EFFECTIVENESS, EFFICIENCY, AND PERFORMANCE OF A MOBILE, AUTOMATED, OPTICAL BIOPSY TECHNOLOGY FOR ESOPHAGEAL CANCER SCREENING: A CLINICAL STUDY IN BRAZIL AND THE UNITED STATES

Short Title: AI-mHRME in Brazil and the United States for Esophageal Cancer Screening

Protocol Number: H-53483

National Clinical Trial (NCT) Identified Number: NCT06435286

Principal Investigator: Mimi Tan, MD, MPH

IND Sponsor: Mimi Tan, MD, MPH

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Title page	Changed the Principal Investigator, IND Sponsor to Mimi Tan	New Sponsor-Investigator for IND
5 Study Population, 5.5 Strategies for Recruitment and Retention	Changed the information that the research population will be identified by Dr. Mimi Tan at both Baylor St. Luke's Medical Center (BSLMC) and at Ben Taub Hospital (BTH)	Mimi Tan is the new Baylor College of Medicine Principal Investigator
10.1.5 Key Roles and Study Governance	Changed the Baylor College of Medicine Principal Investigator and corresponding contact information to Dr. Mimi Tan	Mimi Tan is the new Baylor College of Medicine Principal Investigator

10.1.7 Clinical Monitoring	**Changed the Baylor College of Medicine Principal Investigator for clinical monitoring to Dr. Mimi Tan**	**Mimi Tan is the new Baylor College of Medicine Principal Investigator**
10.1.11 Publication and Data Sharing Policy	**Changed the Baylor College of Medicine Principal Investigator to Dr. Mimi Tan**	**Mimi Tan is the new Baylor College of Medicine Principal Investigator**

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the National Cancer Institute's (NCI) Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. The protocol and the consent form must be approved before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

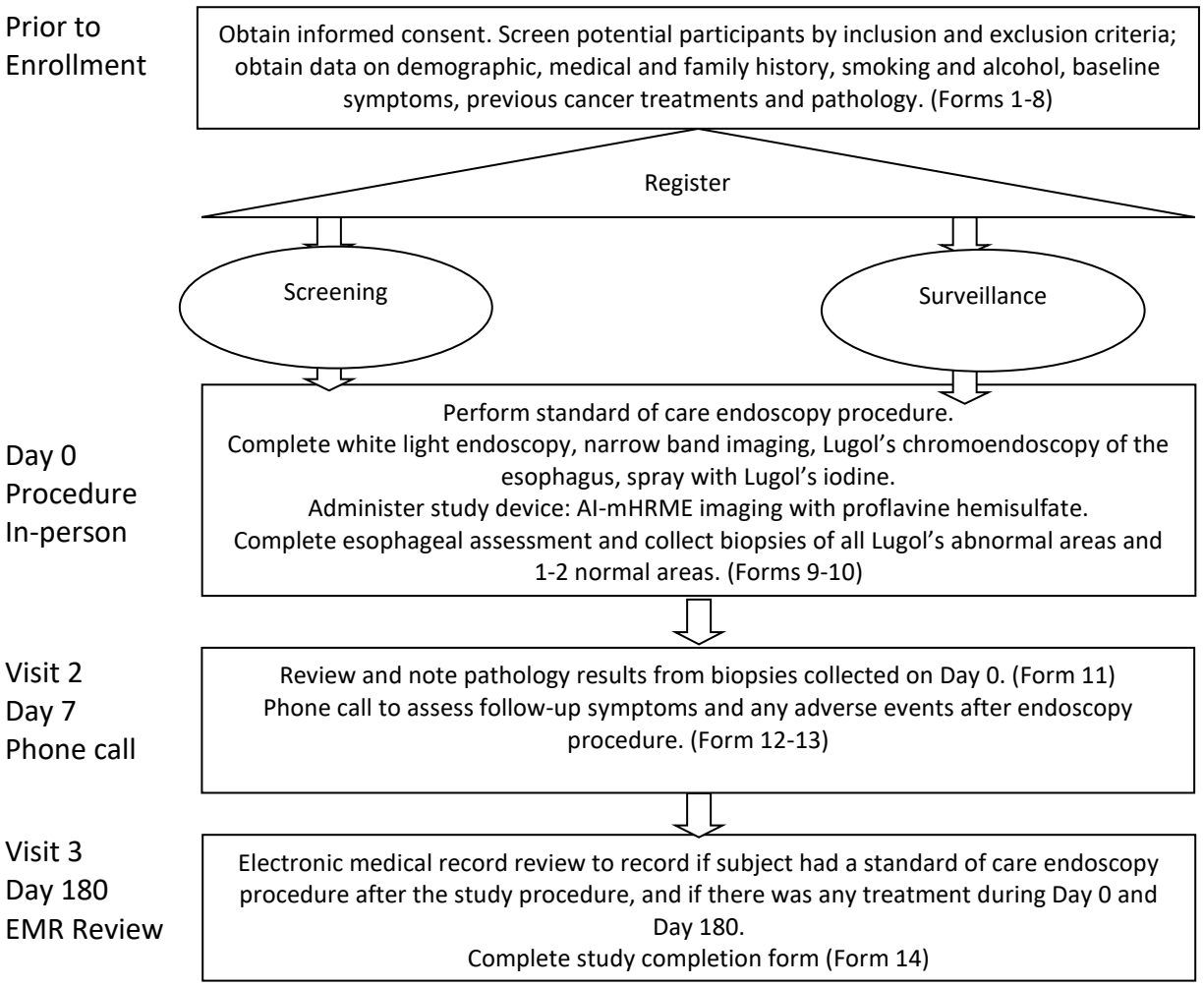
1.1 SYNOPSIS

Title:	Effectiveness, Efficiency, and Performance of a Mobile, Automated, Optical Biopsy Technology for Esophageal Cancer Screening: A Clinical Study in Brazil and the United States
Study Description:	We will develop and evaluate the diagnostic performance, efficiency, and impact of the computer-assisted AI-based HRME in diverse clinical environments in the US and Brazil to facilitate implementation. The main hypothesis of the research study is that AI-mHRME will increase LCE accuracy. Additional exploratory hypotheses include that AI-mHRME will (1) increase AI-mHRME accuracy in novice endoscopists and be non-inferior to expert endoscopists, (2) increase user confidence among expert and novice endoscopists, and (3) increase LCE efficiency and impact by reducing biopsies and second procedures.
Objectives:	**Primary Objective**: To evaluate the specificity among novices using AI-mHRME during esophageal cancer screening and surveillance. Our goal is to increase the specificity among novices using AI-mHRME without significant reduction in sensitivity. **Secondary Objectives**: (A) To assess the diagnostic performance of AI-mHRME; (B) To evaluate provider confidence (experts and novices) in clinically interpreting mHRME (pre- and post-use of AI-mHRME); (C) To assess the clinical impact following AI-mHRME;

	(D) To determine the diagnostic yield of AI-mHRME (E) To determine procedure efficiency of AI-mHRME, including subjects saved any biopsy and biopsy efficiency.
Endpoints:	Primary Endpoint: Specificity (SP) of AI-mHRME in novices Secondary Endpoints: (A) Sensitivity, specificity, positive predictive value, negative predictive value (SN, SP,PPV, NPV) of AI-mHRME; (B) Provider (experts and novices) reported confidence level (low or high) in pre- and post- use of AI-mHRME; (C) Change in clinical plan (biopsy vs. no biopsy vs. treat) post-AI mHRME; (D) Truly neoplastic biopsies/total biopsies obtained; (E) Subjects saved any biopsy: number of patients correctly saved any biopsy; Biopsy efficiency: the total number of biopsies saved.
Study Population:	*Specify the sample size, gender, age, demographic group, general health status, and geographic location.* We will enroll 200 subjects. Subjects will be patients for ESCN screening or surveillance who meet inclusion criteria and consent to study participation. Subjects must be 18 years or older, of any gender, race and ethnicity, categorized as ESCN screening or ESCN surveillance, and present to the endoscopy units in Houston, Texas, and Barretos and Sao Paulo, Brazil.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	Baylor St. Luke's Medical Center (private outpatient clinic), Houston, Texas Ben Taub Hospital, Harris Health System (public hospital), Houston, Texas Instituto do Câncer do Estado de São Paulo (public tertiary care hospital), São Paulo, Brazil Hospital de Amor (non-profit tertiary care hospital), Barretos, Brazil
Description of Study Intervention:	High-resolution microendoscope (HRME): A fiber-optic fluorescence microscope intended for *in vivo* imaging of epithelial cell nuclei. The primary optical components are a blue light-emitting diode, excitation filter, dichroic mirror, objective lens, fiber-optic probe, tube lens, emission filter, and high-frame-rate camera sensor. The HRME system includes a laptop computer and user interface/software. Proflavine hemisulfate: 10 ml or 10 cc of sterile 0.01% w/v proflavine hemisulfate solution administered topically using spray cathether onto the esophageal lining through the endoscope biopsy channel during endoscopy.
Study Duration:	3 years / 36 months
Participant Duration:	7 days

1.2 SCHEMA

Esophageal AI-mHRME Study Flow diagram



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Study Procedure, Day 0	Phone Call Day 7 ± 1 day	Medical Record Review Day 180 ± 7 days
Form No. 1: Study Screening	X		
Form No. 2: Study Eligibility Criteria	X		
Registration	X		
Form No. 3: General Subject Information	X		
Form No. 4: Medical history	X		
Form No. 5: Smoking History	X		
Form No. 6: Alcohol History	X		
Form No. 7: Pathology Review	X		
Form No. 8: Baseline Symptoms	X		
Administer study intervention (AI-mHRME and proflavine)	X		
Form No. 9: Endoscopic Assessment	X		
Form No. 10: NBI, Lugol's, and HRME Interpretations and Biopsy Collection	X		
Collect pathology results		X	
Form No. 11: Local and Central Pathology Read		X	
Call participant for follow-up		X	
Form No. 12: 7-Day Follow-Up Symptoms		X	
Form No. 13: Additional Adverse Event Questions		X	
Form No. 14: Study Completion		X	
Form No. 15: 180 Day Follow-Up			X

2 INTRODUCTION

2.1 STUDY RATIONALE

Esophageal cancer is the 6th most common cause of cancer-related mortality worldwide. While esophageal squamous cell neoplasia (ESCN) carries a significant global burden, those in certain underserved geographic regions (South America, eastern Africa, eastern Iran, and northern China) have exceptionally high incidence and mortality rates due to a lack of endoscopic screening capacity. While endoscopy with Lugol's chromoendoscopy or "digital" chromoendoscopy has shown high sensitivity (>95%) for screening, specificity is poor (<60%), and false-positive results abound due to confounding inflammatory areas. As a result, the standard-of-care endoscopy produces many unnecessary biopsies, increasing the risk and cost of endoscopic screening and surveillance.

In our ongoing R01 project, we developed and validated a mobile, high-resolution microendoscope (mHRME) for screening and surveillance of ESCN. Despite over two years of COVID-19 delays, which significantly impacted the Chinese sites, we completed (1) a randomized, controlled clinical trial (USA and China; n=916) of mHRME with visual interpretation in patients undergoing screening or surveillance for

ESCN, (2) deep-learning software algorithms for automated detection of neoplastic images, and (3) a pilot trial (n=41) of the software-assisted mHRME in Brazil in January 2022. The trial revealed higher specificity for qualitative (visual) interpretation by experts but not novices in the surveillance arm (100% vs. 19%, p<0.05). In the screening arm, diagnostic yield (neoplastic biopsies/total biopsies) increased 3.6 times (8 to 29%); 16% of patients were correctly spared any biopsy, and 18% had a change in clinical plan. In a single-arm pilot study, we evaluated an artificial intelligence-based mobile HRME (AI-mHRME) in 41 Brazilian participants. This pilot study in January 2022 confirmed that quantitative interpretation (AI-mHRME) doubled diagnostic yield, improved endoscopists' confidence, and had a significant clinical impact (change of clinical plan in 64%).

This study will build on this valuable global data to *optimize an AI-mHRME and evaluate its clinical impact and implementation potential* in the USA and Brazil's ethnically and socioeconomically diverse populations.

2.2 BACKGROUND

ESCN Worldwide: Esophageal cancer is the 6th most common cause of cancer-related mortality worldwide.[1] Despite advances in chemoradiation therapy, 5-year survival remains <15% due to diagnosis at an advanced, incurable stage.[1] Indeed, survival drops markedly once the tumor has breached the mucosal layer, reinforcing the importance of early detection.[2] While the disease carries a significant global burden (including the USA), specific geographic areas (South America, Iran, China) have exceptionally high incidence rates. The reasons for this are multifactorial (smoking, alcohol, low socioeconomic status, thermal injury/hot beverages, nutritional deficiency).[3] In Brazil, age-standardized incidence rates are as high as 9.2 per 100,000 people.[4] Endoscopic screening and surveillance protocols have been implemented with limited success, and the incidence-to-mortality ratio remains a dismal 1:1.[1] In 2019 alone, the WHO reported over 8,000 deaths due to esophageal cancer in Brazil.[4,5]

Current Screening for ESCN and Its Limitations: At present, the 'gold standard' endoscopic evaluation for squamous neoplasia involves Lugol's iodine staining (Lugol's chromoendoscopy, LCE) of the esophagus with targeted biopsy of unstained (abnormal) areas. Screening is performed in high-risk patients, e.g., those with a history of head and neck cancer, positive balloon cytology, or living in high-prevalence global areas.[6,7] While LCE is the most sensitive (>97%) method for ESCN detection, specificity is poor (<60%);[8,9] areas of inflammation appear unstained and indistinguishable from cancer, leading to high false-positive rates. As a result, patients undergo multiple unnecessary biopsies with subsequent increases in cost and risk. Narrow-band imaging (NBI) has been proposed as a 'red flag' alternative to LCE that does not require a dye. While the sensitivity of this method (90.5%)[10] has approached that of LCE (>97%), more extensive trials are needed, and the technology is not available in many centers. LCE remains the gold standard for esophageal screening but is limited by its high false-positive rates.

Confocal microendoscopy, a high-resolution imaging technique that evaluates the mucosa at a 1 µm resolution and 1100X magnification, has revolutionized the endoscopic surveillance of cancer by allowing endoscopists to see neoplasia at a subcellular level.[11] When confocal microscopy is coupled with wide-field LCE, accuracy rates reach 95% with marked improvement in specificity.[12] Nonetheless, the technology is expensive ($150,000-$250,000) and requires considerable infrastructure and intravenous contrast injection. Lastly, the interpretation of images calls for a *high level of user training and expertise*. As such, confocal platforms are confined to tertiary centers in high-resource settings.

Artificial Intelligence (AI) and Esophageal Cancer Screening – Acceptability and Barriers: Recently, researchers have used AI-based algorithms to overcome the many pitfalls of ESCN diagnosis.[13,14] Indeed, many AI systems have diagnostic capabilities comparable or superior to expert endoscopists; however , AI algorithms that use microscopic imaging typically have higher specificity than AI algorithms that use "red flag" imaging (e.g., NBI).[11] Using AI as a diagnostic aid increases the accuracy of endoscopists, especially those with less experience[15,16] *Thus, AI can potentially enhance the diagnosis of ESCN and improve non-experts' diagnostic performance*. Nonetheless, there are several potential barriers to the implementation of AI, including patient mistrust,[15,16] lack of local expertise,[17] lack of AI knowledge among medical professionals,[18] regulatory barriers,[19] and AI bias (e.g., skewed or less accurate algorithms due to lack of diverse and representative population data).[20,21]

Need for Low-Cost, High-Resolution Imaging: Given the limitations of existing approaches, the limited availability and high cost of current confocal platforms, and the uniformly poor prognosis of esophageal cancer when it has breached the epithelium, there is a great need for accurate and accessible technologies that improve early detection. A reliable, robust, low-cost method of identifying ESCN at an early, removable stage would improve existing endoscopic strategies and enable *more accurate and selective biopsies*. Additionally, the ability to delineate normal from neoplastic mucosa in real time (with computer algorithms) can *reduce the number of patients lost to follow-up, improve user expertise and confidence, and facilitate immediate, minimally invasive endoscopic therapy*, such as endoscopic mucosal resection, a less costly alternative to surgery with far less morbidity.[22]

Most AI research is severely limited by (1) lack of heterogeneity in patient data and (2) outcomes that are not clinically relevant (e.g., ROC curves rather than clinical impact analysis). There is an excellent need for evaluations of AI-based approaches that study human-machine interaction, clinical impact, and results in diverse patient populations. Our proposed trial (diverse settings, patient, and user/stakeholder evaluations) will break new ground and help address these gaps. Our study endpoints (i.e., provider confidence, clinical impact, procedure efficiency, etc.) directly address the issues of clinical relevance and potential for dissemination.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Many side effects go away soon after the endoscopy procedure, but in some cases, side effects may be serious, long-lasting, or permanent and may even cause death. The participant must tell the study staff about any side effects that they may have had, even if they do not think they are related to the procedure.

2.3.1.1 IMMEDIATE RISKS

Immediate risks from the study procedure with proflavine and HRME:

- **Allergic reaction (anaphylaxis)**. There is the possibility of a severe allergic reaction to Proflavine Hemisulfate, the contrast dye, in which the participant may have difficulty breathing and may have blood pressure drop. If this were to occur, there are procedures in place to treat the subject immediately during endoscopy.
- **Specimen Imaging Probe (AI-mHRME)**: There are no known risks from using the imaging probe (AI-mHRME).

- **Anesthesia**. There may be additional risks from the added time of additional sedation, such as decreased blood pressure. If this were to occur, there are procedures in place to treat the subject immediately during endoscopy.
- **Aspiration**. Inhaling fluid into the lungs during endoscopy might cause lung inflammation. Safeguards to prevent this from happening while the participant is under anesthesia will be in place during and after the procedure, and the participant's breathing and other vital signs will be carefully monitored. If this were to occur, there are procedures in place to treat the subject immediately during endoscopy.
- **Biopsies**. There is the possibility of some hoarseness, sore throat, difficulty and/or pain in swallowing, bleeding or infection, as well as discomfort at the surgical site. One to two biopsies will be collected from LCE normal areas in the esophagus for the study procedure. This additional biopsy does not increase or change the risks due to biopsy collection.
- **Pregnancy**. Insufficient information is available on the use of Proflavine in pregnancy. Drugs can have harmful effects on the fetus at any stage of pregnancy. A patient will not be enrolled in the study or have a standard of care endoscopy performed if they are pregnant.
- **Loss of Privacy**. Subjects will be asked to give consent on the day of their procedure. Subjects will be taken to a private area to discuss the study information. All sensitive information will be requested from the subjects, and only what is required to perform a standard endoscopy. Subjects will be given a study ID number for all forms, images, and communications. All data will be removed of identifiers and coded. Source documents of all PHI will be redacted before being sent from outside sites for data monitoring/data entry in the database. All PHI collected on BCM subjects will be stored in locked cabinets or password-protected files/computers where only the PI and study coordinator can see the names. All case report forms will use the assigned subject ID. Since the subject participation is only for one visit, there will be limited opportunity for privacy interests to arise between study recruitment and the end of the study. The only extra privacy intrusion will be an additional phone call within 7 days of the procedure to ensure that the subject has not suffered any adverse events. Only the study coordinator and/or the PI will contact the subject during the follow-up. Information about the study will only be discussed with the subject, and messages containing identifiers of the subject's participation will not be left on voice-mail messages.

There are immediate, but very rare, risks of standard of care endoscopy procedure overall with or without the study procedure:

- **Bleeding**. The risk of bleeding complications after an endoscopy is increased if the procedure involves removing a piece of tissue for testing (biopsy) or treating a digestive system problem. In rare cases, such bleeding may require a blood transfusion.
- **Infection**. Most endoscopies consist of an examination and biopsy, and risk of infection is low. The risk of infection increases when additional procedures are performed as part of the standard of care endoscopy. Most infections are minor and can be treated with antibiotics. The study doctor may give the subject preventive antibiotics before their procedure if they are at higher risk of infection.
- **Aspiration**. See above.
- **Biopsies**: See above.
- **Tearing of the gastrointestinal tract**. A tear in the subject’s esophagus or another part of their upper digestive tract may require hospitalization, and sometimes surgery to repair it. The risk of this complication is very low — it occurs in an estimated 1 of every 2,500 to 11,000 diagnostic upper endoscopies. The risk increases if additional procedures, such as dilation to widen their esophagus, are performed.

- **A reaction to sedation or anesthesia**. Upper endoscopy is usually performed with sedation or anesthesia. The type of anesthesia or sedation depends on the person and the reason for the procedure. There is a risk of a reaction to sedation or anesthesia, but the risk is low.

Symptoms after a standard of care endoscopy procedure to look out for include:

- Fever
- Chest pain
- Shortness of breath
- Bloody, black or very dark colored stool
- Difficulty swallowing
- Severe or persistent abdominal pain
- Vomiting, especially if your vomit is bloody or looks like coffee ground

2.3.1.2 LONG-TERM RISKS

There are no long-term risks associated with standard of care endoscopy procedures, and there are no known long-term risks of one-time exposure to proflavine.

2.3.2 KNOWN POTENTIAL BENEFITS

2.3.2.1 IMMEDIATE POTENTIAL BENEFITS

Subjects may not directly benefit from participating in this research. However, the AI-mHRME may detect an area of neoplasia not detected by standard-of-care endoscopy and Lugol's chromoendoscopy. The results from the AI-mHRME and the pathology from the endoscopy and study procedures will allow study doctors and investigators to better understand the changes in the esophageal squamous cells.

2.3.2.2 LONG-RANGE POTENTIAL BENEFITS

Long-range potential benefits to the clinical study include a societal benefit. The potential societal benefit will be to help increase the specificity of Lugol's chromoendoscopy when combined with AI-mHRME, allowing for earlier detection of esophageal cancer or pre-cancerous lesions and quicker treatment time. This may potentially increase survival rates for esophageal cancer as well. Societal benefits will be lower endoscopy and screening costs, time saved, and quicker turnaround for treatment.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The incremental risks of proflavine and AI-mHRME added to the standard of care endoscopy with white light and Lugol's iodine are minimal. The principal investigator has had NO serious adverse events in >1000 subjects imaged with the HRME and proflavine. No risks related to either proflavine or AI-mHRME have been noted. White light endoscopy is the standard of care, and all subjects will be undergoing this standard of care procedure so that ALL patients will be receiving (at minimum) the standard of care.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
To evaluate the specificity among novices using AI-mHRME during esophageal cancer screening and surveillance. Our goal is to increase the specificity among novices using AI-mHRME without significant reduction in sensitivity.	Specificity (SP) of AI-mHRME in novices
Secondary	
(A) To assess the diagnostic performance of AI-mHRME;	(A) Sensitivity, specificity, positive predictive value, negative predictive value (SN, SP,PPV, NPV) of AI-mHRME;
(B) To evaluate provider confidence (experts and novices) in clinically interpreting mHRME (pre- and post-use of AI-mHRME);	(B) Provider (experts and novices) reported confidence level (low or high) in pre- and post- use of AI-mHRME;
(C) To assess the clinical impact following AI-mHRME;	(C) Change in clinical plan (biopsy vs. no biopsy vs. treat) post-AI mHRME;
(D) To determine the diagnostic yield of AI-mHRME	(D) Truly neoplastic biopsies/total biopsies obtained
(E) To determine procedure efficiency of AI-mHRME, including subjects saved any biopsy and biopsy efficiency.	(E) Subjects saved any biopsy: number of patients correctly saved any biopsy; Biopsy efficiency: the total number of biopsies saved.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a single arm, single visit, non-randomized phase 2 study. We plan to enroll 200 participants in the USA (n=50) and Brazil (n=150). We will recruit persons at risk for ESCN undergoing a previously scheduled endoscopic screening and surveillance examination.

Screening patients enrolled in this study are defined as patients at high risk of ESCN who have never undergone endoscopy. Surveillance patients enrolled in this study are defined as patients with known esophageal dysplasia but not known cancer.

Both male and female patients will be enrolled in the clinical trial. ESCN typically develops over time and in individuals over the age of 40 and those with a longstanding history of smoking or alcohol usage. In a retrospective review of our institutional databases, the disease was not encountered in a pediatric population; hence, we will not enroll individuals less than 18 years of age. In the Esophageal AI-mHRME clinical trial, participation in the study will be a clinic visit, with a phone call 7 days after the visit for follow-up.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Selection of Control Group

There is no control group in the study. However, all individuals for this study will undergoing standard-of-care (SOC) endoscopy with Lugol's chromoendoscopy (LCE) and artificial intelligence-based mobile high-resolution microendoscope (AI-mHRME) imaging with proflavine hemisulfate. Subjects enrolled and imaged with AI-mHRME during SOC endoscopy with LCE will have biopsies collected from LCE abnormal areas and LCE normal areas, thereby serving as their own controls. This selection is based on the current clinical practice for esophageal squamous cell neoplasia (ESCN) screening and surveillance, which, despite high sensitivity (>95%), suffers from low specificity (<60%), leading to excessive false positives and unnecessary biopsies. By using this SOC approach as the control, we can directly compare the clinical impact and efficacy of the AI-mHRME in real-world settings.

No placebo or historical control was chosen because (1) the ethical considerations surrounding the need for effective cancer screening methods in high-risk populations and (2) the availability of a well-established, albeit suboptimal, standard screening approach. A placebo control would be inappropriate due to the established benefits of endoscopic screening, and historical controls may not account for evolving clinical practices and technological advancements.

Study Design Justification

The main hypothesis of the research study is that AI-mHRME will increase LCE accuracy. Additional exploratory hypotheses include that AI-mHRME will (1) increase AI-mHRME accuracy in novice endoscopists and be non-inferior to expert endoscopists, (2) increase user confidence among expert and novice endoscopists, and (3) increase LCE efficiency and impact by reducing biopsies and second procedures.

To evaluate these hypotheses, we will conduct a single-arm, non-randomized, phase 2 study. We plan to enroll 200 participants across two sites: the USA (n=50) and Brazil (n=150). Participants will be individuals at risk for ESCN undergoing a previously scheduled endoscopic screening and surveillance examination.

This design allows for direct assessment of AI-mHRME's real-world performance in diverse populations while mitigating ethical concerns that would arise from withholding standard-of-care screening from high-risk individuals. The observational structure ensures that AI-mHRME's effectiveness can be evaluated without altering participants' clinical care.

Potential Challenges with the Control Group

The use of SOC endoscopy and normal biopsy collection as the control poses several known challenges:

1. **Variability in Operator Expertise**: Diagnostic accuracy with LCE is highly dependent on the skill and experience of the endoscopist. This may introduce heterogeneity, particularly in resource-limited settings where access to expert endoscopists is limited.

2. **Confounding by Inflammation**: LCE is known to highlight inflammatory areas, leading to an increased rate of false positives. This inherent limitation of the control method may amplify the observed benefits of AI-mHRME but must be carefully considered in interpretation.

3. **Potential for Verification Bias**: Since biopsy decisions are influenced by endoscopists' visual assessment, differences in biopsy rates between AI-mHRME and LCE could introduce bias in diagnostic yield comparisons.

This study is designed to rigorously assess AI-mHRME's ability to improve ESCN screening and surveillance by reducing unnecessary biopsies, increasing diagnostic yield, and enhancing clinician confidence. The within-subject comparator and phase 2 study design provide a robust framework for evaluating AI-mHRME's clinical impact while addressing potential limitations through careful study execution and stakeholder engagement. The findings will inform future implementation efforts, with implications for AI-assisted cancer screening across diverse populations and healthcare settings.

4.3 JUSTIFICATION FOR DOSE

Proflavine hemisulfate solution for optical imaging contains 0.01% (0.1 mg/mL) proflavine hemisulfate in water. The proflavine solution will be prepared in 10 mL of water and stored in a dispending syringe and amber-colored bag at 4°C. Proflavine solution is administered during the endoscopy procedure after the Lugol's iodine has been sprayed and before the AI-mHRME device is used for imaging of the esophagus. The proflavine solution is dispensed into the esophagus by connecting it to a spray catheter in the biopsy channel of the endoscopy. Approximately 5-7 mL of the proflavine solution in the syringe will be sprayed in the esophagus. The planned maximum dosage of proflavine solution will be 10 mL, and it will be a one-time dosage during the endoscopy procedure and study procedure.

Proflavine solution will be sprayed using the spray catheter in the esophagus the same way that Lugol's iodine is sprayed in the esophagus during standard of care endoscopy procedures.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if they have completed all phases of the study including the last follow-up chart review on day 180 shown in the Schedule of Activities (SoA), Section 1.3.

The end of the study is defined as completion of the last follow-up chart review shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Patients will be ***eligible*** for the Esophageal AI-mHRME clinical trial using the following ***inclusion*** criteria:

- Patients scheduled for endoscopy procedures at the following sites:
  - Baylor St. Luke's Medical Center (BSLMC), Houston, Texas, US
  - Harris Health Systems Ben Taub General Hospital (BTH), Houston, Texas, US
  - Instituto do Câncer de Estado de Saõ Paulo (ICESP), Saõ Paulo, Saõ Paulo State, Brazil
  - Barretos Cancer Center/Hospital de Amor (BCC), Barretos, Saõ Paulo State, Brazil
- Patients or their legally authorized representative must be informed of the investigational nature of this study and must sign and give written informed consent per institutional and federal guidelines;
- Patients undergoing routine (standard of care) LCE screening for esophageal squamous cell carcinoma (ESCC)

  - Patients with known history of head/neck squamous cancer (e.g., laryngeal and hypopharyngeal cancer, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, oral and oropharyngeal cancer, or salivary gland cancer)
  - Heavy smoking and/or alcohol use
  - Dietary or geographic risk factors for ESCC
- Patients undergoing follow-up surveillance for ESCC (e.g., prior low-grade dysplasia or indefinite for dysplasia)
- Patients are 18 years or older.
- Patients who have never been enrolled in a study with AI-mHRME and proflavine hemisulfate.

5.2 EXCLUSION CRITERIA

For the Esophageal AI-mHRME clinical study, patients will be ***ineligible*** using the following ***exclusion*** criteria:

- Allergy or prior reaction to Proflavine or Iodine;
- Current active esophageal cancer not amenable to endoscopic therapy;
- The patient is unable to undergo routine endoscopy with biopsy;
  - Women who are pregnant or breastfeeding;
  - Prothrombin time (PT) greater than 50% of control; prothrombin time test (PTT) greater than 50 sec, or international normalized ratio (INR) greater than 2.0;
  - Inability to tolerate sedated upper endoscopy due to cardio-pulmonary instability or other significant medical issues;
  - Patients with known, untreated esophageal strictures, prior partial esophageal resection, or altered anatomy preventing passage of the endoscope;
  - Patients who have previously been enrolled in a study with AI-mHRME and proflavine hemisulfate.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable.

5.4 SCREEN FAILURES

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a clinical reason in which they cannot undergo an endoscopy procedure at date of consent and enrollment may be rescreened if they are eligible for endoscopy at a later date.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

All patients scheduled for standard-of-care (SOC) upper endoscopy will be screened for eligibility for the study at each clinic site and date. The research population will be identified from patients at Baylor St. Luke's Medical Center (BSLMC) and at Ben Taub Hospital (BTH) by Dr. Mimi Tan in Houston, Texas; at Instituto do Câncer do Estado de São Paulo (ICESP)/Faculdade de Medicine by Dr. Fauze Maluf-Filho in São Paulo, and at Barretos Cancer Center (BCC)/Hospital de Amor by Dr. Elisa Ryoka Baba. Each patient will be given information regarding the study and will be given the appropriate amount of time to weigh the risks and benefits of the study carefully. If the patient agrees to consent to participate in the study, they will be counseled regarding all alternatives to study enrollment and regarding the right to withdraw consent at any time. In addition, the patient will be reassured that participation in the clinical trial will not affect future medical care. The consent form will be the only document linking the participant and the research, which will be filed in the subject's medical record.

In the US, screening will be performed through a secure electronic medical record system and in Brazil screening will be done during the scheduled visit for the SOC endoscopy procedure in the São Paulo clinic. The study investigators and staff will ensure that personal information is kept confidential. All participant information will be stored securely in locked file cabinets in areas with access limited to study staff. To maintain participant confidentiality, all reports, study data collection, and administrative forms will be identified only by a coded number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Since Drs. Tan, Maluf, and Baba are medical providers at BSLMC and BTH, ICESP, and BCC, respectively, the temporary access to patients' PHI to determine eligibility for the US and Brazil study sites does not constitute more than minimal risk. It will not adversely affect the privacy rights and welfare of the individuals covered by the waiver.

Patients who fit inclusion criteria will be consented into the research study. Once the patient consents, they will be enrolled in the study. Patients who do not consent to the study will only have their names and demographic information recorded so that the research coordinators will not re-contact them. All this will be kept in a screening log that only the site PIs and site study staff have access and will not be shared outside of each study site.

The study PIs anticipate a higher enrollment of men than women as esophageal squamous cell carcinoma is more prevalent among men compared to women. Additionally, every year there is a higher incidence of men with ESCC compared to women in both the US and Brazil. However, they are committed to making every effort to enroll as many women as possible while meeting the recruitment targets. Additionally, the study sites expect a high proportion of minorities to be enrolled due to the diverse racial and ethnic populations living near the sites, in particular at BTH, ICESP, and BCC.

The study aims to enroll participants from diverse racial and ethnic backgrounds, reflecting the populations of the metropolitan areas where the study sites are located: BSLMC and BTH in Houston, Texas, ICESP in São Paulo, BCC in Barretos, Brazil. We anticipate a significant enrollment of individuals from Black, Hispanic/Latino, and mixed-race backgrounds.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Proflavine Hemisulfate

Proflavine (also known as proflavin, acridine-3,6-diamine, or 3,6-diaminoacridine) is an acridine derivative with bacteriostatic properties, used as a topical antiseptic in wound dressings and in umbilical cord care. As a fluorescent nucleic-acid binding dye, proflavine is an effective optical contrast agent for visualizing cell nuclei. Proflavine is the principal component of acriflavine which has been used for fluorescent imaging in the European, Asian and Australian gastrointestinal literature without any adverse effects noted.[23,24]

Proflavine hemisulfate salt hydrate is commercially available as a red/orange powder from chemical manufacturers and suppliers including Sigma-Aldrich (St. Louis, MO) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The pharmaceutical preparation of proflavine solution for optical imaging is a formulation containing 0.01% (0.1 mg/mL) proflavine hemisulfate in water. The resulting proflavine solution is a yellowish liquid. This is lower than the concentration of the proflavine component in commercial triple dye, 0.11% (w/v) [Kerr Triple Dye, VistaPharm]. The quantity of solution used for imaging is likely to be no greater than that used in neonatal care (0.65 ml per single-use swab). Investigational in vivo human studies of confocal microscopy for gastrointestinal cancer currently use topical acriflavine at 0.05% concentration.[24] The additional exposure to light which will occur during imaging can also be compared to that received by newborn babies undergoing phototherapy for jaundice. The high-resolution fiber-optic microendoscope proposed for use here delivers 1.2 mW of 455 nm light to the tissue through a 0.8 mm diameter fiber-optic bundle, corresponding to an irradiance level of 240 mW/cm^2. The American Academy of Pediatrics defines intensive phototherapy as a spectral irradiance of at least 30 $\mu W/cm^2$ per nanometer over the 430-490 nm spectral band, equivalent to a total irradiance of 1.8 mW/cm^2.[25] Although the irradiance level is 133 times higher with the fiber microendoscope system, a typical 10-minute imaging session is 144 times shorter than a typical 24-hour (1440-minute) phototherapy incubation, leading to an approximately equivalent light dose in each scenario.

Artificial Intelligence Mobile High-Resolution Microendoscope (AI-mHRME)

The AI-mHRME is a fiber-optic fluorescence microscope intended for *in vivo* imaging of epithelial cell nuclei in mucosal tissues. The primary optical components of the AI-mHRME device are a blue LED (light-emitting diode), excitation filter, dichroic mirror, objective lens, fiber-optic probe, tube lens, emission filter, and high-frame-rate camera sensor. The AI-mHRME system also includes a laptop computer and user interface/software.

Illumination light from the blue LED light source passes through the excitation filter, reflects from the dichroic mirror, and is focused by the objective lens onto the proximal end of the fiber-optic probe. A fluorescent contrast agent (0.01% w/v proflavine solution) is applied topically to the tissue to be imaged, and the distal end of the fiber-optic probe is placed in gentle contact with the tissue surface. The illumination light passes through the fiber-optic probe and excites fluorescence, highlighting epithelial cell nuclei. The emitted fluorescent light is collected by the fiber-optic probe and transmitted back through the objective lens, dichroic mirror, emission filter, and tube lens to be imaged onto a high-frame-rate camera sensor. A live video feed is displayed in real time, showing epithelial cell nuclei as bright dots. The system has a circular field of view of approximately 800 µm diameter and lateral resolution of approximately 4 µm. Depending on the system configuration, individual images and/or video may be collected (with or without the use of a foot pedal) and may be quantitatively analyzed in real time by an automated algorithm or may be saved for later analysis.

AI-mHRME Non-significant risk (NSR) justification

In correspondence between Rice University and the FDA for the investigational device exemptions (IDE) for HRME on 20 Dec 2010, the following was issued in a letter from the FDA:

> *FDA had determined that the use of HFR-HRME in proposed clinical investigations is a nonsignificant risk (NSR) device study because it does not meet the definition of a significant risk (SR) device under § 812.3(m) of the investigational device exemptions*

(IDE) regulation (21 CFR 812, available on the internet at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/showCFR.cfm?CFRPart=812).

An IDE application is not required to be submitted to, or approved by, FDA for a NSR study. A NSR study is, however, subject to the abbreviated requirements described in§ 812.2(b) of the IDE regulation. The abbreviated requirements stipulate that the sponsor of the investigation must label the device in accordance with§ 812.5; obtain institutional review board approval of the investigation as a NSR study; ensure that each investigator obtains informed consent from each subject under the investigator's care; comply with the monitoring requirements of§ 812.46; maintain records required under§ 812.140(b)(4) and (5) and file the reports required under § 812.150(b)(1) through (3) and (5) through (10); and ensure that participating investigators maintain the records required by§ 812.140(a)(3)(i) and file the reports required under§ 812.150(a)(l), (2), (5) and (7).

Under the abbreviated IDE requirements, a sponsor must also comply with the prohibitions against promotion and other practices as identified in§ 812.7. According to this section of the regulation, the sponsor of a NSR study, investigator, or any person acting for or on behalf of the sponsor or investigator is prohibited from promoting or test marketing the investigational device until after FDA has approved the device for commercial distribution; commercializing the device by charging a price greater than that necessary to recover the cost of manufacture, research, development, and handling; unduly prolonging the investigation; and representing the investigational device as being safe or effective for the purposes for which it is being investigated.

6.1.2 DOSING AND ADMINISTRATION

Proflavine hemisulfate solution for optical imaging contains 0.01% (0.1 mg/mL) proflavine hemisulfate in water. The proflavine solution will be prepared in 10 mL of water and stored in a dispending syringe and amber-colored bag at 4 degrees C. Proflavine solution is administered during the endoscopy procedure after the Lugol's iodine has been sprayed and before the AI-mHRME device is used for imaging of the esophagus. The proflavine solution is dispensed into the esophagus by connecting it to a spray catheter in the biopsy channel of the endoscopy. Approximately 5-7 mL of the proflavine solution in the syringe will be sprayed in the esophagus. The planned maximum dosage of proflavine solution will be 10 mL, and it will be a one-time dosage during the endoscopy procedure and study procedure.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Proflavine hemisulfate will be purchased by the principal investigator and research staff from Sigma-Aldrich Inc. and distributed to the four study sites and their corresponding research pharmacies. The PI's research coordinator (RC) will place the order with the vendor. Once the proflavine shipment arrives, the RC will coordinate with the study sites and research pharmacy for delivery of proflavine.

For BSLMC and BTH in Houston, Texas, the RC will hand carry the bottle of proflavine to the Research Pharmacy at BSLMC and to the IDS at BTH. For ICESP and BCC, the RC will arrange with the coordinators

at ICESP and BCC to ship the proflavine bottles from Houston, Texas, to ICESP in São Paulo, Brazil. Both bottles will be shipped to ICESP, and ICESP will arrange for one bottle of proflavine powder will be delivered to BCC in Barretos, Brazil.

Proflavine hemisulfate will be kept in the original bottle in powder form at each research pharmacy and prepared on an as-needed basis for the study procedures. The research pharmacists will monitor the initial amount of proflavine and how much has been used after each batch of proflavine syringes are made. Approximately 2-3 months before a year has passed from proflavine delivery and opening of the bottle at each research pharmacy, the pharmacists should reach out to the site RCs to request a new bottle of proflavine hemisulfate powder. The site RCs will inform the site PIs and they will determine to purchase additional bottles of proflavine.

Any unused product, once a bottle of proflavine expires or is not used up by the end of the study period, will be disposed of according to manufacturer's protocols and the research pharmacy's protocols.

Waste treatment methods according to Safety Data Sheet from Sigma Aldrich Inc.: Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact [Sigma Aldrich Inc.] there if you have further questions.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Manufacturer
Sigma-Aldrich Inc.

Formulation
Proflavine hemisulphate salt hydrate
3,6-Diaminoacridinehemisulfate salt
$C_{13}H_{11}N_3 \cdot 0.5\ H_2SO_4 \cdot xH_2O$

Appearance
Form: Powder
Color: Faint Orange to Red-Brown to Brown

Packaging
Amber/brown glass bottle with a red plastic cap

Labeling
White sticker with compound name "Proflavine hemisulfate" and catalog number "P2508-10G."

6.2.3 PRODUCT STORAGE AND STABILITY

According to the manufacturer Sigma Aldrich Inc., proflavine hemisulfate powder is stable for one year from the date of shipment from the manufacturer. Study staff and research pharmacists must take note of when the proflavine hemisulfate was shipped from the manufacturer, when it arrived at the study site, and when it was first opened in the pharmacy. In its powder form, proflavine hemisulfate is stored in an amber-colored glass bottle at room temperature.

Proflavine hemisulfate solution is stored at 4°C and protected from light in an amber or light-blocking bag. It can be stored at the research pharmacy and must be kept cold during transport to the endoscopy suites (if the location of the endoscopy and the pharmacy are in two separate buildings), and then kept cold at 4°C in the medications fridge in the endoscopy suite until it is ready for use.

The prepared proflavine hemisulfate solution is stable at 4°C refrigeration for up to 14 days. After 14 days, if it is not used in the study procedure, the prepared proflavine solution must be discarded in biohazard waste in either the pharmacy or the endoscopy unit, if it was stored for use at the medication fridge in endoscopy.

6.2.4 PREPARATION

Preparation of Proflavine Hemisulfate at the Research Pharmacy at each study site:

Reagents:

1. Proflavine Hemisulfate in powder form
2. Sterile water

Equipment:

1. Hot plate
2. Magnetic stirrer
3. Thermometer

Supplies:

1. Weight boat
2. Scoop
3. Sterile 10 mL/cc syringes

Procedure:

1. Open and weight out 10 mg of the Proflavine Hemisulfate powder stored at room temperature.
2. Prepare a solution of 0.01% (w/v) proflavine hemisulfate by dissolving 10 mg of proflavine in 100 mL of sterile water for injection.
3. Heat the solution to 40 degrees C. Shake or vortex the solution until the proflavine is completely dissolved (about 15 minutes).
4. Transfer 10 mL of the prepared solution to a syringe and place each syringe individually in an amber/brown bag.
5. The solution should be stored at 4°C and protected from light.
6. The solution is stable for up to 14 days in refrigeration.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

There are no methods of randomization and blinding used in this study design. Research doctors and site PIs will know the assignment of study participants as this is a single arm study and all study participants will receive the intervention of HRME imaging and proflavine hemisulfate.

6.4 STUDY INTERVENTION COMPLIANCE

Study intervention compliance will be assessed on a quarterly basis. To assess study intervention compliance, the site RCs will complete the Case Report Form (CRF) section on HRME Procedure

completion. If HRME procedure was not completed, then it will be documented on the CRF if there was a clinical reason or technical reason for incomplete HRME procedure. If there are technical reasons, those will be used to calculate study intervention compliance.

6.5 CONCOMITANT THERAPY

Not applicable.

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION OR WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Upon completion of the study imaging and endoscopy procedure, subjects will be contacted to screen for symptoms and adverse events after 7 days from the endoscopy procedure. Adverse events or serious adverse events that occur during the follow-up period will be recorded regardless of relatedness to the study procedure. The follow-up will be conducted by a phone call; if clinically indicated, a clinic visit should be performed along with relevant lab work.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If the patient is pregnant.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- If the participant is unable to receive proflavine hemisulfate and HRME during endoscopy procedure.

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Participants who sign the informed consent form but do not receive the study intervention (proflavine and HRME) may be re-scheduled at a later time depending on inclusion and exclusion criteria.

7.3 LOST TO FOLLOW-UP

This is a single-visit study. A participant who completed all study procedures on Day 0 will be considered lost to follow-up if they cannot be contacted via phone call for the Day 7 follow-up symptom data collection and adverse event reporting.

The following actions must be taken if a participant fails to be contacted after the study procedure:

- The RCs will attempt to contact the participant up to three (3) times.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable on Day 7 during the follow-up phone call, they will be considered lost to follow-up, they are not withdrawn from the study. In such cases, the participant who is lost to follow-up will have their medical chart reviewed at Day 180.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

For enrolled subjects, data will be collected via medical record review, including review of pathology for any previous upper endoscopy procedures and any prior treatment for esophageal pre-cancer or cancer. After six months of completing the endoscopy and HRME with proflavine study procedure, subjects will have their medical record reviewed for any follow-up standard of care endoscopy procedures to determine treatment, if any, and if pathology was done on any biopsies or specimens collected during that subsequent procedure.

Survey and demographic data will be collected for all patients via interview, including demographics; medical history; tobacco and alcohol use; previous endoscopy procedures and corresponding pathology results; previous treatments for esophageal pre-cancer or cancer; and baseline symptoms prior to the current endoscopy procedure with HRME and proflavine.

During the endoscopy procedure, the study clinician will perform the standard of care endoscopy. The RC will record the start and end time of the entire endoscopy procedure, and the white light endoscopy features—level of gastroesophageal junction, presence and grade of esophagitis; and number of visible lesions (ulcers, nodules, and/or masses).

During the standard of care endoscopy, the study clinician will use NBI to assess the esophagus for any abnormal areas. If they observe any abnormal areas, for each area the RC will record the level and quadrant; if it is a visible lesion (ulcer or nodule), size and Paris classification; the study clinician's read of the abnormal area (non-neoplastic or neoplastic); the study clinician's confidence in their read (low or high confidence); the study clinician's theoretical plan (no biopsy, biopsy, ablate, endoscopy mucosal resection [EMR] or endoscopic submucosal dissection [ESD]); and start and end time of NBI. Once recorded for all abnormal areas under NBI, the study clinician moves on to the do same assessment for LCE with Lugol's iodine. The RC will record the read, confidence, and plan for all Lugol's abnormal areas, and the start and end time of LCE. Once the study clinician has completed LCE assessment, they move on to the study procedure with HRME and proflavine. The RC records the amount of proflavine used to spray in the esophagus, and the study clinician observes all Lugol's abnormal areas and at least one Lugol's normal area with HRME. The RC records the study clinician's read, confidence, and plan. Afterwards, the RC saves the HRME image and records the image number, score and read. After the HRME score is generated, the RC records the study clinician's read, confidence, and plan post HRME score. This is repeated for 1-2 Lugol's normal areas with HRME imagining and data recording. The RC will also collect the start and end time of all the HRME imaging. Lastly, the study clinician will collect biopsies from the Lugol's abnormal and normal areas that were imaged with HRME, and the RC will record the biopsy jar label and start and end time of biopsy collection.

Pathology of esophageal biopsy specimens will be graded by Drs. Rosen, Sobroza, and Reis at BSLMC/BTH, ICESP, and BCC, respectively. Dr. Rosen will also serve as the pathologist for central pathology review. Non-neoplastic biopsies are those biopsies with a diagnosis of normal, esophagitis, and/or low-grade

dysplasia. Neoplastic biopsies are those biopsies with a pathology read of medium-grade dysplasia, high-grade dysplasia, or cancerous.

This study has study procedures in a clinic-visit. Participants will be called on day 7 after the procedure to ask about symptoms and adverse events. To ensure that we can contact the patients by phone, we will request at least 2 phone numbers from each patient, their address as 2 phone numbers and addresses from emergency contacts.

8.2 SAFETY AND OTHER ASSESSMENTS

Upon completion of the study imaging, subjects will be contacted to screen for adverse events after 7 days from the endoscopy procedure. Patients with abnormal clinical findings will be followed until the condition resolves or stabilizes. Adverse events or serious adverse events that occur during the follow-up period will be recorded regardless of relatedness to the study procedure. The safety follow-up may be conducted by a phone visit; if clinically indicated, a clinic visit should be performed along with relevant lab work.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

We will use Common Terminology Criteria for Adverse Events (AEs) v5.0. Serious adverse events (SAE) will count as Grade 3 (Severe or Medically Significant, but not Immediately Life Threatening: Hospitalization or Prolongation of Hospitalization Indicated; Disabling; Limiting Self Care ADL), Grade 4 (Life-threatening consequences; urgent intervention indicated), or Grade 5 (Death related to AE).

Upon completion of the study imaging, subjects will be contacted to screen for adverse events after 7 days from the endoscopy procedure. Patients with abnormal clinical findings will be followed until the condition resolves or stabilizes. Only AEs or SAEs related to the study procedure that occur during the follow-up period will be recorded. The safety follow-up may be conducted by a phone visit; if clinically indicated, a clinic visit should be performed along with relevant lab work.

We anticipate most AE/SAEs will be related to the upper endoscopy procedure rather the HRME and proflavine hemisulfate.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic reaction to proflavine hemisulfate requiring intensive treatment in an emergency room or at home, severe aspiration of fluids during endoscopy procedure that requires hospitalization in the ICU, or endoscopic perforation and/or uncontrolled bleeding from endoscopy procedure that requires hospitalization and blood.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

We will use the Common Terminology Criteria for Adverse Events (AEs) v5.0 definitions for severity of events.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect. Only AEs that are deemed related to the study procedures will be reported.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

The site PI and the coordinating center PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

RCs will record all reportable events with start dates occurring any time after the study procedures with HRME and proflavine are completed on Day 0. During the Day 7 phone call, RCs will inquire about the occurrence of AE/SAEs. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

The PI must report any study related AE to the BCM IRB within 5 business days. Study related AEs will be reported to the IRB annually at renewal. AEs will be reviewed annually by the IRB. Any study related AE that is reportable to IRB will also be reported to the DLDCCC Data Review Committee (DRC) via the Patient Safety Officer at *dldcc-pso@bcm.edu*.

Principal investigators must report to the BCM IRB and the external IRB of Record (if applicable according to procedure) as soon as possible, but in all cases within 5 business days of any of the following events:

1) Event (including but not limited to on-site and off-site adverse event reports, injuries, side effects, breaches of confidentiality, deaths, or other problems) that occurs any time during or after the research study, which in the opinion of the principal investigator meets all the elements a) through c) below:
    a. Suggests that the research places one or more participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. A new or increased risk may be defined as one that requires some action (e.g., requiring a significant and usually safety-related, change in the protocol such as revising inclusion/exclusion criteria or including a new monitoring requirement, informed consent, or investigator's brochure, modification of the consent process, or informing participants.)
    b. Unexpected/Unanticipated (in terms of nature, severity, or frequency given:
        i. The research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and,
    c. The characteristics of the subject population being studied. Related or possibly related to the participation in the research procedures:

i. 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
ii. An event is "related to the research procedures" if in the opinion of the principal investigator, it was more likely than not to be caused by the research procedures or if it is more likely than not that the event affects the rights and welfare of current participants

d. Note: If an event, in the opinion of the principal investigator, does not meet ALL of the elements a) through c) above, the PI is not required to make a prompt report to the IRB. However, such events may require reporting to the sponsor.

2) Changes made to the research protocol without prior IRB review to eliminate apparent immediate harm to a research participant(s)
   a. Note: For protocol deviations that do not require prompt reporting, at time of continuing review the investigator will inform the IRB of its own quality monitoring processes by which deviations were identified, and process changes to prevent unintended variances.
3) Other unanticipated event, incident, or problem that is related to the research and that indicates that participants or others might be at new or increased risks:
   a. Any event that requires prompt reporting to the IRB according to the research protocol or plan or the sponsor
   b. Any accidental or unintentional change to the IRB-approved research protocol (PI self-report of non-compliance) or plan that involved risks or has the potential to recur.
   c. Any publication in the literature, safety monitoring report, interim result, or other finding that indicates an unexpected change to the risks or potential benefits of the research. For example:
      i. An interim analysis indicates that participants have a lower rate of response to treatment than initially expected
      ii. Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected
      iii. A paper is published from another study that shows that an arm of your research study is of no therapeutic value
4) Any complaint of a participant that indicates unanticipated risk or that cannot be resolved by the research team
5) Protocol violation (meaning an accidental or unintentional change to the IRB approved protocol) that placed one or more participants at increased risk, or has the potential to occur again
6) Any instance of non-compliance including PI self-reports
7) Any suspension or termination of research approval
8) Unanticipated adverse device effect (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 (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.)
9) Unauthorized disclosure of Protected Health Information (PHI) or breach of electronic security (these events should concurrently be reported to the BCM Privacy Officer and IT Security)

Any AE that is reportable to the BCM IRB must also be reported by the BCM PI to the FDA via safety reports and in the annual FDA IND report. Unexpected fatal or life-threatening adverse drug experiences will be

reported within 7 calendar days. Serious and unexpected adverse drug experiences will be reported within 15 calendar days.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The PI and study team will monitor 100% of SAEs. SAEs in the study will use the same guidelines for reporting as AEs outlined above.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Not applicable.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable

8.3.9 REPORTING OF PREGNANCY

Not applicable. This study will not enroll people who are pregnant.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers UP involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("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); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, 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 (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report UPs to the reviewing IRB and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;

- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the DCC/study sponsor within <insert timeline in accordance with policy> of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within <insert timeline in accordance with policy> of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the OHRP within 7 days of the IRB's receipt of the report of the problem from the investigator.

An investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)), A sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the FDA and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Not applicable.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

This is a multi-center, single-arm, non-randomized, phase 2 study designed to evaluate the use of AI-mHRME as a diagnostic aid in high risk patients undergoing screening or surveillance for esophageal cancer. Our goal is to enhance the specificity using AI-mHRME, particularly among novices, and to determine the accuracy, impact and effectiveness of AI-mHRME.

9.2 SAMPLE SIZE DETERMINATION

We plan to enroll 200 patients across the USA and Brazil sites. The expected prevalence of neoplasia is 8% in the screening population and 50% in the surveillance population; thus, among 200 subjects (140 in screening and 60 in surveillance), we expect 158 subjects enrolled in the study with no evidence of biopsy-confirmed neoplasia. Assuming 80% of endoscopists are novices, 126 patients without neoplasia will be examined by novice endoscopists. The sample size of 126 pairs of novice interpretations pre- and post-AI-mHRME will provide 90% power to detect an improvement in the specificity of 20% (80% vs. 60%) by McNemar's test of equality with 0.5 discordant pairs and a 5% two-sided significance level.

Clinical impact is a key of AI-mHRME. In the Brazil trial, native endoscopists chose to "biopsy or treat" for 70% of subjects with benign biopsies. Assuming a plan change rate of 30% after AI-mHRME interpretation,

the proportion of non-neoplasia subjects recommended for biopsy/treat will reduce to 55% after using AI-mHRME software algorithm. The study will have 95% power to detect the difference of 15% in pre and post plans (70% vs 55%) at a 5% two-sided significance level.

9.2.1 ANALYSIS OF THE PRIMARY ENDPOINT

The primary endpoint is specificity of AI-mHRME in novices. Specificity will be calculated for novices pre- and post- AI-mHRME assessments with 95% confidence intervals. The paired data will be compared using McNemar's test to assess difference in specificity.

9.2.2 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The secondary endpoints include the following:

1) **Performance Characteristics**, including sensitivity, specificity, positive and negative predictive value (SN, SP, PPV and NPV) will be estimated for endoscopist's interpretations and AI-mHRME along with 95% confidence intervals for overall and by clinician level. These measures will be compared to gold standard pathology. Analysis will be performed on both a per-patient and per-biopsy basis.

2) **Clinician Confidence level** pre- and post- use of AI-mHRME will be recorded. Changes in confidence level will be summarized for all users (experts and novices) and by clinical level with proper descriptive statistics and presented in cross tables.

3) **Clinical Impact** will be estimated as the percentage of patients with a correct change in clinical plan (biopsy vs. no biopsy vs. treat) along with 95% confidence intervals post-AI-mHRME for all users (experts and novices). Additionally, changes in clinical plan at pre and post- AI-mHRME will be presented in cross tables.

4) **Diagnostic Yield** will be calculated as the number of biopsies with neoplastic pathology divided by the total number of biopsies obtained along with 95% confidence intervals.

5) **Procedure Efficiency** will be assessed by subjects saved any biopsy and biopsy efficiency.

   a) **Subjects saved any biopsy** will be assessed by the number of patients whose biopsies are correctly changed from neoplastic (positive) to non-neoplastic (negative), with each biopsy showing negative pathology.

   b) **Biopsy efficiency** will be determined by the number of biopsies that can be potentially avoided as a result of correctly changing from neoplastic to non-neoplastic with the addition of AI-mHRME.

9.2.3 SAFETY ANALYSES

All patients who start study procedures will be included in the safety analysis. Safety data will be assessed through descriptive summaries of AEs. AEs will be tabulated by body system, preferred term, severity and relationship to study procedures. All AE data will be listed by patient to evaluate severity and causality.

9.2.4 PLANNED INTERIM ANALYSES

No interim analysis is planned.

9.2.5 SUB-GROUP ANALYSES

No sub-group analyses are planned.

9.3 POPULATIONS FOR ANALYSES

Safety population includes all patients who start any study procedures regardless of completeness.

Accuracy and effectiveness population will be used for evaluating diagnostic performance, diagnostic yield, and procedure efficiency.

> **Patient-level population** includes all patients who complete the AI-mHRME and have both interpretations and corresponding gold standard histopathology results available.
>
> **Biopsy-level population** includes all biopsies from patients who complete the AI-mHRME and have both interpretations and corresponding gold standard histopathology results available.

Confidence level population includes biopsies from patients who complete the AI-mHRME and have confidence level collected.

Clinical impact population includes patients who complete the AI-mHRME, have both interpretations and corresponding gold standard histopathology results available and clinicians make a treatment plan (no biopsy, biopsy or treat).

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: H-53483 Consent Form.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator or research staff will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about

it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The consent process will be conducted in the patient's preferred language. There is a full-length consent form in English, Spanish, and Portuguese. To facilitate the consent process and discussions, medical interpreters are available to assist the research staff and patients

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator, funding agency, the IND sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB, and sponsor and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at Baylor College of Medicine, the coordinating center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Baylor College of Medicine research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Baylor College of Medicine.

Certificate of Confidentiality (COC)

To further protect the privacy of study participants, a COC will be issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, COC help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at Baylor College of Medicine.

When the study is completed, access to study data will be provided through Baylor College of Medicine.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	Site PI - ICESP	Site PI – Barretos
Mimi Tan, MD, MPH	Fauze Maluf-Filho, MD	Elisa Ryoka Baba, MD, PhD
Baylor College of Medicine	Instituto do Cancer do Estado de Sao Paulo	Barretos Cancer Hospital / Hospital de Amor
Attn: Arlene Zamora, Gastroenterology, Ben Taub Hospital, 1504 Taub Loop, Houston, TX 77030	Av. Dr. Arnaldo, 251 São Paulo Brazil 01246-000	Rua Anterior Duarte Villela, 1331 Bairro Dr. Paulo Prata Barretos São Paulo Brazil
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In Brazil, the highest ethical authority for research involving human subjects is the National Research Ethics Committee (REC, Conep). Established in 1996, it is a central regulatory committee which coordinates a communication network of 836 institutional RECs. Conep is tasked with defining operational criteria for local RECs and setting all standards that guide the development of research involving human subjects in Brazil.

Currently, the entire ethical review process of any research involving human beings in Brazil is carried out within a digital platform called Plataforma Brasil (https://plataformabrasil.saude.gov.br/login.jsf). The registration and management of investigators, registration of research projects, and upload of all documents related to the research project are done on the platform, which creates a specific, individualized identifier for each project. The project evaluation process itself is also fully remote, with opinions based on the evaluation meetings held at the local RECs.

The ethical approval for this clinical trial was first submitted to ICESP, and once approved, was reviewed by the National Body. After the final approval from the National Body, the clinical trial ethics submission was sent to Barretos Cancer Hospital for an expedited approval process as institutions can rely on final approvals from other institutions such as ICESP.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of an Independent Safety Monitor at the University of Michigan, Kirsten Tuck, for the data collection, consent, and enrollment at Baylor College of Medicine (BSLMC and Ben Taub Hospital). For data collection done at ICESP and Barretos Cancer Hospital, the BBCM research staff will provide monitoring and oversight.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study for the Brazil sites will be performed by the Principal Investigator's, Mimi Tan, research staff at Baylor College of Medicine.
- Monitoring for this study for the BCM sites (BSLMC and Ben Taub) will be done by an outside independent monitor.
- Monitoring will be done by Dr. Mimi Tan's research team on site once a year and centralized at BCM research office using electronic/scanned CRFs. Monitoring will be done throughout the study at 25% enrollment, 50% enrollment, 75% enrollment, and 100% enrollment of study participants. The monitoring will be comprehensive, with 100% of the data and consent forms monitored. The BCM research team will prepare a monitoring report after each monitoring session and provide it to the PI and local PIs.
- Independent audits may be conducted by the FDA to ensure monitoring practices are performed consistently across all participating sites.

10.1.8 QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

Each clinical site will perform internal quality management of study conduct, data collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Advarra OnCore, a 21 CFR Part 11-compliant data capture system provided by the Baylor College of Medicine. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance (QA) and Quality Control (QC), section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation. All deviations must be addressed in study source documents, reported to NCI Program Official and Baylor College of Medicine. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 10 years after the completion of the primary endpoint by contacting Mimi Tan, Baylor College of Medicine.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of people who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, people who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable.

10.3 ABBREVIATIONS

ADL	Activities Daily Living
AE	Adverse Event
AI	Artificial intelligence
AI-mHRME	Artificial intelligence mobile High resolution microendoscope
ANCOVA	Analysis of Covariance
BCC	Barretos Cancer Center/Hospital de Amor
BSLMC	Baylor St. Luke's Medical Center
BTH	Ben Taub Hospital
CFR	Code of Federal Regulations
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DRC	Data review committee
eCRF	Electronic Case Report Forms
EMR	Electronic Medical Records
ESCC	Esophageal squamous cell carcinoma
ESCN	Esophageal squamous cell neoplasia
ESD	Endoscopic Submucosal Dissection
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HFR-HRME	High Frame Resolution-HRME
HRME	High resolution microendoscope
ICESP	Instituto do Câncer de Estado de Saõ Paulo
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
ID	Identification
IDE	Investigational Device Exemption
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LCE	Lugol's chromoendoscopy
LED	Light-Emitting Diode
LSMEANS	Least-squares Means
mHRME	Mobile HRME
MOP	Manual of Procedures
NBI	Narrow band imaging
NCI	National Cancer Institute
NCT	National Clinical Trial
NIH	National Institutes of Health
NPV	Negative predictive value
NSR	Non-Significant Risk
OHRP	Office for Human Research Protections

PI	Principal Investigator
PPV	Positive predictive value
PT	Prothrombin Time
PTT	Prothrombin Time Test
QA	Quality Assurance
QC	Quality Control
RC	Research coordinator
REC	Research Ethics Committee, Conep
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SN	Sensitivity
SOA	Schedule of Activities
SOC	Standard of care
SOP	Standard Operating Procedures
SP	Specificity
SR	Significant Risk
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2.0	6 November 2025	Changed the Baylor College of Medicine Principal Investigator and the IND Sponsor-Investigator from Sharmila Anandasabapathy to Mimi Tan	There is a change of the Baylor College of Medicine Principal Investigator and the IND Sponsor-Investigator for the study.

11 REFERENCES

1. Khushalani N. Cancer of the esophagus and stomach. Mayo Clin Proc. 2008 Jun;83(6):712–722. PMID: 18533089
2. Korst RJ, Rusch VW, Venkatraman E, Bains MS, Burt ME, Downey RJ, Ginsberg RJ. Proposed Revision Of The Staging Classification For Esophageal Cancer. The Journal of Thoracic and Cardiovascular Surgery. 1998 Mar;115(3):660–670.
3. Hongo M, Nagasaki Y, Shoji T. Epidemiology of esophageal cancer: Orient to Occident. Effects of chronology, geography and ethnicity. J Gastroenterol Hepatol. 2009 May;24(5):729–735. PMID: 19646015
4. Barrios E, Sierra MS, Musetti C, Forman D. The burden of oesophageal cancer in Central and South America. Cancer Epidemiology. 2016 Sep;44:S53–S61.
5. Oesophagus cancer [Internet]. Mortality DB. [cited 2025 Jun 4]. Available from: https://platform.who.int/mortality/themes/theme-details/topics/indicator-groups/indicator-group-details/MDB/oesophagus-cancer
6. Dawsey SM, Fleischer DE, Wang GQ, Zhou B, Kidwell JA, Lu N, Lewin KJ, Roth MJ, Tio TL, Taylor PR. Mucosal iodine staining improves endoscopic visualization of squamous dysplasia and squamous cell carcinoma of the esophagus in Linxian, China. Cancer. 1998 Jul 15;83(2):220–231. PMID: 9669803
7. Muto M, Hironaka S, Nakane M, Boku N, Ohtsu A, Yoshida S. Association of multiple Lugol-voiding lesions with synchronous and metachronous esophageal squamous cell carcinoma in patients with head and neck cancer. Gastrointestinal Endoscopy. Elsevier; 2002 Oct 1;56(4):517–521.
8. Inoue H, Rey JF, Lightdale C. Lugol chromoendoscopy for esophageal squamous cell cancer. Endoscopy. 2001 Jan;33(1):75–79. PMID: 11204992
9. Yoshida Y, Goda K, Tajiri H, Urashima M, Yoshimura N, Kato T. Assessment of novel endoscopic techniques for visualizing superficial esophageal squamous cell carcinoma: autofluorescence and narrow-band imaging. Dis Esophagus. 2009;22(5):439–446. PMID: 19191860
10. Lecleire S, Antonietti M, Iwanicki-Caron I, Duclos A, Lemoine F, Pessot FL, Michel P, Ducrotté P, Di Fiore F. Lugol chromo-endoscopy versus narrow band imaging for endoscopic screening of esophageal squamous-cell carcinoma in patients with a history of cured esophageal cancer: a feasibility study. Dis Esophagus. 2011 Aug;24(6):418–422. PMID: 21309914
11. Liu H, Li YQ, Yu T, Zhao YA, Zhang JP, Zuo XL, Li CQ, Guo YT, Zhang TG. Confocal laser endomicroscopy for superficial esophageal squamous cell carcinoma. Endoscopy. © Georg Thieme Verlag KG Stuttgart · New York; 2009 Feb 12;41:99–106.
12. Pech O, Rabenstein T, Manner H, Petrone MC, Pohl J, Vieth M, Stolte M, Ell C. Confocal laser endomicroscopy for in vivo diagnosis of early squamous cell carcinoma in the esophagus. Clin Gastroenterol Hepatol. 2008 Jan;6(1):89–94. PMID: 18063417
13. Liu Y. Artificial intelligence-assisted endoscopic detection of esophageal neoplasia in early stage: The next step? World J Gastroenterol. 2021 Apr 14;27(14):1392–1405. PMCID: PMC8047537
14. Huang LM, Yang WJ, Huang ZY, Tang CW, Li J. Artificial intelligence technique in detection of early esophageal cancer. World J Gastroenterol. 2020 Oct 21;26(39):5959–5969. PMCID: PMC7584056
15. Young AT, Amara D, Bhattacharya A, Wei ML. Patient and general public attitudes towards clinical artificial intelligence: a mixed methods systematic review. Lancet Digit Health. 2021 Sep;3(9):e599–e611. PMID: 34446266
16. Longoni C, Bonezzi A, Morewedge CK. Resistance to Medical Artificial Intelligence. Journal of Consumer Research. 2019 Dec 1;46(4):629–650.
17. Guo J, Li B. The Application of Medical Artificial Intelligence Technology in Rural Areas of Developing Countries. Health Equity. 2018 Aug 1;2(1):174–181. PMCID: PMC6110188

18. Ahmed Z, Bhinder KK, Tariq A, Tahir MJ, Mehmood Q, Tabassum MS, Malik M, Aslam S, Asghar MS, Yousaf Z. Knowledge, attitude, and practice of artificial intelligence among doctors and medical students in Pakistan: A cross-sectional online survey. Ann Med Surg (Lond). 2022 Apr;76:103493. PMCID: PMC8928127
19. Harvey HB, Gowda V. How the FDA Regulates AI. Acad Radiol. 2020 Jan;27(1):58–61. PMID: 31818387
20. Carter SM, Rogers W, Win KT, Frazer H, Richards B, Houssami N. The ethical, legal and social implications of using artificial intelligence systems in breast cancer care. Breast. 2020 Feb;49:25–32. PMCID: PMC7375671
21. Coiera E. On algorithms, machines, and medicine. Lancet Oncol. 2019 Feb;20(2):166–167. PMID: 30583847
22. Spechler SJ. Dysplasia in Barrett's esophagus: limitations of current management strategies. Am J Gastroenterol. 2005 Apr;100(4):927–935. PMID: 15784042
23. Kakeji Y, Yamaguchi S, Yoshida D, Tanoue K, Ueda M, Masunari A, Utsunomiya T, Imamura M, Honda H, Maehara Y, Hashizume M. Development and assessment of morphologic criteria for diagnosing gastric cancer using confocal endomicroscopy: an ex vivo and in vivo study. Endoscopy. 2006 Sep;38(9):886–890. PMID: 16981104
24. Polglase AL, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM. A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. Gastrointest Endosc. 2005 Nov;62(5):686–695. PMID: 16246680
25. Maisels MJ, McDonagh AF. Phototherapy for Neonatal Jaundice. New England Journal of Medicine. Massachusetts Medical Society; 2008 Feb 28;358(9):920–928.