Medtronic

Statistical Analysis Plan

Clinical Investigation Plan Title	ASSESSMENT OF A MINIMALLY INVASIVE ESOPHAGEAL CYTOLOGY COLLECTION SYSTEM IN PATIENTS WITH BARRETT'S ESOPHAGUS OR GERD SYMPTOMS (CASE II)
Clinical Investigation Plan Identifier	COVB2710467
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Sponsor/Local Sponsor	Medtronic MITG RGI Gastrointestinal & Hepatology 540 Oakmead Parkway, Sunnyvale, CA 94085
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1 Version History

Version	Summary of Changes	Author(s)/Title
Draft 1.0	• First draft, new document	Haiying Lin
1.0	• Updated to reflect the protocol revision(s) (i.e. adding interim analysis) • Revised as part of global SOP harmonization activity	Haiying Lin
2.0	• Migrated to new template version A • Section 4 table updated to align with CIP Rev G • Updated to reflect change of sample size from 120 to 275	Alex Shih

2 List of Abbreviations and Definitions of Terms

Abbreviation	Definition
AE	Adverse event
BE	Barrett's esophagus
C1	One circumferential centimeter of BE
eCRF	Electronic case report form
EAC	Esophageal adenocarcinoma
EC	Ethics committee
EGD	Esophagogastroduodenoscopy
GEJ	Gastroesophageal junction
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
H&E	Hematoxylin and eosin
HGD	High-grade dysplasia
HIPAA	Health Insurance Portability and Accountability
IRB	Institutional review board
LGD	Low-grade dysplasia
M3	A total BE segment length of at least three centimeters
MedDRA	Medical dictionary for regulatory activities
PDT	Photodynamic therapy
PHI	Protected health information
RFA	Radiofrequency ablation
SAE	Serious adverse event
TFF3	Trefoil factor 3
TGF	Top of gastric folds
VAS	Visual analog scale

- For baseline indicator variables, "unknown" responses will be counted as not having the characteristic and will be included in the denominator. Missing values will not be counted in rate denominators.
- Days to (event) = (event) date – procedure date.
- Definitions and formulas for calculated Impact of Events Scale, Intrusion and Avoidance can be found at the Horowitz,M.,Wilner,N. & Alvarez,W. Impact of event scale: a measure of subjective stress . Psychosom Med 1979; 41: 209-18 document.

3 Introduction

This statistical analysis plan describes the planned statistical analysis to support the interim analysis and final clinical study report (CSR) for the B-271 Cytosponge study based on the clinical investigational Plan (CIP), dated 1 December 2017, version G.

4 Clinical Investigation Plan Summary

Title	ASSESSMENT OF A MINIMALLY INVASIVE ESOPHAGEAL CYTOLOGY COLLECTION SYSTEM IN PATIENTS WITH BARRETT'S ESOPHAGUS OR GERD SYMPTOMS (CASE II)
Clinical Study Type	U.S. Pilot Study
Product Name	Cytosponge™ Cell Collection Device
Sponsor	Medtronic MITG RGI Gastrointestinal & Hepatology 540 Oakmead Parkway, Sunnyvale, CA 94085 Attn: Shirin R. Hasan, Director, Global Clinical Affairs
Indication under investigation	Post-market approval
Investigation Purpose	To assess the utility of the Cytosponge device as a non-endoscopic method for collecting surface cells from the esophagus in patients with BE and GERD
Product Status	510(k) clearance
Primary Objective(s)	• To assess the acceptability of a novel, minimally invasive esophageal mucosal sampling technique, the Cytosponge 1) in subjects undergoing surveillance of BE who have had at least a C1 or M3 segment confirmed (or medically suspected), and 2) in subjects with GERD undergoing screening for BE Patients who are undergoing ablative therapy on the day of Cytosponge testing will have their acceptability data analyzed separately. Based on previous data, we hypothesize that the sponge-based sampling technique will be associated with low levels of subject distress, and will be preferred by subjects, when

	compared to standard sedated upper endoscopy, for surveillance of their esophageal mucosa. *1)* To assess the adequacy of cytology samples obtained by Cytosponge in this population after 1 sampling, or after 2 samplings if first sample is inadequate. Based on previous data, we hypothesize that the Cytosponge will harvest adequate amounts of esophageal cells to perform centrifugation, pelleting, sectioning and staining for trefoil factor 3, a reliable biomarker of intestinal metaplasia.
Secondary Objective(s)	1) To assess the operating characteristics of this technique against a gold standard of upper endoscopy with biopsies for endoscopic surveillance in subjects with BE who demonstrate an adequate sample on Cytosponge assessment. 2) To assess the operating characteristics of Cytosponge against the worst ever histology documented in the subject. Patients who are undergoing ablative therapy on the day of Cytosponge testing must have biopsies within 2 months prior to surgery. *Because Cytosponge provides a "field" sampling of esophageal tissue, it may provide a more comprehensive assessment of minute fields of dysplasia. If this is so, the Cytosponge may avoid sampling error associated with random clinical samples as currently performed.* 3) To assess the operating characteristics of Cytosponge on the basis of baseline histology. *To date, no data is available regarding the accuracy of Cytosponge in subjects with BE and more advanced disease (low-grade dysplasia and high-grade dysplasia). These subjects are at greatest risk for progression to cancer. We plan to collect pilot data on operating characteristics of the assay by degree of baseline dysplasia. We hypothesize that Cytosponge will perform with similar operating characteristics in this group compared to non-dysplastic BE.* 4) To assess the degree of mucosal abrasion following Cytosponge administration, using a standardized scale. *Based on previous data, we hypothesize that mucosal damage due to abrasion by the Cytosponge will be minor. Further, the distal extent of the abrasion will correlate with the presence of columnar cells in the sample.*

	5) To collect and analyze safety measures of Cytosponge use in the target population. *The Cytosponge has been found to be safe and well-tolerated when administered in a primary care setting. We will continue collecting safety data as part of this project. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).*
Study Design	Multicenter, cross-sectional clinical trial
Sample Size	Up to 275 subjects with an enrollment ratio of at least ≥50% BE and ≥25% GERD
Inclusion/Exclusion Criteria	Inclusion Criteria: 1) Male or female subjects, age 18 and above. 2) Able to read, comprehend, and complete the consent form. 3) Clinically fit for an endoscopy. 4) a) Previous confirmed diagnosis of Barrett's esophagus with intestinal metaplasia, and Prague classification of at least one circumferential centimeter of BE or a total BE segment length of at least 3 centimeters (C1+ or CXM3+) (BE arm) . OR b) If the subject does not have documented Prague Classification prior to screening, but the PI is convinced that the subject will meet the inclusion criteria based on previous documentation (for instance, mention of "long-segment BE," they may enroll the subject in the study at their discretion. The study upper endoscopy must confirm that the subject has C1+ or CXM3+ (BE arm). If (C1+ or CXM3+) is not observed at the time of study endoscopy, the subject may still be enrolled but not included in the data analysis with the BE cohort. The data may be analyzed in a separate cohort. OR c) Self-reported heartburn or regurgitation on at least a monthly basis for at least 6 months (GERD arm). Exclusion Criteria: 1) Individuals with a diagnosis of an oropharynx, esophageal or gastro-esophageal tumor, or symptoms of dysphagia. 2) Any history of esophageal varices, stricture, or prior dilation of the esophagus. 3) Current use of anti-thrombotics (anti-coagulants and anti-platelet drugs) that cannot be safely discontinued for the appropriate drug-specific interval in the peri-administration period. Depending on the particular agent or reason for the anti-thrombotic therapy, it may not be necessary to discontinue anti-thrombotic agents. There could be

	circumstances where the drug may not need to be discontinued if the risk of bleeding is considered negligible (e.g. daily aspirin therapy). Physicians should use their clinical judgement and should consult guidelines such as those provided by the ASGE. 4) Known bleeding disorder. 5) Individuals who have had a myocardial infarction or any cardiac event < 6 months prior to enrollment. 6) Individuals who have had a cerebrovascular event < 6 months prior to enrollment in which swallowing was affected. 7) Prior ablative or resection therapy of the esophagus including radiofrequency ablation (RFA), photodynamic therapy (PDT), spray cryotherapy, endoscopic mucosal resection, and other ablation therapies. 8) Any history of esophageal surgery, except for uncomplicated fundoplication. 9) Do not need upper endoscopy as part of patient management. 10) Pregnancy
Study Procedures and Assessments	This is a cross-sectional study of subjects with Barrett's esophagus (BE) to assess the utility of the Cytosponge device as a non-endoscopic method for collecting surface cells from the esophagus. This study will enroll 2 groups of subjects: 1) Subjects presenting for routine endoscopic BE surveillance examinations or planned ablation 2) Subjects with gastroesophageal reflux disease (GERD) symptoms undergoing upper endoscopy for screening for BE. After informed consent, and on the same day as the endoscopic procedure, the subject will undergo administration of the Cytosponge device and complete a questionnaire and a visual analog pain scale (VASThe subject will then undergo routine upper endoscopy, with assessment of BE (where applicable), and routine care biopsies will be taken per accepted surveillance or screening recommendations by performing physician. Photographs of the distal esophagus are encouraged to be taken. For subjects presenting for GERD, a single set of 4 quadrant biopsies (4 pieces of tissue total) of the gastric cardia (TGF+1) will be collected during the endoscopy procedure. The Cytosponge will be placed in fixative and shipped to an accredited pathology laboratory for embedding in paraffin and hematoxylin and eosin (H&E) staining

	to assess the adequacy of the specimen. Further evaluation of the specimen will be performed using trefoil factor 3 (TFF3). If the Cytosponge tissue specimen is inadequate, the subject will be recalled for a repeat sponge procedure. All study-specific gastric cardia biopsies, as well as any routine care tissue biopsies will undergo standard processing and H&E staining at the home institution, with assessment by gastrointestinal pathologists. Subjects will be contacted via phone 7 days (+/-3 days) after Cytosponge administration to complete additional questionnaire and assess adverse events.
Safety Assessments	Adverse events will be reported by number, severity, and relationship to the study procedures and devices. The Medical Monitor will adjudicate all serious adverse events, unanticipated adverse device effects, and events determined by the reporting investigators to be related to the device (possible, probable, definite and unable to be determined).
Statistics	For the first primary objective, to assess the acceptability of the Cytosponge in subjects with BE, we will assess the distribution of Impact of Event Scale scores, and the intrusiveness and avoidance subscales. We will generate measures of central tendency and distribution of these data. Bivariate analysis will be performed to assess predictors of low tolerance of Cytosponge surveillance, and a logistic regression model will be created to assess these factors while controlling for potential confounders. Data will be compared to population norms in published literature[1] using parametric statistics. Visual analog scale (VAS) scores will be calculated, and measures of central tendency and distribution reported. Subjects' preferences for Cytosponge versus endoscopic surveillance, as well as willingness to undergo the procedure again, will be measured as proportions, with bivariate and multivariate analyses for predictors of preference performed. Subjects who undergo same day ablative therapy will Impact of Event Scale scores evaluated separately. For the second primary objective, to assess the adequacy of samples, sample adequacy will be treated as a dichotomous variable. For purposes of this investigation, an adequate sample will be one in which at least 1 columnar cell is present. Sample adequacy will be presented as a proportion of subjects who fulfill this definition after

	up to 2 total administrations of the Cytosponge, as noted in the methods. For the first three secondary objectives, to assess the operating characteristics of Cytosponge against various gold standards, initially 2x2 tables will be constructed demonstrating Cytosponge and the gold standard findings (Y/N for BE). Both endoscopic evidence of BE of C1 or CXM3 or greater length and pathological confirmation of specialized IM (intestinal metaplasia with goblet cells) will be used in the final diagnosis of BE. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy will be calculated. Because Cytosponge positivity may vary based on the burden of BE, we will perform sensitivity analyses, defining "positive" cases as those with BE of ≥2 cm in length, and then ≥3 cm in length, to assess impact of disease burden on operating characteristics. Multivariate models controlling for age, sex, burden of disease, and other potential confounders will be constructed, to assess the impact of these factors on test accuracy. Although we do not expect to see an association between the degree of dysplasia and Cytosponge positivity, exploratory analyses will be performed using degree of dysplasia as a predictor variable, and Cytosponge positivity as the outcome variable. For the fourth secondary objective, to assess the degree of mucosal abrasion following Cytosponge, endoscopic abrasion scores will be correlated with the presence of columnar cells on the H&E slide made from the Cytosponge samples. To assess safety (fifth secondary objective), the number and percentage of subjects with adverse events related to Cytosponge administration will be summarized by MedDRA system organ class and preferred term overall, and by severity.

5 Determination of Sample Size

This study will enroll up to 275 subjects with an enrollment ratio of at least ≥50% BE and ≥25% GERD. While the operating characteristics of Cytosponge in our patient population are unknown, the numbers for this post-market study were selected for several reasons:

- Approximately 50% of the patient population at the involved tertiary care centers have dysplastic BE (25% low-grade dysplasia, 25% high-grade dysplasia), so assessment of 275 patients should allow an adequate number of subjects with dysplastic disease to assess performance characteristics with reasonable accuracy.
- The study will observe an enrollment ratio of at least ≥50% BE and ≥25% GERD.
- For the safety perspective, with 275 subjects, the probability of observing at least one rare event with a true event rate of 5% is more than 99%. For an event rate as rare as 1%, the probability is still greater than 93%.

6 Statistical Methods

6.1 Study Subjects

6.1.1 Disposition of Subjects

Subject disposition (e.g., number completing the study, number lost-to-follow-up) will be summarized with frequency tables. For subjects exiting the trial, the reason for termination will be presented. For the B-271 Cytosponge study, there is only one planned follow-up at 7 days (+/- 3 days) following the Cytosponge and upper endoscopy procedures.

6.1.2 Clinical Investigation Plan (CIP) Deviations

Discuss potential CIP deviations or violations and how they will be reported.

6.1.3 Analysis Sets

Subjects will be considered enrolled in the study once it has been confirmed that they meet all the inclusion and none of exclusion criteria. Unless otherwise specified, analysis of reported outcomes will include all available data for all subjects enrolled.

6.2 General Methodology

All statistical analyses were performed using SAS statistical software. Descriptive statistics were provided for all variables summarized. The statistics for continuous variables may have included mean, standard deviation, minimum, maximum and the number of observations (N). Categorical variables are described with frequencies and percentages.

6.3 Handling of Missing, Unused, and Spurious Data and Dropouts

No imputation or other adjustment techniques are planned for the missing data to be included in the analyses. All data will be included unless judged to be invalid.

6.4 Demographic and Other Baseline Characteristics

The baseline characteristics, including age at time of procedure, gender, race, ethnicity, weight, height, smoking history, alcohol assumption, and baseline histology of BE and GERD symptoms subjects, will be

summarized. Discrete variables will be presented using frequency distributions and cross tabulations. For continuous variables, statistics will include the number of observations (N), mean, standard deviation, median, minimum, and maximum values.

6.5 Interim Analyses

In an effort to understand the sensitivity and specificity of the test, an interim analysis of the data, based on the statistical methodology outlined above, may be performed after approximately half the study subjects have primary outcomes.

6.6 Evaluation of Objectives

6.6.1 Primary Objectives

The following 2 primary objectives will be assessed:

6.6.1.1 Primary Objective 1

To assess the acceptability of a novel, minimally invasive esophageal mucosal sampling technique, the Cytosponge, in subjects undergoing surveillance of BE, and 2) in subjects with GERD symptoms undergoing screening for BE. Based on previous data, it is expected that the sponge-based sampling technique will be associated with low levels of subject distress, and will be preferred by subjects, when compared to standard sedated upper endoscopy.

Acceptability of the Cytosponge

The acceptability of the Cytosponge will be measured using impact of event scale, a visual analog scale. The scale yields two scores assessing intrusive and avoidance thoughts. Data will be compared to population norms in published literature[1]. The VAS scores for pain will be calculated as well.

We will measure the central tendency and distribution of impact of event scale scores, as well as the intrusiveness and avoidance subscales. Bivariate analysis will be performed to assess predictors of low tolerance of Cytosponge surveillance, and a logistic regression model will be created to assess these factors while controlling for potential confounders.

Procedure preference and acceptability

Subjects' preferences for Cytosponge versus endoscopic surveillance, as well as willingness to undergo the procedure again, will be summarized with frequency and percentages. Univariate and multivariate logistic regression analyses for predictors of the procedure preference will be performed.

6.6.1.1.1 Primary Objective 2

To assess the adequacy of cytology samples obtained by Cytosponge after 1 sampling, and after 2 samplings if first sample inadequate. Based on previous data, it is expected that the Cytosponge will harvest adequate amounts of esophageal cells to perform pelleting, centrifugation sectioning and

staining for TFF3, a reliable biomarker of intestinal metaplasia. For purposes of this investigation, an adequate sample will be one in which at least one columnar cell on H&E staining is present. If the initial administration of the Cytosponge demonstrates an inadequate sample, repeat Cytosponge administration will be performed at 30 days (+/- 10 days) from the initial administration.

Sample Adequacy

Sample adequacy will be treated as a binary variable. It will be presented as a proportion of subjects who fulfill this definition after up to two total administrations of the Cytosponge, as noted in the methods.

All individuals offered the Cytosponge will be included in analyses of acceptability and adequacy of sampling.

6.6.1.1.2 Secondary Objectives

6.6.1.1.3 Secondary Objective 1

To assess the operating characteristics of Cytosponge against a gold standard of upper endoscopy with biopsies for endoscopic surveillance in subjects with BE who demonstrate an adequate sample on Cytosponge assessment. It is expected that the assay will demonstrate both a sensitivity and specificity of > 90% in the detection of BE. Further, we expect higher accuracy in those with a larger burden of disease.

6.6.1.1.4 Secondary Objective 2

To assess the operating characteristics of Cytosponge against the worst ever histology documented in the subject.

6.6.1.1.5 Secondary Objective 3

To assess the operating characteristics of Cytosponge as a function of baseline histology. To date, no data are available regarding the yield of Cytosponge in Subjects with BE and more advanced disease (low-grade dysplasia and high-grade dysplasia). These subjects are at greatest risk for progression to cancer. We plan to collect pilot data on operating characteristics of the assay by degree of baseline dysplasia. It is expected that Cytosponge will perform with similar operating characteristics in this group compared to non-dysplastic BE.

For the secondary objective 1, 2 and 3, to assess the operating characteristics of Cytosponge against various gold standards, two-by-two tables will be constructed demonstrating Cytosponge and the gold standard findings (Y/N for BE). Sensitivity, specificity, positive predictive value, negative predictive value and accuracy will be calculated.

6.6.1.1.6 Secondary Objective 4

To assess the degree of mucosal abrasion following Cytosponge administration, endoscopic abrasion score, a standardized scale, will be calculated. The distal extend of cells collected by recording the location of abrasions as well as their grade. Based on previous data, it is expected that mucosal damage due to abrasion by the Cytosponge will be minor. Further, the distal extent of the abrasion will correlate with the presence of columnar cells on the H&E slide made from Cytosponge samples in the sample.

6.6.1.1.7 Secondary Objective 5

To assess safety, the number and percentage of subjects with adverse events (AEs) related to Cytosponge administration will be summarized by MedDRA system organ class and preferred term overall, and by severity.

6.7 Safety Evaluation

To assess safety, the number and percentage of subjects with adverse events related to Cytosponge administration will be summarized by MedDRA system organ class and preferred term, and by severity.

7 Validation Requirements

Level II validations (i.e. the peer reviewer reviews the code; where appropriate, performs manual calculations or simple programming checks to verify the output) will be performed for analysis outputs.

8 References

1. Briere J, Elliot DM. Clinical Utility of the Impact of Event Scale: Psychometrics in the General Population. Assessment. 1998;5(2):171–180.