TITLE:
Autologous Muscle Derived Cells for Underactive Bladder

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CLINICAL INVESTIGATIONAL PLAN

Autologous Muscle Derived Cells for Underactive Bladder

INVESTIGATIONAL NEW DRUG (IND) NUMBER: 16108

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STUDY OVERVIEW

Rationale or Background

This proposal evaluates the safety of Autologous Muscle Derived Cells (AMDC) in the treatment of chronic Underactive Bladder (UAB).

UAB is caused by deteriorating bladder functioning with incomplete bladder emptying. In addition to incomplete bladder emptying, symptoms of UAB may include urine frequency, urgency, hesitancy (a weak urine stream), difficulty starting and/or stopping voiding, incontinence, nocturia (night time voiding), straining to void and recurrent urinary infections.

UAB is common and no effective pharmacotherapy or cellular therapies are available. Currently, no medications or therapies have proven effective in the long-term treatment of UAB and no known cure exists. Consequently, patients who suffer from UAB are usually managed with clean intermittent self catheterization or indwelling catheters or riskier surgical procedures such as suprapubic catheter or urinary diversion with urostomy and/or must wear adult diapers. [22, 27]

Diokno and associates reported that 22% of men over 60 and 11% of women over 60 reported difficulty emptying.[25] The physical and psychological impact of underactive bladder can be devastating. Since the bladder does not fully empty upon urination, it can quickly fill with urine again and unbeknownst to the individual, can cause overflow and leakage.

The long-term effects of UAB can lead to other conditions as well. For example, urine left behind in the bladder can lead to urinary tract infections which can be extraordinarily painful and, if they become chronic, can lead to kidney damage. Sediments can also accumulate in the bladder, forming bladder stones and blood in the urine. In severe cases, urine left behind in the bladder can build up to a level that causes reflux up the ureters, the tubes that join the kidneys to the bladder, and may cause kidney damage.

UAB patients using catheters to ensure the emptying of their bladders also face long-term medical difficulties, including inflammation and discomfort, the potential for injury, and an increased risk of bacterial infection. Urinary tract infections can lead to kidney damage and blood infections. In addition, for many individuals, the use of catheters is a cause for embarrassment and can negatively impact their work and home life.

For many patients with UAB, the emotional effects of the disease can feel just as devastating as the physical effects. For older patients, it can have a major impact on quality of life. In fact, loss of bladder control is the second most common reason for nursing home placement of the elderly. Finding a way to reduce these bladder issues would allow millions more older adults to remain independent and experience life without the worry and embarrassment associated with bladder control dysfunction.

AMDC are currently under investigation and have been tested in women with SUI (BB-IND 11618; Sponsor: Cook MyoSite Incorporated). William Beaumont Hospital was the primary study site evaluating 4 doses of AMDC (NCT00847535). AMDC have not been

associated with any serious adverse effects (SAEs). Preliminary results indicate that the majority of women reported ≥ 50% improvement in stress leaks after receiving treatment with AMDC[29].

With the approval from the Food and Drug Administration (FDA) we will use AMDC for the treatment of chronic UAB in patients who have deteriorated bladder functioning with incomplete emptying.

OBJECTIVES AND ENDPOINTS

a. Primary Objective and Endpoints

The primary objective of this study is to evaluate the safety of AMDC in the treatment of underactive bladder at 6 months post initial injection.

Primary safety endpoints will be assessed by the following safety measures at these specified timepoints:

- Vital signs at each study visit
- Physical examination at baseline and at 6 months post initial injection
- Urinalysis at each study visit
- Hematology and clinical chemistry assessments at baseline and at 6 months post initial injection
- Post-injection cystoscopy immediately post initial injection and at 6 months post initial injection

Of special interest are adverse events related to the study product or procedures, specifically:

- AMDC
- Biopsy procedure
- Injection procedure

Systemic Safety**:** Will be monitored by a) measuring vital signs (blood pressure, heart rate) at each study visit, and b) performing standard laboratory studies (blood and urine).

Localized Safety: Any local adverse effects (hematuria, micturition pain, urinary tract infection) that may occur after AMDC injection will be properly recorded and managed. Urinalysis will be evaluated for the possibility of urinary tract infection (UTI) at each visit. If the subject develops a UTI, antibiotics will be prescribed by the study physician to treat the infection. Signs and symptoms of wound infection will be described to the subject at the biopsy visit. If the subject suspects an infection he/she will be evaluated by study staff and a treatment plan will be created, as necessary. Initial cystoscopy will be performed immediately after AMDC injection and again at 6 months post injection. Cystoscopy will be performed to visualize the bladder and urethra in order to identify changes in appearance as well as abnormalities. Cystoscopic pictures will be obtained to assess changes over the duration of the study.

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Allergic reactions to the study medications and AMDC may present locally or systemically. Signs of infection including flu symptoms (body aches, nausea, fatigue), itching, and rash. An allergic response or reaction may be initially identified during the study visit; therefore study staff will observe the subject in the research office for 30 minutes after AMDC injection. Signs and symptoms of allergic reaction will also be discussed with the subject and the subject will be instructed to contact the research staff immediately if they suspect an allergic reaction.

b. Secondary Objectives
The secondary objective of the study is to evaluate the safety and efficacy of AMDC in the treatment of underactive bladder at 12 months post initial injection.

Secondary efficacy endpoint measures include:

- Changes in Quality of Life (QOL) determined by responses on symptom questionnaires
    - Patient Global Impression of Improvement (PGI-I)
    - Global Response Assessment (GRA)
    - Underactive Bladder Questionnaire (UAB-Q)
    - Incontinence Questionnaire Lower Urinary Tract Symptoms Long Form Female (ICIQ-FLUTS Long Form)
      Male (ICIQ-MLUTS Long Form)
- Changes in voiding habits (i.e. frequency, urgency, urine volume voided independently, urine volume voided via catheterization) as recorded on the 3-day bladder diary

METHODOLOGY
a. Study Design
This is a prospective, open-label, Phase I, single center study evaluating the safety and efficacy of AMDC as a treatment for chronic UAB.

A maximum of 20 subjects, who satisfy all eligibility criteria, will be enrolled and treated with AMDC. Potential study subjects will be identified by referring physicians from within Beaumont Health System.

Initial screening of available existing records provided by the patient will take place to screen for inclusion criteria (presence of chronic UAB symptoms). Informed consent will be obtained prior to any research activity taking place (first study visit). The study coordinator will obtain informed consent and will explain the study in full including, but not limited to, what is involved, the possible risks, and the possible benefits. The explanation will occur at a level of understanding appropriate for the potential participant. Once the potential subject has had ample time to consider the study, review the consent form and ask questions, he/she will be asked to sign the written consent form in the presence of the consent provider. The subject will receive a copy of the consent form for his/her records.

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b. Proposed Intervention

Prior to beginning AMDC therapy, the study physician (Principal Investigator (PI) or Sub-Investigator (Sub-I) will assess the subject for lower urinary tract abnormalities with a cystoscopic examination. Cystoscopy will also be used to document bladder appearance at baseline. Routine venipuncture will be required for blood and serum assessment including serum chemistry, hematology and blood borne viruses. Serum chemistry includes tests for white blood cell count, red blood cell count, platelet count, hemoglobin, hematocrit, creatinine, urea, and partial thromboplastin time (PTT). Blood borne viruses will also be tested; Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C, and Syphilis. If the biopsy procedure is scheduled more than 30 days after the most recent blood borne pathogen tests were completed, the blood borne pathogen tests must be repeated before the biopsy procedure to confirm the patient's continued eligibility for study participation.

Urine will be collected and evaluated with a dipstick test for blood, glucose, ketones, leukocytes, nitrates and protein. Urinalysis, urine culture, patient symptom report, and/or cystoscopy will be used to identify urinary tract infections, as outlined in Appendix A. If the patient is positive for urinary tract infection at the time of screening, the patient will be excluded from the study and eligible for re-screening after the infection has been treated, UTI status has been re-evaluated (as outlined in Appendix A), and the patient is negative for UTI. Residual urine volume or post void residual (PVR) will be assessed via ultrasound or straight catheterization. Assessment of PVR will be performed in the least invasive manner (via bladder scan) whenever possible. If the bladder scan indicates a high urine residual volume (>150 mL), catheterization may be performed. Since catheterization is a more accurate measurement of residual urine volume, the urine volume collected via catheterization will be recorded, rather than the volume detected via bladder scan (if results differ). Since insertion of a urinary catheter is required for the instillation of the numbing medication pre-injection, PVR will be assessed via catheterization at this visit. A urine pregnancy test will also be conducted for women of childbearing potential. If the urine pregnancy test is positive at any time during the study, the patient will be withdrawn from the study and the event will be reported to the local Investigational Review Board (IRB) and the FDA.

A separate consent will be obtained in order to collect urine for the evaluation of biomarkers. If the participant consents to this part of the study, part(s) of the urine samples will be retained in a repository (storage area for safety and preservation) for future research. The type of testing that may be done on the samples could be useful in understanding underactive bladder. The urine samples will be kept indefinitely and will be labeled with a unique code to protect the participant's identity. A subject may withdraw their sample at any time upon request to study staff. The study participant will not be informed of any results of the analysis of their urine sample. The results will not be placed in the participant's medical record. If/when the results are disseminated to the public via presentations and/or publications, the findings may be available to participants upon request to study staff and/or via internet search. Beaumont's Urology Bench Laboratory will store the urine samples, as well as the data collected from the analysis.

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Bladder diaries and questionnaires will be used to evaluate UAB symptoms. Bladder function will be assessed with cystometrogram and uroflow studies. Concomitant medication information (dosage, route, frequency, start and stop dates, and indication), therapies, and minor procedures done during the course of treatment will be assessed at each visit.

Compassionate use of AMDC to treat UAB was previously approved by the FDA for use in a single patient (IND 15417). The subject received a single treatment of 250 million cells in approximately 30 sites (0.5 ml/injection) to enhance bladder detrusor contractility. To date, there have been no adverse events (AEs) related to the treatment or the cell dose. Therefore, the investigators have determined that a total dose of 250 million cells may be safe.

In this study, all enrolled subjects will receive an initial injection of 125×10^6 AMDC. If after the 6 month visit the study investigator determines that the subject may benefit from undergoing a second treatment, the patient will have the option of receiving another injection of 125×10^6 AMDC. Therefore the total cell dose delivered over the course of the study will not exceed 250×10^6.

The AMDC injection is performed via cystoscope. Visualization of the bladder will occur before the procedure to assess appearance and identify abnormalities, during the procedure, and after the injection is completed. The post-injection cystoscopy will ensure that the injection did not cause excessive bleeding and/or excessive bladder trauma.

The biopsy and culture technique allows AMDC to be produced for multiple injections from a single biopsy procedure. Therefore patients will not have to undergo a second biopsy procedure prior to receiving the second injection.

In order to evaluate the long-term safety profile and clinical outcomes associated with AMDC, all subjects that have not been withdrawn from the study or discontinued study participation will be evaluated for 1 year post initial injection.

<u>Muscle Biopsy Procedure</u>
The biopsy procedure will involve minor surgery, as an outpatient procedure, to collect approximately 50-250 mg of the quadriceps femoris muscle using a sampling needle. Muscle biopsies are obtained approximately 15 cm above the patella from the middle region of the vastus lateralis, and approximately 2 cm away from the fascia using a percutaneous needle biopsy technique. The skin is prepared by shaving a small area at the site of the muscle biopsy and cleaning with an antimicrobial skin wash. The biopsy site is anesthetized with a local injection of 1% lidocaine. A small incision of approximately 5 mm is made in the skin to allow the sterile needle to enter and remove between 50 and 250 mg of muscle tissue.

The Medinvents Spirotome 8 gauge needle will be used for this biopsy procedure. This needle type has been used clinically to safely and successfully procure skeletal muscle tissue and is used according to the manufacturer's instructions. Several passes of the biopsy needle may be required to obtain a satisfactory sample of muscle tissue. The

tissue harvested from the patient's quadriceps femoris is immersed in hypothermic medium within a coded vial and is transported to the cell processing facility in Pittsburgh, PA. The AMDC will be isolated and expanded in culture over several weeks to a final concentration of 125 million ±20% (see Appendix 2 for additional process information).

If the first biopsy does not produce an adequate sample for product isolation, it may be necessary for the patient to return for another biopsy procedure. The risk that the patient will be requested to undergo a re-biopsy procedure is less than 10%. The maximum permissible number of biopsy needle passes and total number of biopsies is contingent upon patient tolerance and the satisfactory procurement of muscle tissue. After the biopsy, the incision is closed and dressed appropriately in accordance with standard physician's care. Non-prescription analgesics may be used to manage post-biopsy pain.

Cell Processing Procedure

Cell processing will begin with isolation of cells from the subject's tissue. The cells will then be grown in culture and expanded in number. The culture process will preferentially expand desirable AMDC, producing a final cell culture that is highly enriched in myogenic cell content. Cell culture will be performed in medium consisting of physiological saline solutions with cell nutrients. Cell processing will follow current Good Manufacturing Practices (cGMP) methodologies to prevent contamination and to preserve tissue function and integrity. These practices will include defined procedures for tissue and cell handling, processing, and identification.

The expanded AMDC will be supplied to the physician frozen in a cryogenic medium containing Human Serum Albumin (HSA), and then thawed and diluted with physiological saline for injection into the subject. Materials contacting subject's periurethral tissue will include the subject's own MDC, the cryogenic medium in which it is suspended and shipped, and the physiological saline used to dilute the mixture for injection.

Isolation and Expansion of the Study Agent

After receipt at the cell processing center, desirable AMDC will be isolated from the tissue biopsy. The isolated AMDC will be expanded in medium consisting of physiological saline solutions with cell nutrients that contain bovine serum. The bovine product used in the culture medium is sourced from countries considered free of bovine spongiform encephalopathy (BSE), and is derived from animals not born, raised, shipped through, or slaughtered in countries where BSE is known to exist. This assures to as great a degree as is possible that the products are BSE-free.

Once the desired cell number has been achieved, quality tests following cGTP guidelines (FDA, 21 CFR Parts 16, 1270, and 1271, Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments) assure the cells meet acceptance criteria for sterility, contaminants, cell viability, and functional integrity. The cells are then suspended in a cryogenic medium containing HSA, sealed in a glass vial, frozen, and shipped to the physician for injection.

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Quality control testing samples consists of AMDC Final Product cells in cryopreservation medium. The sample is collected during packaging and prior to cryopreservation. Lot-release tests are performed to determine the identity, purity, and safety of the AMDC Final Product, and include: sterility, mycoplasma, endotoxin, myogenic content, and myogenic differentiation.

All lot-release test results are available prior to final product release. A quality review of the Product Batch Record, by a Quality Representative, includes a review of all lot-release test results prior to product release. A copy of the signed Certificate of Analysis is included in the shipping container along with the final product. This form indicates the product approval status along with the test completion date, test criteria, pass/fail status, and internal sample batch number of each lot-release test performed. Individual samples for all lot-release testing are removed from a single common sample. This sample of the AMDC Final Product cell suspension is withdrawn during fill. Excess sample used for lot-release testing is retained, cryopreserved, and may be used for repeat testing in cases in which invalid test results are obtained (if applicable).

Transportation and Storage of the Study Agent

The cells must remain frozen and undiluted until ready for use. Specialized packaging for transport from the processing center is certified to maintain appropriate temperature for up to 72 hours. If the cells cannot be injected within that period, the cells should be stored at least -60 °C to –80 °C until the subject is available for injection. The cells are intended for a single use, should be thawed immediately prior to the injection, and should be used within the immediate procedure. Any unused portion shall be discarded.

Injection Procedure

The injection procedure consists of injections of 125 million AMDC in approximately 30 sites (0.5 ml/injection) throughout the bladder to enhance the bladder detrusor contractility. This procedure was utilized in the previous UAB AMDC single subject study and is similar to other bladder injection protocols. The procedure requires local lidocaine anesthesia only.

The materials contacting the bladder wall will include the subject's own AMDC, the cryogenic medium in which it is suspended and shipped, and the physiological saline used to dilute the mixture for injection.

Subjects will receive appropriate antibiotic prior to the injection procedure. After a negative urine analysis, 30 ml of 1% liquid lidocaine will first be instilled, via catheter, into subject's bladder and left for 15 minutes. The lidocaine will then be emptied and the bladder rinsed with 30 ml of sterile saline. The saline and catheter will then be removed.

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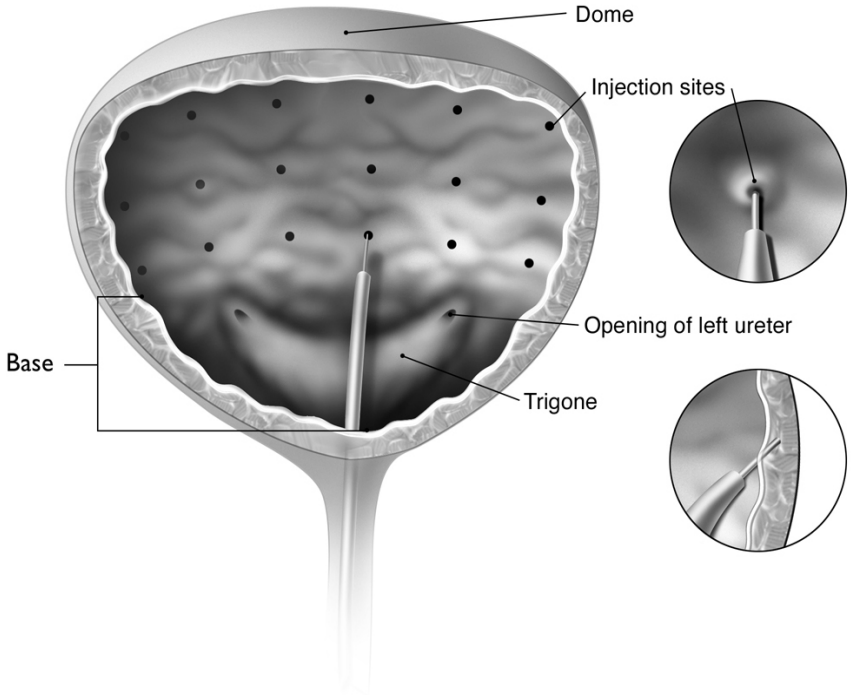
Dose:
Single treatment with an optional second treatment; 125 million cells/2 ml frozen cell suspension will be reconstituted per manufacturer's instructions with 13 ml physiological sterile injectable saline without preservative (15 ml total) and injected 2 mm deep intradetrusor into 30 sites (0.5 ml/site) throughout the bladder. Any unused solution will be discarded. The subject will be monitored in the urology research area for a minimum of 30 minutes after injection.



Planned Duration: AMDC will be injected during one visit (Visit 3) and possibly Visit 7 (approximately 8 months post initial injection). The study requires seven to eight visits to the research office and at least 2 phone contacts for treatment and follow-up over approximately 30 months as outlined below and located in Appendix 3.

c. Description of Research Activities

1. Schedule of Intervention/Activities

The following questionnaires will be completed by the subject during the study:

- 3-Day Bladder Diary
- Incontinence Questionnaire Lower Urinary Tract Symptoms Long Form Module - Female (ICIQ-FLUTS Long Form), Male (ICIQ-MLUTS Long Form)
- Underactive Bladder Questionnaire (UAB-Q)
- Patient Global Impression of Improvement (PGI-I) Scale
- Global Response Assessment (GRA)

Visit 1: Screening
Obtain written informed consent
Demographics (birth date, gender, etc.)
Review medical and surgical history
Review of concomitant medications
Vital signs: blood pressure, heart rate
Baseline testing:

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- Blood collection and analysis; CBC, blood chemistry, PTT, blood borne viruses: (Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C, and Syphilis)
- Urinalysis (dipstick), and culture (if required)
- Urine pregnancy test (if required)

Urine collection for evaluation of biomarkers (presence of inflammatory and neuronal innervation proteins in the urine)
Physical examination
Assessment of post void residual (PVR): measured by bladder scan or catheter
Questionnaire completion (ICIQ, UAB-Q)
Antibiotic (a medicine to prevent infection) given, by mouth, to prevent urinary tract infection
Pressure flow studies
Determination of study enrollment, review inclusion/exclusion criteria
Assess for side effects

Visit 2: Muscle Biopsy
Review of concomitant medications
Vital signs: blood pressure, heart rate
Urine pregnancy test (if required)
Muscle biopsy - After the biopsy surgical glue will be used to adhere steri-strips (surgical tape) to the biopsy site. A gauze pad containing Bacitracin Zinc Ointment, to prevent infection, will then be applied. Finally an ACE bandage will be wrapped firmly around your leg as a protective covering and to reduce possible bruising.Assess for adverse events
Dispense 3-day bladder diary to be completed prior to visit 3; instruct/reinforce maintaining bladder diary

Visit 3: Injection (approximately 10 weeks from visit 2)
Review bladder diary
Assess concomitant medications
Vital signs: blood pressure, heart rate
Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Assessment of post void residual (PVR): measured by bladder scan or catheter
Antibiotic (a medicine to prevent infection) given, by mouth, to prevent urinary tract infection
Flexible cystoscopy
AMDC bladder injection
Assess for adverse events
Dispense 3-day bladder diary to be completed prior to visit 4; instruct/reinforce maintaining bladder diary

24-48 Hours Post-Injection Phone Call
Assess concomitant medications
Assess for adverse events
Review diary completion dates

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Visits 4: 1 Month Post-Injection Follow-up (+/- 7 days)
Review bladder diary
Assess concomitant medications
Questionnaire completion (PGI-I, GRA, UAB-Q, ICIQ)
Vital signs: blood pressure, heart rate
Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Assessment of post void residual (PVR): measured by bladder scan or catheter
Assess for adverse events
Dispense 3-day bladder diary for completion prior to visit 5; instruct/reinforce maintaining bladder diary

Visit 5: 3 Months Post-Injection Follow-up (+/- 7 days)
Review bladder diary
Assess concomitant medications
Questionnaire completion (PGI-I, GRA, UAB-Q, ICIQ)
Vital signs: blood pressure, heart rate
Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Urine collection for evaluation of biomarkers (presence of inflammatory and neuronal innervation proteins in the urine)
Assessment of post void residual (PVR): measured by bladder scan or catheter
Assess for adverse events
Dispense 3-day bladder diary to be completed prior to visit 6; instruct/reinforce maintaining bladder diary

** While an in-person study visit is preferable, this visit may be completed as a phone call visit, if the participant is otherwise unable or unavailable. The diary, questionnaires and urine will be collected via mail.*

In the event the study visit is not conducted in-person, the visit may be conducted by phone and/or mail. Research staff will:
Send the 3- month questionnaires to the participant for completion
Send the 6-month diary to the participant for completion prior to their next visit
Review the diary and questionnaires and completion dates
Request and verify the completed diary and questionnaires have been returned to the research office
Send a Urine Specimen Collection Kit and instructions to the participant. The kit will be utilized to collect urine to perform urinalysis and for the evaluation of biomarkers.
Verify the kit has been returned to the research office.
Assess concomitant medication changes
Assess for adverse events

Visit 6: 6 Months Post-Injection Follow-up (+/- 7 days)
Review bladder diary
Assess concomitant medications
Questionnaire completion (PGI-I, GRA, UAB-Q, ICIQ)
Vital signs: blood pressure, heart rate

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Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Urine collection for evaluation of biomarkers (presence of inflammatory and neuronal innervation proteins in the urine)
Physical examination
Assessment of post void residual (PVR): measured by bladder scan or catheter
Pressure flow studies
Flexible cystoscopy
Antibiotic (a medicine to prevent infection) given, by mouth, to prevent urinary tract infection
Blood collection and analysis; CBC, blood chemistry
Assess for adverse events

If the study physician determines that a second AMDC injection may be beneficial to the subject and the subject agrees to undergo a repeat treatment, the following visits will occur:

Visit 7: Injection #2 (approximately 10 weeks from visit 6)
Assess concomitant medications
Vital signs: blood pressure, heart rate
Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Assessment of post void residual (PVR): measured by bladder scan or catheter
Antibiotic (a medicine to prevent infection) given, by mouth, to prevent urinary tract infection
Flexible cystoscopy
AMDC bladder injection
Assess for adverse events
Dispense:

- Two 3-day bladder diaries; instruct/reinforce maintaining bladder diary
- One set of questionnaires: PGI-I, GRA, UAB-Q, ICIQ (1 of each)
- One postage paid envelope

The Research Nurse Clinician (RNC) will provide completion and study document return instructions. Completion and mailing dates will be provided by the RNC. Diaries and the questionnaires will be completed and returned via U.S. mail at the 1 month post second injection date. A diary will also be completed and returned in person at the 12 months post initial injection visit.

24-48 Hours Post-Injection #2 Phone Call
Assess concomitant medications
Assess for adverse events
Review diary and questionnaire completion dates

1 Month Post Injection #2 Phone Call (+/- 3 days)
Verify completed diary and questionnaires have been returned to research office via U.S. mail
Assess concomitant medications

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Assess for adverse events
Review diary and questionnaire completion dates

The following visits will be conducted for <u>all</u> subjects:

Visit 7 (single injection subjects)/Visit 8 (double injection subjects):
<u>12 Months Post Initial Injection Follow-up (+/- 14 days)</u>
Review bladder diary
Assess concomitant medications
Questionnaire completion (PGI-I, GRA, UAB-Q, ICIQ)
Vital signs: blood pressure, heart rate
Urinalysis (dipstick), and culture (if required)
Urine pregnancy test (if required)
Urine collection for evaluation of biomarkers (presence of inflammatory and neuronal innervation proteins in the urine)
Assessment of post void residual (PVR): measured by bladder scan or catheter
Antibiotic (a medicine to prevent infection) given, by mouth, to prevent urinary tract infection
Pressure flow studies
Flexible cystoscopy
Blood collection and analysis; CBC, blood chemistry
Assess for adverse events

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Schedule of Events

	ALL SUBJECTS							SUBJECTS OPTING FOR 2ND INJECTION (OPTIONAL)			ALL SUBJECTS
Event	Visit 1 Screening	Visit 2 Muscle Biopsy	Visit 3 Injection	Phone Call Post- Inj	Visit 4 1 Month Post-Inj	Visit 5 3 Months Post-Inj	Visit 6 6 Months Post-Inj	Visit 7 2nd Injection	Phone Call Post-Inj #2	Phone Call 1 Month Post-Inj #2	Visit 7/8 12 Months Post-Initial Inj
Visit window			Approx 10 wks from Visit 2	24-48 Hrs	+/- 7 days	+/- 7 days	+/- 7 days	Approx 10 wks from Visit 6	24-48 Hrs	+/- 3 days	+/- 7 days
Informed consent	X										
Medical and surgical history	X										
Vital signs	X	X	X		X	X	X	X			X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X						X				
Residual urine volume	X		X		X	X	X	X			X
Pressure flow studies	X						X				X
Cystoscopy			X				X	X			X
Muscle Biopsy		X									
AMDC injection			X					X			
Antibiotic	X		X				X	X			X
CBC / blood chemistry	X						X				X
PTT	X										
Blood borne virus[4]	X										
Urinalysis[1]	X		X		X	X	X	X			X
Urine culture[2]	X		X		X	X	X	X			X
Urine pregnancy[3]	X	X	X		X	X	X	X			X
Urine biomarker collection	X					X	X				X

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ALL SUBJECTS								SUBJECTS OPTING FOR 2ND INJECTION (OPTIONAL)			ALL SUBJECTS
Event	**Visit 1 Screening**	**Visit 2 Muscle Biopsy**	**Visit 3 Injection**	**Phone Call Post- Inj**	**Visit 4 1 Month Post-Inj**	**Visit 5 3 Months Post-Inj**	**Visit 6 6 Months Post-Inj**	**Visit 7 2nd Injection**	**Phone Call Post-Inj #2**	**Phone Call 1 Month Post-Inj #2**	**Visit 7/8 12 Months Post-Initial Inj**
Visit window			Approx 10 wks from Visit 2	24-48 Hrs	+/- 7 days	+/- 7 days	+/- 7 days	Approx 10 wks from Visit 6	24-48 Hrs	+/- 3 days	+/- 7 days
3 day bladder diary			X		X	X	X			X	X
ICIQ	X				X	X	X			X	X
PGI-I, GRA					X	X	X			X	X
UAB-Q	X				X	X	X			X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X

[1] Urine will be evaluated with a dipstick test for blood, glucose, ketones, leukocytes, nitrates, and protein. If the initial test is positive for leukocytes, further quantitative and diagnostic testing will be performed.

[2] Urine culture will be performed only if qualitative urinalysis is positive for leukocytes (> +2).

[3] If the subject has had a hysterectomy, is post -menopausal, or male, this test is not required.

[4] If the biopsy procedure is scheduled more than 30 days after the most recent blood borne pathogen tests were completed, the bloodborne pathogen tests (i.e. hepatitis B, hepatitis C, HIV, and syphilis) must be repeated before the biopsy procedure to confirm the patient's continued eligibility for study participation.

2. Facilities

All study visits, including biopsy and injection, will occur in the William Beaumont Hospital Urology Research Suite on the Royal Oak campus. The clinical research office is approximately 3,000 square feet of office space consisting of two examination rooms, a consenting/consultation room and a laboratory. Each examination room is equipped with an examination table and appropriate equipment to support clinical trials and provide care including cystoscopy. The laboratory space is capable of conducting urinalysis, urine pregnancy tests, and other minor laboratory procedures. This space includes secure storage for study supplies and records.

Blood collection and analysis will be conducted by the William Beaumont Hospital Outpatient Laboratory. Lab results will be recorded and available in the electronic medical record (EMR).

3. Investigational Product Storage and Accountability

Once the AMDC are ready for injection, they will be shipped frozen and undiluted from the laboratory in Pittsburgh PA to the study site. The research pharmacist will receive the AMDC and evaluate the integrity of the shipment and packaging. Specialized packaging for transport from the processing center is certified to maintain appropriate temperature for up to 72 hours. The cells must remain frozen and undiluted until ready for use. If the cells cannot be injected within that period, the cells should be stored at least -60 °C to –80 °C until the subject is available for injection. AMDC will be appropriately stored in the Investigation Drug Services (IDS) Office in Pharmaceutical Services per policy until the injection visit.

The cells are intended for a single use, should be thawed immediately prior to the injection, and should be used for the immediate procedure. Any unused portion shall be discarded. The IDS will follow a drug accountability plan that includes processes for receiving, storing, dispensing, and final disposition of the investigational product (IP).

4. Criteria for Subject Discontinuation or Withdrawal

The subject may be withdrawn or be discontinued from the study for any of the following reasons:

1. The subject voluntarily withdraws consent.
2. The subject refuses to complete any of the study-related activities.
3. The subject is lost to follow-up.
4. Based on clinical judgment, the investigator may discontinue the subject from participation at any time during the study for any reason (i.e. subject non-compliance). However, if AMDC injection has occurred, the subject will be followed for at least 6 months post-injection.

The subject may also be discontinued from the study, if unable to tolerate treatment. If the biopsy cannot be tolerated, then the procedure will be stopped immediately and the study will be terminated. The subject will be contacted by phone within 24-48 hours for follow-up evaluation. A clinic visit may also be conducted if necessary.

If the injection cannot be tolerated, then the procedure will be stopped immediately. If any of the study product was injected into the bladder, follow up will occur for at least 6 months post-injection.

In order to protect subject safety, enrollment may be suspended or the study may be terminated. Decision to suspend enrollment or terminate the study will be made by the principal investigator with guidance and recommendation from the Medical Safety Monitor. The FDA and IRB will be promptly notified of these actions. If a decision is made to terminate the study, all patients already treated will be followed for 6 months following the injection procedure.

RISKS AND BENEFITS

a. Potential Benefits

This is a prospective, open-label, Phase I, single center study. The treatments described in this protocol are investigational and involve greater than minimal risk to the subject.

The benefits to participating in this study include receiving a treatment that is not currently available to persons suffering with UAB. It is hypothesized that injection of AMDC will reduce UAB symptoms, improve quality of life, and delay or eliminate the need for invasive, surgical treatments in the future. Since this has not been proven, patients may receive no direct benefit from inclusion in this study. Knowledge gained in this study may benefit future patients with chronic UAB.

b. Potential Risks

The study involves procedures that may cause pain and discomfort. Additionally, it is possible, that the subject may experience some side effects. Should any side effects occur, or should the subject experience discomfort or pain, the subject should contact the study doctor and their personal doctor immediately.

Muscle Biopsy Procedure

Most Frequent (occurring more than 10% of the time):

- Bleeding
- Pain
- Muscle spasms and tightness
- Weight-bearing difficulty (experience pain/discomfort when applying weight to the leg)

Less Frequent (occurring more than 1% but less than 10% of the time):

- Wound infection
- Hematoma (bruise)
- Need for re-biopsy due to inability to grow cells from the muscle biopsy tissue

Rare (occurring less than 1% of the time):

- Dizziness and related responses (sweating, anxiety) from the procedure
- Numbing and tingling
- Scarring
- Allergic reaction to numbing medicine

Urinary Catheterization

Less Frequent (occurring more than 1% but less than 10% of the time):

- Temporary inability to urinate
- Bleeding
- Discomfort or pain during catheter insertion
- Mild cramping
- Urinary tract infections

Cystoscopy Procedure

Less Frequent (occurring more than 1% but less than 10% of the time):

- Discomfort or pain
- Mild cramping
- Infection
- Painful or difficult urination

Rare (occurring less than 1% of the time):

- Bleeding (blood in urine)
- Urinary tract infection
- Inability to pass urine after the procedure
- Puncture of the bladder
- Trauma
- Temporary swelling or injury to the urethra
- Overstretching of the bladder
- A change in urinary frequency
- Urgency in urination, including urgency resulting in episodes of urge incontinence
- Feeling of tiredness
- Back pain
- For men, testicular pain and/or temporary swelling of the testes or epididymis

Injection Procedure

Most Frequent (occurring more than 10% of the time)

- Temporary urinary passage (urethral) pain or discomfort
- Temporary bladder pain or discomfort
- Blood in urine

Rare (occurring less than 1% of the time):

- Temporary bleeding at injection site(s) resulting in blood clot(s) in the bladder tissue
- Infection
- Accidental bladder wall puncture resulting in inadvertently injecting AMDC into the lower abdominal area
- Urinary urgency
- Changes in urine frequency

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Investigational Product (AMDC)

Rare (occurring less than 1% of the time):

- Flu like symptoms (nausea, fatigue, body aches)
- Allergic response (cells or components of the final product including: bovine (cow) proteins (used in the process to grow your cells), ampicillin and gentamicin sulfate (antibiotic medications)
    - Risk of allergic response to the cells is expected to be minimal due to the use of your own cells. Risks of allergic response to bovine proteins, ampicillin, and gentamicin sulfate used in AMDC production should be minimal since only trace amounts are expected to be in the final product.
    - Signs/symptoms of an allergic response may include, but are not limited to: swelling, itching, hives, shortness of breath, and nausea

Lidocaine (Numbing Medicine)

1) Used in liquid form pre-biopsy, injected into the skin at the biopsy site,
2) Used in liquid form pre-injection, instilled in the bladder with a catheter
3) Used in gel form pre-cystoscopy, injected into the urethra (urinary passage)

Rare (occurring less than 1% of the time):

- Lightheadedness, dizziness, confusion
- Lowered blood pressure, slowed heart rate
- Swelling or local irritation
- Allergic reaction

Antibiotic (Medicine to Prevent Infection)

Less Frequent (occurring more than 1% but less than 10% of the time):

- Diarrhea
- Nausea and vomiting
- Cramping

Rare (occurring less than 1% of the time):

- Headache
- Itching
- Rash
- Allergic reaction

Risks of Blood Drawing Procedure

Most Frequent (occurring more than 10% of the time):

- Pain
- Discomfort
- Bleeding
- Bruising at the needle puncture site

Rare (occurring less than 1% of the time):

- Blood clot

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- Infection at the needle puncture site
- Feeling lightheaded
- Fainting

There is a rare risk of breach of confidentiality (release of information which personally identifies the participant). Multiple processes are in place to reduce this risk.

Cell Therapy
All cell therapies are thought to involve the possible risk of tumor formation (a nodule or mass-like structure). The true risk of tumor formation is unknown in cell therapy. With the type of cells used in this study, the risks are considered to be extremely low. Testing was carried out on the type of cells used in this study to determine the risk and no evidence of tumor formation was found.

Breach of Privacy and Confidentiality
There is a rare risk of breach of privacy and data confidentiality (release of information which personally identifies the participant). Confidentiality procedures will be strictly adhered to when transferring, managing, and analyzing study data. The subject will be assigned a unique study identification number, research information will be stored in a locked, secure cabinet with access limited to authorized research personnel, and all visits will be conducted in a private area of the clinic.

Unforeseen Risks
Not all possible effects are known. With any therapy, unusual, unexpected or previously unreported side effects may occur. This study includes experimental procedures, therefore not all risks and outcomes can be foreseen.

Methods to Minimize Risk
Only qualified physicians trained in cystoscopy, cystoscopic injection and the use of bladder injection agents will perform the injection procedure. The PI and sub-Investigators have had this experience in private practice utilizing botulinum toxin for bladder injections using a similar approach and needle. Adherence to the treatment protocol is necessary to reduce material- and procedure-related risk. Routine venipuncture will be performed by qualified personnel. Local anesthetic will be used to minimize discomfort. Injection of AMDC will occur under direct vision. After injection, cystoscopy will be used to ascertain the presence of bleeding or puncture. A prophylactic antibiotic will be given to reduce the risk of infection. The patient will be monitored for a minimum of 30 minutes after injection. The risks of incontinence, urinary tract infection, urinary retention, and allergic reaction to the injected reagents will be assessed through follow-up clinical visits and review of the 3-day bladder diary.

ELIGIBILITY CRITERIA

Inclusion

a. Males and females, at least 18 years of age
b. History of UAB for at least 6 months documented in the medical record
c. Recurring UAB symptoms

d. Subjects unresponsive to relief symptoms of UAB with previous use of medications and/or other treatments
e. Voiding difficulty (complains of difficulty emptying the bladder)
f. Post void residual ≥ 150 mL
g. Total UAB Questionnaire Score ≥ 3 or 100% reliant on intermittent self-catheterization to empty bladder
h. Females of child-bearing potential agree to use a reliable form of birth control for the entire study duration
i. Willing and capable of understanding and complying with all requirements of the protocol, including proper completion of the voiding diaries and self-administered questionnaires

Exclusion

a. Pregnant, plans to become pregnant or lactating
b. History of bleeding diathesis, uncorrectable coagulopathy, or would refuse a blood transfusion
c. Currently on anticoagulant therapy
d. Obvious neurological impairment
e. Known allergy or hypersensitivity to bovine proteins or allergens, gentamicin sulfate, ampicillin, and/or lidocaine that medically warrants exclusion as determined by the physician
f. Simultaneously participating in another investigational drug or device study or use of any investigational drug(s) or therapeutic device(s) within 3 months preceding enrollment
g. Has been treated with an investigational device, drug, or procedure for UAB within the last 6 months.
h. Medical condition or disorder that may limit life expectancy or that may cause protocol deviations (e.g. unable to perform self-evaluations and/or accurately report medical history, urinary symptoms, and/or data)
i. History of cancer in pelvic organs, ureters, or kidneys or any cancer that has undergone treatment within the past 12 months
j. Compromised immune system due to disease state, chronic corticosteroid use, or other immunosuppressive therapy
k. History of radiation therapy to the bladder
l. Tests positive for Hepatitis B (Hepatitis B Surface Antigen [HBsAg] and Anti-Hepatitis B Core Total Antibody [Anti-HBc])), Hepatitis C (Hepatitis C Antibody [Anti-HCV]), HIV (HIV Type 1 and 2 Antibodies [Anti-HIV-1, 2]), and/or Syphilis.
m. Abnormal renal function
n. An active urinary tract infection, determined by an investigator, as evidenced by urinalysis, urine culture, patient symptom report, and/or cystoscopy (see appendix A for details)
o. Taking medication(s) that affect urination (e.g. medically necessary, stable drugs) such as prescription drugs, over-the-counter drugs, or dietary supplements, including herbal supplements and those taken with teas
p. Requires concomitant use of or treatment with immunosuppressive agents
q. Pelvic organ prolapse beyond the introitus (e.g., cystocele, rectocele)
r. Abnormal bladder capacity (i.e., less than 100 mL)

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ADEQUACY OF PROTECTION FOR HUMAN SUBJECTS

Investigational Review Board (IRB)

The Institutional Review Board (IRB) must provide written approval of the study protocol and informed consent prior to the initiation of any study related procedures. The PI will supply the required documentation to the IRB for the annual renewal of the protocol, and any approvals of revisions to the informed consent document or amendments to the protocol. The PI will be responsible for providing to the IRB, prompt notification of any new information that may adversely affect the safety of subjects or the conduct of the trial.

Informed Consent

The study coordinator will obtain informed consent and will explain the study in full including, but not limited to, what is involved, the possible risks, and the possible benefits. The explanation will occur at a level of understanding appropriate for the potential participant. Once the potential subject has had ample time to consider the study, review the consent form and ask questions, he/she will be asked to sign the written consent form in the presence of the consent provider and a witness (a Beaumont employee). The subject will receive a copy of the consent form for his/her records.

SUBJECT CONFIDENTIALITY

A report of the results of this study may be published but participants' names will not be disclosed in these documents. The participants' identities may be disclosed to the governing health authorities or the FDA if they inspect the study records. Appropriate precautions will be taken to maintain confidentiality of medical records and personal information.

DATA ANALYSIS AND REPORTING

Descriptive statistics will be reported on clinical and demographic characteristics of patients, as well as for the frequency of AEs and SAEs (both overall and for specific ones of interest). Continuous variables will be summarized using means and standard deviations or median and range, as appropriate. Categorical variables will be summarized with counts and percentages. Assumptions needed for all inference procedures will be checked. Blyth-Still-Casella confidence intervals may be calculated for the proportions of AEs and SAEs. Frequency tables will be presented for the 12 month results on Patient Global Impression of Improvement and the Global Response Assessment. For assessments involving other questionnaires and voiding habits, the pre-treatment assessments will be used as a control for each subject. Analysis may be based on the changes; nonparametric tests (Friedman tests, Wilcoxon signed rank tests) or parametric alternatives may be used. Repeated measures analysis may be used if appropriate.

The primary assessment of safety outcomes will use 6 month outcomes; secondary analyses will use 12 month data. The primary assessments of efficacy will be made at 12 months post initial AMDC injection,

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A description of this study will be available on http://www.ClincalTrials.gov. This site will not include any subject identifiers. After study completion, the posting will include a summary of the results.

Results may also be disseminated in poster or platform presentations at various local, national, and/or international scientific meetings, as well as published in peer-reviewed journals.

DATA SAFETY MONITORING PLAN

Overall, ongoing, safety monitoring will be performed by the study site staff including the principal investigator and sub-investigators. A Medical Safety Monitor will be assigned by the PI. The Medical Safety Monitor (MSM) will be a physician who is not involved in the study and who has no conflict of interest. The MSM will review for SAEs and will determine expectedness of the event. In the event that an AE is unreported and cannot be determined within reportable timeliness, the causality will be determined by the MSM. The MSM may suggest protocol modifications to prevent the occurrence of particular AEs (e.g., modifying the protocol to require frequent measurement of laboratory values predictive of the event or to improve expeditious identification of SAE). In the event that the MSM is unavailable for an extended period of time (i.e. extended vacation, illness, etc.) a back-up MSM will be nominated by the study PI. Safety issues will also be addressed in the annual reports to the IRB and the FDA.

Additional data safety monitoring procedures include:

- Research Administration's Clinical Research Quality and Process Improvement Program (CRQIP) will perform in-house monitoring of the first subject enrolled after the completion of visit 1
- Monitoring of the study will be conducted by the Beaumont Research Coordinating Center (BRCC)
- Safety data review at study team meetings

Adverse Event Reporting

An AE is any untoward medical occurrence in a subject participating in a clinical investigation and which does not necessarily have a causal relationship with treatment. An AE can therefore by any unfavorable and unintended sign (included an abnormal laboratory finding), symptom, or disease temporarily associated with the use of an investigational product (AMDC) or study-related procedure, whether or not related to the product or procedure.

Study subjects will be instructed to report **any** changes in baseline health from the time the subject enters the study through the end of the study regardless if the event is listed as a risk on the informed consent form. Study staff will also inquire about AEs at each visit. All AEs should be recorded on the case report form (CRF) and submitted to the electronic data capture (EDC) system within 10 days of knowledge of the event. The investigator is responsible for reporting AEs to the IRB in accordance with institutional policy.

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All SAEs are to be reported to the Principal Investigator within 24 hours of knowledge of the event. A SAE is defined to include any adverse experience that results in the following:

- Death;
- Life threatening;
- Hospitalization;
- Persistent or significant disability or incapacity;
- Require intervention to prevent death or a life threatening condition.

All such reported SAEs will be investigated by the Medical Safety Monitor in collaboration with the investigator involved as well as with the principal investigator. The principal investigator, as the IND holder, shall, if required according to applicable regulations, report the event to the appropriate regulatory authority in accordance with 21 CFR 312.32. Fatal or life-threatening AEs will be reported to the FDA by telephone or fax within seven days. Unexpected SAEs will be reported to the FDA in writing within 15 days. The principal investigator or designee will notify the IRB of applicable events according to institutional guidelines. AEs identified prior to the end of study will be followed for one month after the last study visit is conducted.

Pre-Existing Conditions

Any chronic or acute sign, symptom, illness, or condition that the subject has at the time of enrollment of this trial that is unrelated to the treatment is considered a pre-existing condition (e.g., asthma, diabetes etc.). Information on pre-existing medical conditions will be obtained at the screening visit. This information will be utilized to assist with identifying changes in health conditions and increase the accuracy of AE reporting. A pre-existing condition should be reported as an AE only if its frequency, intensity or character worsens during the study period.

Determination of Event Causality

Definitely related events are those that the site PI/Sub-I determines are definitely related to the intervention, and for which the PI/Sub-I believes no alternative etiology exists.

Probably related events are those that the site PI/Sub-I believes there is a reasonable likelihood the AE may have been caused by the intervention involved in the research.

Probably not related events are those that the site PI/Sub-I believes there is a reasonable likelihood the AE may have been caused by other factors and not caused by the intervention involved in the research.

Unrelated events are those that the site PI/Sub-I determines are not related to the intervention.

Grading of Adverse Events

The severity of each AE will be categorized using the following criteria:

Grade I (Mild): Transient or mild discomfort; no medical intervention/therapy required

Grade II (Moderate): Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required

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Grade III (Severe): Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
Grade IV (Life-Threatening): Extreme limitation in activity, significant assistance required; significant intervention/therapy required, hospitalization or hospice car probable
Grade V (Death)

Protocol Deviations
The RNC will utilize Beaumont Hospitals, policy 229, "Protocol Deviations" as guidance for IRB reporting. Each event will be reviewed by the PI or his designee (a Sub-I). All reportable and un-reportable deviations will be recorded on a protocol deviation log. The log will aide with identifying trends and recurring issues to be addressed by study staff. The data will be routinely reviewed by the study team, including the PI.

PLAN FOR DATA MANAGEMENT AND MONITORING

Study data will be transcribed by study personnel from the source documents onto the eCRF. Worksheets will be provided for the capture of data for easier transfer to the eCRF. Electronic data capture process for the proposed study will require connecting to the data website via the Microsoft Internet Server. To ensure accuracy and integrity, data entered for each variable will be validated by an electronic audit procedure, which entails a three-step process. The validation rules are to insure that no data is missing, entries are logical, skip patterns are followed, and that non-numerical data entries are appropriate.

The study will be reviewed in accordance with written standard operating procedures consistent with 21CFR812. The BRCC will be assigned to review completed case report forms and/or electronic data entry, data base review, IRB decisions, and investigator and clinical site records at regular intervals throughout the study. Additionally, patient charts, and clinical records will be reviewed to assure protocol adherence. Source documentation may also be verified. Reports from the BRCC will include enrollment data, eCRF completion and delinquencies, query resolutions and delinquencies, protocol deviations, and frequency of unanticipated problems/adverse events. In order to identify, evaluate and prevent adverse events, the total number of events will be reviewed, as well as the details of each event, for example visit number and severity. Data will be reviewed to identify trends and possible concerns. Enrollment data will direct recruitment efforts and assist in study planning. See BRCC monitoring plan for more details.

SUMMARY OF EXISTING STUDY DATA

a. Animal Pharmacology and Toxicology

Nonclinical testing of muscle derived cell (MDC) injection into the lower urinary tract has been conducted in a number of animal studies. The studies summarized here include: 1) a preliminary evaluation of MDC persistence and differentiation after injection in the bladder wall of a mouse model; 2) an assessment of injected MDC persistence as compared to injected bovine collagen in an autologous rat model; and 3) assessment of periurethral injected allogeneic MDCs in rat bladder wall.

Feasibility Testing in an Allogeneic Mouse Model

The feasibility of MDC injection was evaluated in a preliminary assessment of MDC persistence and differentiation into myotubes and myofibers in the bladder wall.[9] In this study, primary MDCs isolated from normal mice were transduced to express the β-galactosidase reporter gene. Twelve adult immunodeficient mice received injections of 1-1.5 x 10^6 MDCs into the right and left lateral bladder walls. The tissue was harvested after 5, 35, and 70 days, and stained for fast myosin heavy chain to assess the formation of muscle fibers, and β-galactosidase to mark the injected cells.

A large number of cells expressing β-galactosidase were observed in the bladder wall at each time point. Many myotubes and myofibers expressing β-galactosidase and positively stained for fast myosin heavy chain were also seen in the bladder wall at 35 and 70 days after injection, demonstrating feasibility and persistence of injected MDC cells through 70 days.

Persistence of MDCs vs. Bovine Collagen in an Autologous Rat Model

The persistence of MDCs as compared to bovine collagen was assessed after injection into the bladder or urethral wall in an autologous rat model.[10] In this study, 12 female Sprague-Dawley rats received injections into the bladder or urethra. Six animals received autologous MDC injection and six received collagen injection. Three animals from each group were sacrificed at three days and at 30 days after injection, and tissues were prepared for histopathological examination. Slides were evaluated for the presence of MDCs by staining for fast myosin heavy chain and β-galactosidase, for the presence of collagen by Tri-chrome staining, and for evidence of inflammation or tissue damage by microscopic examination.

At the three and 30-day time points, a large number of cells expressing β-galactosidase were observed in the bladder and urethral wall of the MDC-injected animals. Moreover, injected MDCs comprised a mass that protruded toward the lumen of the bladder and urethra. The persistence of injected MDCs and collagen was similar at three days. However, at 30 days only scant bovine collagen was detectable at the injection site in the collagen-injected animals while there was significant MDC persistence at the injection site in the MDC-injected animals. Measurement by cell mediated reporter gene expression indicated approximately 88% MDC survival at 30 days.

At three and 30 days post-injection, the measured diameters of nodules created by injection averaged 1.0 ± 0.05 mm and 0.84 ± 0.08 mm for MDC, respectively. The diameter of nodules by collagen injection was 0.78 ± 0.07 mm at three days post-injection, and decreased to 0.15 ± 0.05 mm at 30 days post injection. The three day post-injection sizes were not significantly different between MDC and collagen injection; however, at 30 days post-injection, the mean size of remaining collagen nodules was significantly smaller than for MDCs (P<.05).
Histopathological examination revealed no evidence of inflammation (platelets, macrophages, and monocytes) or tissue damage at the injected site. Importantly, no MDCs (as evidenced by fast myosin heavy chain staining) were observed in the area outside the injection.

Testing in an Allogeneic Rat Incontinence Model

Testing was performed in a rat incontinence model in which the sciatic nerve on each side was transected distal to its origin from the vertebral column and proximal to the pudendal nerve branch. Animals were evaluated for the effect of periurethral injected allogeneic MDCs on leak point pressure, local histopathology, and the potential for immune response.[11]

In this study, cells for injection were isolated from skeletal muscle of normal female rats and purified in MDC content using the pre-plate technique. Fifteen six-week old normal female rats were evenly divided into three experimental groups: a control group had a sham operation without injections, a denervated group had periurethral saline injections, and a denervated group had periurethral MDC injections. Leak point pressure (LPP) was measured at one and four weeks following injection. Following the final LPP measurement, the injection sites were harvested and processed for histopathological assessment.

The potential for immune response was evaluated in four additional animals; two received bladder wall injections of normal satellite cells, and two received bladder wall injections of MDCs. Two weeks after injection the injection sites were harvested and processed for immunohistochemical evaluation of the presence of CD8 lymphocytes.

Results of testing provided evidence of the effectiveness of MDC injection. At one week, the measured LPP was not significantly different between control and saline injection groups, but was significantly greater in the MDC group as compared to control (*P*=.001). At four weeks, the LPP was significantly decreased in the saline injected group vs. control (*P*=.01), while the LPP was significantly increased in the MDC injected group vs. control (*P*=.001). These results are summarized in the table below.

LPP MDCs vs. Control

Group	1-week LPP ($cm \cdot H_2O$)	4-week LPP ($cm \cdot H_2O$)	Difference ($cm \cdot H_2O$)
Control	25.2 ± 1.9	25.8 ± 2.5	0.6
Saline injection	28.6 ± 0.8	18.6 ± 5.2	-10.0
MDC injection	36.7 ± 2.3	44.1 ± 6.6	7.4

Results of histopathological assessment showed both smooth and skeletal muscle contained in the urethral sphincter of control animals, with the smooth muscle cells in tightly packed bundles. As expected, sections from the saline injected denervated rats showed cell atrophy, while the samples from the MDC animals showed increased dorsolateral skeletal muscle classes with variable fiber orientation at the injection sites.

Immunohistochemical evaluation detected CD8 lymphocytes throughout the injection site in sections from animals injected with normal satellite cells; however, no CD8 lymphocytes were observed in the MDC-injected bladders, demonstrating that MDC injected cells did not trigger an immune response, despite their allogeneic nature.

In a more recent study, MDC doses of 1 x 10^5, 1 x 10^6 and 1 x 10^7 were injected into the urethral wall in the rat incontinence model.[12] No adverse events related to treatment

were noted in any of the animals in the MDC injected group. Animals injected with 1 x 10^6 and 1 x 10^7 doses showed significant increase in LPP over sham operated animals.

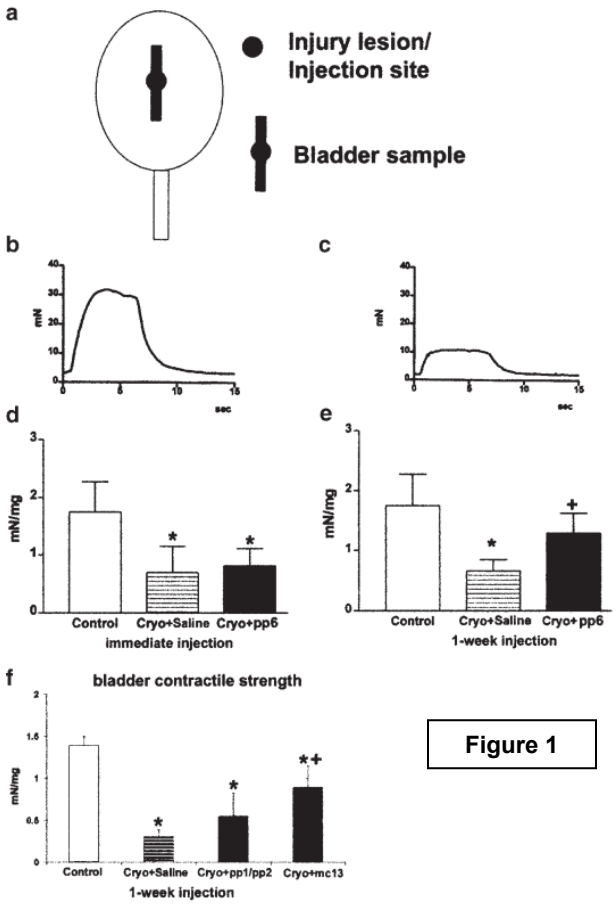

Figure 1

<u>Testing in Rat model of Underactive Bladder</u>

We have demonstrated that MDC transplantation increased muscle contractility in the cryoinjured bladder detrusor model [26, 28]. We have (1) demonstrated the feasibility and survival of MDC injection into the bladder wall; (2) established improved detrusor contractility with MDC injection in a bladder injury model; (3) revealed the maturity of the b-galactosidase expressing myofibers in the injured bladder by demonstrating the presence of neuromuscular junctions based on the accumulation of acetylcholine receptors (AChRs) in small segments of their membrane; and (4) demonstrated the possiblity of MDC differentiating into a smooth muscle lineage when injected into the bladder wall (Figures 1-3). Our preclinical data support that autologous MDC injections can be used as a nonallergenic agent to improve bladder contractility. Thus, transplantation of AMDCs from skeletal muscle might be a promising treatment strategy for patients with UAB.

Figure 1: Physiological improvement of the injured bladder via muscle-derived cell implantation. Schematic representation of the cryoinjury model and location of the bladder strips (a). A representative contractile curve of bladder strips evoked by electrical stimulation (20 Hz 80 shocks) of control (b)and 30-s cryoinjured (c) rat bladders. Cryoinjured rat bladders with immediate saline and pp6 injection both resulted in a significant (∗P <0.05)decrease of contractile responses to electricalfield stimulation when compared with the control bladder (d). However, the pp6 injected at 1 week aftercryoinjury in the rat bladder displayed a significant improvement in bladder contractility versus cryo+saline (1 week) injection (+P<0.05) for up to 80% of the normal baseline level, which was not significantly different from the control group. (e). The cryoinjured mouse bladder injected with mc13 at 1 week after injury significantly improved bladder contractility (+P 0.05) in contrast to that observed with pp1/pp2 (f). Compared with control; +compared with cryo+saline.

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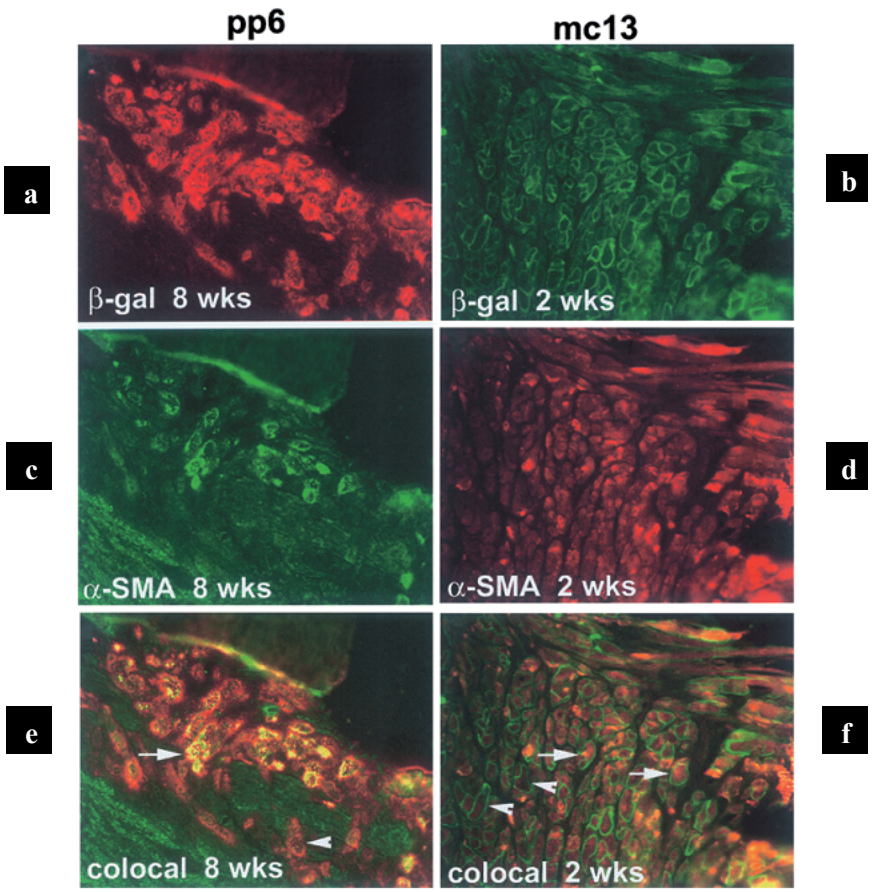

Figure 2

Figure 2: Expression of -SMA in the injected muscle cells at the injected site. Bladders at 8 weeks (pp6) and 2 weeks (mc13) after injection were stained for□b-galactosidase (a, b) and SMA (c, d). Although some of the cells expressing □b-galactosidase did not colocalize with SMA (arrowheads e, f), many of the injected MDC expressed both markers (arrows e, f) showing their differentiation into the smooth muscle lineage. Magnification a–f,×200

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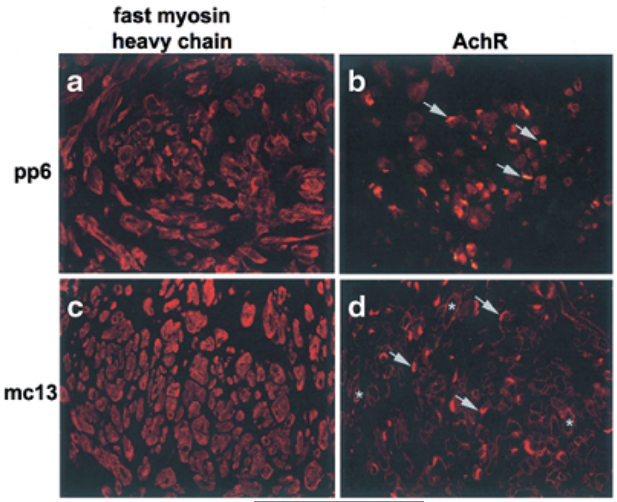

Figure 3

Figure 3: The muscle-derived cells differentiated into myotubes and myofibers with neuromuscular junctions in the injected bladders. Myotubes and myofibers expressing fast myosin heavy chain (a, c) were also found to express AChR clustered in their membranes at 15 days after injection with pp6 (b) and mc13 (d) suggesting that the myofibers became innervated (arrows). However, it has also been observed that in many myotubes and myofibers, the AChR expression was diffused throughout the myofibers, which is suggestive of the lack of innervation of these myofibers (stars, d). Magnification a–d,×400.

The Dose-Effect Relationship Between Cell Dose and Resultant Myofibers

Praud and colleagues demonstrated that the number of myofibers formed from muscle cell transplantation reaches a plateau. Once the plateau is reached, additional cells do not efficiently increase the number of myofibers formed.[13] In fact, these additional cells may adversely impact overall survival of the implanted cells by overwhelming the available local nutrient supply leading to cell death and increased local inflammation. This principal has been demonstrated in encapsulated myoblast delivery devices and in studies of cardiomyocyte transplantation.[14, 15] Thus, the over-delivery of cells may be counterproductive to regeneration due to the possibility of disruption and demise of cells that might otherwise survive. Based on these observations, it is postulated that transplants with lower initial cell counts might improve overall cell survival, and increase the number of viable myofibers formed from the transplanted cells.

Summary

Non-clinical testing has demonstrated MDC persistence and differentiation into myotubes and myofibers after injection in the bladder wall of a mouse model. Testing in an autologous rat model has demonstrated improved cell persistence and bulking as compared to injected bovine collagen, and an absence of cell migration. Testing in a rat incontinence model has demonstrated significant improvement in LPP, increased muscle mass, and no evidence of an immune response as determined by the absence of CD8 lymphocytes. Testing in model of underactive bladder demonstrated improved detrusor contractility.

Additional testing (unpublished) has demonstrated significant autologous MDC survival at six months after injection into the bladder wall in an immunodeficient mouse model, and evidence of increased LPP in an immunodeficient rat incontinence model after

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injection of human MDCs. Higher doses of MDCs have not been associated with adverse events. However, there may be a plateau beyond which additional cells do not improve myofiber content. In fact, lower initial cell doses may improve overall cell survival and improved myofiber content.

b. Experience in Humans

Indication: Underactive Bladder

Compassionate use of AMDC to treat UAB was previously approved by the FDA for use in a single patient by Dr. Ananias Diokno, William Beaumont Hospital (IND 15417). The subject received a single treatment of 250 million cells in approximately 30 sites (0.5 ml/injection) to enhance bladder detrusor contractility in September 2013. He has completed the 6 month post-injection follow-up visit. There were no changes in any of the specified safety measures from baseline to 6 months post AMDC injection. Specific safety measures evaluated include: vital signs, residual urine volume, and urinalysis. No study-related adverse events have been reported by the subject. At each study visit the subject denied experiencing any problems related to urinary functioning including gross hematuria, urgency, frequency and signs/symptoms of infection (i.e. odor, cloudiness, or dysuria). Additionally there have not been any significant changes in laboratory results post AMDC injection. Therefore, the injection of AMDC into the bladder appears to be safe.

Indication: Stress Urinary Incontinence in Women

AMDC has been evaluated in patients in clinical trials for urinary sphincter repair (USR) in female patients with stress urinary incontinence, for anal sphincter repair (ASR) in patients with fecal incontinence, and cardiac tissue repair (CTR) in patients following myocardial infarction (sub-acute), and chronic ischemic heart failure. AMDC products have been used safely in over 300 patients to date in clinical studies approved by FDA and Health Canada. The clinical pharmacology of AMDC is currently under investigation with clinical experience above and below the dose of 150 ± 20% x 10^6. AMDC-USR is expected to improve continence in female patients with stress urinary incontinence. Clinical data collected are suggestive of a safe toxicity profile with injections into the urethral sphincter up to 200 x 10^6 cells. Additionally, in two separate studies, a Phase II dose escalation and a Phase III confirmatory study have been conducted where patients have received a second treatment dose approximately 3-6 months after their initial treatment with AMDC ranging from 1-150 x 10^6 cells per treatment. The Phase III study is ongoing and data is still being collected, but data from the phase II study has been collected, analysed, and reported. No SAEs have been reported from the Phase III confirmatory study to date that have been attributed to the AMDC product (150 x 10^6 cells at 1 or 2 treatments).

<u>Study Code: MCMT- Feasibility Study</u>

In an initial clinical feasibility study in Canada, 8 subjects have been treated with doses of 18-22 x 10^6 AMDC. All subjects have completed the study without experiencing any serious, related adverse events associated with the use of AMDC. [Reference Health Canada File #9427-C2135-21C, Control# 087205 (Original CTA), Control# 089071 (CTA-A), Control# 095823 (CTA-A) and Control# 101701 (CTA-A)]

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Study Code: MCDR- Dose Effect Study

A multicenter dose escalating study demonstrating feasibility of this treatment is completed. A summary of results from the dose escalating clinical study in Canada is presented below. [Reference Health Canada File #9427-C2135-21C, Control# 103555 (Original CTA), Control# 116233 (CTA-A), Control# 117540 (CTA-A)]

In the study, 38 female subjects with SUI, who demonstrated no improvement with standard therapy for at least 12 months, were enrolled in the three parts of this study. 20 subjects were stratified between two sites. Ten subjects in each site were randomized to five arms to receive, in a double-blind fashion, an injection of one of five doses from 1, 2, 4, 8 to 16 x 10^6 cells of autologous MDC obtained from skeletal muscle biopsies. The second part of the study enrolled nine subjects, three in each arm, who were randomized to receive one of three stepped doses of 32, 64 or 128 x 10^6 cells. In the third part of the study, a second group of 9 subjects (3 at each dose level) received one of 3 doses of 16, 32 or 64 x 10^6 cells. The injections of these additional subjects were performed using ultrasound guidance to assure placement in the muscle of the urethral sphincter mechanism. All subjects were followed for one year after their last treatment and monitored closely for any adverse events. For the analysis, doses of 1-16 x 10^6 cells were grouped and analyzed as the “low dose” group while doses of 32, 64 or 128 x 10^6 cells were grouped and analyzed as the “high dose” group.

The first patient was enrolled in the study on September 6, 2006; the last patient completed follow-up on April 29, 2010. Mean patient age was 50 years (range 30-73). A total of 38 patients were enrolled; 32 (84.2%) elected to receive a second AMDC treatment following their initial 3-month follow-up. Thirty-three patients (4 with 1 treatment; 29 with 2 treatments) completed the study; 4 patients withdrew and 1 patient was lost to follow-up. From discussions with physicians, it appears that all patients received periurethral injection of AMDC. There have been no serious, related adverse events associated with the use of AMDC. Minor events occurred at similar rates among all treatment groups and included pain and bruising at the muscle biopsy site, pain at the injection site, mild self-limiting urinary retention, and urinary tract infection.

Treatment affected the amount of leakage during a 1-hour pad test and frequency of diary-reported stress incontinence episodes. Of patients with ≥1 g baseline pad weight who received 2 AMDC treatments, 61.5% (8/13) of low dose patients and 88.9% (8/9) of high dose patients had ≥50% reduction in pad weight with ~30% of both dose groups dry (i.e., <1 g pad weight) at 18 months. Similarly, 53.3% (8/15) of low dose patients and 77.8% (7/9) of high dose patients who received 2 AMDC treatments and who had at least 1 baseline stress leak reported ≥50% fewer stress leaks at 18 months. Quality of life was also affected with ≥50% improvement in IIQ-7 and UDI-6 scores reported at 18-month follow-up for 26.3% (5/19) of low dose patients and 50.0% (5/10) of high dose patients who received 2 AMDC treatments. Improvement may be related to cell dose since a higher proportion of high dose patients than low dose patients experienced ≥50% reduction in pad weight (88.9% vs. 61.5%), ≥50% reduction in diary-reported stress leaks (77.8% vs. 53.3%), 0-1 leaks (88.9% vs. 33.3%), and ≥50% improvement in IIQ-7 and UDI-6 scores (50.0% vs. 26.3%) at 18 months. Valsalva LPP improved for 25.9% (7/27) of 2 treatment patients and an additional 14.8% (4/27) of this patient subset no longer experienced detectable valsalva or cough-induced leakage at 3 months following the second AMDC treatment. However, a standardized method to

assess valsalva LPP was not provided in the protocol and variability in methods between sites and operators may have affected the measurements. Since limited data are available for single treatment patients, the effect of a single dose of AMDC on SUI symptoms cannot be determined.

Study Code: IND1- Dose Effect Study

This prospective, multi-center, multi-country dose escalation trial was initially designed to evaluate the 12-month safety and potential efficacy of 3 different doses (10, 50, 100 x 10^6) of AMDC for treatment of SUI in adult females. In a multicenter, multi-country study [Reference Health Canada File #9427-C2135-21C, Control# 126664 (Original CTA), Control#140159 (CTA-A)]; FDA File #BB-IND-11618 (Original IND)], a fourth dosing group (200 x 10^6) was added (to FDA approved portion only).

Sixty-four women received intrasphincteric injection of AMDC in this study; 16 received 10 million cells, 16 received 50 million cells, 24 received 100 million cells, and 8 received 200 million cells. Fifty-nine patients completed 12-month follow-up; 4 patients withdrew from the study and 1 patient was lost to follow-up.

To assess potential efficacy of AMDC treatment, patients served as their own controls with quantitative and qualitative measures of incontinence assessed before treatment and compared to the same measures at various times after treatment. Diary-reported stress leaks over 3 days and 24-hour pad weight tests were used as quantitative measures of incontinence, while Stamey score and scores from validated quality of life questionnaires (i.e., UDI-6, IIQ-7, GQOL, PGI-S, and PGI-I) served as qualitative measures of incontinence. Potential efficacy was evaluated for all patients, patients with ≥3 stress leaks and ≥3 g pad weight at baseline, and patients maintained on no or stable doses of medications known to affect LUTS. Similar trends for efficacy outcomes were observed for each group.

Twelve months following AMDC treatment, median stress leaks reported over 3 days were significantly lower for all dose groups compared to baseline. Additionally, the percentage of patients who experienced ≥50% reduction in stress leaks increased with increasing AMDC dose and ranged from 53% (8/15) in the 10 x 10^6 dose group to 88% (7/8) in the 200 x 10^6 dose group at 12-month follow-up. Of patients with ≥3 stress leaks and ≥3 g pad weight at baseline who were treated with 200 x 10^6 AMDC, 100% (6/6) had ≥50% reduction in stress leaks and 50% (3/6) were dry (i.e., zero stress leaks over 3 days) at 12-month follow-up.

Similarly, the percentage of patients with ≥50% reduction in pad weight increased with increasing AMDC dose at 12-month follow-up. While only 20% (3/15) of patients in the 10 x 10^6 dose group met this end point, 75% (6/8) of patients in the 200 x 10^6 dose group had ≥50% reduction in pad weight.

The 200 x 10^6 dose group also had the highest percentage of patients with negative pad tests (i.e., <1.3 g pad weight) at 12-month follow-up with 63% (5/8) of patients meeting this endpoint. When only patients with ≥3 stress leaks and ≥3 g pad weight at baseline were considered, 83% (5/6) of patients in the 200 x 10^6 dose group had ≥50% reduction in pad weight and 67% (4/6) of these patients had negative pad tests at 12-month follow-up. In contrast, 0% (0/13) of patients in the 10 x 10^6 dose group, 30% (3/10) of

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patients in the 50 x 10^6 dose group, and 28% (5/18) of patients in the 100 x 10^6 dose group had negative pad tests at 12-month follow-up.

Study Code: CTHM- Dose Effect Study
This multicenter, nonrandomized, Canadian study (Reference Health Canada File #9427-C2135-21C, Control# 134363 [Original CTA], Control#136972 [CTA-A], Control#144277 [CTA-A]) examined the safety and potential efficacy of a single dose of 200 million AMDC in 16 women with stress urinary incontinence. Subjects received intrasphincteric injection of AMDC via the transurethral route and were followed for one year after treatment.

Sixteen patients were treated, and 1 patient withdrew from the study prior to 12-month follow-up. No serious treatment or procedure-related adverse events were reported.

To assess potential efficacy of AMDC treatment, patients served as their own controls with measures of incontinence assessed before treatment and compared to the same measures at various times after treatment. Diary-reported stress leaks over 3 days and 24-hour pad weight tests were used as quantitative measures of incontinence. Diary and pad test data were available for 14 of the 16 treated patients at 12-month follow-up.

The frequency of stress urinary incontinence episodes and 24-hour pad weight decreased following AMDC treatment. From baseline to 12-month follow-up, the median number of stress leaks reported over 3 days decreased from 12 leaks to 4 leaks (p=0.02), while the mean 24-hour pad weight decreased from 116 g to 52 g (p=0.06). Additionally, at 12-month follow-up, 71% (10/14) of patients had ≥50% reduction in stress leak frequency and 29% (4/14) of patients reported no stress leaks over 3 days. Although only 2 patients (14%) had negative pad tests (i.e., <1.3 g pad weight) at 12-month follow-up, 57% (8/14) of patients had ≥50% reduction in pad weight.

Study Code: UIAD- Dose Effect Study
The study is a randomized, double-blind, placebo-controlled, multicenter study to determine the effectiveness and safety of AMDC in the treatment of SUI in female patients. The study will randomize patients to receive one of two doses (placebo and 150 x 106 cells) and either one or two treatments. Allocation ratio within dose will be 2:1 (cells: placebo), while one and two treatments are equally allocated (1:1). The study will treat a total of 246 patients (164 treated with 150×106 AMDC and 82 treated with placebo). This is a multicenter, confirmatory Canadian study [Reference Health Canada File #9427-C2135-21C, Control# 144379 (Original CTA), Control#147259 (CTA-A)].

The study will evaluate the injection of AMDC compared to a placebo dose, with the hypothesis that one or two treatments of AMDC are statistically superior to placebo at 12 months following the initial treatment. The primary effectiveness measure will be based on reduction in the number or size of leaks due to stress incontinence episodes, as recorded in a diary and pad test, respectively. The primary safety measure will be the incidence of treatment-related serious adverse events (SAEs) and the incidence of protocol-defined treatment or procedure-related adverse events. Placebo injected patients will be given the opportunity to receive the 150 x 106 cell dose following the 12 month evaluation.

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At the time of this report, 63/246 patients have been treated and follow up is ongoing. There have been no serious, related adverse events associated with the use of AMDC. Efficacy data has not yet been tabulated.

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APPENDIX A:
Underactive Bladder Study Clinical Algorithm for Determining Subject UTI Status