

TECR & ECM

Investigational Plan

A prospective feasibility study to evaluate the safety and efficacy of transoral endoscopic circumferential esophageal resection with extracellular matrix (ECM) placement to treat Barrett's esophagus with high-grade dysplasia (HGD)

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1. BACKGROUND AND EARLIER EFFORTS

In patients diagnosed with Barrett's esophagus (BE) with high-grade dysplasia (HGD) esophagectomy has been recommended as a standard of care for treatment. However, esophagectomy is associated with high mortality and morbidity rates even in experienced centers, and is too invasive for those with HGD because lymph node involvement is unlikely (<5%) (1,2). With the development of surveillance programs, the number of patients with early stage disease has increased. Additionally, interest of less invasive, incisionless (endoscopic), and esophageal preservation therapies has grown (2). Currently available endoscopic techniques such as photodynamic therapy, radiofrequency ablation, endoscopic mucosal resection (EMR), and endoscopic submucosal dissection (ESD) have significant limitations including compromised histological assessment, stricture formation, sampling error leading to missed synchronous or metachronous lesions, requirement of life-time surveillance endoscopy, and subsequent interventions. To overcome these drawbacks, we have demonstrated the feasibility of transoral endoscopic circumferential long-sleeve resection (TECR) in the pre-clinical setting as both a diagnostic and therapeutic approach (3).

Removal of the entire segment of abnormal mucosa is believed to be the best approach in curing HGD. Biologic scaffold materials composed of a xenogeneic extracellular matrix (ECM) have been investigated extensively in the context of regenerative medicine for their ability to modify the default tissue healing response in the esophagus (4-6). In a pre-clinical model, critically sized, full circumferential defects could be repaired with minimal stricture formation if adjacent autologous muscle tissue was placed in direct apposition to the ECM scaffold at the time of surgery (4). A follow-up pre-clinical study of esophageal transection that was designed to reinforce the anastomosis of a "gastric pull-up" procedure showed restoration of a mature epithelium and regeneration of muscle tissue that bridged the gap between the native muscle tissue on either side of the surgical transaction site and prevented stricture (6). Most recently reported, the use of ECM has been extended to a pre-clinical model of aggressive endoscopic endomucosal resection such as that proposed for treatment of Barrett's esophagus, demonstrating that ECM successfully minimizes or prevents stricture formation by remodeling the default tissue via neo-epithelialization rather than scar formation (6).

Based on these experiences, we performed this endoscopic resection with ECM placement on 5 patients with HGD or T1a EAC, and the outcomes of these 5 patients were published (7). All patients developed mild segmental strictures, which were easily dilated and became stable quickly. During 4-24 months follow-up, restoration of normal mature squamous epithelium and return to a normal diet without significant dysphagia were reported for all patients. Two of 5 patients had a small focus of recurrent Barrett's esophagus but no recurrence of cancer was observed. Furthermore, we successfully performed anti-reflux surgery (laparoscopic fundoplication) to treat gastroesophageal reflux disease (GERD), the root cause of Barrett's esophagus, on 3 patients who had undergone this endoscopic resection, and all reflux symptoms have been eliminated without any complications (8).

Background on Investigational use of Esophageal Stent: As indicated by our previous application of this study design and supported by other literature, we believe using a fully

covered self-expanding metal stent (FCSEMS) for placement of the ECM poses minimal risk to subjects while providing the necessary, temporary support for the ECM to incorporate with the resected area (7, 9-14). Removability of such stents has been well documented in several animal and human models. One retrospective case series found that 95.3% of FCSEMS in 31 people were removed without complication while another found that 100% of stents in 24 patients were successfully removed without complication (9,10). Another retrospective study analyzed FCSEMS placement in 79 patients with benign disease. The investigators observed low incidence of stent induced ulceration and concluded removability was easily demonstrated (11). The only U.S. prospective clinical trial evaluating the safety of FCSEMS placement and removal concluded tissue reaction was common yet clinically insignificant in a majority of patients. Additionally, removal using rat-toothed forceps was deemed “very easy or easy” in 83% of cases with zero complications recorded overall (11).

In addition to our pre-clinical studies (4,5) a study conducted on 8 Yucatan pigs demonstrated a lack of tissue embedment and granulation and concluded that FCSEMS could be successfully removed without trauma (15).

Furthermore, we believe using self-expanding plastic esophageal stents for our purpose could be detrimental to a subject due to the high rate of stent migration and difficult deployment in narrow areas (9,13,16,17). Although plastic stents are currently FDA accepted for benign use with removal, we believe the potential for requiring dilation in order to deploy the stent as well as an inability to collapse the stent for removal could damage the ECM and result in increased risk to subjects.

Summary: Based on our prior research and experience with TECR and ECM placement, we propose to utilize this novel technique as an alternate treatment for patients with esophageal High Grade Dysplasia. This esophageal-preserving approach is externally incisionless and less invasive, whereas the current standard of care, esophagectomy, is highly invasive and may be too aggressive a treatment for those with HGD.

Endoscopic circumferential esophageal resection could be a one-step therapeutic approach to removing dysplastic tissue. ECM is a promising material to promote reconstitution of the resected esophageal epithelium; potentially preventing or minimizing stricture formation for advanced dysplasia. This combined approach could successfully treat patients, preserve the esophagus, reduce incidence of stricture formation, and result in less morbidity and mortality. The results of this study will potentially change the current approach to treat patients with HGD. The outcomes of this feasibility study could also lay the groundwork for future studies utilizing this approach to treat more advanced T1a esophageal adenocarcinoma patients.

2. PURPOSE

Evaluate the safety and efficacy of transoral endoscopic esophageal circumferential resection (TECR) with ECM placement for the treatment of BE with High-Grade Dysplasia (HGD).

2.1 Primary Objectives

2.1.1 Primary Efficacy Objectives

1. To evaluate incidence of stricture formation requiring dilation ($\geq 30\%$ luminal diameter reduction with dysphagia) following TECR with ECM placement. Evidence of stricture formation will be confirmed endoscopically at 2 weeks, and endoscopically and through barium swallow at Month 1, Month 3, Month 6, Month 9, and Month 12 post procedure, and the proportion of subjects with and without stricture formation at the trial endpoints will be compared to historical data.
2. To evaluate incidence of recurrence of BE with HGD through 12 months following TECR with ECM placement. Incidence of disease recurrence will be confirmed endoscopically with pathology confirmed biopsies at 2 weeks, Month 1, Month 3, Month 6, Month 9, and Month 12 post procedure, and the proportion of subjects with and without disease recurrence at the trial endpoints will be compared to historical data.

2.1.2 Primary Safety Objectives

1. To demonstrate the acute safety of TECR with ECM placement for treatment of BE with HGD by evaluating all serious system and procedure related adverse events occurring in the first 2 weeks post procedure.
2. To demonstrate the long-term safety of TECR with ECM placement for treatment of BE with HGD by evaluating all study related adverse events occurring more than 2 weeks post procedure through 12 months post procedure.

2.2 Secondary Objectives

1. To record the incidence of stent migration at 2 weeks post procedure. Stent migration will be determined endoscopically, and defined as any movement from initial deployment location greater than 1cm.
2. To record incidences of poor stent integrity during removal (e.g. stent fracture).
3. To record the number of subsequent follow up treatment interventions (aside from study-related follow up time points) required post-procedure through 12 months.

2.3 Other Data Collected

The following data will be collected and summary observations will be compiled:

- Medical History and Demographics
- Clinical Data
 - Physical Examination

- Vital Signs
- Positron Emission Tomography/Computed Tomography (PET/CT) Scan
- Endoscopic Ultrasound (EUS)
- Barium Swallow Test (BaSW)
- Upper Endoscopy (EGD) with biopsy
- Upper Endoscopy (EGD) photos of inside of esophagus
- Endoscopic Mucosal Resection (EMR) for tissue grading
- Chest X-ray (may be required)
- Electrocardiogram (ECG) (may be required)
- Pulmonary Function Test (PFT) (may be required)
- Blood Testing (CBC, platelet count, chemistry, and electrolyte panel)
- Balloon Dilatation frequency and outcomes
- Subject Questionnaires
 - Dysphagia Severity Questionnaire
 - General Quality of Life as Assessed by the SF-36
 - GERD-HRQL
- TECR with ECM Placement Procedure Data
 - Mapping and Dimensions of Esophageal Segment to be Removed
 - ECM Device Size Information
 - Final Mapping and Location of ECM Placement
 - Stent size and associated information

3. PROTOCOL

3.1 Trial Summary

This is a single-center, prospective, single arm study involving 10 patients with an established diagnosis of BE with HGD. Table 1 outlines the procedures to be conducted at each subject visit. Potential subjects will be prescreened to assess eligibility and must meet inclusion criteria. In order to make this initial qualification, pathology results of biopsies and EMR collected during initial EGD will be made available to the Investigator prior to patient consent. Following informed consent and as part of routine care, all potential participants will undergo endoscopic ultrasound (EUS) and a PET/CT scan to confirm that there is no lymph node involvement or other metastatic lesions prior to the procedure. Clinical data will be collected at baseline to assess the subjects' medical status including: demographics, medical history, physical examination, vital signs, and blood testing. Cardiac and pulmonary clearance will be obtained if needed based on medical history and will include a chest x-ray, ECG, and pulmonary function test. In addition, the subjects will complete three questionnaires prior to the procedure: dysphagia severity questionnaire, SF-36, and GERD-HRQL.

At the time of the procedure, participants will undergo TECR with ECM placement using a fully covered self-expanding metal stent to temporarily hold the ECM in place. Prior to hospital discharge, a barium swallow test (BaSW) will be performed at Day 1 following the study procedure to evaluate the passage of contrast through the GEJ. If the BaSW results in suspicious findings of esophageal perforation or mucosal necrosis, an upper endoscopy will be performed

for further evaluation. The first primary safety endpoint for this study will be assessed following the procedure and BaSW and before discharge from the hospital. At this time point any adverse events will be assessed and recorded.

All subjects will undergo the same follow-up procedures, including questionnaires and post-procedure EGDs performed at Week 2, Month 1, Month 3, Month 6, Month 9 and Month 12 to visually assess tissue healing, recurrent disease, and if stricture formation is present. Biopsies will be taken during follow-up EGDs so that pathology can rule out recurrence of BE and HGD. Subjects will have a barium swallow x-ray at Month 1, Month 3, Month 6, Month 9, and Month 12 to evaluate the potential presence of stricture formation.

During Week 2 EGD (14 days \pm 4 days from day 0), the stent will be removed during the upper endoscopy. If at any time point a subject has dysphagia with stricture formation (30% reduction in esophageal luminal diameter), routine dilation using a balloon catheter will be performed during the EGD. Validated questionnaires including dysphagia severity questionnaire, SF-36 and GERD-HRQL will be administered to objectively assess the severity of symptoms and quality of life based upon self-reported data. Additionally, adverse events will be assessed and recorded at all follow-up time points.

After completion of the Month 12 visit, subjects will be followed on a routine care basis at the Esophageal & Lung Institute.

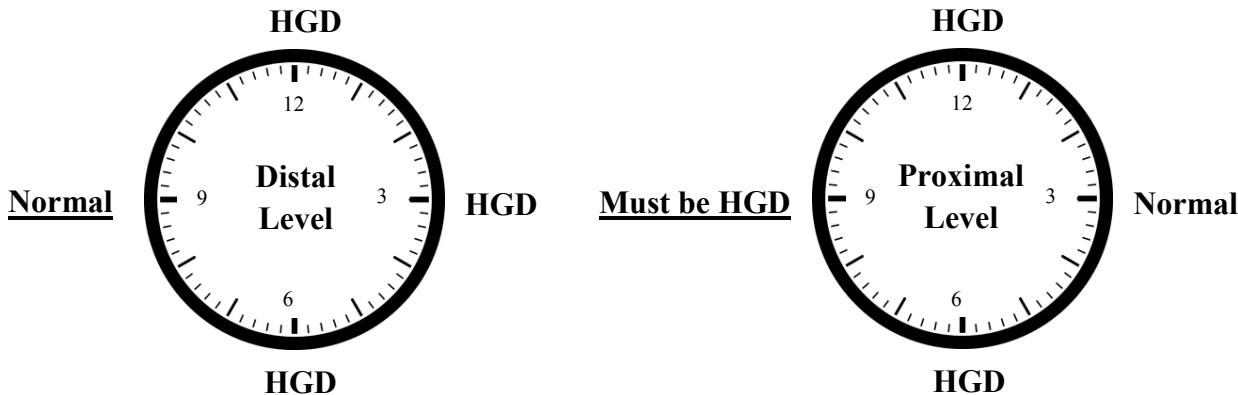
3.2 Subject Inclusion and Exclusion Criteria

3.2.1 Inclusion Criteria

To be eligible for this trial, subjects must meet all of the following inclusion criteria:

1. Be at least 18 years of age and no more than 80 years of age.
2. Have an established diagnosis of HGD
 - a. Specific diagnosis and grading will be determined by pathology review of biopsy tissue collected during baseline EGD as part of a patient's standard of care.
3. Have no evidence of lymphovascular invasion.
4. Have no lymph node or other metastatic involvement based on EUS and FDG-PET/CT.
5. Diameter of affected tissue must warrant circumferential excision
 - a. Subjects must have biopsy confirmed HGD in three of four esophageal quadrants at two levels spaced two centimeters apart (minimum of 6/8 biopsies indicating HGD). If two biopsies are normal (contain no HGD) they must be located on separate levels in two different quadrants.

Example of Acceptable Inclusion Pathology Results:



5. Must be an appropriate or reasonable surgical candidate.
6. Have demonstrated an understanding and signed an approved informed consent form for participation in this study.

3.2.2 Exclusion Criteria

If any of the following criteria are met, subjects are not eligible for this study:

1. Have lesions into or deeper than mucosal layer (superficial (T1a) Esophageal adenocarcinoma).
 - a. Those requiring endoscopic submucosal dissection (ESD) are not eligible for this study
2. Have presence of lymphovascular invasion.
3. Require resection length longer than 10 cm.
4. Have any lymph node or other metastatic involvement based on EUS and FDG-PET/CT.
5. Have history of any kind of previous esophageal surgery (i.e. anti-reflux surgery).
6. Are pregnant or planning to become pregnant.
7. Have coagulation disorders.
8. Have a known hypersensitivity to porcine-based materials.
9. Have an uncontrolled comorbid medical condition that would adversely affect participation in the trial.
10. Has a clinically significant psychological illness that in the physician's opinion would prohibit the subject's ability to meet the protocol requirements.
11. Are unable or unwilling to provide informed consent and/or fulfill the protocol follow-up requirements.

3.3 Visit Windows

The following are allowable date ranges for each follow-up period:

Time Point	Date Range
Screening Evaluation and Baseline	Chart Review and Date of Informed Consent up to Day 0
Clearance tests (optional): ECG, PFT, CXR	Within 30 days prior to Day 0
TECR with ECM Placement (Day 0)	Within 60 days of the Screening Evaluation
Day 1	24 hours following Day 0 \pm 6 hours
Week 2	14 days following Day 0 \pm 4 days
Month 1	30 days following Day 0 \pm 7 days
Month 3	90 days following Day 0 \pm 15 days
Month 6	180 days following Day 0 \pm 30 days
Month 9	270 days following Day 0 \pm 30 days
Month 12	360 days following Day 0 \pm 30 days

3.4 Procedures

3.4.1 Devices and Support

3.4.1.1 ECM

The ECM device being used in this study, provided by ACell, Inc., will be MatriStem® Surgical Matrix PSMX. MatriStem® Surgical Matrixes have been granted FDA 510(k) clearance. MatriStem® devices are medical devices that maintain and support a healing environment through constructive remodeling. The MatriStem® device is a complex protein scaffold obtained from the tissue of porcine (pig) bladders, which has a structure nearly identical to that of human tissue. The use of porcine tissue is an established practice and a variety of porcine tissues have been implanted in humans for medical purposes for more than 40 years. MatriStem® is indicated for surgical, traumatic, and chronic wounds as well as soft tissue reinforcement. MatriStem® medical devices are available only by prescription through a United States physician, hospital or clinic. Only a physician can determine the best treatment for patients.

The following device will be used :

1. MatriStem® Surgical Matrix PSMX – 6 layers – 10cm x 15cm

This biologic scaffold material, composed of a xenogeneic extracellular matrix, will be used to prevent or minimize stricture formation. The ECM will be held in place using a stent.

Per FDA 21 CFR 812.36(c)(ii), the intended use of this device for this study is to cover the resected area of the esophagus to promote tissue healing and to prevent stricture formation.

3.4.1.2 Stent

The stent being used in this study is the WallFlex™ Fully Covered Esophageal Stent (Boston Scientific Corporation). WallFlex™ Fully Covered Stents have been granted FDA 510(k) clearance. Additionally, the WallFlex™ Fully Covered Esophageal Stent has received the CE Mark to treat benign esophageal strictures in Europe.

The WallFlex™ Fully Covered Esophageal Stent is nitinol mesh with a silicone inner lining along the entire length. The silicone lining is intended to prevent tissue ingrowth and food impaction. The WallFlex™ stent has a dog-bone shape with flared ends in order to reduce the risk of migration. WallFlex™ Fully Covered stents are contained within an 18.5 French, low profile delivery system and can be reconstrained up to two times during deployment for positional accuracy. A Teflon® coated polyester removal suture is present on the proximal end to collapse the stent for removal. This stent is available in several diameter and length combinations. All product related description and data is available on the device label and product brochure. Appropriate stent size will be determined on an individual subject basis prior to the procedure.

Per FDA 21 CFR 812.36(c)(ii), the intended use of this device for this study is to hold the ECM in place over the resected area while incorporation of tissue occurs. This stent will provide temporary support of the ECM (14 days ± 4 days).

3.4.2 Device Accountability / Handling and Storage

Devices will be controlled through a Device Accountability Log maintained at the Esophageal & Lung Institute, Clinical Research Department. All serial numbers, lot numbers, expiration dates, date used (or attempted), subject ID, and any comments will be recorded in the Device Accountability Log. The ECM will be provided by ACell, Inc., and the stent will be purchased through a grant supported by ACell, Inc.

3.4.3 Investigators and Facilities

Investigator surgeon(s) performing this procedure are advanced interventional endoscopists who have executed this procedure in animal models (including porcine models) and human models (6). Two investigator surgeons will be involved. Both surgeons have extensive experience with the experimental technique (TECR & ECM placement) as well as components of this procedure including stent removal as part of their standard surgical practice. The investigators consider this procedure to be an extension of current practices. Additionally, all study events will occur in facilities equipped with capabilities for performing all procedures.

3.4.4 Subject Enrollment Procedure

Subjects are enrolled in this study when they have signed the approved informed consent form.

Documentation of the informed consent process will be recorded in the subject's medical record when they are enrolled in the study with the date of entry in the study.

If the inclusion and exclusion criteria during the screening are not met, the subject will not undergo the study procedure even if they have signed an informed consent form. The investigator will also inform the subject that he/she will not undergo the procedure even though they signed the consent. Ineligible subjects will have the option to undergo alternative treatments. The reasons for ineligibility will be captured for all subjects who signed consent.

3.4.5 Informed Consent

3.4.5.1 Obtaining Informed Consent

Subjects will be recruited from the Allegheny Health Network (including hospitals and outpatient centers) through the Esophageal & Lung Institute. No patient will be excluded on the basis of gender, race, or ethnicity. Prior to enrolling in to the study, the potential subject will review the informed consent form with the Investigator or other IRB approved member of the research team (appointed designee). The background of the proposed study, the procedures, the follow-up schedule and all potential benefits and risks will be carefully explained to the subject. Both the subject and Principal Investigator, or appointed designee, will sign, date and time the Institutional Review Board (IRB) approved study-specific Informed Consent Form.

3.4.5.2 Requirements for Informed Consent

In order to obtain informed consent, each subject must be informed about the investigation, and must sign, date and time an agreement acknowledging that participation is voluntary. A signed informed consent will acknowledge subject's receipt of study information, and consent documentation in the medical record will reflect the subject's willingness to participate in the study.

3.4.6 Subject Demographics

At the screening evaluation/baseline visit, the following subject demographic information will be collected:

- Date of Birth
- Gender
- Racial Background
- History of Tobacco and Alcohol Use

3.4.7 Medical History

At the screening evaluation/baseline visit, the investigator will review and document the subject's general medical history as well as a more thorough evaluation of their esophageal disease history. The following specific information will be collected:

- History and severity of BE with HGD.

- Confirmation of negative lymph node involvement or other metastatic disease(s).
- Pathology reports of biopsy samples confirming diagnosis.
- Previous treatments
- History of stricture formation
- Medications (Current and concomitant)

3.4.8 Vital Signs

Vital signs will be collected at all scheduled follow-up visits. Collected vital signs will include:

- Pulse
- Height
- Weight
- Blood Pressure

3.4.9 Routine Care Testing Data Used

3.4.9.1 Esophagogastroduodenal (EGD) Endoscopy with Biopsy and Images

EGD will be performed to make/confirm a diagnosis of BE with HGD. The EGD will be used to visualize the lining of the esophagus, stomach, and duodenum. Biopsies of suspected areas will confirm the presence and extent of BE with HGD recurrence. Biopsies will be taken circumferentially and submitted to pathology with “o’clock” identifiers (ex: 34 cm 6 o’clock, 7 o’clock etc.) in order to assess the circumferential extent of dysplastic tissue. Only those with dysplastic tissue warranting circumferential removal of tissue will be eligible for the study. Subjects must have biopsy confirmed HGD in three of four esophageal quadrants at two levels spaced two centimeters apart (minimum of 6/8 biopsies indicating HGD). If two biopsies are normal (contain no HGD) they must be located on separate levels in two different quadrants. The EGD with biopsy collection will be performed under IV sedation and as part of routine care.

EGD with biopsy will take place at each time point following TECR & ECM placement in order to assess disease recurrence.

Photos will be taken of the inside of the esophagus at baseline EGD, 2 weeks, 1 month, 3months, and 12 months.

3.4.9.1.1 Histological Evaluation of Tissue Biopsy

All biopsy tissue samples collected during EGD will be sent to a board certified pathologist employed by the Allegheny Health Network for evaluation and final post-operative diagnosis. The pathologist will visually analyze the biopsy sample to classify the tissue into dysplastic grades. Potential subjects for this study will not be approached to consent until a pathology report is provided to the PI. This report is typically made available to physicians within one week of sample collection. Only patients with tissue grades (HGD) who meet the study inclusion criteria are eligible for this study.

3.4.9.2 Endoscopic Mucosal Resection (EMR)

EMR will be performed at the time of preliminary diagnostic EGD in addition to biopsy tissue collection to assess the depth of dysplastic infiltration and to exclude potential subjects with submucosal tissue involvement. EMR will be performed under IV sedation and as part of routine care. If the specimens by EMR show a submucosal invasion or cancer, participation in this study will not be offered as described in 3.4.4.

3.4.9.3 Barium Swallow Test (BaSW)

BaSW will be performed as part of routine care to see the movement of the esophagus, aid in the visualization of the anatomical outline of the esophagus, and to examine the upper digestive tract. Subjects who undergo TECR with ECM placement will have a follow-up BaSW on Day 1 post-operatively. This will help assess whether perforations or abnormalities exist in the resected area of the esophagus.

Additional barium swallow x-ray tests will occur at Month 1, Month 3, Month 6, Month 9, and Month 12 within each study visit window to evaluate whether or not an esophageal stricture is present. BaSW will take place at least 24 hours prior to a scheduled EGD so that the results can be made available to the Investigator prior to EGD. Barium swallow tests may also be used outside the set study windows if a subject has complaints of dysphagia. The findings and degree of luminal narrowing observed through BaSW will be recorded throughout the study.

3.4.9.4 Endoscopic Ultrasound (EUS)

EUS will be performed to assess for lymph node involvement. In the event of suspicious findings of lymph node involvement (i.e. >1 cm, non-compressible, firm, or irregular shape) at the time of EUS, a fine needle biopsy will be obtained for a confirmatory histological assessment. If there is confirm lymph node involvement or metastatic lesions, participation in this study will not be offered as described in 3.4.4.

3.4.9.5 Positron Emission Tomography/Computerized Tomography (PET/CT)

Using [F-18] fluorodeoxyglucose (FDG), PET/CT scan will be performed at assess for lymph node involvement and other metastatic lesions. If there is confirm lymph node involvement or metastatic lesions, participation in this study will not be offered as described in 3.4.4.

3.4.9.6 Computerized Tomography (CT Scan)

CT scans will be obtained for surveillance of metastatic lesions. If suspicious metastatic lesions are found, FDG-PET/CT scan will be considered.

3.4.9.7 Balloon Dilatation of Strictures

It is not expected that every subject who undergoes TECR with ECM placement will require balloon dilation. Routine balloon dilation will be performed during follow-up EGD if stricture formation is present (30% reduction in esophageal lumen and dysphagia determined endoscopically and through barium swallow x-ray). Balloon dilation will be done directly through the working channel of the endoscope by passing the deflated balloon into the lumen of the stricture. The balloon will then be inflated to certain pressure for a given stricture diameter. This will be determined on a subject by subject basis. Therapeutic outcomes and other results following balloon dilation will be recorded.

3.4.9.8 Blood Work

Routine blood work of CBC, platelet count, partial prothromboplastin time (PTT), prothrombin time international normalized ratio (PT INR), chemistry, and electrolyte panels will be reviewed. The exclusion levels are as follows: platelet count less than 150,000/mm³, PTT of 50 seconds or above, or PT INR of 1.8 or above.

3.4.9.9 Chest X-ray (May be required for surgical clearance)

Chest x-rays within 30 days of evaluation will be reviewed for support of cardiac and pulmonary clearance if needed.

3.4.9.10 Electrocardiogram (ECG) (May be required for surgical clearance)

ECG within 30 days of evaluation will be reviewed for support of cardiac and pulmonary clearance if needed.

3.4.9.11 Pulmonary Function Test (PFT) (May be required for surgical clearance)

PFT within 30 days of evaluation will be reviewed for support of cardiac and pulmonary clearance if needed.

3.4.9.12 Questionnaires

At screening/baseline evaluation and at all follow-up time points, subjects will be asked to complete three questionnaires. These validated questionnaires, which are described below, are designed to objectively assess the severity of symptoms and quality of life. These questionnaires are designed to be self-administrated, but will be administered during study visits.

The Investigator or appointed designee will provide the subject instructions on how to complete the questionnaires. If the subject has questions regarding the instrument, the investigator or appointed designee will only discuss the mechanics of filing out the forms and not discuss questions in the questionnaires.

The subject questionnaires are described in detail below:

- 3.4.9.12.1 Dysphagia Severity Questionnaire
This instrument helps measure the level and severity of dysphagia.
- 3.4.9.12.2 Gastroesophageal Reflux Disease-Health Related Quality of Life (GERD-HRQL)
This instrument is reliable and practical tool to measure symptom severity in subjects.
- 3.4.9.12.3 Short Form (SF)-36 health survey
This instrument helps measure overall health and well-being.

3.4.10 TECR with ECM Placement

If a patient meets all inclusion criteria, no exclusion factors are present, and they sign an informed consent form, the subject will undergo transoral endoscopic esophageal circumferential resection (TECR) with ECM placement.

3.4.10.1 TECR

TECR will be performed to resect the entire length and area of the BE lesion in the in-patient setting. A dissection plane between the mucosal and submucosal layer will be created by injection of sodium hyaluronate (SH) (HYALGAN®, Bridgewater, NJ) mixed with indigo carmine and epinephrine into a site immediately distal to the gastroesophageal junction. This site will represent the “goal” or terminus of therapeutic endoscopic mucosal resection (EMR). The normal esophageal mucosa 1cm proximal to the squamocolumnar junction will represent the “entry” or beginning of resection. Circumferential esophageal mucosal resection will then be performed at both of these sites thereby defining the proximal and distal resection margins. More SH will be injected and endoscopic mucosal resection with a needle knife (Olympus America Inc., Center Valley, PA) will be performed to create a submucosal tunnel along the entire length of the longest lesion. The bridging vessels between the mucosal and submucosal layer will be cauterized using hemostat forceps. The creation of 2-3 additional submucosal tunnels between the proximal and distal resection margins will be created leaving the attachments between each tunnel for counter-traction. At the proximal entry point, a circumferential cuff of mucosal tissue will then be developed using standard cap resection, cautery dissection, and/or hydro-dissection with irrigation catheter techniques. Using EMR technique, the remaining attachments will be dissected away from the submucosal later thereby freeing the entire sleeve of tissue. The specimen will then be retrieved through the mouth. Hemorrhage within the submucosal tube will be controlled and the endoscope will be removed.

3.4.10.2 ECM Placement

Following resection, the exposed area will be covered with a 6 ply sheet of ACell MatriStem® Surgical Matrix PSMX ECM (ACell Inc., Columbia, MA). The ECM will be placed and held in position for 14 days (\pm 4 days) using a Boston Scientific WallFlex™ Fully Covered Esophageal Stent (Boston Scientific, Boston, MA).

Stent size will be selected on an individual basis upon endoscopic evaluation of the esophageal diameter for a subject. In prior experiences (7) the Investigators utilized a 23mm diameter, 26mm flare UltraFlex stent. It is expected that most subjects enrolling in this study will require WallFlex™ stent models M00516730 or M00516740 (23mm, 28mm flare, 10 cm and 12 cm length respectively) however smaller versions including an 18mm O.D. with 23 or 25 mm flare diameters will also be available as needed.

The WallFlex™ stent and insertion catheter will act as the vehicle to place the ECM over the resected area. The stent comes preloaded into an insertion catheter from the manufacturer. In order to attach the ECM to the stent, the outer sheath of the catheter will be pulled back to reveal 1 mm of the distal end of the stent. The ECM will be formed into a pliable tube to a diameter of roughly 3 cm. The exact size of the ECM tube will be determined by the Investigator depending on a subject's esophageal diameter and the stent selected for use. This tubularized ECM will be secured on the distal, exposed margin of the stent with 3-4, absorbable 7-0 Vicryl Rapide (Ethicon, Inc.) sutures; fully covering the collapsed stent and insertion catheter. The ECM will cover the entire length of the WallFlex™ stent so that only ECM contacts the resected area of esophageal mucosa.

The insertion catheter and collapsed stent with ECM attached will be inserted transorally and positioned over the resected area under the guidance of endoscopic and fluoroscopic visualization. The stent will slowly be deployed using fluoroscopic visualization to confirm full ECM coverage of resected area. The stent can be reconstrained up to two times for positional accuracy prior to full deployment. The fully deployed stent will provide enough pressure against the esophageal wall to hold the ECM in place while initial incorporation of tissue occurs (14 days \pm 4 days). Please see MISC FILES "001_TECR and ECM Placement Video" for visually depiction of this procedure. The Investigator will re-introduce the endoscope to ensure the stent is in place, the ECM is fully covering the resection region, and to look for indications of bleeding, esophageal tear, or perforation.

Based upon prior experience using this ECM application technique (7) and device specification data, it is not expected that deployment of the stent will damage the ECM. Six ply MatriStem® Surgical Matrix PSMX ECM, has a tensile strength of greater than 16 N/cm and is manufactured to withstand the human abdominal wall at maximum intra-abdominal physiological pressure (Please see VOL_004 file 007_ACell Device Specifications PSM and PSMX for full report)

3.4.10.3 Post-Operative Care and Dietary Guidelines

Subjects will be held for at least one night at West Penn Hospital and their condition monitored closely post-operatively. Prior to discharge, subjects will undergo a Barium Swallow (BaSW) to ensure there are no leaks from the procedure. Subjects will also receive careful instruction concerning their post-operative care and an at-home dietary plan will be provided and explained. This dietary plan is part of standard patient care for any stent placement procedure and recommends a soft texture with supplemental high-calorie liquid diet until the stent is removed. This diet plan is intended to prevent food-bolus obstruction while providing adequate nutritional intake. Please see VOL_011, file "006_Stent Placement Dietary Plan" for full details.

3.4.10.3.1 Management of Post-Operative Events & Food Bolus Obstruction

Due to the experimental nature of this study, adverse events could occur after a patient is discharged from West Penn Hospital. Patients will receive education related to post-operative complications such as bleeding, extreme pain, fever, and dysphagia, and will be asked to return to the hospital if such events occurs.

As part of pre-discharge dietary counseling, food bolus obstruction will be addressed. If a subject experiences a suspected food bolus obstruction or dysphagia, they will undergo an immediate endoscopic evaluation. If a food bolus obstruction is identified, it will be extracted with precision as to not disturb the esophageal stent and to protect the airway. The stent may need to be repositioned if it has migrated more than 1 cm from its original placement location. This will be done using rat-toothed forceps. The subject will be evaluated following any food bolus obstruction and/or stent repositioning to ensure no tissue damage has occurred. The subject will be re-c counseled by a staff Dietitian following bolus clearance in an effort to avoid the event from reoccurring.

3.4.11 Removal of Stent

At the Week 2 EGD, the stent will be partially collapsed for transoral removal. In order to remove the WallFlex™ Fully Covered Esophageal Stent, the Investigator will grasp the distal end of the stent using rat tooth forceps. Once the distal end is securely held, the Investigator will grasp the Teflon® coated polyester removal suture at the proximal end of the stent using rat tooth forceps and gradually apply tension (7,11,12, Please also see MISC FILES “002_Stent Removal Technique Video”). The gentle tension begins to collapse the stent; reducing the stent diameter and decreasing the contact pressure between the stent and esophageal wall. As the suture is pulled, the stent will begin to collapse, pulling away from the ECM. At 14 days post placement, the ECM is securely incorporated into the native tissue and does not provide a hindrance to stent removal. The stent will be examined for any tissue embedment and will then slowly be removed transorally in a semi-collapsed state. The 7-0 Vicryl sutures originally holding the ECM to the stent maintain 0% of their original BSR after 14 days, thereby providing no additional tether between the stent and ECM.

A note concerning stent removal: Successful removal of fully covered self-expanding metal esophageal stents such as the WallFlex™ Fully Covered Stent is supported by multiple published retrospective and animal studies (7, 9-14). The Investigators of this study have had extensive experience removing esophageal stents including removal following TEGR. The PI and Co-I have both removed stents from individuals undergoing esophagectomy to treat esophageal cancer. In prior experiences with TEGR with ECM placement, stents have been easy to remove and no stent fracturing has occurred.

3.4.11.1 Contingency Plan - Stent Removal Technique

In prior experiences with stent removal post ECM placement, the stents have been able to be removed using rat-tooth forceps as described above. There have been instances during routine clinical care that the Investigators have not been able to remove stents as described. Typically this is because the removal suture is no longer visible. In these cases, the Investigators have utilized an approach in which the stent is pulled into the stomach using retroflexion of the endoscope. In this process, the distal margin of the stent is grasped using forceps while the scope is in retroflexion and the stent is gently pulled into the stomach (grasped from below). The removal suture typically becomes visible once the stent is lowered into the stomach. At that point, the endoscopist has greater maneuverability to partially collapse of the stent using rat-toothed forceps and oral removal can be achieved.

3.4.11.2 Contingency Plan – Removal of a Fractured Stent

In previous experiences with removing esophageal stents, poor stent integrity has not been observed. While stent fracturing has not been observed, a contingency plan for coping with this possible outcome will be employed if needed. Several tools may aid in the removal of broken stent pieces. Rat tooth forceps may be used for larger pieces that do not appear to have any sharp edges that could pose risk of abrasion, laceration, or perforation of the esophagus. An EMR retrieval snare could also potentially be used for larger, smooth pieces. An EMR cap with suction could remove any small pieces. The pieces can be suctioned directly into the cap. The cap provides a protective encasement for small fragments and reduces the likelihood of esophageal damage. A Roth Net may be used for any pieces that fall into the stomach. The major benefit of the Roth Net is the protective netting that may prevent any sharp pieces from lacerating the stomach or esophagus upon removal. All extracted pieces will be reviewed outside the body to ensure no fragments remain in the Subject. The investigator will re-introduce the endoscope and carefully view the esophagus, stomach, and duodenum to further ensure no pieces remain inside the body.

3.4.11.3 Contingency Plan – Esophageal Injury

Serious esophageal injury has not been previously observed in patients who have had esophageal stents removed. Removing esophageal stents typically results in superficial injury to the mucosa and minimal bleeding can be observed. The appearance of the esophageal lining will be documented through endoscopic photographs taken at 2 weeks (stent removal), month 1, and month 3.

In the event serious esophageal injury occurs during stent removal, the following steps will be taken per standard of care measures for esophageal laceration or perforation:

- The patient is first stabilized while sedated during stent removal. Immediate surgical repair may be initiated. The patient will be fully sedated by an anesthesiologist. Debridement of any excess tissue will occur. The muscular layer is incised longitudinally along the muscle fibers above and below the perforation to reveal the entire mucosal injury. The mucosa is then closed with absorbable sutures and the muscularis layer is closed with non-absorbable sutures.

- Some patients may undergo a secondary stent placement using a fully covered plastic stent to seal the tear as opposed to surgical repair.
- The subject will be admitted so that he/she can be monitored for indications of fever, infection, mediastinitis, and other post-operative complications.
- The subject will be NPO for at least 7 days and will be placed on broad spectrum antibiotics for 7-10 days
- The patient will have a barium swallow prior to discharge to ensure no persistent leak remains
- The subject will be placed on a semi-solid diet and followed-up on a consistent basis (1 week post-op, 2 week, 6 week).

3.4.11.4 Evaluation of Esophageal Mucosa following Stent Removal

Immediately following stent removal, the esophageal mucosa will be carefully assessed endoscopically for the following indications:

- Excessive bleeding
- Perforation
- Tear
- Necrosis
- Areas of ECM removed by stent
- Excess/loose ECM
- Visible stricture

3.4.12 Adverse Events (AE)

An adverse event is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally (timing of) associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

An underlying disease that was present at the time of enrollment is not reportable. However, any increase in the severity or seriousness of the underlying disease is to be reported as an adverse event.

Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.

The following list includes, but is not limited to, events that will be considered adverse events for this study:

- 1) stent migration,
- 2) perforation,
- 3) severe pain,
- 4) bleeding,
- 5) fever,
- 6) reflux,
- 7) aspiration and
- 8) severe stricture formation refractory to dilation

Events that do not meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition leading to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.

All adverse events will be assessed and coded in accordance with CTCAE 4.0. Adverse events will be documented through a subject specific AE log and AE CRF.

3.4.12.1 Adverse Event Relatedness

Investigators will assess the potential relationship of an adverse event to the study procedure.

Device related: An adverse event that results from the presence or performance of the devices (ECM and/or Stent) or other component of the system. For example, stent migration due to incomplete expansion.

Procedure related: An adverse event that occurs due to the study procedure. For example, perforation caused by resection or stent removal.

Whether device or procedure related, all adverse events will be classified using the FDA relatedness categories: not related, unlikely related, possibly related, related.

3.4.12.2 Observations and Complications

Investigators will determine whether an adverse event is a complication or an observation.

Complication: An adverse event resulting in death, permanent injury, or requires an invasive intervention to prevent death or permanent injury. Semi-invasive procedures (i.e. blood transfusions) will be considered a complication.

Observation: An adverse event that does not result in death, permanent injury, or requires an invasive intervention to correct.

Invasive procedures performed solely for diagnostic or surveillance purposes are excluded.

3.4.12.3 Serious Adverse Events (SAE)

A serious adverse event is defined as any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

3.4.12.4 Unanticipated Adverse (Device) Events (UAE/UADE)

Per 21 CFR 812.3(s) "an unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects."

If an adverse event is determined to meet the definition of an unanticipated adverse event, the Principal Investigator will immediately conduct an evaluation. An unanticipated adverse device effect that represents an unreasonable risk for subject health or a product performance failure becomes apparent, immediate action will be taken and the appropriate authorities will be notified.

In the event of an unanticipated adverse device effect, which presents an unreasonable risk, for subject's health or a product performance failure becomes apparent, immediate action will be taken and the appropriate authorities will be notified.

Since these are approved devices, any UAE should also be reported to the manufacturer of the product as per user facility reporting requirements at your hospital/institution.

3.4.12.5 Adverse Events Reporting

All adverse events will be reported to the ASRI-WPAHS Institutional Review Board and FDA (as necessary) in a manner consistent with institutional and FDA guidelines (FDA guidelines cited below). In accordance with applicable policies, the investigator will report any adverse effect that is determined to meet the following criteria: *1) associated with the investigational device or, if applicable, other study treatment or diagnostic product(s); 2) a serious adverse effect; and 3) an unexpected adverse effect.*

Applicable adverse effects will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator's receipt of the respective information. Adverse effects which are *1) associated with the investigational device or, if applicable, other study treatment or diagnostic product(s); 2) fatal or life-threatening; and 3) unexpected* will be reported to the IRB within 24 hours of the investigator's receipt of the respective information.

Follow-up information to reported adverse effects will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse effect that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigators will report the adverse effect to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

3.4.12.5.1 FDA Guidelines

The investigators will be required to submit a report of a UAE/UADE to the IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event (§ 812.150(a)(1)).

The sponsor investigator will conduct an evaluation of a UAE/UADE and will report the results of the evaluation to FDA, the IRBs, and participating investigators within 10 working days after notice of the effect (§§ 812.46(b), 812.150(b)(1)).

4. DATA COLLECTION

The following table lists the various intervals at which data is to be collected:

Interval	Data To Be Collected	Time of Collection
Screening/Baseline	ICF Demographics Medical history Prior medications Physical exam Pregnancy test (females) Results from the following: PET/CT Scan EUS BaSW	Prior to Treatment

	EGD with Biopsy EMR Blood Work Chest X-ray (if required) ECG (if required) PFT (if required) Questionnaires	
Treatment	TECR with ECM Placement Documented Dietary Consult	Day 0
Initial Follow-Up	BaSW	Day 1
Stent Removal	Stent Removal EGD with Biopsy EGD Photos Dilation of Stricture if present Questionnaires Vitals	Week 2
Follow-Up	EGD with Biopsy EGD Photos (months 1,3,12) Dilation of Stricture if present Questionnaires Barium Swallow x-ray test Vitals	Month 1, 3, 6, 9, 12
Anytime	Concomitant medications Adverse Event Follow-Up Adverse Event Protocol Deviation/Violation Death Termination	At Time of Occurrence, or When Notified of Event

5. STATISTICAL METHODS AND DATA ANALYSIS

This study is a feasibility study in order to assess general efficacy, collect clinical information, make observations, report adverse and serious events, and to provide the basis for possible future applications of TECR with ECM placement. The proposed sample size of 10 is not sufficient to conduct a comprehensive statistical analysis. However, we set forth efficacy and safety objectives in order to standardize our data collection points and outline specific criteria for consideration. Additionally, we have developed a statistical analysis plan that may be useful in future applications of TECR with ECM placement in the event enrollment is increased or extended to include those with T1a esophageal adenocarcinoma.

5.1 Primary Efficacy Objectives

Efficacy of the TECR with ECM placement will be assessed via two primary efficacy objectives that will assess efficacy of TECR with ECM placement at 2 weeks post-procedure through the study endpoint at 12 months. Both primary objectives will be considered in order to demonstrate overall efficacy of TECR with ECM placement.

5.1.1 *Primary Efficacy Objective 1*

To record incidence of stricture formation requiring dilation, following TECR with ECM placement. Evidence of stricture formation will be confirmed endoscopically at 2 weeks, and endoscopically and using barium swallow test at Month 1, Month 3, Month 6, Month 9, and Month 12 post procedure, and the proportion of subjects with and without stricture formation at the trial endpoints will be compared to historical data.

5.1.1.1 Parameter of Interest

The parameter of interest is the percent of subjects with stricture formation.

5.1.2 *Primary Efficacy Objective 2*

To record incidence of recurrence of BE with HGD through 12 months following TECR with ECM placement. Incidence of disease recurrence will be confirmed endoscopically with pathology reviewed biopsies at 2 weeks, Month 1, Month 3, Month 6, Month 9, and Month 12 post procedure, and the proportion of subjects with and without disease recurrence at the trial endpoints will be compared to historical data.

5.1.2.1 Parameter of Interest

The parameter of interest is the percent of subjects with recurrence of BE.

5.2 Primary Safety Objectives

Safety of TECR with ECM placement will be assessed via two primary safety objectives that will assess the acute safety of the devices and implant procedure, and long-term safety of the treatment technique. Both primary objectives will be considered to demonstrate overall safety of TECR with ECM placement.

5.2.1. *Primary Safety Objective 1*

To demonstrate the acute safety of TECR with ECM placement for treatment of BE with HGD by evaluating all serious adverse events occurring in the first 2 weeks post procedure.

5.2.1.1 Parameter of Interest

The parameter of interest is the proportion of subjects with any serious, acute (within 2 weeks post-procedure) adverse events.

5.2.2. Primary Safety Objective 2

To demonstrate the long-term safety of TECR with ECM placement for treatment of BE with HGD by evaluating all study related adverse events occurring more than 2 weeks post procedure through the study endpoint at 12 months post procedure.

5.2.2.1 Parameter of Interest

The parameter of interest is the proportion of subjects with any serious, long-term (after 2 weeks post-procedure) adverse events.

The endpoint is any serious, long-term (after 2 weeks post-procedure) adverse event attributable to the TECR with ECM placement procedure. All adverse events will be classified according to severity and relatedness, and will be reported to the IRB and/or FDA (as necessary) based on 3.4.12.

5.3 Secondary Objectives

The following secondary objectives will be recorded.

5.3.1 Secondary Objective 1

To record the incidence of stent migration at 2 weeks post procedure. Stent migration will be determined endoscopically, and defined as any movement from initial deployment location greater than 1cm.

5.3.2 Secondary Objective 2

To record the number of subsequent follow up treatment interventions required post-procedure through the trial endpoint at 12 months.

5.3.3 Secondary Objective 3

To record the incidence and nature of poor stent integrity during placement and removal (broken wires, fracturing etc.)

5.4 Size and Duration of Trial

Ten (N=10) subjects will be enrolled in this study. Follow-up on each subject will be 12 months, except in the case of early death, losses to follow-up, or other censoring events.

5.5 Possible Future Analysis

In the event this study is expanded to enroll a greater number of individuals and/or extended to include those with T1a esophageal adenocarcinoma, additional thought has been given to potential statistical analysis for assessing overall efficacy and safety outcomes. Below is a description of potential future statistical analysis based off of an enrollment of 30 or more individuals.

5.5.1 Future General Statistical Methods

All patients that undergo TECR with ECM placement for treatment of BE with HGD and/or superficial (T1a) EAC during the study period will be included in the analysis. There are two primary efficacy endpoints: the proportion of patients with stricture formation requiring dilatation ($\geq 30\%$ reduction in esophageal luminal diameter) and the proportion of recurrence of BE with HGD and/or superficial (T1a) EAC during the 12 months post initial procedure. The primary safety endpoints will be the proportion of patients experiencing either a serious system or procedural adverse event during the 2 week post initial procedure period or any study related adverse event occurring during the 12 months post initial procedure period.

Data analysis will begin with assessment of the normality of the data using the Shapiro-Wilk test and a frequency histogram with normal overlay. Normally distributed data will be reported as means and standard deviations; non-normally distributed data as median (interquartile range). Categorical data will be reported as counts and percentages. Descriptive statistics will be presented for demographic, comorbid, clinical, laboratory and procedural data. One-way repeated-measures analysis of variance with the Tukey post-hoc test will be used to compare baseline and follow-up measures for continuous variables. The non-parametric Friedman test will be used when the dependent variable being measured is ordinal or the data violates the assumption of normality. The rates of all types of adverse events by event type will be summarized using counts, percentages, and graphical displays and reported at their designated follow-up time. Serious system and procedural related adverse events will also be combined and similarly reported. Adverse event and survival curves will be calculated from the date of the initial procedure according to the Kaplan-Meier method. The log-rank test will be used to compare the Kaplan-Meier curves. For event-free and BE-free survival, patients who failed one or both events will be censored at the time of the first failure. Univariate and multivariate Cox proportional hazards regression will be used to identify predictors of adverse events and to determine the hazard rate with a 95% confidence interval. Diagnostic tests of the assumptions of the Cox model will be performed using Schoenfeld residuals. Statistical tests will be two-sided and a value of $P < .05$ will be considered statistically significant. Statistical analyses will be performed using IBM-SPSS Statistics, version 20.0 (IBM-SPSS Inc., Armonk, NY). Graphs and charts will be done using MedCalc, version 13.1.0 (MedCalc Statistical Software bvba, Ostend, Belgium) and Microsoft Excel 2010.

5.5.2 Subgroup Analysis

One subgroup analysis will be performed. The cohort will be subdivided into two groups based on endoscopic biopsy at baseline. Group 1 will consist of patients with High Grade Dysplasia and Group 2 will consist of patients with T1a EAC. Descriptive statistics will be presented for

demographic, comorbid, clinical, laboratory and procedural data. A two-sample t-test will be used to compare continuous variables between groups. A chi-square test or Fisher's exact test will be used to compare categorical variables. A two-way repeated-measures analysis of variance with the Tukey post-hoc test will be used to compare baseline and follow-up measures for continuous variables.

5.5.3 Questionnaire Data Analysis

Descriptive statistics will be provided for items that comprise the Dysphagia Severity Questionnaire, Gastroesophageal Reflux Disease-Health Related Quality of Life Questionnaire (GERD-HRQL), and Short Form-36 Health Survey (SF-26). For all patients, changes in scores from baseline to 12 months post initial procedure will be compared using the paired t-test or Wilcoxon signed-rank test. Separately, changes in scores between patients with High Grade Dysplasia versus T1a EAC will be first compared within each group then between groups using the two-sample t-test or Mann Whitney U test. Internal consistency of the scales, both at baseline and 12-months post initial procedure, will be measured by Cronbach's alpha.

5.5.4 Primary Efficacy Objectives Analysis

All patients that undergo TECR with ECM placement for treatment of BE with HGD and/or superficial (T1a) EAC and who have stent removal at Week 2 with at least one valid follow-up EGD biopsy collection will be included in the primary efficacy analyses. Counts and percentages of patients that have stricture formation requiring dilation will be summarized for each follow-up time and totaled at the study endpoint. At 12-months post initial procedure, the proportions of patients who did and did not have stricture formation requiring dilation will be compared using the chi-square test. Time-to-events will be analyzed by the Kaplan-Meier method and Cox regression. Univariate and multivariate logistic regression will be performed to identify which variables were predictive of stricture formation requiring dilation. Demographic, comorbid, clinical, laboratory and procedural data will be included as covariates in the logistic regression models. Analysis using historical data for stricture incidence is based off of a 41% observation rate (19).

Similarly, counts and percentages of patients with recurrence of BE by 12-months post initial procedure will be reported. At 12-months post initial procedure, the proportions of patients with versus without recurrence of BE will be compared. Time-to-events will be analyzed by the Kaplan-Meier method and Cox regression. Univariate and multivariate logistic regression will be performed to identify which variables were predictive of recurrence of BE. Demographic, comorbid, clinical, laboratory and procedural data will be included as covariates in the logistic regression models.

5.5.5 Primary Safety Objectives Analysis

The analysis will begin with the counts and related percentages of all adverse events by event type (serious system and procedure-related) occurring during the first 2 weeks post initial procedure and later, of all serious system related adverse events occurring more than 2 weeks

post initial procedure through 12 months post initial procedure. Two outcome variables, serious system adverse events and procedure-related adverse events, occurring during the first 2 weeks post initial procedure, will be combined to provide a sufficient number of events for analysis. Time-to-events will be analyzed by the Kaplan-Meier method and Cox regression. Univariate and multivariate logistic regression will be performed to identify which variables were predictive of adverse events during the first 2 weeks post initial procedure and later, of all serious adverse events occurring more than 2 weeks post initial procedure through 12 months post initial procedure. Demographic, comorbid, clinical, laboratory and procedural data will be included as covariates in the logistic regression models.

Statistical analysis submitted by Diane V. Thompson M.S.

5.6 Ancillary Analyses

The following ancillary or supportive analyses will also be performed with data collected in this trial.

- 1) Summarize procedure-related complications and observations
- 2) Summarize the rates of all-cause adverse events

6. RISK ANALYSIS

6.1 Benefits

TECR with ECM placement is a novel endoscopic approach for the treatment of BE with HGD. Direct benefits regarding this procedure would include the following: organ (esophagus) preservation, no external surgical incisions required, less pain, short hospital stay and sooner return of the patient to usual activities compared to alternative surgical treatment options such as esophagectomy.

6.2 Risks

It is anticipated that subjects participating in the study will be exposed to operative and post-operative risks similar to related surgical procedures of the esophagus for the treatment of BE with HGD. Additional risk may include breach of confidentiality as a result of participating in this research study.

Stent removal may pose additional risks that are not fully known at this time. Removal of a fully covered metal stent is not an FDA accepted use of this device.

Potential complications of TECR with ECM placement include, but are not limited to, the following:

- Pharyngolaryngeal pain, temporary abdominal or esophageal pain, post-operative dysphagia due to edema or esophageal stricture, narrowing of the esophagus, nausea, vomiting, musculoskeletal pain (e.g. left shoulder pain), oral or dental injury, and heartburn, are usually mild in severity and may occur in greater than 25% of people.
- Laceration or tear of esophagus, post-operative bleeding, and stent migration are usually moderate to severe in severity and may occur in 1-10% of people.
- This procedure is rarely (less than 1%) associated with severe complications such as pneumothorax, pneumomediastinum, pleural effusion, nerve damage, esophageal perforation, infection in the center compartment of the chest, and arrhythmia. Patients may require conversion to open surgery.
- Failed endoscopic procedure (recurrent disease or narrowing of the esophagus which proves difficult to manage).
- Food bolus impaction is possible due to noncompliance with post-op dietary plan while the stent is in place.
- Allergic reaction to ECM (porcine material). The risks of prolonged contact with porcine derived material such as ECM are not fully known however it is possible that an individual could become sensitized to pork products as a result of this procedure.

Potential complications and complication rates of stent removal post ECM placement are not fully known and may include, but are not limited to:

- Bleeding – bleeding is common however generally subclinical and not severe
- Abrasion – superficial esophageal abrasions are usually visible however they are not typically severe
- Laceration to the esophagus – laceration is rarely observed with stent removal however can pose additional bleeding risk, and pain.
- Perforation of the esophagus

Patients who undergo this procedure will be closely monitored by the investigators on an ongoing basis throughout the study. If an adverse event listed above occurs, the investigator will immediately employ proper management to correct and/or minimize further complications.

Furthermore, EGD, and the study procedure are performed under general anesthesia which poses a potential risk of genetic mutations and birth defects. BaSW and fluoroscopic placement of the esophageal stent expose a subject to small amounts of radiation. The reproductive risks of this procedure for women (and on embryos or fetuses if they become pregnant) are not fully known and are unforeseeable. If subjects are female and of child-bearing potential, subjects will undergo a pregnancy test prior to their participation in the study procedures. Tests have not been done to determine the risk of this procedure to pregnant women. To avoid risks to the fetus, it is important that subjects not be pregnant during this study. It is also advised that subjects not become pregnant for at least six months after the study procedure, as it may impact the outcome of the study procedure. Avoiding sexual activity is the only certain method to prevent pregnancy. However, if subjects choose to be sexually active, they should use an appropriate “double barrier” method of birth control (such as female use of a diaphragm, intrauterine device (IUD),

or contraceptive sponge, in addition to male use of a condom) or the female should be using prescribed “birth control” pills, injections or implants (3.2.1). If subjects choose to be sexually active during this study, pregnancy could still result even with the use of these birth control methods. If subjects have questions, they are encouraged to speak with either the study investigator or their personal physician.

6.2.1 Minimizing Potential Risks

All efforts will be made to minimize these risks by the investigators who are experienced and skilled in endoscopic procedures, clearly defining eligibility criteria to ensure that only the appropriate patients are enrolled and by ensuring that the treatment and follow-up of the patient are consistent with current medical practice. All research procedures will be conducted in the fully equipped facilities, providing access to emergency care and to all necessary precautions to ensure subject privacy. The research procedures will be conducted in a private room, collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, drapes or other barriers will be used for subjects who are required to disrobe.

Stent removal risks are being minimized throughout the study. The Boston Scientific WallFlex™ stent has been carefully selected in order to minimize the risks of tissue ingrowth (7, 9-14) and esophageal damage upon removal. The removal suture will aid in collapsibility of the stent upon removal which will reduce any forceful pulling against the esophageal wall, thusly reducing the likelihood of esophageal abrasion, tear, or perforation. The Investigator will carefully examine the stent for any indications of fracture during and immediately after the removal to ensure no fragments may be remaining in the subject. Data will be collected following each stent removal to document any stent related events. This data will be considered prior to enrolling additional subjects to evaluate the overall safety and potential risk to new subjects.

The Investigator may withdraw a subject from continued participation in this study in the event the procedure cannot be completed (ECM and Stent are not placed), a subject cannot or will not return for stent removal, or if a subject experiences a Serious Adverse Event that compromises his or her ability to undergo follow-up evaluations specified in the protocol.

6.2.2 Potential Risks to Subject Confidentiality

In all clinical studies, confidentiality of protected health information may be breached due to study-related activities beyond those of routine clinical care. This risk will be minimized by not collecting personally identifying information on data collection forms or other study related documentation to be provided to industry supporter (ACell Inc.).

6.2.3 Confidentiality and Data Integrity

Any information about the patient obtained from or for this research study will be kept as confidential (private) as possible. All records related to the patient involvement in this research study will be stored in a locked, limited access file cabinet in the office of the investigators. The subject will not be identified in any publication of research results unless the patient signs a

separate form giving the permission (release). This research study will involve the recording of current and/or future identifiable medical information from the patient hospital and/or other (e.g. physician office) records. The information that will be recorded will be limited to information concerning the procedures performed only for this study. In addition, no personal subject identifiers are to be used in any presentation or publication.

All data will be entered with masked identification and subjects will be numbered. Investigators and Monitors will only have the privilege to read data. The subject identification code list will be stored. All electronic records will identify patients and their enrollment number (e.g., AHN-001) only. There will be no way to determine the patient's identity from the enrollment number except for the identification code list stored; this will protect the subject's identity during data analysis. No patient names will appear on any reports, publications or other disclosures of clinical study outcomes. All research records will be retained for a minimum 7 years following study completion.

6.3 Monitoring

A Monitoring Plan will be used as a guide for performing site monitoring visits. This Monitoring Plan will be reviewed throughout the trial and updated as needed with joint approval by the Sponsor/Principal Investigator. The most recent version of the Monitoring Plan will supersede all prior versions.

The clinical site will be monitored to ensure that the Protocol, Institutional Review Board (IRB) approvals and amendments, Good Clinical Practices (GCP), Food and Drug Administration (FDA) regulations (specifically 21 CFR 812.40, 812.42, 812.43, 812.46, 812.100, 812.110, 812.140 and 21 CFR 50, 54, and 56), and other local laws are adhered to throughout the entire course of the study and required follow-up periods.

The occurrence of adverse events will be monitored for each subject on an ongoing basis throughout the study. Unexpected adverse event will be reported immediately to the West Penn Hospital Institutional Review Board (IRB). If an unexpected adverse event occurs, the investigators will re-assess the risk/benefit ratio of the study and submit any modifications deemed necessary to the IRB.

7. INFORMED CONSENT MATERIALS

All subjects will be given a copy of the Institutional Review Board approved Informed Consent Forms and Study Questionnaires.

8. INSTITUTIONAL REVIEW BOARD

This site will conduct research under the assurance of the Allegheny-Singer Research Institute Institutional Review Board (00007365). IORG# 0006117 and FWA# 00015120.

9. CONTACT INFORMATION

Correspondence for this study should be sent to the Manager of Clinical Research for the Esophageal & Lung Institute at the following address:

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All correspondence will be reviewed with the Principal Investigator and will be addressed accordingly.

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“001_TECR and ECM Placement Video” is provided for this submission by Investigator Toshitaka Hoppo M.D. Ph.D. as a supplement to the publication: Badylak SF, Hoppo T, Nieponice A, et al. Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine approach with a biologic scaffold. *Tissue Eng. Part A* 2011 Jun; 17(11-12):1643-50.

“002_Stent Removal Technique Video” was downloaded as a supplement embedded within the original text in the publication: Eloubeidi MA, Lopes TL. Novel Removable Fully Covered Self-Expanding Metal Esophageal Stent: Feasibility, technique of Removal, and Tissue Response in Humans. *Am. J. Gastroenterol.* 2009 Jun; 104:1374-1381.

Please note, the stent pictured in “001_TECR and ECM Placement Video” is the Boston Scientific UltraFlex™ Esophageal NG stent. This stent has uncovered metal ends. We are proposing to use the WallFlex™ stent which is fully covered. Additionally, the technique pictured is submucosal endoscopic dissection whereas we propose using endoscopic mucosal resection.

11. APPENDICES

- 11.1 Dysphagia Severity Questionnaire 9.24.15**
- 11.2 Gastroesophageal Reflux Disease-Health Related Quality of Life (GERD-HRQL) 9.24.15**
- 11.3 Short Form (SF)-36 health survey 9.24.15**
- 11.4 Case Report Forms (CRF) 9.24.15**
- 11.5 Informed Consent Form 9.24.15**
- 11.6 Monitoring Plan 9.24.15**