

## **INVESTIGATIONAL PLAN**

**PROTOCOL:**                   **Feasibility Study of Intra-cavernosal  
Administration of Non-Expanded Autologous  
Bone Marrow Cells in Treatment of Erectile  
Dysfunction**

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**TRIAL SYNOPSIS:**

<b>STUDY TYPE:</b> Interventional
<b>TITLE OF STUDY:</b> Feasibility Study of Intracavernosal Administration of Non-Expanded Autologous Bone Marrow Cells in Treatment of Erectile Dysfunction
<b>STUDY CENTERS:</b> Single site; LA Biomed, Harbor-UCLA Medical Center
<b>NUMBER OF SUBJECTS:</b> 20 Patients
<b>STUDY DURATION:</b> Study will be concluded 12 months after entry of each patient into the study.
<b>STUDY OBJECTIVE:</b> To evaluate safety and efficacy of autologous bone marrow mononuclear cells concentrated using a closed-system device, the Arteriocyte Magellan® device, and injected intra-cavernously in 20 patients aged 18 to 40 years of age diagnosed with erectile dysfunction. Safety will be determined based on device- and procedure-associated adverse events and blood chemistry measurements at baseline vs. follow up visits at one, three, six, and twelve months post-treatment. Efficacy will be evaluated at baseline and follow up visits by Doppler ultrasound, dynamic infusion cavernosometry, and blood work. The study will determine whether injection of bone marrow cells intra-cavernously is a clinically feasible therapeutic approach for treating erectile dysfunction.
<b>STUDY RATIONALE:</b> Bone marrow is enriched for cells with regenerative potential, including mesenchymal stem cells (MSC), which accelerate healing of damaged tissue. The possibility of using bone marrow cells in the treatment of ED is enticing since stem/progenitor cell populations are known to secrete various growth factors, possess anti-inflammatory activities, and can differentiate into cells of the penile architecture. While the therapeutic activities of MSC have been established in animal models of erectile dysfunction [1-8], this study will be the first to examine the utility of intra-cavernosal injection of stem cells in a clinical setting.  Erectile dysfunction (ED) is characterized by abnormalities of the vascular system; most commonly, venous leakage (or veno-occlusive dysfunction) resulting from loss of integrity of the surrounding corporal smooth muscle [9]. In the penile vascular system, the corporal smooth muscle is responsible for trapping the blood delivered to the corpora cavernosa via the arterial system. In men who develop ED at an early age, loss of smooth muscle mass, rather than arterial dysfunction, is believed to be the major culprit in the inability to maintain or sustain erections [9, 10].

Bone marrow mononuclear cells can be extracted and concentrated in FDA-approved closed system devices and are already being used under the practice of medicine for a variety of indications. Therefore, we are seeking to explore the safety and feasibility of bone marrow cells harvested using Arteriocyte's Magellan® device and administered intracavernously to patients with ED.

#### **STUDY ENDPOINTS:**

##### Primary Outcome Measures:

- Improvement in erectile function as measured by total score in the International Index of Erectile Function [Time frame: Baseline to 6 months]
- Change in Doppler Ultrasound (papaverine induced color duplex Doppler) for evaluating blood flow [Time frame: Baseline to 6 months]
- Change in dynamic infusion cavernosometry that measures veno-occlusive pressure [Time frame: Baseline to 6 months]
- Adverse events [Time frame: Baseline to 12 months]

##### Secondary Outcome Measures:

- Improvement in erectile function as measured by total score in the International Index of Erectile Function [Time frame: Baseline to 12 months]

#### **METHODOLOGY:**

**Pre Screening:** Patients with a medical history indicating a diagnosis of ED will have their records retrospectively reviewed for eligibility in the trial. Prospective patients must have undergone Doppler Ultrasound and/or dynamic infusion cavernosometry during their previous ED diagnosis. Prescreening will confirm ED diagnoses and review patient medical history against all inclusion/exclusion criteria.

#### **Visit 1: Screening Visit/Baseline Assessment:**

- Informed consent process
- Complete physical examination (body system review, vital signs, history)
- Medication use review
- Laboratory testing:
  - Comprehensive Metabolic Panel (CMP)
  - Complete Blood Count (CBC)
  - Urinalysis
  - Prostate Specific Antigen (PSA) (case by case basis only)
- IIEF-5 questionnaire
- Nocturnal penile tumescence
- Doppler Ultrasound
- Dynamic infusion cavernosonometry

**Visit 2: Intracavernosal Injection of Bone Marrow**

- Bone marrow harvest and concentration of stem cells using Arteriocyte's Magellan® device used for bone marrow harvest and concentration of stem cells
- Intracavernosal injection
- Physician monitoring & discharge

**Visit 3: One-Month Follow-Up**

- Limited Physical Exam
  - Vital Signs (blood pressure, heart rate, respiration rate, temperature), body weight.
- Medication Use Review
- Adverse Events Assessment (patient self-reporting of side effects)
- Laboratory Testing: CMP, CBC
- IIEF-5 questionnaire
- Doppler Ultrasound

**Visit 4: Three-Month Follow-Up**

- Limited Physical Exam
  - Vital Signs (blood pressure, heart rate, respiration rate, temperature), body weight.
- Medication Use Review
- Adverse Events Assessment (patient self-reporting of side effects)
- Laboratory Testing: CMP
- IIEF-5 questionnaire
- Doppler Ultrasound
- Dynamic infusion cavernosometry

**Visit 5: Six-Month Follow-Up**

- Limited Physical Exam
  - Vital Signs (blood pressure, heart rate, respiration rate, temperature), body weight.
- Medication Use Review
- Adverse Events Assessment (patient self-reporting of side effects)
- Laboratory Testing:
  - IIEF-5 questionnaire
  - Doppler Ultrasound
- Dynamic infusion cavernosometry

**Visit 6: Twelve-Month Follow-Up conducted via phone conversation**

- Adverse events reporting
- IIEF-5 questionnaire

**SUBJECT POPULATION:**

Men at 18-40 years of age with a previous ED diagnosis will be eligible to participate. ED must have been diagnosed at least 6 months before enrolling in the trial.

Study subjects must have been diagnosed with erectile dysfunction of a veno-occlusive nature, excluding psychogenic, vasculogenic, neurogenic, anatomical/structural, hormonal, and drug-induced causes of ED.

The diagnosis of veno-occlusive ED will be based on physical examination (including heart rate, EKG, and blood pressure monitoring) and medical history, including sexual history, laboratory assessment, IIEF questionnaire scoring, nocturnal penile tumescence, Doppler ultrasonography, and dynamic infusion cavernosometry.

The Principal Investigator or his/her designee must review the medical history and baseline evaluations prior to enrollment of patients to ensure that inclusion/exclusion criteria have been met. The subject must be informed about all aspects of the study and written informed consent must be obtained from the subject prior to study procedures.

**Inclusion Criteria:**

- Men aged 18-40.
- Chronic organic ED duration at least 0.5 years
- Diagnosis of ED based on Doppler Ultrasound and/or dynamic infusion cavernosometry.
- Baseline International Index of Erectile Function (IIEF) score of <21
- Oral medications and intracavernous pharmacological approaches have been deemed ineffective, contraindicated or cannot be tolerated.
- Concurrently undergoing treatment with testosterone.
- Willing to forego any other treatments for ED over the course of the study.

**Exclusion Criteria:**

- Subjects using any medications/drugs with known effects on erectile function within 4 weeks of the study period, including certain antidepressants, antihistamines, diuretics, and beta-blockers.
- Subjects using herbal remedies for addressing erectile dysfunction within one month of study initiation.
- Subjects with penile prosthesis or other urinary prosthesis.

- Subjects with penile anatomical deformities (e.g. Peyronie's disease) or history of priapism.
- Previous penile surgeries for erectile dysfunction, premature ejaculation or penile enlargement.
- Diagnosis of psychogenic ED as determined by nocturnal tumescence testing.
- Presenting with uncontrolled or severe disease, including cardiovascular disease, diabetes, liver disease.
- Uncontrolled hypertension or hypotension (systolic blood pressure  $> 170$  or  $< 90$  mm Hg, and diastolic blood pressure  $> 100$  or  $< 50$  mm Hg)
- Suffered a cardiovascular event within 6 months prior to study initiation.
- Current or previous malignancy other than non-melanoma skin cancer (successfully treated or treatable by curative excision or other local curative therapy).
- Diagnosis of a systemic autoimmune disorder.
- Receiving immunosuppressant medications.

**List of Abbreviations:**

AE	Adverse Event
CBC	Complete Blood Count
CFR	Code Federal Regulation
CMP	Comprehensive Metabolic Panel
DICC	Dynamic infusion cavernosometry / cavernosography
EDV	End Diastolic Velocity
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HSC	Hematopoietic Stem Cells
IEC	Institutional Ethics Committee
IIEF	International Index of Erectile Function
IRB	Institutional Review Board
MSC	Mesenchymal Stem Cells
NPT	Nocturnal Penile Tumescence
PSA	Prostate Specific Antigen
PSV	Peak Systolic Velocity
RI	Resistance Index

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## 1. Study Purpose & Design

This study is designed to assess the safety and efficacy of autologous bone marrow mononuclear cells concentrated by a closed-system device, the Arterioocyte Magellan® Device, and injected intra-cavernously into patients with ED. Specifically, this study will evaluate ED treatment in young 18-40 year old men, a demographic where the etiology of ED is attributable primarily to the loss of corporal smooth muscle [9]. Study endpoints will therefore evaluate the efficacy of intra-cavernosal stem cell administration for ameliorating the veno-occlusive dysfunction in ED patients.

## 2. Background

### 2.1. Erectile Dysfunction: Incidence & Standard of Care

Erectile dysfunction (ED) is characterized by the lack of ability to achieve and maintain penile erection for intercourse. Methods used to quantify ED include the Erectile Function Visual Analog Scale (EF-VAS) and the International Index of Erectile Function (IIEF-5) [11, 12], however clinically it is primarily diagnosed based on symptomology. In our aging society, ED is becoming an increasing problem. According to one study 39% of men at age 40 experience symptoms of ED, whereas by age 70 the incidence rises to 67% [13]. In this latter age group, it is believed that 50-85% of ED cases are associated with hypertension, diabetes, cardiovascular disease and dyslipidemia [14]. Overall, it is estimated that 10-30 million Americans suffer from this condition [15].

Today, the first line of therapy for ED is a trial of phosphodiesterase (PDE) 5 inhibitors, drugs that augment the physiologic process of tumescence [16]. During penile erection, neuronally derived nitric oxide stimulates cyclic guanosine monophosphate (cGMP) production in vascular smooth muscle cells of the corpus cavernosum [17]. cGMP acts through a series of intracellular pathways to lower the intracellular level of calcium that, in turn, causes cavernosal smooth muscle relaxation and penile arterial vessel dilation. PDE5 inhibitors, which include sildenafil, vardenafil, tadalafil, and avanafil represent this first-line therapy for various forms of ED including both organic and psychogenic ED.

Unfortunately, up to 50% of patients are either unresponsive to phosphodiesterase-5-inhibitor therapy or do not tolerate adverse effects associated with treatment [18-21]. The primary reason for this unresponsiveness to the PDE5 inhibitors is either the presence of restricted inflow of blood into the cavernosa usually as a result of atherosclerosis of the iliac-pudendal – cavernosal arterial vessels, or nerve damage in which NO cannot be made to initiate the production of cGMP by the corporal smooth muscle, or simply smooth muscle atrophy where the remaining corporal tissue is insufficient to allow tumescence to occur [22]. Adjunctive and/or additive pro-erectile therapies have been used to try to overcome any less than optimum response from the oral PDE5 inhibitors but these have never been accepted as standard of care [23-25]. In addition, it is believed that a normal circulating testosterone level is necessary for the PDE5 inhibitor to work optimally [26,27]. Simply stated, any defect in either one or a

combination of these 4 parameters – the psyche, the nerve, the vascular system (both inflow and outflow), the endocrine (testosterone) system – can lead to ED.

PDE5 inhibitors are known to possess a variety of systemic effects in numerous organ systems; therefore, the long-term effects of PDE5 inhibition are still uncertain. PDE5 inhibitors can induce a variety of adverse effects such as optic neuropathy [28], headaches [29], and various cardiovascular pathologies [30], especially when taken in combination with nitrate medications[31]. In fact, in 1998, the US Food and Drug Administration published a report on 130 confirmed deaths among men who received prescriptions for sildenafil citrate, where causes of death included arrhythmias, sudden cardiac death and hypotension-associated events [32]. Beneficial non-ED uses of PDE5 inhibitors are also known, for example, since PDE5 is expressed in lung tissue, investigators sought to, and succeeded at inhibiting symptomatic pulmonary arterial hypertension in a double blind clinical trial [33] by administration of sildenafil citrate. However, given the various areas in the body that PDE5 is expressed, such as platelets, kidneys, and pancreas [34], it is the belief of some that systemic inhibition of this enzymatic system may have adverse physiologic consequences in the long-run [35].

In addition to oral phosphodiesterase inhibitors, other treatments for ED are increasingly invasive and include vacuum pumps, penile prostheses, and intracavernosal injections with vasodilators, and vascular surgery. Vacuum pumps may be difficult for some men to use, do not allow for spontaneous, natural erections to occur, and may cause penile trauma if used improperly. Implantation of penile prostheses is invasive, expensive, and irreversible and can cause penile deformity. Intracavernosal injections of vasoactive drugs are satisfactory or effective in 30 to 90 percent of men, but they can be associated with pain, priapism, penile hematomas, and fibrosis. Clinical interest in penile revascularization surgery stems from the widely reported link between ED and atherosclerotic vascular disease (examples in [36, 37]). Unfortunately however, the success rate of vascular surgery has been reported to be highly variable and has raised questions concerning the appropriate means for diagnosis of arteriogenic ED, and the safety and feasibility of stent-based therapies [38]. Hence, there is clearly a need for additional treatments for addressing ED.

**2.2. Causes of Erectile Dysfunction**

The causes of ED are diverse, and can be classified as veno-occlusive, vasculogenic, psychogenic, neurogenic, anatomical/structural, hormonal, and drug-induced, as summarized below.

Table 1: Summary of Common Causes and Diagnosis of ED

Type of ED	Cause	Diagnostic Criteria
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Vasculogenic	Blockage of arteries: May be associated with risk or presence of cardiovascular disease, diabetes, hypertension, hyperlipidemia, smoking, major surgery or radiotherapy of the pelvis. Venous leakage: Blood is not effectively trapped in the penis due to loss of corporal smooth muscle mass, causing inability to maintain erections.	Duplex ultrasonography, dynamic infusion cavernosonography, laboratory tests (CMP)
Psychogenic	Generalized (eg. disorders of arousal), situational (stress, partner-related)	History of risk factors such as stress, interpersonal problems; nocturnal penile tumescence test, IIEF questionnaire
Neurogenic	Nerve damage; for example, caused by diabetes. Diseases such as multiple sclerosis, stroke, spinal cord disorders.	Physical examination and medical history.
Anatomic/ Structural	Peyronie's disease, penile fracture, congenital curvature of the penis.	Physical examination and medical history.
Hormonal	Hypogonadism, hyperprolactinemia, thyroid problems, Cushing's disease	Laboratory tests, physical examination, medical history.
Drug	Antihypertensives, antidepressants, antipsychotics, antiandrogens, antihistamines, recreational drugs.	Medication history.

### 2.3. Vascular Dysfunction in the Penis

In the flaccid penis, a balance exists between blood flow in and out of the erectile bodies. Normal erectile function requires a complex set of dynamic neural and vascular interactions. On arousal, parasympathetic activity triggers a series of events starting with the release of nitric oxide and ending with increased levels of the intracellular mediator cyclic guanosine monophosphate (cGMP). Increases in cGMP cause penile vascular and trabecular smooth muscle relaxation. Blood flow into the corpora cavernosa increases dramatically. The rapid filling of the cavernosal spaces compresses venules resulting in decreased venous outflow, a process often referred to as the corporal veno-occlusive mechanism. The combination of increased inflow and decreased outflow rapidly raises intracavernosal pressure resulting in progressive penile rigidity and full erection.

Breakdown of cGMP in the cavernosal tissue is mediated by PDE-5. Increasing the duration of NO signaling by preventing cGMP breakdown is the main mechanism of action for the successful PDE-5 inhibitor class of drugs, which currently are used as first-line treatment of ED [39]. Interestingly, recent studies have shown that these drugs have other beneficial effects such as stimulation of bone marrow endothelial progenitor cell function [40-44], inhibition of smooth muscle cell apoptosis [45, 46], preservation/restoration of function in post-prostatectomy settings [47, 48] and activation of mesolimbic dopaminergic neurons in the CNS to promote sexual behavior [49].

Thus, vascular ED can result from vascular inflow impairment and/or failure of the corpus cavernosum restriction mechanism that is referred to as corporal veno-occlusive dysfunction.

Much attention has been directed to arteriogenic ED; specifically, identifying the relationship between atherosclerosis and development of ED. Interest in this area stemmed from a plethora of studies investigating the putative linkage between ED, endothelial dysfunction, and cardiovascular disease. For example, several studies suggest the ED is actually one of the first signs of impending cardiovascular disease [50-52]. Therefore, one method of treating the cause as opposed to the symptoms is to attempt to heal the endothelium. Since the majority of ED cases were believed to be a manifestation of systemic atherosclerotic disease [36, 37], and various forms of stem cell therapy have shown some efficacy in other manifestation of atherosclerotic disease [53-64], the possibility of applying such regenerative approaches to ED has been considered by investigators in animal models [4, 65-76].

While a great deal of focus has been placed on endothelial dysfunction and poor arterial inflow in ED, studies have demonstrated that venous dysfunction rather than arterial insufficiency is responsible for the majority of ED cases. The majority of patients report having difficulty maintaining or sustaining an erection once the erection had occurred, which is suggestive of venous leakage rather than impaired arterial inflow [9]. Indeed, the corporal smooth muscle mass is responsible for trapping blood in the corporal sinusoids, thereby maintaining rigidity of the penis. A study by Rajfer et al. demonstrated that venous leakage has a high prevalence (68% of men with organic ED) as defined by dynamic infusion cavernosometry [77]. Other early studies confirmed that venous dysfunction accounts for the majority of vascular pathology observed in ED patients [78, 79].

Veno-occlusive dysfunction is a major culprit in the onset of ED symptoms in young men. Loss of corporal smooth muscle mass in young men is likely governed by age- and genetics and may remain clinically asymptomatic until a threshold level of venous leakage is reached or until age-related disorders related to atherosclerosis also restrict arterial inflow [9].

#### **2.4. Cell Therapies for Erectile Dysfunction**

Stimulation of angiogenesis using bone marrow stem cells has been performed in animal models of ischemia, as well as in clinical trials [80]. Kendirci et al used bone marrow cells that were isolated for expression of the p75 nerve growth factor receptor using magnetic activated cell sorting [67]. They chose this population based on possible enhancement of neurogenic potential. Intra-cavernous administration of these cells into a rat bilateral cavernous nerve crush injury model was performed. At 4 week follow up, improvement in erectile function as assessed by mean intra-cavernous-to-mean arterial pressure ratio and total intra-cavernous pressure was assessed. Significant improvements were observed in animals receiving the p75 selected cells as compared to those receiving an equal concentration of bone marrow derived multipotent stromal cells, fibroblasts, or saline. Significantly higher levels of FGF-2 were found in

the cavernosum of animals receiving the p75 selected cells. To our knowledge this is the only animal experiment that utilized bone marrow derived cells without expansion in vitro.

The possibility of using mesenchymal stem cells in the treatment of ED is enticing not only because these cells are known to secrete various growth factors that are beneficial in ED such as IGF-1 [81-83], VEGF [84], and FGF-2 [85], but also because of their anti-inflammatory activities [86], as well as possibility of differentiating into tissue relevant to the penile architecture [87]. To assess whether bone marrow derived MSC had a therapeutic effect on diabetes induced ED, Qiu et al performed intra-cavernous administration of these cells. Four weeks after administration, the ratio of intra-cavernous pressure and mean arterial pressure (ICP/MAP ratio), as well as smooth muscle and endothelial cell compartment was significantly upregulated compared to controls. Cell tracking experiments revealed that the MSC were retained for at least 4 weeks post injection and showed expression of endothelial and smooth muscle cell markers, suggesting the possibility of trans-differentiation [66]. A subsequent study examined long term effects of MSC administration via the intra-cavernous route in aged rats. The study found that the mean cavernous cGMP levels after 3 and 4 months of MSCs transplantation were increased compared with those after 3 or 4 weeks, which were in turn higher than controls. Cavernous tissue ICP measurement showed significant increase in MSCs transplanted groups compared with the controls, which was more significant in the long-term follow up [4]. This suggests that some of the therapeutic effects of regenerative therapy may be observed in a more delayed setting as opposed to some of the previously mentioned gene therapy approaches. Similar therapeutic effects were observed with muscle derived MSC in the aged rat model, however long term follow-up was not performed [72]. Given that MSC may be used clinically in an allogeneic model, a xenogeneic model of human MSC into immune competent rats was performed. Administration of an immortalized human MSC clone into the cavernosum of Sprague Dawley rats resulted in differentiation into endothelial and smooth muscle cells [74]. Non-invasive imaging studies by the same group reported that human MSC may be found up to 12 weeks post injection in the cavernosum of rabbits and rats [73]. In order to augment therapeutic efficacy of MSC, genes for VEGF and eNOS were transfected into MSC for treatment of diabetes and age-associated ED, respectively. In both cases significant improvements in therapeutic efficacy were observed when gene transfected MSC were used in comparison to MSC alone [65, 75].

Adipose tissue derived stromal vascular fraction (SVF) cells represent a potent source of EPC, MSC and hematopoietic stem cells that has been used in clinical pilot trials and is part of veterinary medical practice in the USA [88]. The MSC component from SVF is postulated to possess some unique advantages to bone marrow MSC, such as augmented angiogenic activity, however this is controversial [71]. Several studies have used adipose derived mesenchymal stem cells that were in vitro expanded for the treatment of ED in the cavernosal nerve injury model [68], the hyperlipidemia model [69], and the streptozotocin induced diabetes model of ED [70]. Unfortunately it is still not clear which stem cell source is better since back to back experiments have not been

performed. Given the potent angiogenic characteristics of the endometrial-derived MSC, termed endometrial regenerative cells (ERC) [89], it may be relevant to assess therapeutic effect of these cells in models of ED.

Clinical use of stem cells in treatment of ED has been reported by Bahk et al from Korea who treated 7 patients with diabetes associated ED which was unresponsive to medication for at least 6 months with intracavernous cord blood mononuclear cells. 3 additional patients with similar characteristics were used as controls [90]. No treatment associated abnormalities were reported despite the allogeneic nature of the cells in absence of immune suppression. One month after treatment, morning erections were regained in 3 participants. By the third month post treatment 6 of the 7 patients had regained morning erections. In all patients rigidity increased as the result of cord blood administration, but was not sufficient for penetration. When the patients were administered PDE5 inhibitor before coitus, 2 achieved penetration and experienced orgasm, and maintained for more than 6 months; however, 1 participant could not achieved penetration at ninth month. Interestingly, an increase in sexual desire was reported in 6 of the 7 patients. No improvements were observed in any of the 3 control patients.

Overall these studies support: a) the rational for use of adult bone marrow-derived stem cells in the treatment of ED, and b) preliminary human feasibility of intracavernously delivered bone marrow cells for ED therapy.

## **2.5. Clinical Experience With Intracavernosal Injection of Bone Marrow Cells**

A 35 year-old patient presented to us with a history of erectile dysfunction unresponsive to oral PDE5 inhibitors. The patient was a smoker and had a history of hypercholesteremia, marginal effects from intracorporal PGE1 (Caverject) administration, 2 years ago, but as of 6 months, the treatment had no effect. Psychogenic ED was discounted based on 2 independent nocturnal penile tumescence (NPT) tests, which revealed abnormal findings. Normal was defined as having at least 1 episode of nocturnal erection of at least 10 minutes duration with a 2-cm increase in tumescence of the tip and 3-cm increase in tumescence of the base, together with 70% rigidity in the tip and base using the RigiScan monitoring [91]. Upon discussing with his urologist, the patient was considering a penile prosthesis implant.

The procedure was approved by the institutional review board, the procedure was explained to the patient, and the patient signed informed consent. Tumor markers AFP, PSA, CA19-9, and CEA, hematology, biochemistry panel and coagulation were unremarkable. CT scans of the chest, ultrasound of the abdominal area, and fecal occult test were also unremarkable.

The patient was administered one tablet of Vicodin (7.5 mg hydrocodone) and one tablet of Xanax (1 mg) 30 minutes before the procedure. Local lidocaine was applied topically at the area of bone marrow puncture. A total of 60 ml of bone marrow aspirate was obtained and processed in a closed-system bone marrow concentration device.

Bone marrow mononuclear cells were concentrated to a volume of 2 ml, with 1 ml administered into each cavernosal body using a 25-gauge syringe. A tourniquet was placed around the base of the penis during the injection procedure and held for 5 minutes to allow for maximal retention

No immediate injection-associated adverse events were noted. The patient reported a morning erection 2 days after cell administration. Although angiogenesis could not occur during this short time period, the possibility of bone marrow released nitric oxide stimulating erections via vasodilation may be postulated [92]. Three weeks after treatment, the patient reported erection strong enough for penetration, but did not have ability to sustain the erection until orgasm. At three month follow-up the patient reported having intercourse until orgasm several times and a marked increase in morning erections. Importantly, no adverse effects or ectopic tissue formation was observed at a follow up appointment 3 months post-procedure. These very preliminary results are suggestive of safety and feasibility of this procedure.

### 3. Patient Population

This study will enroll a total of 20 patients previously diagnosed with ED, as confirmed in the screening visit/baseline assessment. All inclusion/exclusion criteria must be met.

## 4. Protocol Procedures

### 4.1. Protocol Procedures Summary

Monitoring Study Plan	Assessments	Screening /Baseline	Treatment (Day 0)	Schedule of Events (Month post- treatment)			
				1	3	6	12
Informed Consent		X					
Complete Physical Exam		X					
Medication use review		X		X	X	X	
Laboratory Testing		X		X	X	X	
IIEF-5 Questionnaire		X		X	X	X	X
Nocturnal Penile Tumescence		X					
Doppler Ultrasound		X		X	X	X	
Dynamic Infusion Cavernosonometry		X			X	X	
Bone marrow harvest			X				
Intracavernosal Injection			X				
Adverse Events Reporting			X	X	X	X	X
On Site Follow-up				X	X	X	
Follow-up Phone Call							X



## **4.1. Acquisition and Delivery of Bone Marrow-Derived Cells**

### **4.1.1. Bone Marrow Aspiration**

Patient will be administered 4mg Morphine Intramuscular (for pain) and 2mg Ativan orally (for relaxation) 30 minutes prior to the procedure. The procedure will be conducted in an examination room. The patient will be placed in a lateral decubitus position with the patient's eyes away from the physician and the knees at a 45-degree angle. The region of the iliac crest (anterior or posterior) from which bone marrow will be harvested will be palpated, and a surgical marking pen will be used to mark the center point of the iliac crest spine (common area for collecting bone marrow due to its prominence and easy access). Under sterile conditions, the area (skin and soft tissues) will be anesthetized by injection with local anesthetic (lidocaine). A sterile drape with a fenestrated opening will be placed over the area to be operated on.

Prior to aspiration, rinse the aspirate syringes to be used in the procedure with 10% heparin solution such that there is contact between all inner surfaces and heparin. All the heparin solution will then be expelled.

A jamshidi-type needle will be used for bone marrow aspiration. It may be necessary to make a small (2-mm) skin incision at the level of needle entry using a scalpel blade. The aspiration needle will be pushed through the dermis and advanced on top of the iliac crest. Once the needle is correctly positioned, it can be pushed through to penetrate the bone and manually advanced between the inner and outer walls of the iliac crest to a depth of 5 cm. Bone marrow is then aspirated into a syringe (10cc) that has been pre-loaded with 3-4 mL of anti-coagulant. To aspirate more marrow, the jamshidi needle can be rotated 45° to reorient the bevel. After a full rotation of the needle at this level, the needle can be withdrawn approximately 1cm toward the surface for further aspiration. If needed, several perforations can be made through the same skin opening, approximately 2cm apart. The marrow should be aspirated in small amounts (approximately 4mL) at each site to avoid contamination with blood [93]. Aspirates will then be pooled for subsequent concentration steps.

Once bone marrow procurement is complete, direct pressure with sterile gauze is applied to prevent bleeding and a small sterile bandage is applied over the needle entry site. If required, the incision is closed with #3-0 nylon.

The target volume of bone marrow aspirate is 20-30mL per patient. It is estimated that approximately 3-5 aspirations will be needed to obtain sufficient bone marrow.

### **4.1.2. Enrichment of Bone Marrow Stem Cells**

Arteriocyte's Magellan® device (Figure 1) will be used for concentrating stem cells from bone marrow aspirate according to the manufacturer's instructions. The Magellan® device is a fully automated and closed system comprising a microprocessor controlled centrifuge and syringe pumps that concentrate specific cellular populations. Bone marrow is dispensed into centrifuge chambers for rapid and automatic enrichment of cellular fractions to yield a bedside prepared product rich in platelets, hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) in as little as fifteen minutes. The Magellan® device yields a 3-10cc injectable unit of platelet and stem cell enriched plasma for the physician's use for intracorporeal injection into the same patient.

According to a published report where bone marrow was enriched with the Magellan® 27 mL of bone marrow yielded approximately  $1.7 \times 10^8$  cells concentrated into 3mL [94].

In the current study, the Magellan® will be used to concentrate a dose of  $10^8$  cells (post-enrichment) into 3 mL plasma for intracavernosal injection from a starting (pre-enrichment) volume of 20-30 mL crude bone marrow. This procedure will be conducted in the examination room at the patient's bedside.

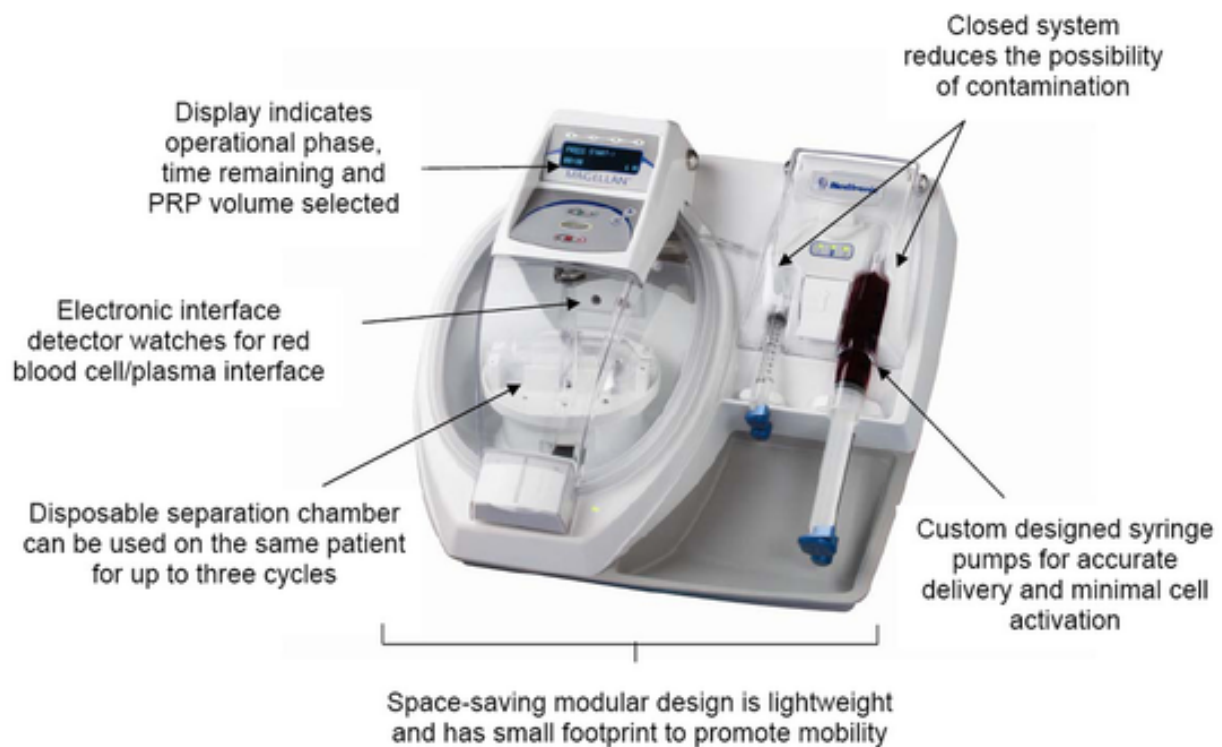


Figure 1: Arteriocyte's Magellan® (Source: <http://www.arteriocyte.com/no.html>; date accessed 12-05-2014) will be used to concentrate bone marrow, performed at the patient's bedside, followed by intracavernosal injection of the cells in the same treatment session.

### 4.1.3. Intracavernosal Injection of Stem Cells

Following extraction of 3 ml of sterile bone marrow concentrate using the Arteriocyte Magellan device, two 1/2-inch 25-gauge needles will be filled with 1.5 ml of the bone marrow concentrate. The injection site will be cleansed with an alcohol swab prior to injection. The cells will be injected into both corpora cavernosum along the dorso-lateral aspect of the proximal third of the penis. Care must be taken to avoid any area where there are visible veins. After the injections, sterile gauze will be placed with pressure on the injection site to prevent bleeding. The area of injection is the dark area in the diagram below (Figure 2).

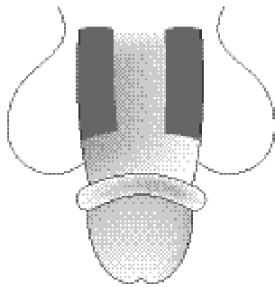


Figure 2: Injection site for intracavernosal bone marrow delivery.

## 4.2. Summary of Patient Windows

Visit	Patient Visit	Patient Window
1	Screening (Baseline)	Approx. 30 days
2	Treatment (Same day bone marrow harvest, processing, and intracavernosal injection)	Within 1 month of screening
4	1 Month (Post-Treatment Visit)	+/- 1 Week
5	3 Month (Post-Treatment Visit)	+/- 1 Week
6	6 Month (Post-Treatment Visit)	+/- 1 Month
7	12 Month (Follow up phone call)	+/- 1 Month

## 4.3. Laboratory Tests

### 4.3.1. Summary of Laboratory Tests

The following laboratory tests will be performed at the Screening Visit:

- **Comprehensive Metabolic Panel (CMP):** This series of blood tests will identify any health problems affecting major organ systems as a component of determining whether patients meet the inclusion/exclusion criteria.

- **Complete Blood Count (CBC):** Blood tests to evaluate patients for underlying medical conditions, including infections and diseases, in order to assess inclusion/exclusion eligibility of patients.
- **Prostate Specific Antigen (PSA):** Blood measurement to rule out conditions such as prostate cancer or benign prostatic hyperplasia. Since these conditions are uncommon in 18-40 year old men, PSA testing will be only ordered by the physician if indicated based on physical examination and/or medical history in the screening visit.
- **Urinalysis:** To provide information concerning the etiology of ED through assessment of the presence of medical conditions such as diabetes, kidney problems, and testosterone deficiency.

#### 4.3.2. Specimen Collection and Preparation

**Blood tests (CMP, CBC):** Blood tests will be performed after an 8-10 hour fast. Only fasting patients are eligible for laboratory testing. The check-in process for the blood draw will include asking the patient whether they have had anything to eat or drink (other than water) within the last 8 hours. If the fast has not been met, laboratory testing will be rescheduled within 48 hours.

A phlebotomist designated by the Investigational Team will collect blood samples by venipuncture in the arm.

A minimum volume = 1 mL and a preferred volume = 2 mL will be collected in serum separator tubes (SST<sup>®</sup>). Serum should be centrifuged and separated as soon as possible after clot formation (preferably within 45 minutes after collection).

Specimens must be properly labeled in the presence of the patient. Specimens should be labeled with patient's identifier number.

Specimens will be stored and transported under refrigeration temperatures for shipping to the designated testing facility.

**Urinalysis:** The opening of the urethra (tip of the penis) should be cleansed with a wipe before collection is begun to minimize contamination of the samples with bacteria from the surrounding skin. Once a stream of urine is established, 10-15 mL can be collected into the sterile specimen cup by directly urinating into the cup. Once an adequate amount is collected, the remaining urine should be voided in the toilet.

Specimens must be properly labeled in the presence of the patient. Specimens should be labeled with patient's identifier number..

### **4.3.3. Procedures for Laboratory Tests**

Specimens (blood and urine) will be stored and transported under refrigeration temperatures for shipping to the designated testing facility where all the testing will be performed.

### **4.3.4. Interpretation of Laboratory Results**

It is the investigators responsibility to assess the clinical significance of any abnormal laboratory values. All abnormal laboratory values will be defined by outside of normal range for the study site laboratory.

Laboratory results will be used to determine whether patients meet the inclusion & exclusion criteria. These tests will provide information concerning the etiology of ED as well as the presence of medical conditions that might preclude the patient from participating in or completing the study.

## **4.4. Physician Assessments**

A complete physical examination will be conducted at the screening visit, as detailed below. Physical examination will evaluate patients for the inclusion/exclusion criteria of the present study; namely, that ED is of veno-occlusive nature and that the patient exhibits no symptoms of uncontrolled diseases that might contribute to erectile dysfunction and/or is projected to interfere with their ability to complete the study.

### **4.4.1. Medical & Sexual History:**

Evaluation will include the following:

- Description of erectile dysfunction
  - Age at onset and duration
  - Association with specific event, if applicable
  - Progression (rapid vs. gradual) of dysfunction
- Quality of erections
  - Duration of ED, degree of ED, erectile spontaneity, erectile sustaining capability, early morning/nocturnal erectile function
- Frequency of dysfunction
  - Mild, moderate, complete absence

Because erectile dysfunction is frequently caused by medication, a review of the patient's medications is essential and should include prescription and over-the-counter medications.

Patient history will include questions pertaining to lifestyle. Excessive and long-term use of a number of substances may also cause erectile dysfunction including cigarette smoking, alcoholism, and recreational drugs; therefore, use of these substances will be considered in diagnosing the etiology of ED.

The patient's social history will be documented to assess life stressors such as change in social status, death of a family member, loss of job, or other family problems, which may have an effect on erectile function. Also, the physician should determine the patient's and their partner's level of understanding of sexual function, and expectations each has with regard to treatment outcome.

#### **4.4.2. International Index of Erectile Function (IIEF-5):**

In addition to the complete medical and sexual history recorded by the physician, each patient will complete the IIEF-5 questionnaire in the screening visit and during follow up visits to provide an objective read-out of changes in erectile function resulting from intracavernosal injection of bone marrow cells.

The IIEF-5 is a multidimensional scale that has been developed to assess sexual function. The IIEF addresses the relevant domains of male sexual function (that is, erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction), is psychometrically sound, and can be self-administered in a clinical setting. The EF domain of the IIEF-5 demonstrates the sensitivity and specificity for detecting treatment-related changes in patients with erectile dysfunction.

The IIEF questionnaire is provided in Figure 3. The questionnaire will be administered, scored and interpreted by the attending physician or designated qualified health care practitioner. According to diagnostic criteria for erectile dysfunction, the IIEF test results for the ED domain are considered normal if the score is  $\geq 22$  out of a possible maximum score of 25. .

### The IIEF-5 Questionnaire

Please encircle the response that best describes you for the following five questions:

1. How do you rate your confidence that you could get and keep an erection?	Very low  1	Low  2	Moderate  3	High  4	Very High  5
2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	Almost never or never  1	A few Times(much less than half the time)  2	Sometimes (about half the time)  3	Most times (much more than half the time)  4	Almost always or always  5
3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated your partner?	Almost never or never  1	A few Times(much less than half the time)  2	Sometimes (about half the time)  3	Most times (much more than half the time)  4	Almost always or always  5
4. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	Extremity difficult  1	Very difficult  2	Difficult  3	Slightly difficult  4	Not difficult  5

5. When you attempted sexual intercourse, how often was it satisfactory for you?	Almost never or never	A few Times(much less than half the time)	Sometimes (about half the time)	Most times (much more than half the time)	Almost always or always
	1	2	3	4	5

Figure 3: International Index of Erectile Function (IIEF-5) Questionnaire (from [95])

#### 4.4.3. Physical Examination:

The physical examination will assess the patient's overall health and contributing factors to ED. Particular attention will be given to the cardiovascular, neurologic and genitourinary systems, as these systems are directly involved with erectile function. The cardiovascular examination should include assessment of vital signs (especially blood pressure and pulse) and signs of hypertensive or ischemic heart disease. Abdominal or femoral artery bruits and asymmetric or absent lower extremity pulses are indicative of vascular disease.

To rule out neurogenic ED, an assessment of sacral cord function is indicated. The superficial anal reflex, indicative of normal function of sacral cord levels S2–4, is assessed by touching the perianal skin and noting contraction of the external anal sphincter muscles. Alternatively, the bulbocavernosus reflex, whereby the contraction of the anal sphincter and bulbocavernosus muscle occurs following the squeezing of the glans penis, can also be evaluated.

Examination of the penis in this patient population should focus primarily on the presence of Peyronie's disease plaques. Assessment of the integrity of the erectile tissue may be gained from stretching the penile shaft. Examination of the testicles is aimed primarily at defining the presence or absence of masses and also to ascertain the testicular volume and consistency. Digital rectal examination will be used to check for abnormalities of the prostate when indicated.

#### 4.4.4. Nocturnal Penile Tumescence (NPT):

To rule out a psychogenic cause of ED, the NPT test will be recommended by the physician following medical history, physical examination and completion of the IIEF questionnaire. Psychological causes of ED are common in the 18-40 year old age category; however, patients with psychogenic ED will NOT be eligible to participate in this study.



The test involves the detection of spontaneous erections and measurement of erection rigidity during the hours of sleep, when anxiety and other distractions are not present. The device, called a RigiScan®, consists of an electronic recorder and two fine, elastic band-like strain gauges, which are worn around the base and near the tip of the penis. These continuously monitor and record penile activity during the assessment.

The NPT test will be performed by the patient at home as a component of initial screening for study eligibility. The duration of the test will comprise one overnight period. The physician or designee will supply the patient with the RigiScan® device and will provide verbal and written instructions on how to conduct the test at home.

Briefly, the RigiScan® device monitors NPT and rigidity through two loops held around the base and tip of the penis. A patient is considered to have a normal NPT if he has at least one erectile episode during 8 h of sleep fulfilling the following criteria: tip and base rigidity  $\geq 70\%$ , an increase in tumescence at the tip  $\geq 2$  cm and at the base  $\geq 3$  cm, and erectile episode  $\geq 10$  min[96].

Results of NPT testing will be analyzed by the physician using RigiScan® software. Summary statistics are provided by the RigiScan® as numerical summaries of erectile events (measured as by a 20% increase in base loop circumference maintained for at least 3 minutes), tumescence (penile circumference), and rigidity activity units for each event separately and as a summary for the night [96].

In this study, the presence of normal nocturnal erectile events will be used to rule out psychogenic ED. Further diagnosis will be required to determine eligibility of patients based on the presence of veno-occlusive ED.

#### **4.4.5. Duplex Doppler Ultrasound**

This test will evaluate penile hemodynamics in patients with erectile dysfunction in order to distinguish arterial dysfunction and veno-occlusive dysfunction. The latter group of patients will be eligible for participation in the trial.

The patient is placed in the supine position and the penis is positioned in its anatomical position along the anterior abdominal wall. Pre-injection measurements are first taken by measuring the inner diameter of cavernosal artery (normal value is 0.3-0.5 mm), baseline peak systolic velocity and end diastolic velocity.

A vasoactive agent (0.6-0.9ml of prostaglandin E1 (PGE-1) 500mcg (1ml) diluted with 9ml of Normal Saline, total 10ml) will be injected intracavernously. The penis will be scanned using color Doppler imaging using a high frequency transducer (7-10 Mhz) and a sampling angle of 30-60 degrees.

Post injection measurements are then taken: inner diameter of cavernosal artery (normal value is 0.6-1.0 mm), peak systolic velocity, end diastolic velocity, visual tumescence and erection.

Results of Doppler ultrasonography will be interpreted by the physician. Peak systolic velocity will be used to diagnose erectile dysfunction arising as a result of arterial abnormalities. Peak velocities <25 cm/sec will be considered indicative of arterial dysfunction. Peak velocities of 25 to 34 cm/s will be considered indicative of some degree of arterial compromise. Peak velocities of >35 cm/s will be considered normal. In cases of borderline PSV values (25 to 34 cm/s), secondary diagnostic criteria for arteriogenic ED will include asymmetry of >10 cm/s in PSV, an increase in the diameter of the cavernosal artery by 75% post-intracavernosal injection, focal stenosis in the cavernosal artery and retrograde arterial flow [97].

Parameters to assess veno-occlusive function are end-diastolic volume and resistance index (RI) of the cavernosal arteries. RI can be calculated as follows.  $RI = (PSV - EDV) / PSV$ . Venous competency is normally indicated by diastolic flow as the erection is developing, which ceases or reverses once erection is achieved. Thus, adequate arterial inflow, a short duration erection, with persistent antegrade flow of >5 cm/s (angle corrected) and/or  $R1 < 0.75$  [98] will be considered indicative of venous leakage.

#### **4.4.6. Dynamic infusion cavernosometry cavernosography (DICC)**

DICC testing will be performed to diagnose veno-occlusive dysfunction. A single dose of vasodilator termed tri-mix (0.2ml of Papaverine 30mg/ml (10ml), Regitine tablet 5mg, and PGE-1 500mcg (1ml) combination) and 0.9ml of PGE-1 500mcg (1ml diluted with 9ml of Normal Saline total 10ml) will be administered by intracavernosal injection into one of the corpora using a 25-gauge needle that is connected to a pressure transducer. After 10 min, a 20-gauge needle will be introduced into contralateral corpus cavernosum.

Saline is then infused into the corpus cavernosum via the 20 gauge needle using an infusion pump device until a full erection is attained. The pressure decay rate is then monitored. The flow rate to achieve erection and flow rate to maintain erection will be recorded. When full erection is attained by saline infusion, the flow will be stopped and intracavernous pressure will be monitored for 30 seconds.

Diagnosis of veno-occlusive dysfunction will be based on the following criteria: an infusion rate greater than  $35 \text{ ml min}^{-1}$  to initiate erection, a maintenance flow rate greater than  $17 \text{ ml min}^{-1}$  at 10 minutes post-trimix injection (at a standard intracorporal pressure of 90 mm Hg ) and a decrease of more than 50 mm Hg in corporal pressure within 30 s following cessation of infusion.

## **5. Study-Related Risks**

### **5.1. Bone Marrow Harvest:**

The likely risks of this procedure are discomfort from the area of aspiration. Unlikely risks could be suppression of breathing from the sedation requiring medication or possibly being placed temporarily on a breathing machine. Highly unlikely risks or severe side effects would be an allergic reaction to sedation/anesthesia.

**5.2. Intra-cavernosal Injection of Vasodilators:**

Papaverine is associated with unlikely risk of hives, bleeding at the site of injection, liver damage (since it is not being administered systemically) and priapism, all of which require immediate medical attention. Common side effects include mild to moderate dull ache at the injection site and a small bruise. Rare side effects include dizziness and headache.

**5.3. Intracavernosal Injection of Stem Cells:**

Common risks associated with this procedure include fibrosis of the penis and mild to moderate pain at the injection site.

**5.4. Venipuncture for Blood Tests:**

The patient will have needles/ tubes put in their veins. The likely side effect is a small discoloration or bruise and pain that may remain at the site for several days. There is a rare risk of infection. There is also the infrequent risk of bleeding and rarely fainting.

**6. Adverse Events**

The Investigators and Study Coordinator are responsible for recording and reporting adverse events observed during the study protocol. An adverse event is any unanticipated or pathological change that the patient experiences during the conduct of the trial. All adverse events related or unrelated to the study will be recorded in the Case Report Form.

Only events that occur after the start of study treatment will be classified as adverse events. An adverse event is any untoward medical event in a subject given any dose of a therapeutic product and that does not necessarily have to have a causal relationship with the use of the product.

Any untoward medical event that occurs outside the period of follow-up defined in the protocol is not considered a protocol adverse event.

Symptoms or medically significant laboratory or instrumental abnormalities of a preexisting disease, such as cancer or other disease, should not be considered an adverse event. New symptoms as well as worsening of existing ones, are considered adverse events.

A pre-existing condition should not be reported as an adverse event unless there has been a substantial increase in severity or frequency of the problem that has not been attributed to natural history.

**6.1. Relationship of Adverse Events to the Investigational Product:**

The investigator should assess the relationship of the adverse event to the investigational product. The relationship should be assessed using the following categories:

- **Definite:** A direct cause and effect relationship between the investigational product and the adverse event exists.
- **Probable:** A direct cause and effect relationship between the investigational product and the adverse event has not been clearly demonstrated, but is likely or very likely.
- **Possible:** A direct cause and effect relationship between the investigational product and the adverse event is not likely, but may exist.
- **Not Likely:** A direct cause and effect relationship between the investigational product and the adverse event has not been demonstrated or is improbable, but not impossible.
- **Unrelated:** The adverse event is definitely not associated with the investigational product.

### **6.2. Serious Adverse Events:**

Each adverse event should be assessed for its seriousness. The definition below should be used for this assessment. Please note that the term “serious adverse event” is not synonymous with a “severe” adverse event, which may be used to describe the intensity of an event experienced by the subject. An adverse event should be classified as serious if it meets any of the following criteria:

- Results in, or contributes to, a death;
- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at risk of death at the time of the event but it does not include an event that, had it occurred in a more severe form, might have caused death);
- Results in permanent disability or incapacity (i.e., permanent impairment of a body function or permanent damage to a body structure);
- Requires in-patient hospitalization or prolongs hospitalization;
- Necessitates medical or surgical intervention to preclude a permanent disability or incapacity; and,
- Results in a congenital anomaly or birth defect.

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event, or the outcome (subject treatment, life-threatening condition, hospitalization, recovery) cannot be determined with the information provided.

### **6.3. Classification of Adverse Event Severity:**

Each adverse event should be assessed for its severity, or the intensity of an event experienced by the subject, using the following:

- **Mild (1):** Discomfort noticed, but no disruption to daily activity.
- **Moderate (2):** Discomfort sufficient to reduce or affect normal daily activity.
- **Severe (3):** Inability to work or perform normal daily activity.

#### **6.4. Reporting of Adverse Events:**

The investigator will assess subjects for the occurrence of adverse events at each study visit. In order to avoid bias in eliciting adverse events, subjects should be asked the following non-leading question: "How have you felt since your last visit?" All adverse events (serious and non-serious) reported by the subject must be recorded on the source documents and case report forms provided by the sponsor.

#### **6.5. Reporting Requirements for Serious Adverse Events:**

The investigator should report any serious adverse events by telephone to the site IRB or IEC immediately after the investigator becomes aware of the event. The site research coordinator should report this adverse event to sponsor by electronic means within 24 hours after receipt of information from the investigator. Subjects experiencing serious adverse events should be followed clinically until their health has returned to baseline status or until all parameters have returned to normal, or have otherwise been explained. The investigator must also comply with any other requirements for reporting Adverse Events, which are imposed by the IRB/IEC. The study's investigator will also provide the Data Safety Monitoring Plan with all serious adverse event reports.

#### **6.6. Stopping Rules**

When an investigator identifies an event potentially associated with a stopping rule, the investigator must notify sponsor immediately.

Safety stopping rules may be invoked upon identification of serious adverse events. If, for any reason, the use of the investigational product is unsafe or inefficacious by the Attending Physician can recommend stopping the study. If enrollment is halted and then restarted, the same rules will apply beginning upon restart.

## **7. Regulatory and Ethical Requirements**

### **7.1. Informed Consent**

Informed consent will be obtained from all subjects prior to study participation. A properly executed, written informed consent in compliance with Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki shall be obtained from each subject prior to entering the study. A copy of the informed consent document to be used will be submitted by the Principal Investigator to their IRB for review and approval prior to the start of the Study. The Principal Investigator shall provide a copy of the signed informed consent to the subject, and a copy shall be maintained in the subject's medical record.

### **7.2. IRB/IEC Approval & Reporting**

Per Title 21 of the US Government Code of Federal Regulations (21 CFR) Parts 50 and 56, the study protocol and the final version of the Informed Consent form will be approved by the institutional review board (IRB) before enrollment of any patients. The opinion of the IRB will be dated and given in writing.

The investigators will ensure that the IRB will be promptly informed of all changes in the research activity and of all unanticipated problems including risk to patients. The investigator will also ensure that no changes will be made to the protocol without IRB approval.

As a part of the IRB requirement for continuing review of approved research, the investigator will be responsible for submitting periodic progress reports to the IRB at intervals appropriate to the degree of patient risk involved, but no less than once per year.

The investigator is responsible for providing the appropriate reports to its reviewing IRB/ICE during the course of the clinical study. This will include the following:

- Informing the IBC or IEC of the study progress periodically as required;
- Reporting any unanticipated adverse effects within 10 working days of first learning of the event;
- Reporting any deviations from the clinical protocol, undertaken to protect the well-being of a subject, within 5 working days after the emergency occurred;
- Reporting the use of the investigational product without obtaining informed consent from a subject within 5 working days of the event; and,
- Providing any other reports requested by the IRB or IEC
- The IRB or IEC must be notified of study completion within 30 days of the final visit of the last subject, and should be provided with a summary of the results by the study investigator.
- In the event that the IRB or IEC require shorter reporting standards the investigator will comply with these local requirements.

### 7.3. Cost & Payments:

The study sponsor will provide the treatment, study related exams specific in the investigational protocol such as, laboratory tests and office visits at no cost to the institution or the patient.

The patient's insurance carrier will be billed for non-related standard-of-care laboratory tests, physical exams, and any office visits that are not required by this study. In the event that the patient's insurance company does not cover any of these charges, the patient will be responsible for costs not covered.

Patient will be reimbursed a specific amount for every completed office visit (below):

Visit	1 (screening)	2 (BM aspiration)	3(month1)	4 (month3)	5 (month6)	Total
Patient stipend	55 \$	150 \$	55 \$	55 \$	55 \$	370 \$

## 8. Data Collection & Reporting

### **8.1. Management of Source Documents:**

During each subject's visit to the clinic, a member of the investigator team participating in the study will record progress notes to document all significant observations and clinical reports with the source documents. All information housed in the source documents will be transposed into the case report forms. In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes.

Case report forms (CRF) will be provided for each patient; patients will not be identified by name on any study documents (or copies) retrieved from the site. Each patient shall be identified by the patient identification number provided by the study coordinator upon entry onto the study.

The data will be entered into a computer database by the study coordinator. Audits of the database against the CRF's will be performed by the Principal Investigator and a sample of the study patient population will be randomly selected for these audits.

Any changes to information in the source documents and case report forms will be initialed and dated in ink on the day the change is made by a site study staff member authorized to make the change. Changes will be made by striking a single line through erroneous data and clearly entering the correct data, (e.g., right data). If the reason for the change is not apparent, a brief explanation for the change will be written in the source documentation by the clinician or investigational team member.

Dated progress notes, case report forms, and laboratory reports will be signed and dated by the Principal Investigator.

### **8.2. Required Records:**

**Prior to participation in the investigation, the investigator must have following documentation:**

- Investigator Agreement, signed by the investigator, which lists any physicians who will be involved in conducting the investigation under the direction of the primary investigator.
- A copy of the primary investigator's curriculum vitae (CV) as well as copies of CVs for any co-investigators.
- A letter signed by the chairperson of the Institutional Review Board (IRB)/IEC of the institution at which the investigation will be conducted, indicating that the IRB has reviewed and approved this investigational plan.
- A copy of the IRB/IEC-approved informed consent document.
- During the study, investigators are required to maintain on file the following accurate, complete and current records relating to this study as described in 21 CFR §812.140. A summary of these records is described below:
- All correspondence and required reports which pertain to the study;

- Records of receipt, use or disposition of the investigational product, including the type and quantity of the product, the dates of receipt, the lot number, the names of all persons who received, used or disposed of each product, and why and how many units of the product have been returned to the sponsor or otherwise disposed;
- Records of each subject's case history and exposure to the investigational product;
- Signed and dated consent forms;
- Relevant observations, including records concerning adverse events, condition of each subject upon entering and results of diagnostic tests;
- Case report forms and corrections to the forms;
- Protocols and amendments;
- Subject recruiting materials;

### **8.3. Reporting of Results:**

The data from the entire study or from subsets of the study may be considered for reporting at a scientific meeting or for publication in a scientific journal, in which case the Principal Investigator will determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues.

The final contents of any manuscript will be reviewed by the Principal Investigator before submission to a peer-reviewed journal. Submitted publications will conform to international standards for biomedical manuscripts, including those regarding authorship.

## **9. Statistical Methods**

### **9.1. Analysis of Safety:**

All adverse events will be reported in terms of severity, possible relationship to administration of the investigational product and consequences. Adverse event rates (percentage of subjects) will be reported for patients treated by intracavernosal injection of bone marrow.

### **9.2. Analysis of Physician Assessments:**

All changes in parameters of erectile function will be compared in baseline vs. follow up visits conducted at 1,3,6 and 12 months post-treatment. Statistical significance of changes will be evaluated using both t-tests and non-parametric tests, since distributional assumptions cannot be verified with the planned sample size.

Statistical analyses will be performed using Statview (SAS Institute Inc.).

## **10. Alterations to Study Schedule**



**10.1. Screen Failures:**

A screen failure subject is one from who informed consent is obtained, but administration of the investigational product was not attempted because it was determined (after the subject signed the informed consent form) that the subject did not meet all of the eligibility criteria. The number of screen failure subjects will be reported, but such subjects will not be included either in the intent-to-treat or the per protocol analysis.

**10.2. Withdrawal Criteria and Procedures:**

All subjects have the right to withdraw without prejudice at any point during the study. The investigator can withdraw any subject from the study at any time if deemed medically necessary; for example, if serious adverse events occur. The reason for the subject's withdrawal should be documented on the appropriate case report form. Withdrawn subjects will not be replaced. The sponsor should be notified promptly when a subject is withdrawn, preferably within 48 hours.

**10.3. Missed Clinic Visits:**

Any subject who does not return for a scheduled follow-up visit will be contacted at least twice by telephone to determine the cause for the missed visit. All attempts to contact these subjects will be recorded in the subject's records. If the subject is contacted, a new visit will be scheduled as soon as possible. Subjects will be excluded from the study if they cannot be rescheduled for follow up visits within the time windows outlined.

**10.4. Protocol Deviations:**

Except for emergency situations, this study should be conducted as described in this protocol. An example of such an emergency situation is one in which the protection, safety and wellbeing of a subject requires a protocol deviation; this deviation would be based upon the judgment of the investigator (or a responsible, appropriately trained professional designated by the investigator). If a deviation from the protocol is necessary to protect the life and physical wellbeing of a subject in an emergency, such protocol deviations must be reported to the sponsor and the reviewing IRB or IEC as soon as possible, but no later than five working days after the emergency occurred. In the event of a significant deviation from the protocol due to an accident or mistake, the investigator or designee must contact the sponsor at the earliest possible time by telephone to discuss the deviation and its impact on the study, and that subject's continued participation in the study. These discussions will be documented by the investigator and the sponsor, and reviewed by the monitor.

**10.5. End of Study:**

All subjects who have signed an informed consent, except for screen failures, will be considered to have enrolled in the study. Subjects who complete the 6-month study duration will be considered to have completed the study. All subjects should however be followed until completing the study follow-up at 12 months after administration of the study product or until study discontinuation for other reasons. The reason for study discontinuation should be documented for each subject.

**10.6. Medication Changes:**

Medications should be documented; if any changes to the medications have been made during the study all study related documents should reflect such changes. Patient must still meet any inclusion/exclusion medication criteria to continue in the study.

## 11. References:

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