Diagnosis and monitoring of eosinophilic esophagitis using the Cytosponge

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DIAGNOSIS AND MONITORING OF EOSINOPHILIC ESOPHAGITIS USING THE CYTOSPONGE

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SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

Name:

Title:

Summary of Changes

Version 4.0 dated 25Sep2020	
Study Summary	Objectives and methodology updated according to changes made to these sections of the protocol.
2.0 Study Objectives	Aim 2 removed as the scope of the study was limited by device availability and aim 2 was unable to be evaluated with available data.
3.1 General Design	Reference to Aim 2 removed.
3.4 Secondary Outcomes	Added “agreement between cytosponge and endoscopic biopsy as measured by kappa.” Revised secondary outcome to clarify measures used to assess acceptability.
6.1 Sample Size Determination	Removed reference to Aim 2.
6.2 Statistical Methods	Removed reference to Aim 2.
Administrative Changes	Administrative changes to protocol version and version dates throughout.
Version 3.0 dated 05Sep2017	
3.1 General Design	Additional analyses on samples has been added.
Administrative Changes	Administrative changes to protocol version and version dates throughout as well as updating table of contents and section numbers.
Version 2.0 dated 05Oct2016	
Study Summary 6.1 Sample Size Determination	Increased enrollment from 100 to up to 150 to account for any potential issues with sample quality and additional safety assessments.
Administrative Changes	Administrative changes to protocol version and version dates throughout as well as updating table of contents and section numbers.
Version 1.0 dated 24Nov2015	Initial Protocol

Table of Contents

List of Abbreviations

Item	Definition
AE	Adverse Event
CRF	Case Report Form
EC	Ethics Committee
EGD	Esophagogastroduodenoscopy
EoE	Eosinophilic Esophagitis
IES	Impact of Events Scale
IRB	Institutional Review Board
VAS	Visual Analog Scale

Study Summary

Title	Diagnosis and monitoring of eosinophilic esophagitis using the Cytosponge
Methodology	This will be a prospective cohort study, with patient enrollment conducted at UNC and Mayo Clinic by Drs. Dellon and Katzka, and further sample analysis performed by Dr. Fitzgerald. In Aim 1, patients with eosinophilic esophagitis (EoE) or suspected EoE will be enrolled, tissue will be obtained from both the Cytosponge and routine care endoscopy, and the methods will be compared for a single time point to determine accuracy of Cytosponge for quantifying esophageal eosinophil counts. The patients who are being treated will be followed and tissue will be assessed over time with both Cytosponge and routine care endoscopy to determine the utility of Cytosponge for monitoring treatment response in patients with EoE. For all patients, safety will be monitored and subjects will complete a survey about the acceptability of Cytosponge (Aim 2).
Study Duration	2 Years
Study Center(s)	University of North Carolina, Chapel Hill, NC Mayo Clinic, Rochester MN
Objectives	*Aim 1:* To determine the accuracy of Cytosponge for quantifying esophageal eosinophil counts in patients with active EoE, as compared to endoscopy with biopsy as the gold standard. *Aim 2:* To determine the safety and acceptability of Cytosponge for patients with EoE.
Number of Subjects	150
Diagnosis and Main Inclusion Criteria	Inclusion Criteria: 1. Able to read, comprehend, and complete the informed consent form 2. Male or female subjects, age 18-80 years, 3. Suspected EoE or has a diagnosis of EoE with current active disease, Exclusion Criteria: 1. History of esophageal stricture precluding passage of the endoscope or sponge, 2. Pregnancy, or planned pregnancy during the course of the study, 3. Any history of esophageal varices, liver impairment of moderate or worse severity (Child's- Pugh class B & C) or evidence of varices noted on any past endoscopy, 4. Any history of esophageal surgery, except for uncomplicated fundoplication 5. History of coagulopathy, with INR>1.3 and/or platelet count of <75,000. 6. Current use of blood thinners such as coumadin, warfarin, clopidogrel, heparin and/or low molecular weight heparin (requires discontinuation of medication 7 days prior to and 7 days after esophagogastroduodenoscopy [EGD] and Cytosponge administration, aspirin use is OK). 7. Are allergic to local anesthetics such as lidocaine (these subjects may opt not to receive the optional lidocaine gargle prior to the Cytosponge administration and still be eligible). 8. Have not fasted the night before administration of the Cytosponge. 9. History of perforation.

Statistical Methodology	*Aim 1:* To test the hypothesis that the Cytosponge accurately quantifies esophageal eosinophil counts in patients with active EoE, the maximum eosinophil counts from the Cytosponge and endoscopic biopsy will be compared by calculating the Pearson coefficient of correlation. In addition, patients will be categorized as having an eosinophil count either above or below 15 eos/hpf (the current diagnostic threshold). Agreement between patients above and below this threshold for Cytopsponge and endoscopic biopsy will be measured using the kappa statistic. If eosinophil counts are not normally distributed, non-parametric methods will be used. *Aim 2:* Summary statistics will be calculated to describe adverse events and the tolerability of the Cytosponge. The proportion of subjects who prefer the Cytosponge will be calculated.

1 Introduction

This document is a protocol for a human research study. The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and applicable federal regulations and institutional policies. All personnel involved in the conduct of this study have completed human subjects protection training.

1.1 Key Roles and Personnel

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1.2 Background

Eosinophilic esophagitis (EoE) is an emerging disease defined by the abnormal presence of eosinophils in the esophageal lining, leading to trouble swallowing (dysphagia), esophageal narrowing (stenosis), and food impaction.[1-3] The incidence and prevalence of EoE have risen dramatically,[4-7] and EoE is now the most common cause of food impaction in patients presenting to emergency rooms.[8, 9] In order to diagnose and monitor EoE, upper endoscopy with esophageal tissue biopsy is currently required. Two upper endoscopies are needed to diagnose EoE, the first one to evaluate symptoms and the second one to confirm the diagnosis after treatment with a proton pump inhibitor (anti-acid) medication.[3] If a patient is treated with a swallowed ("topical") steroid medication such as budesonide or fluticasone, they undergo a third endoscopy to determine whether the medication was effective.[10-14] If they are treated with an

elimination diet, they will require an endoscopy 6 weeks after the initial elimination, and then another one every 4-6 weeks after each food is added back.[15-17] In some cases this can add up to more than 8-10 endoscopies in a single year. This is costly, inconvenient, and the repeated procedures add risk. New diagnostic methods for EoE are urgently needed.

A novel, minimally invasive a simple, non-endoscopic device, termed the Cytosponge, has been developed for endoscopic screening of subjects at risk for Barrett's Esophagus (a precancerous condition that is a complication of reflux disease),[18] by investigators at the University of Cambridge in the U.K. and is readily applicable to EoE. Cytosponge consists of a foam sponge which is contained within a dissolvable capsule and attached to a string (see Figure 1). The capsule is swallowed, dissolves in the stomach, the sponge expands into a sphere, and after 5 minutes is retrieved by pulling the string. As the sponge is withdrawn, cells along the whole length of the esophagus are collected by the foam sphere; this amounts to obtaining an esophageal biopsy tissue sample, but without doing the endoscopy. The foam sphere, now containing cells, is placed into preservative fluid and processed using standard pathology methods readily available everywhere. Our team has published data about the safety, acceptability, and accuracy of this technique in Barrett's,[18-21] and we have preliminary data in EoE patients. Of note, Cytosponge differs substantially from the Esophageal String Test (EST), which is also under development for EoE.[22] The EST uses a dissolvable capsule and a string to collect fluids secreted from the esophageal lining, but does not capture tissue. Moreover, the EST requires a specialized lab to run advanced tests to measure factors secreted from eosinophils. In contrast, because Cytosponge obtains tissue, it can be used anywhere with existing and inexpensive pathology techniques.

Figure 1: The Cytosponge. The left image shows the expanded sponge. The right image shows the capsule. Both have the string in place.

Figure 2 CytospongeTM (left) and Cytosponge I (right) for comparison

Medtronic, formerly Covidien GI Solutions, has developed a more refined version of the Cytosponge I (referred hereafter as the Cytosponge™ or "Cytosponge"). The Cytosponge was developed from the Cytosponge I specification and design, with the additional priority of a more reproducible manufacturing process, standardization of dimensions, and other quality related features (Figure 2). Because we seek to assess a tool for widespread clinical usage, this study will use the Cytosponge which will be purchased from Covidien GI Solutions (now part of Medtronic).

The current endoscopic methods for diagnosing and monitoring treatment response in EoE are costly, inconvenient, and risky. Novel diagnostic methods are needed, and the minimally-invasive Cytosponge holds great promise. It has been shown to be safe and accurate in Barrett's esophagus, it has the advantage (over the string test) of obtaining a true tissue sample, and our preliminary data supports its further study in EoE. The proposed prospective cohort study, conducted by experts in esophageal diseases and EoE, will assess the accuracy of Cytosponge compared to endoscopy and biopsy in EoE, and determine the safety and acceptability of this technique. Use of the Cytosponge would fundamentally change the paradigm for clinical management of EoE by allowing collection of non-

endoscopic esophageal biopsies, thus minimizing the need for invasive testing. It would also facilitate future genetic, mechanistic, and pathogenesis research in EoE.

The specific goals of this study are to assess the safety, acceptability, and accuracy of Cytosponge for diagnosis and monitoring of EoE in comparison to endoscopy and biopsy as the gold standard. To achieve these goals, we will recruit subjects with EoE from both the University of North Carolina (Dr. Dellon) and from the Mayo Clinic in Rochester where Cytosponge has already been used for EoE (Dr. Katzka). Our central hypothesis is that the tissue obtained using Cytosponge will show an eosinophilic infiltrate similar to that seen on endoscopic biopsies in patients with active EoE, will have utility to monitor tissue eosinophilia in patients being treated for EoE, and will be safe, acceptable, and preferable for patients when compared with endoscopy. This research will impact clinical practice by fundamentally changing the way EoE is diagnosed and monitored, decreasing the need for costly and invasive endoscopic procedures. Moreover, because this technique readily obtains esophageal tissue, it could greatly facilitate genetic, mechanistic, and pathogenesis research in EoE. providing less invasive and more cost-effective surveillance of this large and growing patient population.

1.3 Rationale

PRELIMINARY RESULTS

Our research team has extensive experience with EoE diagnosis, treatment, and Cytosponge

Drs. Dellon (from UNC) and Katzka (from Mayo Clinic) are recognized experts in diagnosis and treatment of EoE. At both centers, their clinical practice and research endeavors focus on esophageal diseases and EoE in particular. They each have a large EoE patient population and have demonstrated the ability to recruit patients for EoE studies. Dr. Fitzgerald (from Cambridge, UK), also a Gastroenterologist who is expert in esophageal diseases, has developed the Cytosponge, shown its great utility in diagnosis and surveillance of Barrett's esophagus (see below), and is committed to extending its application into EoE.

Cytosponge has been shown to be safe and have great diagnostic utility in Barrett's esophagus

The safety, feasibility, and acceptability of using Cytosponge in a primary care setting to screen for Barrett's esophagus has been shown by Dr. Fitzgerald's group.[18-21] In the largest published study to date,[18] 99% of the 504 subjects successfully swallowed the Cytosponge and there were no serious adverse events. In an interim analysis of 751 patients in a follow-up study, it is similarly well-tolerated.[21] The tissue samples obtained from these patients were more than adequate to evaluate for Barrett's esophagus, and the Cytosponge gave very similar results compared with endoscopy and biopsy as the gold standard.[18, 21] Moreover, the tissue that was obtained was readily amenable to standard pathologic analysis techniques, including regular hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC).[18, 19, 21] These data show that in a large cohort of patients, Cytosponge was safe, well-tolerated, effective for obtaining tissue samples that are easily processed, and effective for diagnosis and surveillance of Barrett's esophagus compared with endoscopy and biopsy.

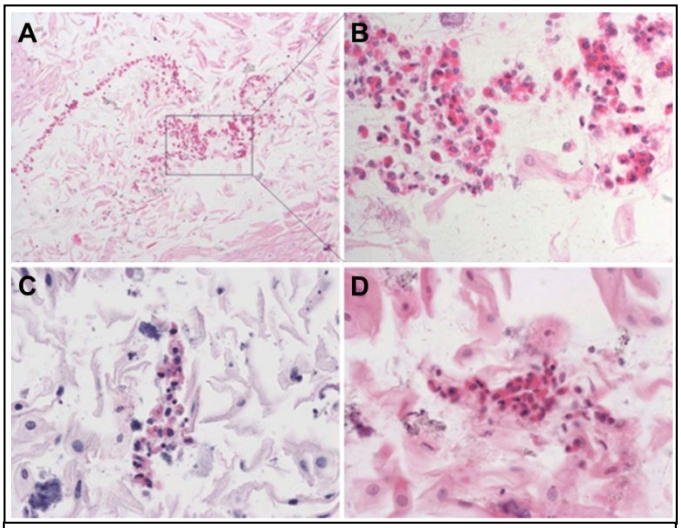


Figure 3: Examples of esophageal tissue samples from EoE patients using Cytosponge. Low power **(A)** and high power **(B)** views from the same patient show multiple bright red eosinophils that are easy to see on routine exam. Examples from two other patients are seen in **(C)** and **(D)**.

In a pilot study, Cytosponge shows great promise for use in EoE

Given the success of Cytosponge in Barrett's esophagus and the need for esophageal biopsy tissue for diagnosis and to monitor treatment response in EoE, Drs. Katzka and Fitzgerald conducted a pilot study

of Cytosponge in EoE. A total of 20 EoE patients, some with active disease and some with disease in remission to provide a range of disease severity and esophageal eosinophilic infiltration, swallowed the Cytosponge for tissue sampling prior to undergoing standard endoscopy and biopsy. All patients, even those with esophageal strictures, were able to swallow the Cytosponge without problems, and there were no serious adverse events. The first main result was that the tissue samples were adequate for analysis with standard pathology H&E staining (a stain on which eosinophils are bright pink in color; see Figure 3). The second main result was that Cytosponge performed very well compared with endoscopy and biopsy, with a sensitivity of 100% (the Cytosponge picked up all of the cases of EoE that were also found by endoscopy) and a specificity of 70% (there were some cases felt to be EoE on the Cytosponge that were not felt to be EoE on endoscopy). These preliminary data show that the Cytosponge holds great promise for diagnosis and monitoring of treatment in EoE. If tissue can be obtained with this minimally invasive technique, it would fundamentally change the paradigm for clinical management of EoE by minimizing the need for invasive endoscopies.

1.4 Device Description

The Cytosponge™ Cell Collection Device (Cytosponge) is intended to collect surface cells from the esophagus. The device consists of a swallowable capsule, which dissolves in the body cavity, releasing a self-expandable sponge. The sponge is then retrieved from the esophagus using an attached cord. During the retrieval process, the sponge collects cells from the most superficial layer of the esophageal mucosa. Once removed from the body cavity, the sponge and cells are retained for investigation and/or testing.

Figure 4 Cytosponge with planned packaging and retrieval cord

The Cytosponge™ Cell Collection Device (Cytosponge) received 510(k) clearance from the FDA on November 26, 2014 (K142695). The Cytosponge ™ Cell Collection device is a Class II product under 21 CFR 874.4710 esophagoscope (flexible or rigid) and accessories. This study uses the Cytosponge in accordance with its labeling and is therefore exempt from an IDE.

1.4.1 Prior Utilization

Dr. Fitzgerald and colleagues administered Cytosponge I to 504 patients in a primary care setting and found it to be safe and well-tolerated. Of these patients, 501 (99%) were able to successfully swallow the capsule. Unsurprisingly, given pill-swallowing difficulty in the general population, 3 patients were unable to swallow the pill, feeling it was too large. No adverse events were noted, and patients demonstrated a low level of anxiety associated with the test. These 504 administrations are documented in a report in the British Medical Journal[30]. These investigators have, since this investigation, administered the Cytosponge to an additional 831 patients as part of the BEST2 study (a multicenter, prospective study to determine whether BE patients can be risk stratified using the Cytosponge) and an interim data analysis shows similarly excellent safety and tolerance profile, with no adverse events reported. [31] Overall, to date, there have been 1,335 documented administrations of the sponge, with no adverse events. Several hundred additional uses of the device have occurred in Cambridge, UK, without adverse event (personal communication, Dr. Fitzgerald), but have not yet been reported in the peer-reviewed literature.

Cytosponge is extremely similar to Cytosponge I. Although no major complications have been reported with this device, there are several risks associated with the administration of this device. There is the possibility of aspiration whenever instrumentation of the esophagus or stomach is performed. Because the device does not render the upper esophageal sphincter incompetent, this risk is expected to be minimal. Additionally, although the sponge is soft and non-abrasive, subjects could bleed from any mucosal surfaces of the mouth, stomach or esophagus which come in contact with it, and/or experience a sore or irritated throat following administration. Finally, to date there have been >1,000 administrations

and detachment of the sponge from the string has occurred in less than 1% of cases. Should the sponge detach from the string, it will be retrieved during the routine care upper endoscopy immediately following administration.

The current study has been designed to minimize occurrence of these theoretical risks. Specifically we plan to exclude those patients who:

- Are unable to discontinue clopidogrel, and/or warfarin for 7 days prior and 7 days after procedure,
- Have a history of esophageal stricture that precludes passage of an adult endoscope,
- Have any history of esophageal varices, liver impairment of moderate or worse severity (Child's-Pugh class B & C), or evidence of varices noted on any past endoscopy,
- Have any history of esophageal surgery (except uncomplicated fundoplication), and history of coagulopathy, with INR >1.3 and/or platelet count of <75,000.

Cytosponge administration will occur after an overnight fast to minimize the possibility of aspiration of any gastric contents. Every administered sponge will be assessed post-procedure for signs of fracture or incomplete retrieval of the sponge. In the unlikely case of incomplete retrieval, the sponge will be retrieved with a Roth net at the standard of care endoscopy which will routinely immediately follow the administration of the sponge per the study protocol. Any bleeding noted, either clinically following the sponge administration or due to blood on the sponge itself, will be similarly investigated, and, as necessary, treated during the subsequent endoscopy. Because study inclusion/exclusion criteria are designed to exclude those at highest risk for a bleeding complication, the risk of bleeding in this study should be extremely low.

2 Study Objectives

Aim 1: To determine the accuracy of Cytosponge for quantifying esophageal eosinophil counts in patients with active EoE, as compared to endoscopy with biopsy as the gold standard.

Aim 2: To determine the safety and acceptability of Cytosponge for patients with EoE, all patients who are enrolled in both Aims 1 and 2 will be monitored.

3 Study Design

3.1 General Design

This is a prospective longitudinal cohort study, with patient enrollment conducted at UNC and Mayo Clinic by Drs. Dellon and Katzka, and further sample analysis performed by Dr. Fitzgerald. In Aim 1, patients with EoE will be enrolled, tissue will be obtained from both the Cytosponge and endoscopy, and the methods will be compared for a single time point. For all patients, safety will be monitored and subjects will complete a survey about the acceptability of Cytosponge (Aim 2).

Subject recruitment will be from consecutive eligible patients presenting for endoscopy as part of their routine care. Potentially eligible subjects will be contacted by telephone in advance of their procedure and their interest in study participation assessed. Subjects interested in participating will be asked to present to the endoscopy unit prior to their scheduled procedure. At that time, inclusion and exclusion criteria will be reviewed, and eligible subjects will give informed consent. Subjects will then undergo the Cytosponge assay.

After the Cytosponge has been removed, the patient will undergo standard upper endoscopy and biopsy, as clinically indicated. During this exam, endoscopic features of EoE will be recorded, including rings, furrows, white plaques, decreased vascularity, and strictures. The severity of the endoscopy findings will be measured using the recently validated EREFS scoring system.[23] Four esophageal biopsies will be taken both from the distal (5 cm above the gastro-esophageal junction) and proximal (15 cm above the gastro-esophageal junction) esophagus. This number of biopsies has been shown to maximize the diagnostic sensitivity for EoE.[24]

Enrolled subjects will be administered the Cytosponge during the enrollment visit. After enrollment, if subjects receive treatment for their EoE and return to the enrolling site for an upper endoscopy as part of their standard of care, then subjects will be asked to repeat the Cytosponge procedure prior to each follow-up standard of care endoscopy.

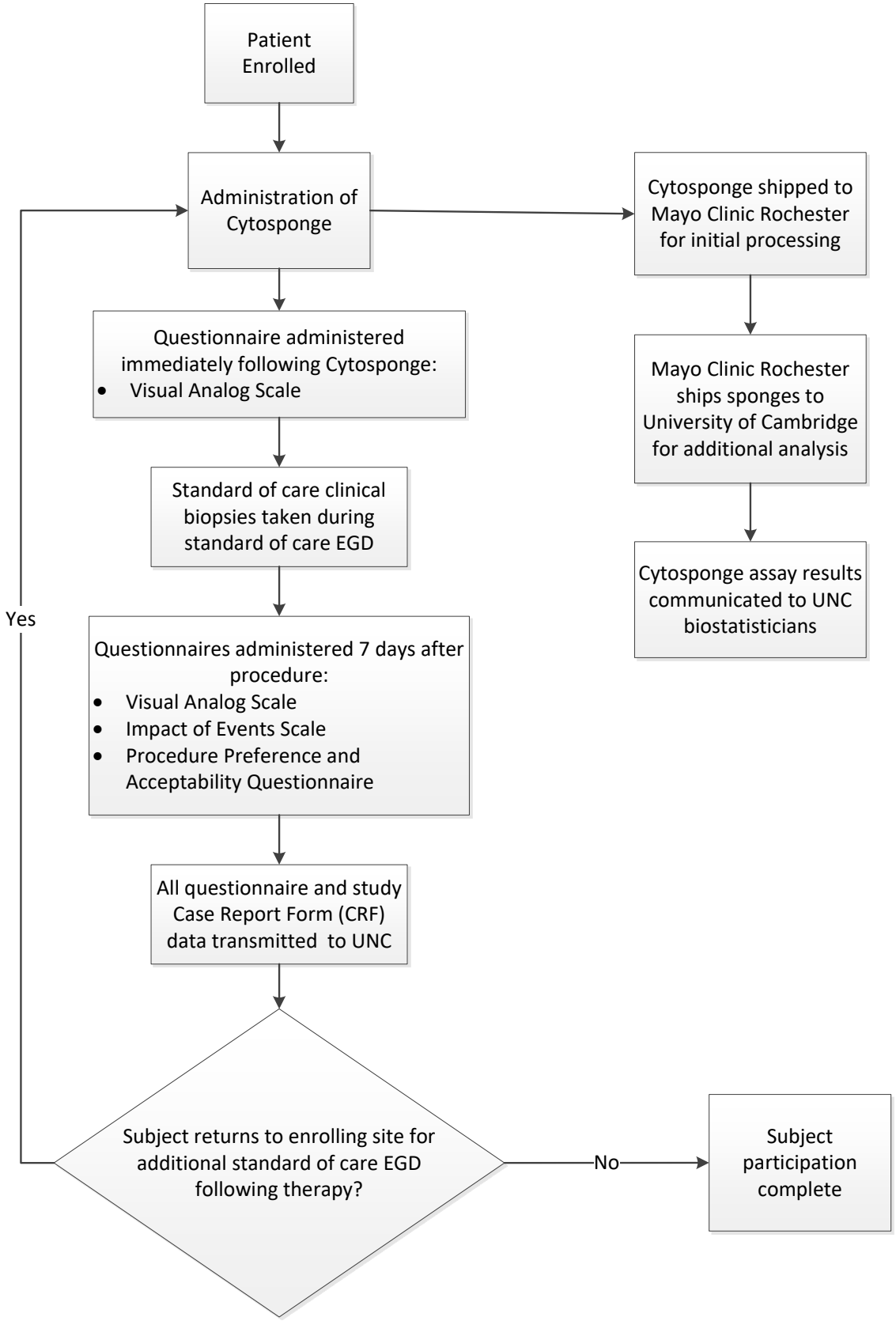
Histology and eosinophil counts
All tissue samples from the Cytosponge and endoscopy will be coded with a subject's ID number, but will otherwise be masked for all clinical data, including EoE activity, symptoms, patient characteristics, and treatments prescribed. Using the paraffin blocks, pathology slides will be cut and the tissue processed with routine H&E staining. The slides will then be digitized, and using the Aperio ImageScope (Aperio Technologies, Vista, CA), the maximum eosinophil density (eosinophils/mm^2 [eos/mm^2]) will be determined using our previously validated protocol.[25] For purposes of comparison to previous studies, eosinophil density will then be converted to eosinophil counts (eos/hpf) for an assumed hpf size of 0.24 mm^2, the size of an average field as reported in the literature.[26] The study pathologists from UNC and Mayo Clinic will review the specimens from their sites, and the study pathology from Cambridge will provide a second review of all specimens to ensure the most accurate quantification of eosinophil counts possible. Importantly, no record of the results of the standard endoscopy and biopsies will be provided to those assessing the Cytosponge results and these individuals will be masked to all clinical data. Similarly, the pathologists interpreting the histological specimens from the endoscopy will have no knowledge of the outcomes of the Cytosponge assay. Only the study biostatistician will have access to these data.

In addition, we would plan to perform special staining and analysis of the existing biopsy and sponge samples with the goal of determining if the diagnostic accuracy of this test can be improved. In particular we will examine markers of eosinophil function, activation, and inflammation, such as eosinophil peroxidase (EPX), a granule protein that clearly identifies intact eosinophils, as well as extracellular EPX deposition suggestive of degranulation. This can be detected with immunohistochemistry.

Safety and accessibility assessments
Patients will be assessed at multiple points to determine the safety of the Cytosponge in EoE. Research staff will assess for any symptoms or events as soon as the sponge capsule is swallowed, as well as immediately after the expanded sponge is removed. Subjects will also be contacted 7 days after the endoscopy to assess for adverse events.

Primary assessment of acceptability will be via the Impact of Events Scale. This widely used scale was developed to assess the distress associated with a specific life event. It includes measures of both the intrusiveness of the event, and any avoidance responses by the subject in response to the event. Final scores are between 0-75, with low scores demonstrating a low impact of the event. The scale will be administrated 7 days after the sampling in order to allow the subjects to have time to reflect on the experience and to compare with the EGD. Secondary acceptability outcomes will include a visual analog scale of acceptability of the Cytosponge, performed immediately after the Cytosponge is administered and again 7 days after. Also, the subject will be asked whether he/she would be willing to repeat the assay, and, assuming similar accuracy between Cytosponge and upper endoscopy, whether he/she would rather undergo surveillance by Cytosponge or standard EGD with biopsies.

3.2 Protocol Map



3.3 Primary Outcomes

The primary outcome variables will be sensitivity and specificity of the cytosponge, as compared against the gold standard of the presence of EoE as detected by upper endoscopy with biopsies.

3.4 Secondary Outcomes

1. Agreement between cytosponge and endoscopic biopsy as measured by kappa.
2. Acceptability of cytosponge compared to endoscopy, as rated on visual analog scale.
3. Acceptability of cytosponge as measured by the Impact of Events Scale.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Able to read, comprehend, and complete the informed consent form
2. Male or female subjects, age 18-80 years,
3. Suspected EoE or has a diagnosis of EoE with current active disease,

4.2 Exclusion Criteria

1. History of esophageal stricture precluding passage of the endoscope or sponge,
2. Pregnancy, or planned pregnancy during the course of the study,
3. Any history of esophageal varices, liver impairment of moderate or worse severity (Child's- Pugh class B & C) or evidence of varices noted on any past endoscopy,
4. Any history of esophageal surgery, except for uncomplicated fundoplication
5. History of coagulopathy, with INR>1.3 and/or platelet count of <75,000.
6. Current use of blood thinners such as coumadin, warfarin, clopidogrel, heparin and/or low molecular weight heparin (requires discontinuation of medication 7 days prior to and 7 days after esophagogastroduodenoscopy [EGD] and Cytosponge administration, aspirin use is OK).
7. Are allergic to local anesthetics such as lidocaine (these subjects may opt not to receive the optional lidocaine gargle prior to the Cytosponge administration and still be eligible).
8. Have not fasted the night before administration of the Cytosponge.
9. History of perforation.

4.3 Subject Recruitment and Screening

Potential subjects will be identified prior to or during their GI clinic or procedure visits at their enrolling institutions. All subjects will be screened and enrolled using EC/IRB-approved and HIPAA compliant methods.

An investigator, study coordinator, or other qualified personnel will obtain written informed consent prior to any study procedures. Potential subjects will have an opportunity to carefully review the consent form. The details of the study will be reviewed verbally, and all questions will be answered to the satisfaction of the patient. Only adults with the ability to consent will be eligible for enrollment in this study. After the subject signs the consent, a copy of the signed consent will be provided to the subject. Once written consent has been obtained, the coordinator will collect demographic and historical information from the patient pertaining to history esophageal conditions and procedures received.

The consent process will be documented by the coordinator in the patient's study file.

4.4 Early Withdrawal of Subjects

Subjects will be considered to have completed the study after completion of the last study visit (follow-up phone call). Subjects may be withdrawn prior to this for any of the following reasons:

- Death, or
- Lost to Follow-Up, or

- Withdrawal of consent, or
- Discontinuation by the investigator.

Documentation must be maintained at the site for any subject withdrawals. Subjects unable to complete Cytosponge administration will be withdrawn from the study (discontinued by investigator). Three attempts at contact using two different methods are required prior to determination that the subject is lost to follow-up. Attempts at contact must be with certified letters OR documented telephone contact. If a subject is withdrawn prior to completion of the study, the site should complete and submit a change of status case report form. See section 8.3 for additional information on case report forms. Subjects who withdraw after completion of the initial Cytosponge administration will not be replaced. Withdrawn subjects will not be followed, unless they have an active adverse event (AE) at the time of withdrawal. Subjects withdrawn while experiencing an adverse event will be followed until resolution of the AE.

5 Study Procedures

Assessment	Screening/Enrollment Visit	Follow-Up Phone Call (7 days +/- 2 days after Enrollment)	Follow-Up Cytosponge Administration[1]	Follow-Up Phone Call (7 days +/- 2 days after each follow-up Cytosponge)
Informed Consent Form	X			
Demographics	X			
Medical History	X			
Inclusion/Exclusion Criteria	X			
Cytosponge Administration	X		X	
Routine Care Endoscopy with Biopsy	X[2]		X[2]	
Visual Scale	X[3]	X	X[3]	X
Impact of Events Scale		X		X
Procedure Preference and Acceptability Questionnaire		X		X
Adverse Events[4]	X	X	X	X

[1] After enrollment, if subjects receive treatment for their EoE and return to the enrolling site for an upper endoscopy as part of their standard of care, then subjects will be asked to repeat the Cytosponge procedure prior to each follow-up standard of care endoscopy.
[2]Routine care biopsies should be taken during the endoscopy following the Cytosponge as this is considered standard of care for the target population. No research-specific biopsies will be obtained during the procedure.
[3] The VAS should be administered immediately following completion of the Cytosponge and prior to the upper endoscopy.
[4]Only those events potentially related to participation in this research study must be reported. See section 7.2 for definition of a reportable adverse event.

5.1 Screening/Enrollment

During screening/enrollment, eligibility is assessed and those eligible and interested in participating are consented on the study. Once consent is obtained, subjects will undergo administration of the Cytosponge, complete a questionnaire and proceed with routine care upper endoscopy immediately following completion of the Cytosponge in which biopsies are taken for clinical purposes and sent to pathology.

5.1.1 Assessments

The following will be completed during the screening/enrollment visit:

- Eligibility review

- Informed consent
- Cytosponge administration
- Visual Analog Scale
- Adverse event assessment
- Routine care upper endoscopy with biopsy
- Enrollment Case Report Form (CRF): This captures demographics including race, ethnicity, gender, and year of birth, relevant EoE medical history including documentation of endoscopic procedures received to date as well as pathology findings and endoscopic history related to current diagnosis.

5.1.2 Consenting Procedure

If a subject is screened eligible and interested in the study, then the subject will be consented on the study prior to any study procedure. Written informed consent will be obtained by qualified study personnel. Documentation of the consent process will be maintained in the subject's research record.

Subjects will be given ample time to review the consent document and ask any questions they may have. A copy of the written consent form will be provided to the subject and the original maintained in the subject's research record.

If subjects meet all inclusion and none of the exclusion criteria and consent to the study, then they will be enrolled in the study. Subjects will be assigned a unique subject code. Each institution will be provided a list of subject codes to use.

5.1.3 Cytosponge Administration

The Cytosponge™ device (referred to hereafter as the "Cytosponge") will be supplied by Covidien GI Solutions to the participating sites. Study sites will be responsible for storage and accountability of the device. The Cytosponge lifetime/use by date will be confirmed on the product packaging. The device received FDA 510(k) clearance on November 26, 2014 (K142695). The Cytosponge device consists of a spherical 3.0 cm diameter reticulated polyester foam compressed and encapsulated in a standard vegetarian capsule (size 00).

Subjects will undergo administration of the Cytosponge™ according to the IFU. Briefly, subjects will be placed in the seated position and will swallow the capsule with 150 – 250 mL of water. Additional water may be used if necessary. The sponge is attached to a length of suture material which passes out through the capsule. The suture is affixed to a retainer card which is held by the subject or administrator to prevent inadvertent swallowing of the suture. The string is to be held without tension as peristalsis and gravity advance the capsule into the stomach.

The capsule dissolves in the stomach, allowing the sponge to expand to its full size. Seven minutes and 30 seconds to ten minutes after ingestion, the sponge is then withdrawn by gentle traction on the suture, collecting cells from the lining of the esophagus in passing.

After retrieval, the string is cut and the retrieved foam sphere containing the cytological specimen is immersed in fixative and stored refrigerated (1° to 12°*C* [34° to 54°F]) until shipped. Samples will be shipped in batches to the Mayo Clini for initial processing, then batch shipped to the Fitzgerald lab in Cambridge for additional analysis. On arrival at the lab, the fixative is spun in a centrifuge, and the pelleted cells are embedded in paraffin using standard techniques. The blocks are sectioned and stained and processed with routine H&E staining.

If a subject fails to swallow the Cytosponge, the subject will be asked to swallow again. Subjects who are willing to try again will be asked to wait 5 minutes before the Cytosponge is presented to them again. Subjects will be able to try up to three times before they are classified as "Cytosponge swallowing failure" and discontinued by the investigator.

5.1.4 Routine Care Endoscopy with Biopsy

After Cytosponge administration is complete, subjects will undergo routine care upper endoscopy, with assessment of EoE, and biopsy. During this exam, endoscopic features of EoE will be recorded, including rings, furrows, white plaques, decreased vascularity, and strictures. The severity of the endoscopy findings will be measured using the recently validated EREFS scoring system.[23] Four esophageal biopsies will be taken both from the distal (5 cm above the gastro-esophageal junction) and proximal (15 cm above the gastro-esophageal junction) esophagus as part of their standard of care for EoE. The clinical tissue biopsies will undergo standard processing and H&E staining at the home institution, with assessment by expert gastrointestinal pathologists.

5.1.5 Adverse Event Assessment

Subjects should be assessed for any adverse events that occur before, during, or after Cytosponge administration. Only those events that are potentially related to participation in this research study must be reported to the lead site. See section 7.2 for the definition of a reportable adverse event. Sites are responsible for following local IRB/EC guidelines for reporting adverse events to their local IRB/EC.

5.2 Follow-Up Phone Call

Subjects will be contacted 7 days (+/- 2 days) following the Cytosponge and upper endoscopy procedures. During this phone call, adverse events will be assessed and subjects will complete questionnaires.

5.2.1 Assessments

The following data will be collected from subjects during the follow-up phone call:

- Visual Analog Scale
- Impact of Events Scale
- Procedure Preference and Acceptability Questionnaire
- Adverse event assessment
- Follow-Up Case Report Form (CRF): This captures relevant information for questionnaire completion and assessment of adverse events.

5.2.2 Impact of Events Scale

The impact of events scale will be completed with the subjected during the follow-up phone call and measures subjective distress related to administration of the Cytosponge.

5.2.3 Procedure Preference and Acceptability Questionnaire

The procedure preference and acceptability questionnaire will be completed with the subject during the follow-up phone call. This assessment collects subject preference for the Cytosponge or traditional upper endoscopy as well as willingness to undergo the procedure again.

5.2.4 Follow-up Phone Call Adverse Event Assessment

During the follow-up phone call, subjects should be assessed for any adverse events that have occurred since administration of the Cytosponge. Only those events that are potentially related to participation in this research study must be reported. See section 7.2 for the definition of a reportable adverse event. Sites are responsible for following local IRB/EC guidelines for reporting adverse events to their local IRB/EC.

5.3 Follow-Up Cytosponge Administration

After enrollment, if subjects receive treatment for their EoE and return to the enrolling site for an upper endoscopy as part of their standard of care, then subjects will be asked to repeat the Cytosponge procedure prior to each follow-up standard of care endoscopy.

Patients will be followed and tissue will be assessed over time with both Cytosponge and routine care endoscopy; samples will be taken at up to 6 time points.

5.3.1 Assessments

The following will be completed during each follow-up Cytosponge administration

- Re-affirmation of consent
- Cytosponge administration
- Visual Analog Scale
- Adverse event assessment
- Routine care upper endoscopy with biopsy
- Follow-Up Case Report Form (CRF): This captures demographics including race, ethnicity, gender, and year of birth, relevant EoE medical history since enrollment including documentation of endoscopic procedures received, pathology findings and EoE treatment(s) received since enrollment.

5.4 Follow-Up Phone Call

Subjects will be contacted 7 days (+/- 2 days) after the follow-up Cytosponge and upper endoscopy procedures. During this phone call, adverse events will be assessed and subjects will complete questionnaires. The same assessments are completed during the follow-up phone call following a follow-up Cytosponge administration as were completed during the initial Cytosponge administration. See section 5.2 for details.

5.5 Subject Participation Completion

Subject participation is considered complete when the subject no longer returns to the enrolling site for clinical care of their condition, or prior to that time if the subject withdraws for any reason (see section 4.4).

6 Statistical Plan

6.1 Sample Size Determination

With 60 subjects enrolled for Aim 1, we will be able to detect a correlation between the Cytosponge and endoscopy eosinophil counts as low as R = 0.40 with a power of 0.9 and an alpha of 0.05. This sample size would also have a power of 0.8 to detect an agreement (kappa) of as low as 0.7 (compared to a null of 0.4).

We will allow for enrollment up to 150 subjects across both sites to account for any potential issues with sample quality and additional safety assessments.

6.2 Statistical Methods

Aim 1: To test the hypothesis that the Cytosponge accurately quantifies esophageal eosinophil counts in patients with active EoE, the maximum eosinophil counts from the Cytosponge and endoscopic biopsy will be compared by calculating the Pearson coefficient of correlation. In addition, patients will be categorized as having an eosinophil count either above or below 15 eos/hpf (the current diagnostic threshold). Agreement between patients above and below this threshold for Cytopsponge and endoscopic biopsy will be measured using the kappa statistic. If eosinophil counts are not normally distributed, non-parametric methods will be used. Separate analyses will be performed for patients with active EoE and patients undergoing treatment.

Aim 2: Summary statistics will be calculated to describe adverse events and the tolerability of the Cytosponge. The proportion of subjects who prefer the Cytosponge will be calculated.

We predict that the Cytosponge will be safe and well-tolerated in patients with EoE, and that they will prefer this minimally invasive method to endoscopy with biopsy. We also predict that the eosinophil counts obtained with Cytosponge will strongly correlate with the counts from endoscopy with biopsy in both patients with active EoE.

6.3 Subject Population for Analysis

The population whose data will be subjected to the study analysis will include all subjects enrolled in this study that completed the Cytosponge administration and have pathology results available from the subsequent routine care endoscopy with biopsy.

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following completion of the biomarker panel.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 Reportable Adverse Events

For this study, only those events that are related to participation in the study must be reported. This includes events related to:

- Cytosponge administration
- Questionnaire administration

- Any adverse event that may be **related** to participation in this study or use of the Cytosponge device (possibly, probably, or definitely related)
- Any adverse event in which the Cytosponge may **have caused or contributed** to the event.
- Any event required to be reported to the FDA and/or manufacturer per 21CFR803 including:
    - Device-related deaths;
    - Device-related serious injuries.

****Deaths due to the expected progression of disease do not need to be reported as adverse events but should be reported as an outcome for the patient.*

7.2.1 Reporting Timeline

Serious adverse events (meeting the definition of a reportable AE) or unanticipated problems involving risk to subjects or others must be reported within **24 hours** of learning of the event. To report such events, sites must complete the Reportable Event case report form. In addition to completion of the CRF, sites must also fax or email the form to +1 919 843-2508 or cedas@med.unc.edu. If for any reason the form cannot be completed within 24 hours, a phone call should be made to the lead site +1 919 966-7655 to meet the reporting timeline. In the case of a telephone report, sites must still complete the reportable event form at the earliest possible opportunity, and no later than 72 hours following learning of the event.

All other reportable events should be reported within **15 days** of learning of the event.

7.2.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events possibly related to the study that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study procedures or study participation should be recorded and reported immediately.

7.2.3 Reporting Adverse Events to Lead Site

All adverse events that meet the criteria of a “reportable” adverse event as described in section 7.2 above must be reported by completing the reportable event form and entering in the study database.

All events will be reported using the National Cancer Institute’s Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A quick reference guide can be accessed from the following website: http://www.acrin.org/Portals/0/Administration/Regulatory/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf

7.2.3.1 Initial Report

If a patient experiences an event that should be reported as described in section 7.2 the site should complete and submit a reportable event form.

If a patient experiences more than one event, sites should report each event using a separate reportable event form.

7.2.3.2 Follow-Up Reports

All reported adverse events should be followed until resolved or a reason documented if resolution will not occur. Any new information or updates to a previously reported event should be reported as a follow-up

to the event. To report a follow-up to an event, sites will update the previously completed electronic reportable event CRF.

7.2.4 Reporting Adverse Events to Local EC/IRBs

Investigators must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible. All investigators are responsible for safety reporting to their local institutional review board (IRB) or ethics committee (EC). Investigators are responsible for complying with their local EC/IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's files. The definition of a reportable event for a local EC/IRB may not be the same as the definition used for this pilot study.

7.2.5 Notifying the FDA

The facility/institution and device manufacturer are required to report events to the FDA as defined per 21CFR803 (medical device reporting).

1. *Reports of death*. Facilities must submit a report to the FDA as soon as practicable but no more than 10 work days after the day that you become aware of information, from any source, that reasonably suggests that the device has or may have caused or contributed to the death of a patient of your facility. Facilities and institutions must report the following to the FDA via an FDA MEDWATCH Form 3500A (this form can be completed on paper or submitted electronically. You may obtain this form from http://www.fda.gov/medwatch/getforms.htm.
2. *Reports of serious injury*. Facilities must submit a report to the manufacturer of the device no later than 10 work days after the day that you become aware of information, from any source, that reasonably suggests that a device has or may have caused or contributed to a serious injury to a patient of your facility.

Facilities and manufacturers are responsible for all other FDA reporting requirement per 21CFR803 including semi-annual reporting. For additional guidance on reporting to the FDA including where to send reports please visit:
http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

To review 21CFR803 please visit:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=803&showFR=1

7.2.6 Lead Site Reporting to Participating Investigators

It is the responsibility of the lead site (UNC) to notify all participating investigators of any adverse event associated with the study that is both serious and unexpected.

7.3 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Case Report Forms

This study will utilize paper case report forms (CRFs). The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, this should be documented in the comments field.

8.3.1 Case Report Form Completion Table

Form	Screening/Enrollment	Follow-Up	PRN (As Needed)
Enrollment CRF	X		
Follow-Up CRF		X	
Reportable Event CRF			X
Change of Status CRF			X

8.4 Records Retention

The data compiled in this study will be stored for a period of at least 2 years following study termination.

9 Study Monitoring, Auditing, and Inspecting

9.1 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC, IRB, the sponsor, the lead site, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted according to the International Conference on Harmonisation Good Clinical Practice (ICH/GCP) as well as US federal regulations (21 CFR parts 803, 812, and 45CFR46), as well as all applicable local and state government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor-investigator, Dr. Evan Dellon, before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. Sample consent forms will be provided by the lead site. These consent forms include a consent for the study as well as a consent for storage of samples for future use. All consent forms will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent forms must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

This study is funded by the CURED (Campaign Urging Research for Eosinophilic Disease) Foundation.

11.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of North Carolina investigators will follow the University conflict of interest policy.

12 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the lead investigator, Dr. Evan Dellon. Any investigator involved with this study is obligated to provide the lead investigator with complete test results and all data derived from the study.

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