The University of Miami Miller School of Medicine
Department of Urology

Clinical Research Protocol

Title:	**A Randomized, Double-Blind, Placebo Controlled Trial on Safety and Efficacy of Autologous Platelet-Rich Plasma for Treatment of Erectile Dysfunction**
Protocol No:	**UM IRB#: 20200373**
Protocol Version:	**Version 1.3** **Version Date: 1/23/2023**
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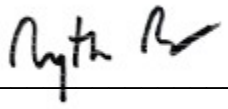
Investigator Signature Page

Title: **A Randomized, Double-Blind, Placebo Controlled Trial on Safety and Efficacy of Autologous Platelet-Rich Plasma for Treatment of Erectile Dysfunction.**

I have read the enclosed protocol. I will ensure the safety of the study subjects enrolled under my supervision, and will provide the sponsor with complete, accurate, and timely information on this study, as outlined in this Protocol. The signature below constitutes approval of this protocol and the attachments and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including local legal and regulatory requirements, applicable US federal regulations and (ICF E6) guidelines. I shall hold strictly confidential all information pertaining to the study, and that this confidentiality requirement applies to all study staff at the site(s) and/or under my supervision.

Ranjith Ramasamy, MD 1/23/2023

Print Name for Principal Investigator Date



Principal Investigator Signature

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List of Abbreviations and Definition of Terms

AE	Adverse Events
BPH	Benign Prostatic Hyperplasia
BRB	Biomedical Research Building
CNS	Central Nervous System
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	Electronic Case Report Form
ED	Erectile Dysfunction
EDITS	Erectile Dysfunction Inventory of Treatment Satisfaction
EDV	End Diastolic Velocity
GCP	Good Clinical Practice
HIPAA	Health Information Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference of Harmonization
ICI	Intracavernosal Injection
IEC	Institutional Ethics Committee
IIEF	International Index of Erectile Function
IIEF-EF	Erectile Function Subdomain Score
IRB	Institutional Review Board
ISCI	Interdisciplinary Stem Cell Institute
MCID	Minimal Clinically Important Difference
mL	Milliliter
ng/dL	Nanograms Per Deciliter
PDE5i	Phosphodiesterase 5 inhibitor
PPP	Platelet Poor Plasma
PRFM	Platelet Rich Fibrin Matrix
PRP	Platelet Rich Plasma
PSV	Peak Systolic Velocity
RCT	Randomized Controlled Trial
SAE	Serious Adverse Events
SEP3	Sexual Encounter Profile
SOP	Standard Operating Procedure
SSRI	Selective Serotonin Reuptake Inhibitor
TE-SAE	Treatment Emergent Serious Adverse Events
UM	University of Miami
VAS	Visual Analogue Pain Scale

Protocol Synopsis	
TITLE	A Randomized, Double-Blind, Placebo Controlled Trial on Safety and Efficacy of Autologous Platelet-Rich Plasma for Treatment of Erectile Dysfunction
SPONSOR	University of Miami, Miller School of Medicine, Department of Urology
PHASE OF STUDY	Phase I
STUDY THERAPY	Autologous Platelet-Rich Plasma (PRP)
STUDY DESIGN	Randomized, Double Blind, Placebo Controlled, Pilot Trial
ROUTE OF ADMINISTRATION	Intracavernosal Injection
SUBJECT POPULATION	80 male subjects between the age of 30-75 with erectile dysfunction of organic origin
STUDY OBJECTIVES	Primary: To investigate and compare the treatment efficacy of PRP vs placebo treatment in men with mild-moderate ED, as measured by IIEF. Secondary: To study incidence of adverse events and safety of PRP injection treatment in men with mild-moderate ED, as measured by IIEF.
INVESTIGATION PLAN	80 men with Erectile Dysfunction (ED) of organic origin, that meet all of the inclusion and none of the exclusion criteria, will be randomized to receive, intracavernosal injection of either PRP or placebo in a 1:1 double-blinded fashion: Group A (40 subjects): Autologous PRP All subjects in this group will receive 2 sessions of autologous PRP with 30 ± 7 day treatment interval. 5mL of PRP will be injected at each session. Group B (40 subjects): Placebo (Normal Saline) All subjects in this group will receive 2 sessions of normal saline with 30 ± 7 day treatment interval. 5mL of normal saline will be injected at each session. - Patients will be followed for outcome assessments at 1, 3, and 6 months post final injection.
DURATION OF STUDY	9 months (1 month screening, 2 therapy sessions 1 month apart, 6 months follow-up)
DEFINITION OF ENDPOINTS	Primary Endpoint (Efficacy) - The percentage of subjects in each group who attain MCID in IIEF-EF domain from baseline to 4 weeks after final treatment. Secondary Endpoints (Efficacy) - The percentage of subjects in each group who attain MCID in IIEF-EF domain from baseline to 12 & 24 weeks after final treatment. - The difference between groups in change of IIEF-EF score from baseline to 4, 12, & 24 weeks after final treatment. - Differences in Doppler ultrasound parameters (PSV and EDV) from baseline to 24 weeks after final treatment. Secondary Endpoints (Safety) - Incidence of adverse events in all patients during the study period.

INCLUSION CRITERIA	1. Be Male 2. Be 30 to ≤75 years of age (inclusive). 3. Be able to provide written informed consent. 4. Have a diagnosis of ED due to organic origin for at least 6 months prior to consent. 5. Sexually active in a stable, heterosexual relationship of more than three months duration. 6. IIEF-EF score 11-25 at screening (even if taking a single PDE5i). 7. Agree to attempt sexual intercourse at least 4 times per month for the duration of the study without being under the influence of alcohol or recreational drugs. 8. Agree to comply with all study related tests/procedures.
EXCLUSION CRITERIA	1. Previous penile surgery of any kind (except circumcision and condyloma removal), such as penile lengthening, penile cancer surgery, penile plication, grafting. 2. Previous history of priapism or penile fracture 3. Abnormal morning serum testosterone level defined as a value lower than 300 ng/dL (±5%) (indicative of untreated hypogonadism), or greater than 1197 ng/dL (±5%). 4. Current or previous hormone usage, other than prescribed testosterone, clomiphene or thyroid medication. (Subjects with prior or current use of hormonal treatment for prostate cancer are also excluded.) 5. Psychogenic ED as determined by study investigator. 6. Anatomical (Peyronie's Disease or penile curvature that negatively influences sexual activity) or neurological abnormalities in the treatment area. 7. Patients using ICI for management of ED 8. Patients with generalized polyneuropathy, or neurological conditions irrespective of cause, such as severe diabetes, multiple sclerosis or Parkinson's disease. 9. Have a serious comorbid illness/condition/behavior that, in the opinion of the investigator, may compromise the safety or compliance of the subject or preclude successful completion of the study. 10. History of consistent treatment failure with PDE5 inhibitors for therapy of ED. 11. Any history of significant psychiatric disease, such as bipolar disorder or psychosis, greater than one lifetime episode of major depression, current depression of moderate or greater severity. Patients who are currently using SSRI or psychotropic medications. 12. Hemoglobin a1c >9%.

1. Protocol Title

A Randomized, Double-Blind, Placebo Controlled Trial on Safety and Efficacy of Autologous Platelet-Rich Plasma for Treatment of Erectile Function

2. Background

2.1 Platelet-based therapies

Platelet-derived therapies are a growing trend across multiple medical and surgical specialties including dermatology, ophthalmology, cardiology, colorectal surgery, plastic surgery and mainly orthopedics [1-2]. One of the most well described platelet-based therapies is autologous platelet-rich plasma (PRP) [3]. PRP is derived from the centrifugation of whole blood with a separator gel to remove the red and white blood cells. The resulting supernatant has a greater than four-fold increase in platelets and other plasma proteins [1]. This concentrate is then administered via injection. PRP has been frequently used for orthopedic conditions such as bone and soft tissue trauma, inflammatory conditions, and chronic pain syndromes [3-4]. Evidence suggests that platelets play an important role not only in coagulation but also in regulation of body metabolism, promotion of the wound healing, tissue regeneration, vascular remodeling and inflammatory/immune responses through secretion of growth factors, cytokines and chemokines [4-5]. These biologically active proteins include transforming growth factor-β, platelet-derived growth factor, platelet-derived epithelial growth factor, insulin- like growth factor, vascular endothelial growth factor, basic fibroblast growth factor, as well as many others [6]. When platelets are activated, they release these growth differentiation factors, facilitating even nerve repair and regeneration [7-8]. Growth factors act locally and are implicated in many aspects of natural wound healing, including chemotaxis, cell proliferation, cell differentiation and angiogenesis. They also control and conduct synthesis, modification and degeneration of extracellular matrix proteins. Coordination of these cellular and molecular processes is integral to proper wound healing and tissue regeneration [9]. The key role of platelets in these processes makes them an attractive candidate for therapies aimed at accelerating natural healing, as well as tissue regeneration.

Autologous blood-based biomaterials are promising therapeutic options for varied pathology. Rapid generation of therapeutic material following collection allows for point-of- care therapy [10]. Furthermore, an autologous therapy avoids the need for immunosuppression and eliminates concerns of rejection. Newer strategies to prolong the anti-inflammatory and wound healing properties of platelets have focused on creating a fibrin matrix (platelet rich fibrin matrix, PRFM) to bind the platelets and prevent extravasation from the site of injection, thereby addressing the concern of early washout with PRP [11]. In addition, PRFM offers a potential scaffold for tissue ingrowth and may allow continued release of platelet-related factors for a longer duration. Across multiple disciplines, PRP has been used both as a primary treatment modality and as a supplement to other therapies in hopes of supplementing wound healing, tissue regeneration, and angiogenesis. Although most of the studies focusing on PRP injections have been relatively small and heterogenous, they largely support the concept of administration in terms of safety, while efficacy remains uncertain. Finally, the concept of

autologous therapy has be shown in the real world that it is particularly attractive to patients [10].

2.2 Platelet-rich plasma treatment for erectile dysfunction

Erectile dysfunction (ED) affects as many as 1 in 4 men, and evidence indicates that incidence is rising [12-13]. The pathophysiology is multifactorial, but the most common pathophysiology, vasculogenic erectile dysfunction, has as first sign endothelial dysfunction secondary to inflammation [14]. The most common treatments for ED aim to improve endothelial function through augmentation of the nitric oxide pathway [15]. Return of potency after surgical injury of CNS partially depends on axon regeneration in the remaining neural tissue [16]. The process of regeneration and functional recovery of peripheral nerve is slow and is influenced by many factors [17] such as extracellular matrix, neurotrophic factors, and cellular components [18]. Recent advances in the understanding of molecular pathways and their physiological role demonstrate that growth factors are an important part of the development, maintenance, and regeneration of the nervous system [19]. Various growth factor neuromodulatory strategies, including insulin-like growth factor (IGF-I) and neurotrophic factors, are investigated to identify agents that may have neuroprotective and regenerative function after the occurrence of peripheral nerve injury. Accumulating evidence indicates that neuroimmunophilin ligand (such as FK506) plays a significant role in neural regeneration and upregulation of neuronal nitric oxide synthase (nNOS), as well as in the recovery of erectile function after CN injury occurrence [20-21]. FK506 neuroprotection after CN injury is mediated by antioxidative/nitrosative and anti- apoptotic pathway [22]. When platelets are activated, they release many kinds of growth differentiation factors and a few types have been found to facilitate nerve repair and regeneration. Moreover, corporeal dysfunction due to smooth muscle atrophy or other inracavernosal pathology can lead to corporo-venous occlusive erectile dysfunction despite a normal arterial inflow. Rejuvenating the Corporeal tissues with PRP, which is well known for its growth and healing factors, is a possible modality as a potential treatment for erectile dysfunction according to Alkhayal et al [23]. In their retrospective study examining the efficacy of one intra-cavernosal PRP injection to 40 ED patients, they reported that mean IIEF-5 score before treatment was 13 (5-20) and post treatment IIEF-5 = 17 (7-24), ($p < 0.001$). Other studies have shown similar results with minimal side effects, no serious adverse reactions and potential efficacy (Table 1) [23-26].

Human Studies of PRP for ED							
Author	**Year**	**Number of Patients**	**Patient Population**	**Administration**	**Followup**	**Adverse Events**	**Conclusion**
Banno [26]	2017	N=9	Penile Rehab	PRP ICI into Pnies with Vacuum Device	4 weeks	No AEs reported	PRP may represent a safe and viable option as a supplementary therapy for penile rehabilitation. Particularly notable is the prospect of zero side effects.
Matz [24]	2018	N=16	Erectile dysfunction, Peyronies Disease	PRP with CaCl ICI	15.5m	Minor: Bruising 1, Mild Pain 4. Major: none	PRFM injections for ED, PD, and female SUI are feasible and safe. Although the limited data is suggestive of efficacy, a placebo control will be required in subsequent efforts for confirmation
Epifanova [25]	2019	N=10	Erectile Dysfunction	6 injections PRP with CaCl2 ans Shockwave	60 Days	No AEs reported	There were no serious adverse events as well as severe adverse events. Erectile dysfunction symptoms in all men participated in the study significantly decreased after treatment with PRP-therapy and extracorporeal shockwave therapy
Alkhayal [23]	2018	N=40	Erectile Dysfunction	1 injection PRP	> 1 month	No AEs reported	Platelet rich plasma is a safe and efficacious option for penile rejuvination and improvement of erectle dysfunction

Table 1: Summary of published studies and abstracts

To date, there are no treatments that address the underlying cause of endothelial dysfunction, although LIST treatment for ED has shown promising results. Platelet-derived therapies targeting inflammation and promoting tissue/nerve regeneration and may represent a potential treatment option towards this direction. Despite growing evidence to the efficacy of PRP, no randomized placebo-controlled studies exist.

3. Study Objectives

3.1 Primary Objective:

To investigate and compare the treatment efficacy of PRP vs placebo treatment in men with mild-moderate ED, as measured by IIEF.

3.2 Secondary Objective:

To study the adverse events and safety of the PRP treatment in in men with mild-moderate ED, as measured by IIEF.

4. Study Endpoints

4.1 Primary Endpoint:

- The percentage of subjects in each group who attain MCID in IIEF-EF domain from baseline to 4 weeks after final treatment.

4.2 Secondary Endpoints (efficacy):

- The percentage of subjects in each group who attain MCID in IIEF-EF domain from baseline to 12 & 24 weeks after final treatment.
- The difference between groups in change of IIEF-EF score from baseline to 4, 12, & 24 weeks after final treatment.

- Differences in Doppler ultrasound parameters (PSV and EDV) from baseline to 24 weeks after final treatment.

4.3 Secondary Endpoints (safety):

- Incidence of adverse events in all patients during the study period. Anticipated adverse events that will be recorded at every visit are the following
    - Pain
    - Bruising
    - Swelling
    - Edema
    - Allergy
    - Penile Fracture
    - New Penile Curvature

4.4 Exploratory Endpoints:

- Change in stretched penile length from baseline to 24 weeks after final treatment.
- The percentage of subjects in each group who attain MCID in IIEF-EF domain from baseline to 4 weeks after final treatment.

5. Study Location

The study will be funded by the Department of Urology and coordinated by the University of Miami (UM), Miller School of Medicine, Department of Urology. The UM Interdisciplinary Stem Cell Institute (ISCI) will also support the project (logistics, quality control, management, storage of specimens and data). All study visits will be carried out in the Department of Urology clinics, (ADDRESS: 1150 NW 14th Street, Suite 309, University of Miami Miller School of medicine, Miami, FL. & Lennar address) PRP preparation will take place in the clinic's andrology lab.

5.1 Additional Study Sites

No additional study sites.

6. Study Population

80 males with ED of organic origin. All patients will be regular PDE5i users/responders. After a 1-month wash-out period for PDE5i, men with ED will be evaluated by IIEF-EF domain and eligible patients will be randomized to one of 2 treatment groups with an equal allocation ratio (1:1). Participants who do not adhere to the 1-month wash-out period, or do not meet the IIEF-EF domain score will be considered a screen failure and will not receive the study therapy.

6.1 Inclusion criteria: In order to participate in this study, a patient must:

1) Be Male
2) Be 30 to 75 years of age (inclusive).

3) Be able to provide written informed consent.
4) Have a diagnosis of ED due to organic origin for at least 6 months prior to consent.
5) Sexually active in a stable, heterosexual relationship of more than three months duration.
6) IIEF-EF score 11-25 at screening (even if taking a single PDE5).
7) Agree to attempt sexual intercourse at least 4 times per month for the duration of the study without being under the influence of alcohol or recreational drugs.
8) Agree to comply with all study related tests/procedures.

6.2 Exclusion criteria: In order to participate in this study, a patient must not:

1) Previous penile surgery of any kind (except circumcision and condyloma removal), such as penile lengthening, penile cancer surgery, penile plication, grafting.
2) Previous history of priapism or penile fracture
3) Abnormal morning serum testosterone level defined as a value lower than 300 ng/dL (±5%) (indicative of untreated hypogonadism), or greater than 1197 ng/dL (±5%).
4) Current or previous hormone usage, other than prescribed testosterone, clomiphene or thyroid medication. (Subjects with prior or current use of hormonal treatment for prostate cancer are also excluded.
5) Psychogenic ED as determined by study investigator.
6) Anatomical (Peyronie's Disease or penile curvature that negatively influences sexual activity) or neurological abnormalities in the treatment area.
7) Patients using ICI for management of ED
8) Patients with generalized polyneuropathy, or neurological conditions irrespective of cause, such as severe diabetes, multiple sclerosis or Parkinson's disease.
9) Have a serious comorbid illness/condition/behavior that, in the opinion of the investigator, may compromise the safety or compliance of the subject or preclude successful completion of the study.
10) History of consistent treatment failure with PDE5 inhibitors for therapy of ED.
11) Any history of significant psychiatric disease, such as bipolar disorder or psychosis, greater than one lifetime episode of major depression, current depression of moderate or greater severity. Patients who are currently using SSRI or psychotropic medications.
12) Hemoglobin a1c >9%.

7. Identification and Enrollment of Subjects

7.1 Recruitment and Pre-screening

The research coordination team may develop materials to aide in recruitment. This may include, but is not limited to, informational videos and brochures which provide education about erectile dysfunction and include information about the study; physician referral letter templates which can be used to promote awareness of the study in the urology community; flyers/posters which can be utilized at approved clinic locations and as part of health fair materials; templates for print advertisements which can be utilized in

newsprint and media campaigns. Not all materials have been developed prior to trial initiation, however each of these recruitment methods will be reviewed and approved by the IRB prior to use.

Pre-screening of subjects includes reviewing medical records and imaging studies for inclusion/exclusions prior to consent. From the review of subjects' medical records and imaging studies on file, subjects who are determined to have a diagnosis of erectile dysfunction of organic origin, and have mild-moderate erectile function as assessed by IIEF-EF, as stated in Section 6.1, and also do not have evidence in their medical record of study exclusions stated in Section 6.2, are eligible to be consented to the study.

Investigators will inform research staff of potential participants who have been identified through pre-screening so the subjects can be approached to discuss the study and conduct the informed consent session. No study procedures will take place prior to the subjects signing of the informed consent form.

7.2 Payment to Subjects

No payments will be made to subjects as compensation for their participation in this study. The subjects will not be asked to pay for the treatments or participation in study either.

7.3 Informed Consent

7.3.1 Consenting Process

All subjects must provide written consent to participate in this study. An informed consent form (ICF) will be given to each subject. The ICF will contain all United States federally required elements, all International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) required elements, and Health Insurance Portability and Accountability Act Authorization (HIPAA) information in language that is understandable to the subject. The informed consent includes descriptions of all study related procedures, all possible risks to participant, and the time commitment involved with participating. All consent forms will have IRB approval. The ICF and review must be in a form understandable to the subject. Translation of ICFs will be done in accordance with local IRB procedures.

Potential participants will be approached by one of the study investigators or research coordinators. Information regarding study participation will be provided to the potential participant prior to consent. Subjects will be given ample time to review the ICF and ask questions before signing. The Investigator or designee and the subject must both sign and date the ICF after review, and before the any study procedures are performed. The subject will receive a copy of the signed and dated form, and the original will be retained in the site study files. The research staff member obtaining consent will document the informed process in the subject's chart for monitoring purposes. The Investigator or his/her designee must emphasize to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

7.3.2 Non-English Speaking Subjects

In addition to English-speaking subjects, Spanish-speakers will also be included in the trial. A certified translated version of the IRB approved English consent form will be made

available to non-English speakers. Spanish version questionnaires and other patient-facing materials will also be made available.

7.4 Withdrawal Criteria

All subjects who either screen fails, is withdrawn from the study, or has completed all visits should be de-enrolled from the research database within 2 business days, in accordance with university policies (or respective institutional policy).

Subjects may be withdrawn from the study for any of the following reasons:

a) A subject does not meet the eligibility criteria (the subject will be considered a screen failure).

b) A subject withdraws consent

c) A subject expires during protocol participation from causes other than the study treatment (not due to adverse events)

d) At the discretion of the principal investigator for issues of non-compliance, or other behavioral factors.

8. Procedures Involved

72 men with Erectile Dysfunction (ED) of organic origin, that meet all of the inclusion and none of the exclusion criteria, will be randomized to receive either PRP, or placebo in a 1:1 double-blinded fashion.

a) Group A (40 subjects): Autologous PRP injection. All subjects in this group will receive 2 sessions of autologous PRP penile injection with 30 ± 7 day treatment interval. 10mL of PRP will be injected at each session.

b) Group B (40 subjects): Placebo (Normal Saline). All subjects in this group will receive 2 sessions of normal saline penile injection with 30 ± 7 day treatment interval. 10mL of normal saline will be injected at each session.

8.1 Schedule of Events

The time and events table for the conduct of this study is shown below:

Table1:

Study Phase		Screening	Injection		Follow Up		
Time Point		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Visit Window		0 - 28days prior to therapy	Day 0	Day 28 ± 7	**Week 4** Day 30 ± 7	**Week 12** Day 90 ± 7	**Week 24** Day 180 ± 7
Informed Consent		**X**					
Demographics		**X**					
Medical History		**X**					
Physical Exam		**X**					
Doppler Ultrasound		**X**					**X**
Concomitant Medications/Therapy		**X**	**X**	**X**	**X**	**X**	**X**
Review Adverse Events			**X**	**X**	**X**	**X**	**X**
Randomization			**X**				
Study Therapy Injection			**X**	**X**			
Questionnaires	IIEF	**X**			**X**	**X**	**X**
	SEP3	**X**			**X**	**X**	**X**
	EDITS				**X**	**X**	**X**
	VAS		**X**	**X**			
Laboratory Testing	Hematology	**X**					
	Chemistry	**X**					
	HbA1c	**X**					
	Testosterone	**X**					

8.2 Description of Study Procedures

8.2.1 ***Informed Consent:*** Refer to Section 7.3 for details regarding the informed consent process. Study procedures will be completed only after participants have signed the informed consent documentation.

8.2.2 ***Demographics:*** Demographic characteristics will be recorded including: date of birth, gender, marital status, race and ethnicity. If Medical history has been recorded as part of their participation in another study those source documents may be requested and used for the purpose of this research study.

8.2.3 ***Medical History:*** Assessment of current and past medical, surgical, and social history will be conducted.

8.2.4 ***Physical Examination:*** Genitourinary/Reproductive system physical exam will be performed.

8.2.5 ***Doppler Ultrasound:*** Penile Doppler ultrasound will be performed as standard of care for the management of erectile dysfunction. Results from within the past 12 months can be extracted from the medical record for use in this study.

8.2.6 ***Concomitant Medications:*** Review of current use of prescription and over the counter (OTC) medications. Participants must discontinue use of PDE5i medications throughout the duration of the study.

8.2.7 ***Review Adverse Events:*** Refer to Section 12 for description and reporting of adverse and serious adverse events

8.2.8 ***Randomization:*** Refer to Section 9.3 for details about randomization plan

8.2.9 ***Study Therapy Injection*****:** Approximately 120mL of blood will be collected from participants for preparation of investigational product. Refer to Section 9.2 for description of IP preparation and administration.

8.2.10 ***International Index of Erectile Function (IIEF) Questionnaire*****:** This validated 15-item self-evaluation scale provides pre and post treatment clinic evaluations of erectile function, orgasmic function, sexual desire, satisfaction in sexual intercourse and general satisfaction. If the subject has completed the IIEF questionnaire within the past 6 months, those results may be requested and used for the purpose of this research study. Questionnaires will be completed prior to therapy at injection visits.

8.2.11 ***Sexual Encounter Profile (SEP3) Questionnaire*****:** This 5-item questionnaire is completed after each sexual intercourse attempt. Participants will be asked to complete the short version consisting of question #2 and #3 only.

8.2.12 ***Erectile Dysfunction Inventory of Treatment Satisfaction (EDITS) Questionnaire*****:** This is a psychometrically sound questionnaire used to assess satisfaction with medical treatment modalities for erectile dysfunction.

8.2.13 ***Visual Analogue Pain Scale (VAS)*****:** The visual analog scale (VAS) is a validated, subjective measure for acute and chronic pain. Scores are recorded by making a handwritten mark on a line that represents a continuum between “no pain” and “worst pain”.

8.2.14 ***Laboratory Testing:*** Hematology, chemistry, hemoglobin A1C, and serum testosterone, lab test results will be recorded at screening. If any standard of care labs tests have been performed within 12 months of visit date, those results may be used for the purpose of this study.

8.3 Description of Study Visits

8.3.1 Visit 1: Screening Visit

Visit 1 (0 – 28 days before therapy): The basic work-up will take place, including medical and sexual history, as well as necessary lab tests (testosterone, prolactin, hematology, chemistry), if not available during the last 12 months prior to screening. Subjects will complete the IIEF-ED questionnaire. We will ensure that patients have 4 attempts for intercourse, which they will record on the SEP diaries. PDE5i use is prohibited throughout the study. Screening visit can occur on same day as first injection. Refer to table 1 for procedures to be performed at the Screening visit.

8.3.2 Visit 2-3: Injection Visits (Day 0 & Day 28)

All participants will complete the IIEF questionnaire, and SEP diaries will be evaluated. Upon evaluation of eligibility, subjects will be randomized to one of the 2 parallel study groups, using an online program, and initiate treatment on the same day.

There will be 2 treatment visits for all Groups with 28 ± 7 days treatment interval for all subjects. Subjects will complete a Visual Analogue Scale (VAS) pain score after the end of each treatment. Refer to table 1 for all procedures to be performed at the Intervention Visits.

8.3.3 Visit 4-6: Follow Up Visits (Week 4, 12, 24) Post-Injection

Follow up visits will occur within 30 ± 7 days of final injection (V3). Refer to table 1 for procedures to be performed at the follow up visits visit.

9. Investigational Product

9.1 Preparation of IP

Autologous Platelet-Rich Plasma (PRP) is prepared by taking 120 mL aliquots of anticoagulated blood (120 mL whole blood and 16 ml anticoagulant citrate dextrose formula A) obtained from each treatment subject by venipuncture. Each aliquot is processed by an autologous platelet separator (Arthrex Angel, Arthrex Inc., Naples, Florida) to yield 3-10 mL of PRP from each subject. Platelet poor plasma (PPP) can be added to PRP to achieve the desired injection volume. This system is FDA approved by 501k for “clinical laboratory or intraoperatively at the point of care for the safe and rapid preparation of platelet poor plasma and platelet concentrate (platelet rich plasma) from a small sample of whole blood or a small mixture of blood and bone marrow”. For patients randomized to the control arm, they will still have their blood drawn, however it will not be processed to make PRP.

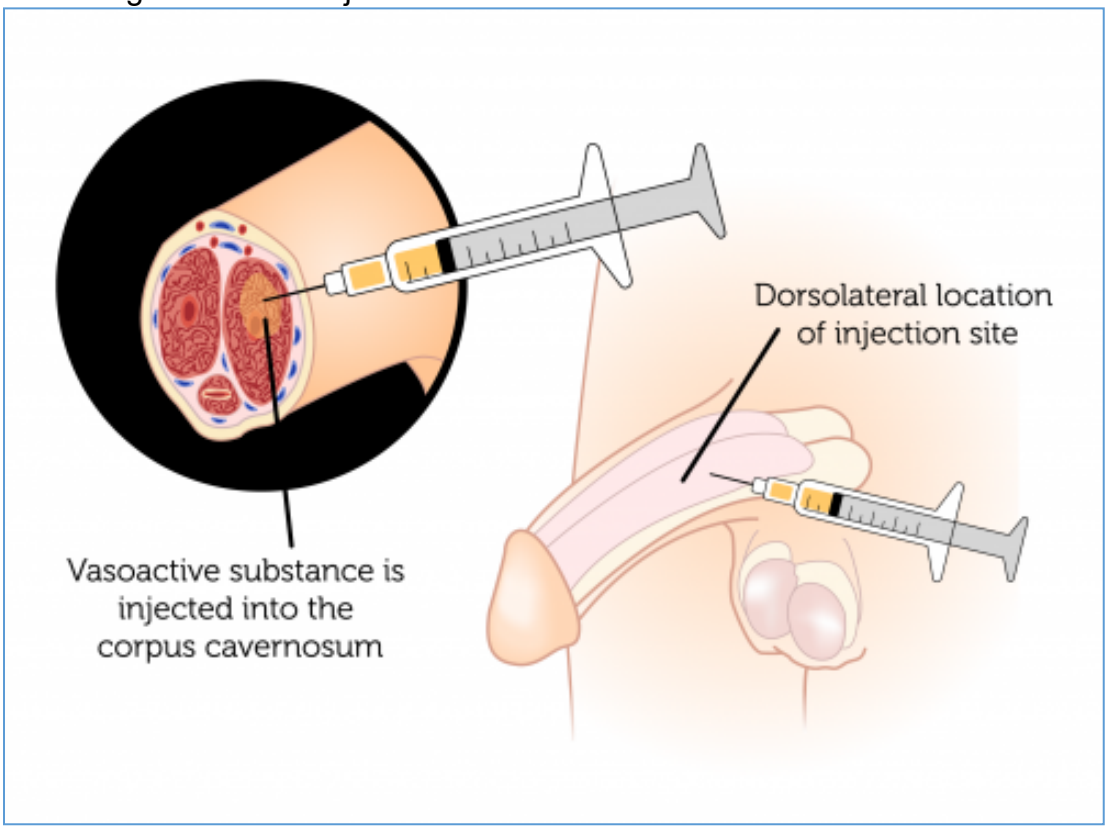
9.2 Administration of IP

The patient will be positioned supine. A sterile field will be developed with Betadine prep of the genitalia, sterile gloves and a sterile extremity drape. A total of 20 ml of 1% lidocaine will be drawn up using an 18-gauge needle and will be subcutaneously administered using a 27-gauge needle into the subcutaneous tissue at the base of the penis to obtain a dorsal nerve block. A 1/4-inch Penrose drain will be used as a tourniquet by placing it at the base of the penis and maintaining it in place under tension by a sterile clamp. A maximum of 5mL of PRP will be infused steadily over a two-minute period – approximately 2.5 ml each into the right and left corpus cavernosum. The infusion will be performed at this slow speed (each side over 2 minutes of infusion) to minimize injury to

the platelet cells. In the control arm, men will be administered injectable normal saline that will be injected in a similar manner as the PRP.

Following administration of PRP or normal saline, compression of the penis will be achieved with a clenched fist for 20 minutes. At 20 minutes, the tourniquet will be removed, and a compressive dressing will be placed around the penile shaft. The patient will be instructed to remove the compression bandage in 4 hours and to contact the study coordinators if he experiences any problems.

Figure 1: Diagram of PRP Injection



9.3 Randomization

On Day 0 (V2), participants who remain eligible for the trial will be randomized to one of two groups in a 1:1 ratio. The randomization sequence will be computer generated by the study coordinating team.

9.4 Blinding

The study will remain double-blind by having both blinded and unblinded study teams. All subjects, active-therapy and placebo groups will undergo blood collection procedure on day of intervention visits. Preparation of PRP and normal saline injections will be conducted only by unblinded research team members. In the interest of safety, the study product must be inspected to ensure it is free of air bubbles, clumping, etc.

The 10cc syringes used will be colored and covered in both groups in order to ensure the double-blindness of the study.

If for important medical reasons unblinding of additional team members is thought to be necessary, the Investigator may identify the treatment assignment by obtaining randomization records from unblinded study team.

10. Data Management

Data will be entered from source documents into eCRFs which are maintained in an online research database. The investigator will ensure data integrity by confirming the CRF's are attributable, legible, contemporaneous, original, accurate, and complete.

10.1 Case Report Forms (CRFs)

The Investigator or designee must record all required subject data, and an explanation must be documented for any missing data. This study will use electronic case report forms (eCRF) for data entry and database storage.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, and records kept at medical technical departments involved in the clinical trial. All corrections to study data will be made by drawing a single line through the information to be corrected without obscuring it. All corrections will be initialed, dated and explained, if necessary, in accordance with good clinical practice (GCP) guidelines.

10.3 Study Monitoring, Auditing, and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents. The investigator will ensure the capability for inspections of applicable study-related facilities.

11. Data and Specimen Banking

11.1 Storage of Data

Data will be maintained on research team hardware, and subject charts will be stored in a filing room maintained by Department of Urology. Clinical follow up and laboratory data will be stored in a locked room, with access only to authorized personnel. It is up to the principal investigator's discretion as to how long non-FDA study essential documents are retained.

11.2 Specimen Banking

Bio-specimens will be reserved for future testing and stored at -80 degrees Celsius in equipment maintained by ISCI in the Biomedical Research Building (BRB) (address: 1501 NW 10th avenue Miami FL 33136). Each specimen must have a corresponding requisition form to track the chain of custody from time of collection to time of processing and/or storage.

12. Safety Monitoring and Reporting

12.1 Adverse Events (AEs)

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study. The event does not need to have a causal relationship with treatment. Common Terminology Criteria for Adverse Events Version 5 (CTCAE) will be used for describing AEs. All urological and/or reproductive system AEs will be recorded. For all other AEs, only events greater than grade 2 will be recorded.

Adverse Events Reported N=75	
Pain	4
Bruising	1
Swelling	0
Edema	0
Allergy	0
Penile Fracture	0
New Penile Curvature	0

We anticipate there to be few adverse events related to the study. Based on the four published studies and abstracts, including 75 men, only minor adverse events were reported (Bruising 1, Mild Pain 4). No serious adverse events were observed [23-26] (Table 2)

12.2 Serious Adverse Events (SAEs)

A serious adverse event (SAE) is defined as an AE which, in the view of the Investigator results in: 1) Death; 2) a life-threatening event (i.e. an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe); 3) inpatient hospitalization of > 24 hours or prolongation of existing hospitalization; 4) a significant disability/incapacity; or 5) a congenital anomaly/birth defect. Other important medical events may be considered SAEs if, in the opinion of the Investigator, they jeopardize the subject or require intervention to prevent one of the other outcomes listed above. Based on prior investigations, we do not anticipate any SAEs related to the study procedures [23-26]

Treatment Emergent Serious Adverse Events (TE-SAEs)

A treatment emergent serious adverse event (TE-SAE) is defined as any serious adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. For the purposes of safety reporting, “a reasonable possibility” means there is evidence to suggest a causal relationship between the study product/procedures and the adverse event.

12.3 Reporting Responsibilities of the Investigator

Safety monitoring and reporting will be conducted in accordance with all university and departmental standard operating procedures (SOP). The study investigators will report to a surgeon monitor (**Dr. Nicholas Hauser)** who is not directly involved in the study, and has been delegated by the sponsor to ensure data quality and subject safety. The investigators will conduct continuous reviews of the safety data, keep track of SAEs and TE-SAEs, which will be discussed at research committee meetings once every 3 months.

Common Terminology Criteria for Adverse Events, Version 5 (CTCAE v5.0) will be used to assess severity of adverse events. All grade 3-5 adverse events, regardless of

association with the IP, will be entered into study database and reviewed at research committee meetings. In addition, all SAEs will be entered into research database and reviewed by the surgeon monitor on an ongoing basis. If a death occurs within 30 days of investigational therapy, and is determined to be related to the study, the investigators will notify the Urology Department Chair **(Dr. Dipen Parekh)** within 1 business day. If an increase in the frequency of grade 3 or 4 adverse events is noted in the study, a report will be submitted to the Department Chair at the time the increased rate is identified. If at any time the principal investigator stops enrollment or stops the study due to safety issues, the Department Chair will be notified within 1 business day and a formal letter will be sent to the Department Chair to be received within 10 business days.

13. Statistical Considerations

13.1 Data Analysis Plan

Analysis plan: Continuous data will be analyzed using ANOVA with repeated measures, or student's T-Test (where applicable) in order to compare differences between the treatment and control groups. For categorical data, Fisher's Exact test to determine differences between groups. Statistical significance will be evaluated using $\alpha=0.05$.

13.2 Sample Size

Inclusion Criteria are men with IIEF scores >11 which includes men with mild (IIEF 16-21) and mild/moderate (12-16) Erectile dysfunction. Minimal clinically important differenced (MCID) for these groups are an increase of 2 for mild and 5 for moderate ED. Previous studies on the effect of placebo show about a 15% improvement in men with mild to moderate ED. Therefore, 15% of the placebo group is expected to meet MCID. Results from the PRP safety study by Matz et al. 2018, which showed a mean improvement in IIEF of 4.14 points in seven patients with ED after PRP. In the interventional group, we expect 50% to meet MCID. At 80% power, we will therefore need 30 patients in each group to detect a difference of 35% between treatment and placebo arms.

$$\text{Sample size} = \frac{2(Z_{\alpha/2} + Z_{\beta})^2 P(1-P)}{(p_1 - p_2)^2}$$

$28.08 = (2*(1.96+0.84)^2 * (0.25*(1-0.25)) / (0.35^2)$

Assuming attrition of approximately 20%, an additional 6 participant slots will be added to each group for a total sample size of 80 subjects.

14. Risks to Subjects

We anticipate there to be few adverse events related to the study. Based on the four published studies and abstracts, including 75 men, only minor adverse events were reported (Bruising 1, Mild Pain 4). No serious adverse events were observed [23-26] (Table 2). After literature review, we believe this product to be of non-significant risk to patients (NSR) and for investigational use in a clinical study protocol.

Based on published studies, we will record anticipated adverse events to patients include: local injection cite reaction, penile plain and penile bruising. Additionally, we anticipate

patients may experience, penile swelling, allergic reaction, local infection (though none reported in previous studies) and will record them at every visit systematically.

15. Potential Benefits to Subjects

The potential benefits for men randomized to the treatment arm will include improvement in erectile function and sexual performance.

16. Privacy & Confidentiality

Each subject's protected health information will remain strictly confidential and shall be excluded from the database. Patients deemed eligible to participate in the study following evaluation of inclusion/exclusion criteria will be assigned a unique study participant identifier number (ex.: 01-PRP01-001). The first two characters indicate the enrolling site, next 5 characters describe the study and phase, and final 3 characters are the unique identification number assigned to subjects in sequential order. Only each participant's initials, assigned study participant identifier number, and date of birth may be documented in the database. The Investigator will retain a cross-referencing record of each subject's name and assigned identifier number. All study data and results will be stored in the electronic database. Each study subject will give explicit consent for representatives of the IRB/IEC and regulatory authorities to inspect and verify each subject's medical records and collected information. In turn, each study subject will be assured that all their personal information will be maintained in the strictest of confidence, and in compliance with HIPAA, and all other federal and local laws regulating privacy and data protection.

17. Ethical Considerations

17.1 Regulatory Authority Approval

This study will be conducted in accordance with Good Clinical Practice (GCP) requirements described in the current revision of International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) Guidelines and all applicable regulations, including current United States Code of Federal Regulations (CFR), Title 21, Parts 50, 54, 56, and Title 45, Part 164. Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki. This study will also be carried out in accordance with local legal requirements.

According to FDA guidance issued to institutional review boards and investigators, we will use PRP for ED (an indication not approved on the labeling) while simultaneously "bearing the responsibility to be well informed about the product, base its use on firm scientific rationale and on sound medical evidence, and to maintain records of the product's use and effects."

We believe that according FDA regulation**,** the clinical investigation of PRP for ED does not require submission of an IND / IDE since all six of the following conditions are met:

i. it is not intended to be reported to FDA in support of a new indication for use or to support any other significant change in the labeling for the drug;

ii. it is not intended to support a significant change in the advertising for the product;

iii. it does not involve a route of administration or dosage level, use in a subject population, or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

iv. it is conducted in compliance with the requirements for IRB review and informed consent [21 CFR parts 56 and 50, respectively];

v. it is conducted in compliance with the requirements concerning the promotion and sale of drugs [21 CFR 312.7]; and

vi. it does not intend to invoke 21 CFR 50.24.

17.2 Ethics Approval

The investigators agree to conduct the study in compliance with the protocol, current good clinical practices, and all applicable (local, FDA) regulatory guidelines and standard of ethics.

It is the Investigator's responsibility to ensure that, prior to initiating this study, this protocol is reviewed and approved by the appropriate local and/or external IRB. The composition and conduct of this committee must conform to the United States CFR. The IRB/IEC must also review and approve the site's informed consent form (ICF), other written information provided to the subject.

If it is necessary to amend the protocol or the ICF during the study, the Sponsor-Investigator will be responsible for ensuring that the IRB/IEC reviews and approves these amended documents. An IRB/IEC approval of the amended protocol and/or ICF must be obtained in writing before implementation of the amended procedures and before new subjects are consented to participate in the study using the amended version of the ICF.

17.3 Conflict of Interest

Any investigator who has a conflict of interest with the study must have the conflict reviewed by a properly constituted Conflict of Interest Committee with the Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All investigators will follow the University of Miami's (or applicable institution's) conflict of interest policy.

18. Publications

The preparation and submission of manuscripts for publication that contain results from this study shall comply with all applicable privacy laws and in accordance with processes determined by the University of Miami, Miller School of Medicine.

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