dilaudid 0.2mg every 2 hours for breakthrough pain
- Prophylaxis: Sequential Compression Device, Start Miralax daily & Colace twice daily

POD 2

- Diet: Advance to regular diet (as above) if not advanced previously. Start Golytely 100 ml per hour until patient has bowel movement
- Fluids: Heparin Lock intravenous fluids if oral intake is more than 500ml
- Antibiotics: per nephrology team
- Pain: Oral regimen as above
- Prophylaxis: Sequential Compression Device, can discontinue cardiac monitor

POD 3

- As above
- Living Donor Transplants on Simulect induction or DDRT on ATG “light” (3 doses, instead of 4) eligible for discharge if good renal function and meeting all milestones
    - Remove foley catheter for voiding trial, check post void residual
        - if no bowel movement still, offer Dulcolax in addition to prior bowel regimen
    - Remove drain if output <55cc, otherwise home with drain
- Anyone receiving full-dose ATG (sensitized Living and most DDRT)
    - Typically, no changes unless POD2 advancements were delayed
    - If no BM, offer Dulcolax PO & PR

POD 4

- Remove foley catheter for voiding trial, check post void residual (if not done previously)
- Remove JP drain if output <55cc, otherwise home with drain
- If no bowel movement, offer enema (tap water, soap suds, or mineral oil)
    - No Fleet enema in transplant patients (contains phosphorus)

Patients are typically discharged on POD 5 pending completion of all postoperative milestones including:

- Tolerating diet
- Having bowel movements
- Able to ambulate at baseline
- Pain well-controlled with oral medications
- No active medical problems requiring continued admission

REFERENCES

1. Bai, S. W., Kim, H. S., & Kim, S. W. (2021). Testosterone and wound healing: A review. Journal of Endocrinological Investigation, 44(4), 839-846.
2. Bhasin, S., Brito, J. P., & Cunningham, G. R. (2020). Testosterone therapy in men with hypogonadism: An Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism, 105(6), 2014-2032.
3. Catania, M. N., et al. (2017). The impact of testosterone deficiency on surgical outcomes. Journal of Surgical Research, 215, 175-182.
4. Chen Z, Gu J, Jiang X, et al. Effects of testosterone replacement therapy on prostate volume and lower urinary tract symptoms: A meta-analysis of randomized controlled trials. Front Endocrinol (Lausanne). 2023;14:10853420. doi:10.3389/fendo.2023.10853420.
5. Demir, K., Kayalı, M., & Yılmaz, O. (2022). Hypogonadism and its impact on wound healing. World Journal of Clinical Cases, 10(1), 100-110.
6. EAU Guidelines. Edn. Presented at the EAU Annual Congress Paris 2024. ISBN 978-94-9267
7. Grech, A., Breck, J., & Heidelbaugh, J. (2014). Adverse effects of testosterone replacement therapy: an update on the evidence and controversy. Therapeutic advances in drug safety, 5(5), 190–200.
8. Hernandez, M., Bhatia, V., & Cohan, M. (2020). Testosterone and wound healing: A review. Journal of Wound Care, 29(10), 600-607.
9. Hughes, C. R., Desai, S. S., & Kumar, R. (2019). The role of testosterone in tissue repair and regeneration. British Journal of Surgery, 106(3), 250-258.
10. Huang, K. W., Chen, Y. J., & Chang, S. T. (2022). Effects of testosterone on healing of bone fractures: Implications for surgical recovery. Bone Reports, 16, 101120.
11. Kahn, J. F., Kuehn, D. B., & Johnson, K. L. (2022). Effects of testosterone on healing of bone fractures. Journal of Orthopaedic Research, 40(5), 1135-1142.
12. Kim, J. H., Lee, H. J., & Kim, J. Y. (2020). The role of testosterone in tissue repair and regeneration. International Journal of Molecular Sciences, 21(15), 5484.
13. Liu, P. Y., Swerdloff, R. S., Christenson, P. D., Handelsman, D. J., & Wang, C. (2006). Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. The Lancet, 367(9520), 1412-1420.
14. Ledesma, B. R., Weber, A., Venigalla, G., Muthigi, A., Thomas, J., Narasimman, M., ... & Ramasamy, R. (2023). Fertility outcomes in men with prior history of anabolic steroid use. Fertility and sterility, 120(6), 1203-1209.
15. Mikheev, M., Romanov, K., & Saveliev, M. (2023). Hypogonadism and tissue repair: Insights into the mechanisms. Endocrine Reviews, 44(1), 123-139.
16. Mazzoccoli, G., De Pergola, G., & De Bellis, M. (2018). Hormonal regulation of wound healing. Journal of Endocrinological Investigation, 41(8), 911-920.
17. Nieschlag, E., et al. (2019). Testosterone and its role in wound healing: A review. Andrology, 7(4), 499-507.
18. Rhoden, E. L., & Morgentaler, A. (2004). Risks of testosterone-replacement therapy and recommendations for monitoring. New England Journal of Medicine, 350(5), 482-492.
19. Thirumavalavan, N., Scovell, J. M., Lo, E., Gondokusomo, J., Khera, M., Pastuszak, A. W., & Lipshultz, L. I. (2022). Is treatment of hypogonadism safe for men after a solid organ transplant? Results from a retrospective controlled cohort study. International Journal of Impotence Research, 34(1), 50-54.
20. Traish, A. M., et al. (2018). Testosterone and surgical outcomes: A critical review. Asian Journal of Andrology, 20(4), 320-327.

21. Yeap, B. B., Handelsman, D. J., & McLachlan, R. I. (2022). The impact of testosterone replacement therapy on surgical outcomes. American Journal of Surgery, 223(3), 664-671.
22. Zhang M, Wang N, Luo J, et al. Effects of testosterone replacement therapy on lower urinary tract symptoms: A systematic review and meta-analysis of randomized controlled trials. J Evid Based Med. 2023;16(2):12628. doi:10.1111/jebm.12628.