Protocol

Title:	**B**arrett's o**ES**ophagus **T**rial **4** (BEST4): A Randomised Controlled Trial Comparing capsule sponge Test Screening to Usual Care
Brief Title:	BEST4 Screening Trial
Version:	7.0 10 February 2025
IRAS Number:	332589
ISRCTN Number:	88972567
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Sponsors:	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge
Funders:	Cancer Research UK NIHR HTA NHS Commissioners

1. Protocol Signatures

I give my approval for the Protocol entitled 'Barrett's oESophagus Trial 4 (BEST4): A Randomised Control Trial Comparing capsule sponge Test Screening to Usual Care' version V6.0, dated 16 October 2024.

Chief Investigator

Name: Professor Rebecca Fitzgerald

Signature: Rebecca C. Fitzgerald
Rebecca C. Fitzgerald (Feb 19, 2025 18:44 GMT)

Date: 19-Feb-2025

Site Signatures

I have read the attached Protocol entitled "Barrett's oESophagus Trial 4 (BEST4): A Randomised Control Trial Comparing capsule sponge Test Screening to Usual Care" version V7.0 dated 10th February 2025 and agree to abide by all provisions set forth therein.

I agree to comply with the conditions and principles of Good Clinical Practice as outlined in the UK Medical Devices Regulations 2002 (SI 2002 No 618, as amended) (UK MDR 2002), Clinical investigation of medical devices for human subjects — Good clinical practice (ISO 14155:2020), the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, the General Data Protection Regulation (UK GDPR), the Data Protection Act 2018 and any subsequent amendments of the clinical trial regulations, the Sponsor's standard operating procedures, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical trial without the prior written consent of the Sponsor(s).

Principal Investigator

Name:

Signature: Date:

2. Key Trial Contacts and Protocol Contributors

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Contents

Abbreviations

AE	Adverse Event
BO	Barrett's oesophagus
CDMA	Clinical Data Management Application
CE	Conformité Européenne
CI	Chief Investigator
CIMG	Clinical Investigation Management Group
CPTU	Clinical Trial Prevention Unit
CRF	Case Report Form
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed consent form
IFU	Instructions for Use
ISF	Investigator Site File
ISO	International Organisation for Standardisation
ISRCTN	International Standard Registered Clinical/social sTudy Number
MHRA	Medicines and Healthcare products Regulatory Agency
NDRS	National Disease Registration Service
NDBO	Non-dysplastic Barrett's Oesophagus
OAC	Oesophageal adenocarcinoma
PI	Principal Investigator
PPI	Patient and Public Involvement
QMUL	Queen Mary University of London
REC	Research Ethics Committee
RRDN	Regional Research Delivery Network
SAE	Serious Adverse Event
TMF	Trial Master File
TMG	Trial Management Group

TSC	Trial Steering Committee
UKCA	United Kingdom Conformity Assessed

3. Trial Summary

Trial Title	Barrett's oESophagus Trial 4 (BEST4): A Randomised Control Trial comparing capsule sponge Test Screening to Usual Care
Short Title	BEST4 Screening Trial
Trial Design	A Randomised Control trial to evaluate the effectiveness of the capsule sponge test offered to patients on medication for heartburn symptoms and to investigate whether it could improve OAC-associated mortality and morbidity. In addition to the trial, a Heartburn Health programme will be established to create a Bioresource of patients with Gastro-oesophageal reflux disease (GORD). This Protocol is part of the BEST4 Platform which includes the BEST4 Surveillance Trial and the BEST4 Screening Trial.
Trial Participants	**Inclusion Criteria:** **BEST4 Heartburn Health Programme and Screening Trial:** • Able to give electronic informed consent • Males aged 55-79 years and Females aged 65-79 years • Have a mobile phone number • Self confirmed symptoms of heartburn, acid reflux or indigestion In addition, for Screening Trial: • Self confirmed regular use (at least biweekly) of a prescription or over the counter acid-suppressant medication use over 6 months (this information will be provided by the participant when they complete the Heartburn Health Invitation and Consent). **Exclusion Criteria for capsule sponge procedure:** • Patient reported previous diagnosis of Barrett's oesophagus (BO) confirmed on histology • Patient reported upper Gastro Intestinal (GI) endoscopy in the last 3 years • Patient reported Cytosponge/capsule Sponge procedure in the last 3 years

	• Patient reported symptoms meet the guidelines for an urgent endoscopy referral according to National Institue for Health and Care Excellent (NICE) guidelines (dyspepsia with acute GI bleed, dyspepsia fail to respond to Proton Pump Inhibitors (PPI) or Histamine 2 Receptor (H2R) antagonist with negative Helicobacter Pylori test, unintentional weight loss, persistent vomiting, iron deficiency anaemia, epigastric mass, suspicious barium meal indicating risk of cancer) • Patient reported diagnosis of an oropharyngeal, oesophageal or gastro-oesophageal tumour (T2 staging and above), or symptoms of dysphagia • Patient reported difficulty in swallowing due to a known cerebrovascular accident or neurological disorder • Patient reported oesophageal varices, cirrhosis of the liver, portal hypertension • Patient reported prior endoscopic (photodynamic therapy or radiofrequency ablation) or surgical intervention to the oesophagus • Inability to (or have not skipped medication on **the evening before and/or on the day of*** the capsule sponge test) temporarily discontinue anti-thrombotic medication prior to procedure as per the guidance in the PIS • Known pregnancy • Lack capacity to provide informed consent • Procedural: having eaten or drank within the last 4 hours *depending on type and dosing regime of anti-thrombotic medication participant is taking
Planned Sample Size	120,000 in total. Approximately 40,000 participants in the intervention arm and 80,000 in the control arm.
Trial Duration	Total Screening Trial Duration: 12 years Active recruitment of participants: 27 months Passive follow-up (undertaken outside of the trial): Identification of cancer diagnosis and death up to 12 years from trial intervention start date via the National Cancer Registration & Analysis Service (NCRAS).

	Heartburn Health Programme Duration: 12 years
Primary Objective	To evaluate the effect of screening with the capsule sponge test in patients taking medication for reflux symptoms and efficacy on oesophageal adenocarcinoma associated morbidity and mortality.

4. Flow Chart

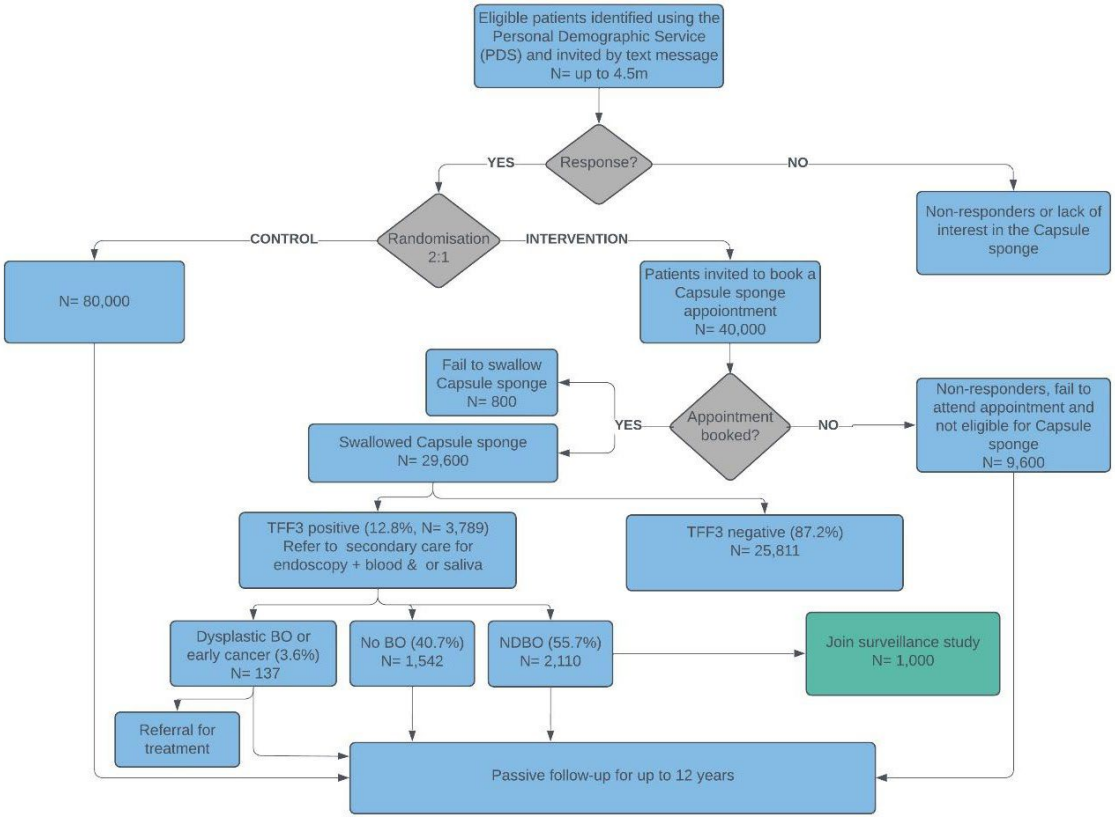

Figure 1 *BEST4 Screening Trial Summary*

5. Introduction

5.1. Background

According to global estimates for cancer, oesophageal cancer is ranked as the 7th most common cancer and 6th overall in mortality (1). Among Western countries, the incidence of the subtypes oesophageal adenocarcinoma (OAC) has risen markedly over the past 3 decades and OAC is more predominant than squamous cell carcinoma (2). The rise in the incidence of OAC has been accompanied by an almost parallel increase in deaths associated with OAC, with the average 5-year survival <20% (3). Although recent trends have shown that the incidence of OAC may be starting to plateau, a sizeable proportion of patients still present with late-stage disease and 40% of patients harbour distant metastases at index presentation (4). This undoubtedly has contributed to the poor overall survival associated with OAC.

Barrett's oesophagus (BO) is currently the only known precursor for OAC and contributes up to a 30-fold increase in the risk of developing OAC compared with the general population (5). The precipitant for the development of BO is chronic exposure to gastro-oesophageal reflux (GORD), wherein the native squamous lining of the distal oesophagus undergoes a metaplastic transformation to columnar epithelium containing goblet cells, called intestinal metaplasia (IM).

In patients destined to develop OAC, BO progresses through pathologically discernible changes from non-dysplastic BO (NDBO) to low-grade dysplasia (LGD), high-grade dysplasia (HGD), intramucosal carcinoma (IMC) and invasive adenocarcinoma. Due to the high mortality associated with OAC, there have been efforts to identify patients with BO and monitor them for evidence of dysplasia or early OAC requiring pre-emptive treatment to prevent advanced OAC. Randomised controlled trials have shown substantial and durable response rates for endoscopic therapy, and substantially improved quality of life compared with the chemo/radiotherapy and oesophagectomy regimes required for advanced cancer (23). There is no coordinated screening programme currently in the UK; the British Society of Gastroenterology (BSG) recommend that endoscopic screening can be considered in patients with chronic GORD symptoms and multiple risk factors [at least three of age ≥50, Caucasian, male, sex, obesity or having a positive family history (1st degree relative with BO or OAC)] (6). However, the underlying issue is that most cases of BO remain undiagnosed in the community.

5.1.1. Challenges with Endoscopic screening

Chronic gastroesophageal reflux disease (GORD) is common and affects up to 20% of the Western population. Approximately 8% of those with chronic GORD will develop BO (10, 11). It has been shown that there is significant variation in clinical

practice among primary care colleagues. A lower rate of referral for endoscopy at some General Practices is associated with late-stage presentation and poorer outcomes for OAC (12). Furthermore, it is estimated that over 80% BO is undiagnosed in the community and this is the key to identifying individuals with changes (dysplasia and cancer), that can be treated endoscopically before they progress to symptomatic disease.

Although the BSG guidelines provide recommendations for the targeted screening of patients at high risk of harbouring BO and subsequent surveillance of those with a confirmatory diagnosis of BO, this strategy is variably implemented and has several limitations. First, endoscopy is expensive with an NHS tariff of £885 per procedure and more when multiple endoscopies are required (13). Further, this cost does not include indirect costs such as lower productivity due to absence from work. Secondly, endoscopic services are often primarily based in secondary care and are not easily accessible. Thirdly, endoscopies are often performed by a specialist and require rigorous training. Fourth, this procedure is somewhat invasive with a small risk of potentially serious complications from bleeding and perforation as well as risk associated with sedation, especially in older patients. Fifth, even with the most vigorous surveillance endoscopy, the rates of post[1]endoscopy OAC (defined as OAC/dysplasia within 1 year after index endoscopy) may be as high as 14% (14, 15). Further, the average person with BO would require around 10 of these procedures throughout their lifetime, therefore the cost associated with this strategy is substantial, in addition to limited capacity. In one study from the USA, the incremental cost-effectiveness ratio with this strategy was estimated to be $600,000 per quality-adjusted life-year (QALY) saved (16). These limitations make the use of endoscopy as a screening tool for BO unrealistic and not cost effective.

Since it is not feasible to endoscope all individuals with heartburn a practical solution is required which is acceptable to patients and clinicians, accurate enough to improve outcomes, and affordable for the healthcare system. Over the past 18 years, the Fitzgerald laboratory has developed and rigorously tested a novel, minimally invasive cell-sampling device which was trade marked by the Fitzgerald laboratory and called the Cytosponge (Figure 2). The concept has been adopted by several companies and so the term capsule sponge will be used from here on. The capsule sponge cell collection device is composed of a compressed mesh and encapsulated in a vegetarian capsule and attached to a string. When swallowed, the capsule passes into the stomach and within 7 minutes, the capsule dissolves to release a spherical mesh of 3cm in diameter. The device is then withdrawn by a nurse/ practitioner by pulling onto the string, taking a few seconds, sweeping the mesh over the entire length of the oesophagus and retrieving up to 1 million cells (17). The sponge containing the cells is then immersed into SurePath Preservative fluid and regfrigerated until transported to the laboratory for processing. The cells are then embedded into paraffin, sectioned and stained for

analysis. To aid the histopathological analysis and make it as objective as possible, the Fitzgerald laboratory has also identified an accompanying biomarker assay for BO called Trefoil Factor 3 (TFF3) which is expressed within the goblet cells of BO with a sensitivity of 80% (increasing to 90% when the sample passes the quality control check for columnar cells) and specificity of 92-94% for BO (18). Further, we have shown in a randomised controlled trial (BEST3) that the Cytosponge-TFF3 test can detect 10x more BO than GP usual care, including cases with dysplasia and early stage cancer (11). This capsule sponge test therefore bypasses many of the limitations of the current endoscopic screening paradigm for BO due to it being easy to administer, less invasive for the patient, less dependent on the skills of the administrator compared with endoscopy and more cost-effective.

5.2. Rationale for Trial and Importance of the Research Question

As a result of the unmet need, a less invasive, and more affordable alternative to endoscopy is required to screen an at-risk population and subsequent surveillance of those with a diagnosis of BO. Further, it is required to show that we cannot only detect more BO but also prevent morbidity and mortality assoicated with late stage cancer detection. The BEST4 Screening Trial is therefore designed to:

- Demonstrate that the capsule sponge test can be used to screen for BO-related neoplasia which could lead to early detection and a decrease in OAC-associated morbidity and mortality as compared with standard clinical care. Importantly, it will also provide evidence about whether it could lead to a health economic benefit.
- Establish a long-term bioresourse of a cohort with GORD (including many with BO) who are willing to participate in research, consent to access of their medical records and willing to provide longitudinal clinical samples to help towards the understanding of the neoplastic process in OAC and lead to advances in prevention, early detection, and management of these patients.

5.2.1. Safety data

Extensive information has been collected on safety and tolerability of capsule sponges from several trials in the primary and secondary care setting (11, 17, 18) and from clinical implementation piloting across the NHS (primarily in secondary care but also in a mobile diagnostic unit in rural settings), (21). In this trial, the capsule sponge test will be performed in previously trialled settings with the appropriate safety measures and in line with the requirements for safety monitoring.

In a systematic review of five prospective studies (prior to BEST3) which performed >2600 Cytosponge (a particular capsule sponge) procedures across the UK, USA and Australia, the Cytosponge was demonstrated to have a high safety

profile with only two reported adverse events, a case of minor pharyngeal bleed and a case of Cytosponge detachment (19). The case of a minor bleed was managed conservatively and the case of Cytosponge detachment was managed with an endoscopy, which easily retrieved the Cytosponge. Both cases did not result in any long-term sequalae. In the BEST3 randomised study, among 1654 patients who swallowed the Cytosponge, there was one case of Cytosponge detachment requiring endoscopic removal. More recently the real-world implementation of reports of >10,000 procedures was reported (21). Overall, combining the results of all Cytosponge studies to date, the rate of Cytosponge detachment is <1:2000 procedures. The most commonly reported side effect is a mild and transient sore throat, affecting up to 4% of those who swallow the capsule sponge (11).

6. Trial Design

6.1. Statement of Design

This is a randomised controlled clinical trial being conducted in the community setting within Mobile Diagnostic Units, for patients on medication for heartburn symptoms, to evaluate whether an offer of a capsule sponge test could improve OAC-associated mortality and morbidity. The morbidity reduction would come from earlier stage diagnosis meaning that systemic chemotherapy and oesophagectomy can be obviated, since early disease can be treated endoscopically.

In addition, to establish a BEST4 Platform for basic, clinical, and epidemiological research into BO and OAC. This would include a longitudinal bioresource of capsule sponge (including supernatant), saliva and blood samples in patients undergoing endoscopy; and the opportunity for trials of lifestyle interventions for patients with GORD and chemoprevention among patients with BO. This is essential to ensure that the research community at large can benefit from this big investment.

6.2. BEST4 Platform

The BEST4 platform consists of the BEST4 Screening Trial, the Heartburn Health Programme (included in this Protocol) and the BEST4 Surveillance Trial (IRAS: 331813).

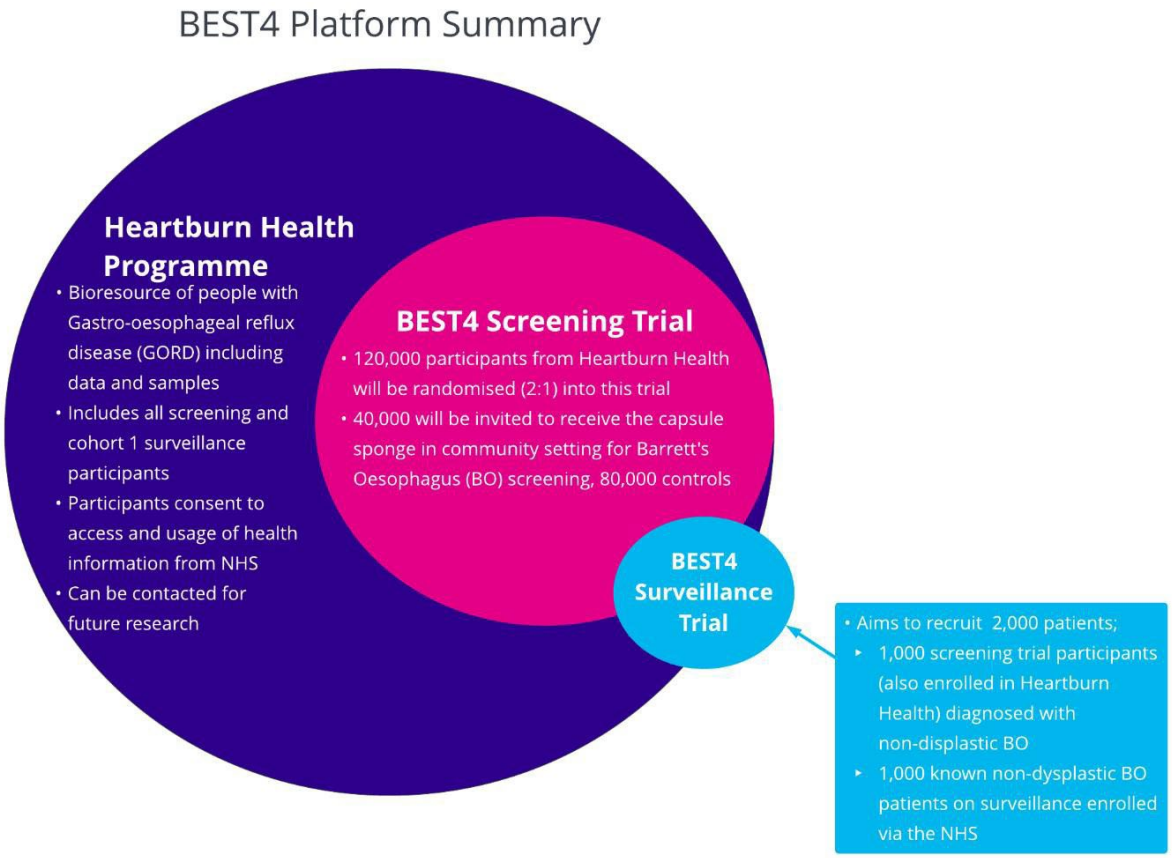

Figure 2 *BEST4 Platform Summary*

6.3. Heartburn Health Programme

The Heartburn Health Programme will primarily establish the Bioresource within the BEST4 Platform. In addition, due to the Screening Trial's modified Zelen-design (see section 6.4) the programme acts as the initial participant identification mechanism (two-staged approach, section 9.4) and seeks consent for passive follow-up data collection required for the BEST4 screening trial participants (section 9.7).

6.4. BEST4 Screening Trial – modified Zelen design

The Zelen design has been chosen to facilitate participation in clinical trials in which individuals in the control arm would receive standard of care treatment. Its key innovation is to randomise individuals prior to consent and only to seek consent from those in the intervention arm. Use of the Zelen design in trials with poor adherence to randomised treatment allocation results in a serious loss of power (22). The 'Zelen Design within a Cohort Study' tries to overcome this

weakness and to alleviate ethical concerns regarding lack of consent from the control arm. Individuals are first recruited and consent to be part of a cohort trial (The Heartburn Health Programme) from which they may be invited to participate in further clinical trials. They are also questioned regarding their interest in participating in trials of certain types of interventions. Only those who consent to join the cohort and express an interest in the intervention are randomised using a Zelen design. Participants in the cohort do not provide explicit additional consent before randomisation. Those randomised to the intervention will need to consent to the intervention before receiving it. The Screening trial will be conducted using such a design. Individuals in the Heartburn Health cohort may be randomised and will then become part of the screening trial. Participants randomised to the intervention arm (invitation for a capsule sponge test) will need to provide consent before receiving a capsule sponge examination. Participants randomised to the control arm (passive data follow-up only) will not provide additional consent for their follow-up during screening trial.

6.5. BEST4 Surveillance Trial (Not included in this protocol IRAS: 331813)

The BEST4 surveillance trial will recruit from 2 cohorts. Patients enrolled in the BEST4 Screening Trial who are reported TFF3 positive on their capsule sponge and subsequently diagnosed with NDBO on a confirmatory endoscopy will be contacted by local site staff with information about the surveillance trial. In total, up to 1,000 such patients will be enrolled onto the surveillance trial (Cohort 1) and will remain in the Heartburn Health Programme. Cohort 2 consists of patients with a prior diagnosis of NDBO who are due for (endoscopic) surveillance. They will not be consented to the Heartburn Health Programme.

6.6. Participating site model

EMS Healthcare headquarters will act as the single participating site and will identify an internal Prinicpal Investigator to oversee all trial activities for the 5 mobile units acting as satellite sites. The Prinicipal Investigator will onward delegate some investigator functions to a nurse lead at each mobile unit satellite site as specified on the master EMS HQ delegation log.

Secondary care endoscopy units, particpating in the BEST4 Surveillance trial, will be identified to support triggered Screening trial activities (removal of device in the event of a detachement and referral endoscopies for positive result partcipants). Each secondary care site will identify a Prinicipal Investgator and dedicated team to support these activities.

6.7. Number of participating regions

We plan to recruit patients from approximately 4 NIHR Regional Research Delivery Networks (RRDNs) initially, with the flexibility to expand this number if necessary to achieve our recruitment target, potenitally extending recruitment to include the devolved nations. Each RRDN involved will be covered by a Mobile Diagnostic Unit to facilitate Screening trial recruitment and visits.

Up to 18 secondary care hospitals participating in the BEST4 Surveillance Trial, within our targetted RRDNs, will support triggered Screening trial activities. This will include managing endoscopy referrals triggered by the screening process, removal of detached devices and collecting blood and saliva samples from participants.

6.8. Mobile Diagnostic Units (MDU)

The mobile unit model, provided by EMS Healthcare, will comprise of 2 clinical spaces per unit and will facilitate ease of access for patients and participant recruitment, whilst maintaining patient safety and meeting UK clinical facility standards. MDU design has proven successful in lung CT screening and the NHS-Galleri trial of GRAIL's multi-cancer early detection blood test which was also run by the CRUK Cancer Prevention Trials Unit.

6.9. Number of Participants

All participants will first be enrolled on to the BEST4 Heartburn Health Programme, which will also include particpants who do not go on to be enrolled into the Screening trial. Therefore we plan to enrol $\geq$120,000 to BEST4 Heartburn Health. Up to 4.5 million patients will be invited to particpate to reach the recruitment target.

The Screening Trial will enrol 120,000 participants who will be randomised on a 2:1 ratio to control (80,000) or intervention arm (40,000), respectively.

6.10. Participant Trial Duration

Screening Trial participants randomised to the intervention arm will be offered a baseline visit at which they will be screened using a capsule sponge. A positive capsule sponge test will trigger an either urgent or or routine referral for a clinical endoscopy by the trial team.

Active trial participation in the screening intervention arm will be dependent on the result of the baseline capsule sponge. Participants who test positive for TFF3 biomarker only will be referred for an endoscopy via a routine referral to be completed within 8 (+4 weeks), participants who test positive for TFF3, P53 and/or

aytipia will be referred for an ensdocopy via the urgent suspected upper GI pathway. Therefore, active trial participation for the TFF3, P53 and/or aytpia positive participants will be as per current NHS cancer waiting times targets for diagnostic test and appropriate follow up or discharge back to GP care. For particpants with TFF3 positive only sponge results, their active particpation will be 8(+4) weeks and appropiate follow up or discharge back to GP care. For participants with negative baseline sponge results, their active participation in the trial will be approximately 8 weeks, allowing time for the results to be conveyed to the participant and to their GP.

All participants will remain in the Heartburn Health Programme whilst this protocol remains open, unless they wish to withdraw.

6.11. Protocol Objectives

Table 1 *BEST4 Screening, Endpoints and Measures*

Objective	**Endpoint**	**Measure**
Primary		
To evaluate the effect of screening patients on medication for reflux symptoms with a capsule sponge test on oesophageal adenocarcinoma associated morbidity and mortality (Efficacy)	Incidence of stage II+ or fatal oesophageal adenocarcinoma (OAC). Death from oesophageal adenocarcinoma (OAC), including those arising at the gastro-oesophageal junction.	Primary measures: Kaplan-Meier estimate of the cumulative incidence of stage II+ or fatal adenocarcinoma (OAC) in the control and intervention arms (separately) at 4.5 years, 6.0 years, and 8.5 years. We will test for a difference between the control and intervention arms using a log-rank test. Kaplan-Meier estimate of the cumulative risk of death from oesophageal adenocarcinoma (OAC) in the control and intervention arms (separately) at 6 years, 7.5 years, and 12 years (log-rank test). We will

		test for a difference between the control and intervention arms using a log-rank test. Secondary measures: Hazard ratio for risk of stage II+ or fatal adenocarcinoma (OAC) in the intervention arm compared to the control arm at 4.5 years, 6.0 years, and 9.0 years. Hazard ratio for risk of death from oesophageal adenocarcinoma (OAC) in the intervention arm compared to the control arm at 6 years, 7.5 years, and 12 years. The complier average causal effect (hazard ratio) of screening on stage II+ or fatal OAC at 4.5 years, 6.0 years, and 9.0 years. The complier average causal effect (hazard ratio) of screening on OAC mortality within 6 years, 7.5 years and 12 years.
Create a cohort of individuals with heartburn who are	Number recruited providing consent for data use and contact	Minimum of 120,000 into the Heartburn Health programme

willing to participate in research		
Secondary		
To evaluate the effect of screening with capsule sponge to patients with reflux symptoms on oesophago-gastric cancer mortality.	Death from oesophago-gastric cancer.	Hazard ratio for risk of death from oesophago-gastric cancer within in the intervention arm compared to the control arm. This will be calculated at 12 years and at 6 and/or 7.5 years if the corresponding test for a difference in OAC mortality between the trial arms is statistically significant. Both intention-to-treat and complier average causal effects hazard ratios will be estimated.
To evaluate the detection of Barrett's neoplasia by the capsule sponge-TFF3 test when used in targeted screening	Dysplastic BE and stage I OAC	Proportion of screened individuals with dysplastic BE or intramucosal OAC diagnosed via endoscopy triggered by a positive screen
To evaluate the health economic impact of screening with the capsule sponge-TFF3 test in patients on medication for reflux symptoms	Incremental cost-effectiveness	Screening cost Incremental cost QALYs gained Life-years gained Incremental cost per QALY gained Incremental net monetary benefit Probability that screening with the capsule sponge-TFF3 test is cost-effective

	Budget impact	Total national budget impact of conducting one round of Cytosponge-TFF3 screening in the UK
To estimate uptake to screening with capsule sponge (in those expressing interest in participating in the trial) and reasons for lack of screening	Non-response to screening invitation. Failure to attend for screening. Failure to swallow capsule sponge	Proportion of randomised participants that make an appointment for screening. Proportion of participants with a screening appointment who attend for screening. Proportion of participants attending for screening who swallow the capsule sponge.
To estimate uptake for endoscopy	Non-attendance at endoscopy appointment	Proportion of participants referred for endoscopy for a positive screen who attend their endoscopy appointment.
Exploratory		
Detection of other clinically important conditions by the capsule sponge		Proportions of screened participants with different pathologies diagnosed as a result of screening.
To study the determinants of the natural history of Barrett's and to evaluate other early detection technologies		Potential biomarkers in blood and saliva samples from patients referred for capsule sponge triggered endoscopy (N= 2,100)
To explore the potential for better targeting of individuals at risk of	Prevalence of (screen-detected) BO or OAC	Logistic regression of diagnosed BO or OAC in screened individuals on

Barrett's oesophagus (BO) using a simple questionnaire		factors such as body mass index (BMI), smoking status (current, ex, never) and amount (pack-years), duration and severity of heartburn etc.
To explore the potential of patterns in methylation of DNA extracted from cell blocks to predicted TFF3 positivity and screen-detected BO or OAC	TFF3 positive on slide from cell block Screen-detected BO or OAC Risk of OAC during follow-up of TFF3 negative individuals by methylation score	Area under the receiver operating characteristic (ROC) curve of TFF3 positivity by methylation score Area under the ROC curve of screen-detected BO or OAC by methylation score Cumulative incidence of OAC by methylation score in individuals negative for TFF3 at baseline
To explore the potential of proteins including glycoproteins in the supernatant to act as biomarkers of: a) sample adequacy, and b) TFF3 positivity on cell block, and c) screen-detected BO or OAC	TFF3 and other protein related markers presence in the supernatant	The extent to which lack of proteins in the supernatant can be used to predict lack of columnar cells in the cell block Concordance between TFF3 on the cell block and protein markers, including TFF3, in the supernatant Risk of screen-detected BO or OAC (among those with TFF3 on the cell block) as a function of Barrett's related proteins in the supernatant

7. Selection and Withdrawal of Participants

7.1. Inclusion Criteria for the Heartburn Health Programme

To be included in the protocol the participant must*:*

- Have given electronic informed consent to participate
- Males aged 55-79 years and Females aged 65-79 years (sex at birth)
- Have a mobile phone number
- Patient reported symptoms of heartburn, acid reflux and indigestion.

7.2. Inclusion and Exclusion Criteria for the BEST4 Screening Trial

To be included in the Screening Trial the participant must*:*

- meet same criteria as 7.1
- Confirmed regular use (at least biweekly) of a prescription or over the counter acid-suppressant medication use in the last 6 months (this information will be provided by the participant when they complete the Heartburn Health Invitation and Consent).

Exclusion Criteria for the Capsule Sponge procedure:

The presence of any of the following will preclude participant inclusion:

- Patient reported previous diagnosis of BO confirmed on histology
- Patient reported upper GI endoscopy in the last 3 years
- Patient reported Cytosponge/capsule sponge procedure in the last 3 years
- Patient reported symptoms meet the guidelines for an urgent endoscopy referral according to NICE guidelines (dyspepsia with acute GI bleed, dyspepsia fail to respond to PPI or H2R antagonist with negative Helicobacter Pylori test, unintentional weight loss, persistent vomiting, iron deficiency anaemia, epigastric mass, suspicious barium meal indicating risk of cancer)
- Patient reported diagnosis of an oropharyngeal, oesophageal or gastro-oesophageal tumour (T2 staging and above), or symptoms of dysphagia
- Patient reported difficulty in swallowing due to a known cerebrovascular accident or neurological disorder
- Patient reported oesophageal varices, cirrhosis of the liver, portal hypertension
- Patient reported prior endoscopic (photodynamic therapy or radiofrequency ablation) or surgical intervention to the oesophagus
- Inability to (or have not skipped medication on **the evening before and/or on the day of*** the capsule sponge test) temporarily discontinue

anti-thrombotic medication prior to procedure as per the guidance in the PIS
- Known pregnancy
- Lack capacity to provide informed consent
- Procedural: Having eaten or drank within the last 4 hours

*depending on type and dosing regime of anti-thrombotic medication participant is taking

7.3. Participant Randomisation

Approximately 120,000 participants will be randomised. iPLATO, a trusted NHS primary care digital patient engagement provider, will invite participants to join Heartburn Health and transfer the consented participant data to CPTU REDcap database. Identification of participants from Heartburn health for inclusion and randomisation in the BEST4 Screening Trial will be handled by CPTU. Participants who meet BEST4 eligibility criteria, and criteria for invitation to a BEST4 Screening trial, will be individually randomised using a 2:1 control to intervention ratio following the BEST4 Randomisation Working Instruction, in accordance with the CPTU SOP for randomisation.

There will be no formal upper limit to the number of patients randomised per RDN, although a limit may be agreed for each unit location so that the appointments are manageable.

7.4. Participant Procedural Withdrawal Criteria

If a participant presents at their appointment with clinical contraindications (as per the exclusion criteria), participants will not receive a capsule sponge test and will be encouraged to visit their Primary Care health provider in the instance of concerning symptoms.

7.5. Withdrawal of Consent

Participants may withdraw their consent to continued participation in the Heartburn Health Programme and/or (if appropriate) the Screening Trial at any time. If the participant explicitly states their wish to withdraw consent, the Clinical Co-ordinating and CPTU team will be responsible for documenting this in participants REDCap notes and completing the withdrawal of consent eCRF which will notify the CPTU.

No further Protocol procedures or future Programme activities will be undertaken and no new data will be collected from the time of withdrawal. Data and samples

collected up to the time of consent withdrawal will be included in the data reported for the Screening Trial.

7.6. Criteria for Participant Replacement

Participants who withdraw from the trial for whatever reason i.e. consent withdrawal or withdrawal due to clinical reasons, will not be replaced.

8. Medical Device

8.1. Medical Device Details

EndoSign® is a capsule sponge cell collection device and is a CE-marked single-use, non-sterile, 3cm diameter, polyester, medical grade sphere on a string, compressed within a vegetarian capsule manufactured by Cyted Ltd. The device is classified as Class 1 as defined in the EU Medical Device Directive 93/42/EEC of 14 June 1993. The device is a minimally invasive way to perform a preliminary oesophageal cell collection. Samples can be used for cytological and histological review to assess patients suspected of oesophageal pathologies.

8.2. Legal Status of Medical Device

EndoSign®, manufactured by Cyted Ltd is a CE and UKCA marked class 1 device which is commercially available and is to be used within its licenced use in the UK for BEST4.

8.3. Medical Device Supply

Full details of supply, storage, management, returns and destruction are included in the Device Management Plan. It is the responsibility of the Principal Investigator (PI) or delegated individual at each participating site to maintain adequate stock levels. An agreement outlining the supply of the device for this trial will be in place with Cyted Ltd and the required organisations.

8.4. Medical Device Packaging and Labelling

Devices will be labelled by the manufacturer with a batch number and expiry date.

Figure 3 *Endosign Device labelling and packaging*

8.5. Instructions for Use

All use of the devices will follow the instructions provided by the device manufacturer, as per the Instructions for Use (IFU).

8.6. Storage Conditions

Devices should be stored at room temperature (prior to use) according to instructions issued by the device manufacturer.

8.7. Medical Device Accountability, Returns and Destruction

A Device Management Plan will be developed for BEST4 detailing how devices will be accounted for and managed by Mobile Diagnostic Unit sites.

8.8. Contraindications

Participants receiving anti-thrombotic medication will discontinue their medication if required following the medical guidance in the PIS. A PT-INR test may be conducted on patients on Warfarin prior to the capsule sponge procedure, in line with local arrangements. Other restrictions are listed in section 6.

9. Procedures and Assessments

9.1. Trial Setup

The central trial team will identify the participating NIHR Regional Research Delivery Networks (RRDNs). The selection of regions is guided by the funding model, specifically tailored to support NHS Research part B and service support related costs.

The RRDNs will actively assist in the selection of suitable locations for the Mobile Diagnostic Unit to be placed to optimise participant searches and recruitment. These units will act as participating sites and play a vital role in accommodating the Screening Trial activities. The Mobile Diagnostic Units will be strategically located within a defined radius of the BEST4 Surveillance Trial secondary care endoscopy units which have been identified to also support the Screening Trial.

9.2. Recruitment strategy

Our recruitment strategy follows a two-phase approach utilising a 'Zelen design within a cohort study' (section 6.4). Initially, participants will be identified and consented to the Heartburn Health Programme. Subsequently, eligible individuals for the Screening Trial will be identified from the Heartburn Health Programme cohort. Eligible participants will be randomised, with only those in the intervention arm providing additional consent. Participants in the control arm will not receive further communication regarding the Screening Trial.

Recruitment will span 32 months and will be closely monitored to ensure alignment with recruitment targets outlined in the BEST4 recruitment plan. The recruitment targets are based on an 80% appointment uptake capacity across the five mobile units, with a staggered start-up to allow adequate time for staff training and process optimisations.

Presently, electronic participant identification and consent are the only methods feasible for the Heartburn Health Programme due to the trial's scale and resources available. This design aligns with current routine digital communication practice observed in primary care. We will continuously monitor participant uptake and explore the implementation of a non-digital invitation mechanism if necessary.

9.3. Heartburn Health Programme

We plan to build a cohort of participants with GORD, who have consented to longitudinal health-related data collection and to be contacted further regarding research opportunities within the Heartburn Health Programme. Programme activities will be conducted as per the Heartburn Health Programme Management Plan.

9.4. Participant Identification for Heartburn Health Programme

The Heartburn Health Programme's objective is to enrol a minimum of 120,000 participants. We estimate this will require the identification and invitation of approximately 4.5 million patients. Potential participants will be identified via centralised NHS identification using the Personal Demographic Service (PDS).

NHS Digitrials recruitment service will facilitate access to PDS to identify eligible individuals using GP post code sector across participating RRDNs. Once an eligible cohort is identified, NHS Digitrials will securely extract and share patient details with iPLATO, a trusted third-party responsible for sending text invitations.

Eligible individuals will also be identified using a research participant bioresource, once identified patient details will be shared with iPLATO, a trusted third-party responsible for sending text invitations.

Patients who have applied for National Data Opt-Out will automatically be excluded prior to PDS data extraction. NHS Digitrials will implement the trial specific opt-out within 24 hours of data extraction from PDS.

Written informed consent will not be sought prior to data extract. The legal basis for disclosure of personal information without prior consent will be research in the public interest as a non-commercial research institution and a section 251 exemption will be applied (see 15.5).

Participants will be made aware of the programme via one of the following routes:

SMS text message: iPLATO will send a text message invitation to potential participants, inviting them to join the Heartburn Health Programme. The trial team will determine the optimal size and frequency of the text message batches for each region.
myGP app: In addition to an SMS text message, participants with the myGP app will receive an iPLATO advertisement to join the programme. The advertisement will guide participants to the invitation URL link for the Heartburn Health Programme.

9.5. Opt out of Heartburn Health Programme invitation

Prior to contacting participants, fair processing notices will be displayed on the trial website and on social media. The coordination of these notices will be overseen by the CPTU BEST4 central trial team. These notices will be publicised for a minimum of 4 weeks prior to messaging. The fair processing notice will detail the eligibility criteria for the programme. Additionally, they will provide guidance for individuals who meet the criteria but do not wish to be contacted.

If an individual chooses not to be informed about the programme, they will have the option to opt out of data usage via a user-friendly online form accessible via a URL link or a QR code on posters. To ensure accessibility for all, a contact number for the BEST4 Screening team will also be provided, allowing those facing digital exclusion to complete the online opt-out form over the phone with the assistance of the clinical team.

9.6. Participant Identification for BEST4 Screening Trial

Approximately 120,000 participants will be enrolled onto the BEST4 Screening Trial, 40,000 intervention and 80,000 control cohorts. Upon consenting to join the Heartburn Health Programme, via the iPLATO survey, participants will be asked to answer a short set of health/eligibility questions and whether they would be interested in having a capsule sponge test if it was available in their local area. Participants which express interest and meet BEST4 Screening Trial criteria will be randomised into the control or intervention arm.

A proportion of participants will remain in the Heartburn Health Programme only, if they do not express interest in a capsule sponge test or meet the BEST4 Screening and capsule sponge eligibility criteria.

9.7. Consent

Informed electronic consent will be obtained from each participant in a two-phased approach. Each participant will be informed about the Programme and as appropriate the Trial, what to expect regarding their participation and possible risks and side effects associated with joining the Heartburn Health Programme and as appropriate, the capsule sponge test, via multimedia information including a short explanatory video about the test.

Should a participant require a translation of the Heartburn Health Programme or Screening Trial documentation, this will be accessible in the provided languages linked to the electronic invitations. Should a Screening Trial participant require a verbal translation by an approved interpreter/translator at the time of their capsule sponge appointment, it is the responsibility of the investigator or designee to use an approved translator services.

The screening electronic consent form and any change made during the trial, must be approved by the REC/HRA. Electronic consent will be sought and stored in REDCap. If e-consenting is unavailable, a paper version will be used as an alternative. In this instance, the investigator will retain the original of each participant signed consent form and stored securely by mobile unit sites in their local site file and a copy uploaded in the trial Clinical Data Management Application (CDMA) in REDCap.

In certain cases, such as participants requesting a physical copy of their consent form, EMS will have access to and store patient identifiable data captured on the consent form on to their laptop computers used for the trial. Further guidance on how this will be deleted is documented in the eConsent SOP.

Any new information which becomes available, which might affect the participant's willingness to continue participating in the trial, will be communicated to the

participant as soon as possible. If applicable, participants will be asked to re-consent.

9.7.1. Consent design

The initial phase will seek consent for participants to register to the Heartburn Health Programme, consisting of long-term data collection and future contact. They will also consent to potentially being a control in a future trial with interventions offered to members of the Heartburn Health Programme. Participants who consent to the Heartburn Health Programme will automatically enter the second phase; assessment of eligibility for the capsule sponge test accompanied with assessment of willingness to have the procedure 'if it became available in their local area'. Participants are intentionally not given detailed information on the Screening Trial, to minimise dissapointment bias, if they were assigned a control subject.

Those who consent to the Heartburn Health Programme, express interest in the capsule sponge, and meet the BEST4 Screening criteria will be randomised. Participants randomised to the intervention arm will receive the Screening Trial information by email and be asked to book an appointment. Consent will take place at the time of their capsule sponge appointment. As per the Zelen Design, those randomised to usual care will not be contacted further regarding their involvement as a control.

9.7.2. Consent for BEST4 Heartburn Health Programme

Firstly, for all participants, electronic, individual level consent will be obtained to join the Heartburn Health Programme, via statements embedded within the SMS invitation. Alternatively a self enrolment form will be available on the Heartburn Health website for potential participants to consent. All participants will consent to long-term, related health data collection and future contact for participation in future research opportunities within the Platform. Electronic patient facing documentation will be presented prior to consenting and a contact telephone number for the Cambridge Clinical Team will be available to discuss any Programme queries.

9.7.3. Consent for BEST4 Screening Trial

Patients will receive information about the trial prior to consent when they receive a text message inviting them to book a capsule sponge appointment. Electronic (nurse present), individual-level consent (intervention arm only) will be obtained before carrying out any procedures at the appointment (including swallowing a capsule sponge or receiving a result informed endoscopy). All participants receiving a capsule sponge test or endoscopy as part of the trial will be individually consented to have the procedures and for the associated clinical data to be

collected. Participants will have the opportunity to discuss the nature and objectives of the trial, and possible risks associated with their participation with a EMS Unit staff (at the time of their appointment at the mobile unit) and if required a member of the Cambridge Clinical team (prior to their appointment via telephone).

Participants randomised to the control arm will not be notified.

9.8. Participant enrolment

Following consent, data captured by iPLATO during the invitation process will be imported into the relevant CRFs in the BEST4 CDMA in REDCap to record participant enrolment and consent to the Heartburn Health Programme and Screening Trial.

9.9. Screening Trial Baseline Assessments (Day 0)

Assessments performed at baseline will be completed in accordance with the schedule of events table (see **Table 2**).

Participants will receive the capsule sponge test at baseline on the day of their booked appointment.

9.9.1. Medical History

Mobile Unit staff will be responsible for confirming and reporting relevant medical history information for all participants at baseline via the relevant CRFs in the BEST4 CDMA. Upon medical history assessment, if a nurse deems the participant ineligible for the capsule sponge test, they will not be offered a capsule sponge test and will simply be passively followed within the trial.

9.9.2. Height and Weight Measurements

Mobile Unit staff will be responsible for collecting height and weight measurements of all participants where possible at baseline and entering in the relevant CRFs in the BEST4 CDMA. Should mobile unit sites be unable to collect these measurements during the visit, it will be acceptable for participants to self-report both height and weight measurements for the purpose of the trial. Self-reported measurements will be denoted in the relevant CRFs in the BEST4 CDMA.

9.10. Screening Trial capsule sponge Test Procedures

9.10.1. Participant preparation

Participants will be asked to not eat or drink for 4 hours prior to their appointment. Participants will also be advised in the Participant Information Sheet they can continue to take their daily prescribed medication. Specific instructions, will also be available in the PIS for participants on anticoagulation medication. For participant queries related to this, a contact telephone number will be available for the Cambridge Clinical Team.

Before the procedure, the site staff are responsible for confirming participant eligibility for the capsule sponge procedure, particularly whether the patient has any swallowing difficulties or other clinical contraindications which would put their safety at risk. If the participant has any dysphagia or otherwise clinically unsuitable, they will not be eligible to receive the capsule sponge test. Participants presenting with alarming symptoms will be advised to contact their GP.

9.10.2. Capsule sponge Test Administration

The capsule sponge test will be performed by a trained nurse or healthcare professional that has been assessed and deemed competent by the Cambridge Clinical Team to carry out the procedure independently. The capsule sponge test will be administered according to the manufacturer's instruction for use (IFU).

Once collected, the sponge is then placed in a sample collection pot containing preservative and then dispatched from the mobile unit sites to the central, accredited Cyted laboratory for analysis.

If participants are unable to swallow the capsule sponge on their first attempt, they will be offered a second attempt. A maximum of 2 attempts is allowed per participant.

9.10.3. Post-procedure and assessments

Following the procedure, the participant will be given instructions regarding follow-up arrangements and who to contact if there is a query or problem post procedure. In the event of a sponge detachment or an obvious bleed or other emergency, the nurse will immediately inform the local endoscopy service as per the emergency detachment procedure and local arrangements. A specific emergency SOP will be provided to the Mobile Units outlining the procedure that should be taken in the event of a detachment. Adverse events should be recorded as outlined in section 10.4.1.

9.10.4. Capsule sponge Test results

All capsule sponge tests will be processed by the Cyted laboratory and analysed for H&E and TFF3 as per the BEST4 Laboratory Manual. Positive or equivocal TFF3 samples will also be analysed for p53. Cyted will report sponge results (TFF3, atypia and if required p53) within 14 days of the sample collection date to the BEST4 CDMA. The University of Cambridge Clinical Team will be responsible for centrally reviewing the sponge results in the BEST4 Trial Database and arranging appropriate follow-up.

9.10.5. Capsule sponge Test result communication

All participants will be informed about the results of their sponge test. The University of Cambridge clinical team will be provided with letter template result wording which can be adapted when required. Results being sent via text will have a standard format.

If the participant receives a negative result, they will be informed via a text message.

If the participant receives a negative result with other clinical findings (non-relevant to the trial), an initial inadequate result and decline a repeat test or have a second inadequate result, they will be sent a letter, and an email will be sent to their GP. An 'inadequate' result is a sample which is unable to be analysed due to an insufficient cell collection.

If the participant receives any of the following result from the capsule sponge test an endoscopy referral will be triggered and the patient will be contacted:

Routine endoscopy referral within 8 weeks (+ 4 weeks):

- TFF3 positive

Suspected Cancer Urgent referral:

- TFF3 positive and one of the options below:
    - p53 is aberrant in squamous/columnar cells
    - p53 equivocal in squamous/columnar cells
    - Squamous/columnar atypia of uncertain significance/equivocal
    - "dysplasia" is mentioned e.g dysplasia is suspected, dysplasia is present, high-grade dysplasia, dysplasia difficult to grade

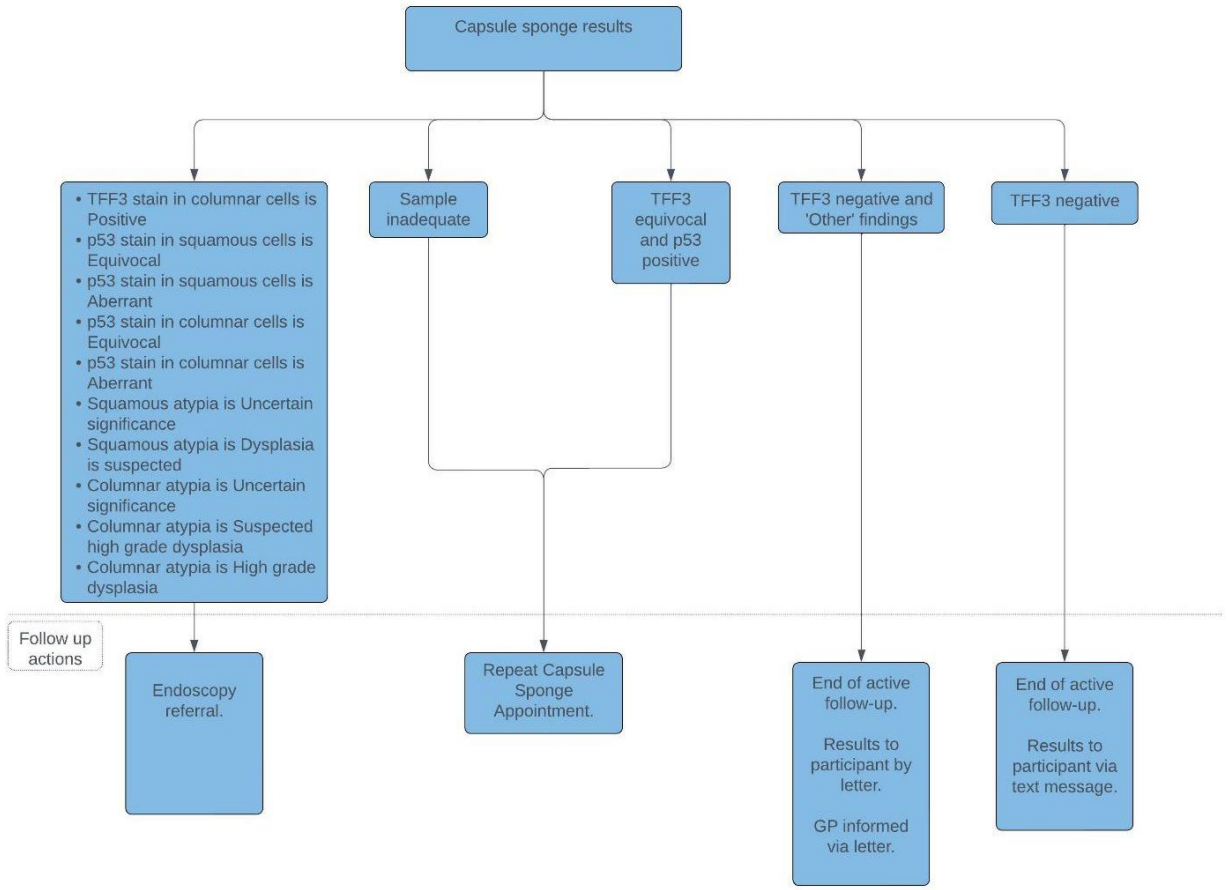

Figure 4: *Flow diagram for Capsule Sponge result reporting and endoscopy referral*

If the participant accepts the invitation to endoscopy, a referral will be requested by Cambridge Clinical Team via NHS electronic referral system at their local participating Secondary Care site. The Cambridge Clinical Team will inform the participants GP if an electronic referral has been made via email or letter. The local Secondary Care team will report the outcome of the endoscopy result as per Standard of Care. If a patient refuses the invitation to endoscopy, the Cambridge Clinical Team will inform their GP of their results by a letter and advise as appropriate.

As it is standard practice to share research results with patients registered GP Practice, we will adopt the same approach in this trial. The Cambridge Clinical team will include sponge results and as appropriate together with endoscopy referrals and outcomes to the participants GP in the methods described above. We will seek consent from participants to share their test results with their GP.

9.10.6. Repeat capsule sponge test

In cases where the capsule sponge test is reported by Cyted as inadequate or the sample fails in processing or TFF3 equivocal, the participant will be invited for a repeat test by text message.

The Clinical Team at Cambridge will be responsible for flagging such cases to iPLATO who will then be responsible for contacting the participant to arrange a repeat capsule sponge procedure. This should be repeated as soon as possible. Repeat capsule sponge procedures will be reported by the team using the relevant CRFs in the BEST4 Database.

Should participants refuse or ignore the invitation to return for repeat sponge procedure then they will not receive further trial interventions. They will still be included in the passive follow-up and data analysis. The Cambridge Clinical team will be responsible for informing their GP regarding insufficient results. These outcomes will be reported by the Cambridge Clinical team using the relevant CRFs in the BEST4 CDMA.

9.11. Endoscopy procedures

All endoscopy procedures will take place under standard clinical consent processes and will not involve the collection of additional research biopsy samples. Standard templates for feeding back clinically relevant diagnoses should be used depending on prevailing trust requirements.

Secondary Care sites will be provided with instructions and be responsible for the collection of research blood and saliva samples prior to the participants endoscopy. Consent for the collection of these samples is taken at the participants baseline visit. Sample collection kits and return sample shipment arrangements will be provided by the University of Cambridge.

9.11.1. Endoscopy results

Secondary Care sites will be responsible for reporting endoscopy results to participants as per usual local procedures and for reporting outcomes of participant endoscopies via the relevant CRFs in the BEST4 database. Endoscopy findings will be clinically managed according to the current (at that time) standard of care guidelines.

Participants who receive a non-dysplastic Barrett's Oesophagus diagnosis will be approached to join the BEST4 Surveillance Trial up until the recruitment target (1000) for this arm is met.

All participants with NDBO will be encouraged to participate in routine surveillance provided by the NHS.

Participants with dysplasia will be discussed at the multi-disciplinary team meeting as per best practice and should be offered endoscopic treatment via the NHS.

9.12. Schedule of Assessments

Table 2 *BEST4 Screening Trial Schedule of Assessments*

Assessment	**Screening/ Identification**	**Baseline**	**Follow-up visit (as required)**	**Long term follow-up**
Visit	N/A	**Day 0**	**Month 2 (+/- 1 month)** **(Follow-up visit for capsule sponge positive participants only)**	
Centralised search for potentially eligible patients in Participating RRDN using Postcode Sector	X			
Text message invitation to Heartburn Health Programme	X			
Heartburn Health Programme Informed consent	X			
Self-assessed eligibility assessment	X			
Screening Trial activities only				
Randomisation	X			
Online booking capsule sponge appointment within 4 weeks of invitation	X			
Confirm capsule sponge procedure eligibility		X		
Screening Trial Informed consent		X (intervention cohort)		
Demographics	X			
Medical history (inlcuding smoking status)		X (intervention cohort)		
Medication review		X (intervention cohort)		
Height and weight measurement		X (intervention cohort)		

Vital signs		X (intervention cohort – as required per clinical staff discretion)		
Capsule sponge administration		X (intervention cohort)		
Adverse event assessments		X (intervention cohort)	X (triggered positive sponge result)	
Result SMS message (Negative & repeat cases)		X (intervention cohort)		
Result letter (positive & other clinical findings cases)		X (intervention cohort)		
Endoscopy clinical consent			X (triggered positive sponge result)	
Endoscopy (Excess treatment)			X (triggered positive sponge result)	
Blood collection			X (triggered positive sponge result- high risk)	
Saliva sample collection			(x)	
Immunohistochemistry on capsule sponge (H&E,TFF3)		X (intervention cohort)		
Biomarker analysis on capsule sponge (p53,atypia)		X (triggered positive TFF3)		
Passive follow-up via NCRAS				X(annually for 12 years)

9.13. Summary of Assessments

Prior to the capsule sponge procedure, vital signs wil be recorded for participants based on clinical staff discretion upon their clinical assessment. These will include: blood pressure and pulse. Particpants on anticoagulation medication, will be assessed to ensure they have followed the relevant stopping guidelines as per their medication.

9.14. End of Trial

The active part of the Screening trial will end once recruitment has concluded, referral endoscopies performed as required, all results have been received/recorded in the trial database and participants and GPs have been informed of results. To ensure all data collection and all pre-specified data and sample analysis has completed, we anticipate this will be around 6 months after the final trial visit has taken place. There will be no further follow-up visits. Participants who go on to be diagnosed with Barrett's will be clinically managed by their local Secondary Care team.

After this, the Screening trial will enter a long-term follow up phase, where collection of passive follow-up data will continue to be carried out for up to 12 years from trial recruitment start date.

The Heartburn Health programme recruitment will end once the Screening trial has concluded recruitment and will continue as a standalone bioresource. Participants will consent to their data and (if appropriate) samples being used and to be contacted for future approved research indefinitely after end of trial.

9.15. Long-Term Follow-up Assessments

After the trial has ended, we will continue to passively observe those enrolled in the trial through National Cancer Registration and Analysis Service (NCRAS). We will seek permission from NCRAS to extract annual oesphageal cancer diagnosis and death data for all participants enrolled in the trial for up to 12 years from trial recruitment start date, with the exception of those who have withdrawn consent. The collection of this follow up data will be coordinated by CPTU as outlined in the Data Management Plan.

10. Assessment of Safety

10.1. Definitions

10.1.1. Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence, unintended disease or injury, or any untoward clinical sign, including abnormal laboratory findings, in subjects, users or other persons.

A pre-existing condition must not be recorded as an AE or reported as an SAE unless the condition worsens during the trial and meets the criteria for reporting or recording in the relevant CRF.

10.1.2. Serious adverse event (SAE)

A serious adverse event (SAE) is defined as any AE that:

- led to death
- led to serious deterioration in the health of the subject that either resulted in:
    - a life-threatening illness or injury[1]
    - a permanent impairment of a body structure or a body function
    - inpatient hospitalisation or prolonged existing hospitalisation[2]
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
    - chronic disease
- led to foetal distress, foetal death or a congenital abnormality or birth defect

[1] The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[2] A planned hospitalisation for pre-existing condition, or a procedure required by the trial, without a serious deterioration in health, is not considered to be a SAE.

10.2. Assessment of causality

The assessment of relationship of adverse event to the trial is a clinical decision based on all the information available at the time of the event. The following categories will be used to define the causality of the adverse event:

Category	Definition

Definitely:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Possibly	There is some evidence to suggest a causal relationship. However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant events).
Unlikely	There is little evidence to suggest there is a causal relationship. There is another reasonable explanation for the event (e.g. the participant's clinical condition).
Not related	There is no evidence of any causal relationship.
Not Assessable	Unable to assess on information available.

10.3. Expectedness

Category	Definition
Expected	An adverse event which is consistent with the information clearly defined in this protocol.
Unexpected	An adverse event which is not consistent with the information clearly defined in this protocol.

10.4. Recording Adverse Events

All procedures in this trial including the capsule sponge and endoscopy procedures are both routinely used within the NHS either as part of standard of care or via NHS pilots. Therefore, we do not plan to collect AEs, other than the AEs of special interest as detailed below in **Section 10.4.1** occurring within 7 days of receiving the capsule sponge or endoscopy, as well as all SAEs (see **Section 10.5**) whether or not related to the procedure**.** AEs of special interest will be recorded by delegated members at participating sites using the relevant CRFs in the BEST4 CDMA. Participating sites will receive training on the process for recording AEs.

10.4.1. AEs of special interest

The following list are expected adverse effects of receiving the capsule sponge test or endoscopy and will be collected for this trial:

Capsule sponge related:

- Capsule sponge detached from the string while in the patient's oesophagus/stomach
- Inability or difficulty to remove the sponge
- Laceration at the back of the throat

Endoscopy related:

- Bleeding from endoscopy biopsy site (not requiring hospital admission)
- Perforation or tear of the oesophagus (not requiring hospital admission)

10.5. Recording and reporting of SAEs, SARs, and SUSARs

All SAEs occurring from the time of consent until 7 days after receiving the capsule sponge or endoscopy will be collected. The assessment of causality (to determine if an SAE is a SAR) will be performed by the PI and CI or their delegate. An SAE is not required to also be an AESI as outlined in Section 10.4.1 to be reportable. All events meeting seriousness criteria must be reported.

10.5.1. Reporting procedures

Participating sites will receive training on the process for reporting SAEs and a trial specific reporting SOP will be produced for the trial which local site staff will follow. If the sites are informed by a participant about an adverse event that meets the criteria of serious, is not exempt from report, the SAE should be reported to the CPTU (on behalf of the sponsors) within 24 hours using the trial specific SAE form.

Once the SAE form is received by CPTU, this will be sent to the Chief Investigator (or delegate) for review and assessment of causality and expectedness. CPTU QA Manager should also receive the SAE form for their review.

Only SARs i.e. events resulting from trial procedures which are unexpected i.e. not listed in this protocol as expected, will be reported to the REC following their standard SUSAR reporting procedures.

10.5.2. Expected events

Capsule sponge related:

- Laceration at the back of the throat requiring patient hospitalisation
- Obstruction on breathing or airway as a result of the sponge

Endoscopy related:

- Bleeding from biopsy site requiring patient hospitalisation

10.5.3. Exempt events not requiring reporting as SAEs

- Cancer diagnosis (including hospitalisations, life-threatening events or deaths related to a diagnosis)

10.6. Reporting Urgent Safety Measures

The CI will take urgent safety measures, if necessary, to ensure the safety and protection of participants from immediate risks to their health and safety. The measure will be taken immediately. The approval of the REC prior to implementing urgent safety measures is not required. However, the CI will inform the sponsors and REC (via telephone) of this event immediately. The CI will inform the REC in writing within 3 days, in the form of a substantial amendment. The Sponsors will be sent a copy of the correspondence.

10.7. Responsibilities

The CI has overall responsibility on behalf of the sponsor for safety reporting. The CI will ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

11. Storage and Analysis of Samples

All material will be handled in line with the Human Tissue Act 2004. Material transfer agreements will be implemented as required by the Joint Sponsor.

11.1. Capsule sponge samples

As per the BEST4 laboratory manual, all capsule sponge samples will be labelled with trial ID, NHS number, participant initials date of birth and sex. Samples can be stored at room temperature shipment to the central laboratory. All samples will be shipped at room temperature directly from the Mobile Diagnostic Units to the central laboratory to be processed and analysed for TFF3 and H&E. Samples which are TFF3 positive will also be processed for p53 and atypia.

Results will be reported by Cyted within 14 days via electronic data transfer into the BEST4 REDCap CDMA. The Cambridge clinical team will be reporting on all the results and have access to the CDMA. All samples will be pseudonymised by the lab with a unique ID after initial processing.

11.2. Blood and saliva samples

For the BEST4 Screening trial blood and saliva will be collected from patients at a higher risk of developing Cancer. Whole blood will be collected for the bioresource for future research on the following: germline blood variants, cell free nucleic acids, extracellular vesicles, while blood cell genomics, proteomics & transcriptomics.

Samples will be collected from participants based on their capsule sponge result:

- TFF3 positive and Atypia positive and/or p53 positive
    - For all participants: whole blood and saliva sample
- TFF3 positive and Atypia negative and p53 negative
    - 1600 participants: blood and saliva sample*
    - 2052 participants: saliva sample only*

*TFF3 positive and Atypia negative and p53 negative participants will be allocated additional sample collection at each site as per the Secondary Care Operations manual. Samples collected will be kept at specific temperature conditions and transferred to the Cambridge central laboratory for processing.

11.3. Long-term residual sponge material storage

Participant consent will be sought for any residual Capsule Sponge material following the analysis outlined in section 11.1. The residual material, following diagnosis, will be stored long-term in an HTA licenced facility at the University of Cambridge and used in future unspecified research by the university and other organisations (including by overseas organisations and in the commercial sector) in line with patient consent and the appropriate regulatory requirements.

During the trial, the BEST4 Trial Committees will be responsible for reviewing and authorising any analysis (not outlined in this Protocol) involving the residual capsule sponge material.

Once the trial has ended, the University of Cambridge will act as sample custodians and a system will be implemented where a researcher can outline their study and apply for requests for samples (and data) as outlined in **Section 22**

Residual samples from the capsule sponge will consist of; formalin fixed paraffin embedded (FFPE) blocks and slides which will be stored at room temperature and leftover extracted DNA which will be stored in freezers set to -20°C and any electronic images of capsule sponge materials.

The residual material will be stored in an HTA licenced facility at the University of Cambridge and may be used in future unspecified research by the university and other organisations (including by overseas organisations and in the commercial sector) in line with patient consent and the appropriate regulatory requirements. New ethical approval will be secured as required.

11.4. Long-term Saliva and Blood material storage

Saliva and Whole Blood samples (intervention arm only for participants with positive capsule sponge test result) will be pseudonymised and sent directly from hospital sites to the Early Cancer Institute at the University of Cambridge via an arranged courier at +/-4°C.

On arrival, pseudonymised samples will be stored in freezers set to -80°C at the Early Cancer Institute, including at the end of the trial for long term storage.

Once the trial has ended, sample custodianship will rest with the University of Cambridge and a system will be implemented where a researcher can outline their study and requests for data or samples. The release of materials will only be permitted after all planned analysis of the BEST4 screening trial have been performed to ensure that materials are retained for any quality control or additional analyses which inform the primary or secondary endpoints following which, we will consider sharing samples on request by external investigators.

11.5. Protein detection and Methylation analysis

The University of Cambridge will be responsible for performing additional exploratory biomarkers on the material including methylation and protein related biomarker detection on a subset of capsule sponge and supernatant samples to ascertain differential methylation changes and quantification of protein biomarkers indicative of dysplasia. This activity will be exploratory and will not inform patient management. The assay and methods used for the methylation and protein detection will be detailed in a separate Laboratory manual or analysis plan written by the University of Cambridge.

12. Statistics

12.1. Statistical Methods

Data on trial conduct and outcomes will be presented using appropriate descriptive methods. For example, mean and standard deviation (continuous, symmetrically distributed data), median and quartiles (continuous, skewed data) or frequency count and percentage (categorical data).

For Kaplan-Meier estimation of coprimary endpoints, the time origin will be date of randomisation and time-to-event will be calculated, in those who experience the event of interest, as time from date of randomisation to date of event, and in those who do not experience the event of interest, as time from date of randomisation to date of last follow-up. Observations relating to participants who withdraw from the trial before the event of interest will be censored at date of withdrawal. Observations relating to participants who do not withdraw from the trial and at the time of analysis have not experienced the event of interest will be censored at date of last follow up. For estimation of the cumulative incidence of stage II+ or death from OAC, the event of interest will be diagnosis of stage II+ OAC or death from stage I OAC (with time measured to the earliest event) and stage II+ OAC will include diagnosis of OAC with unknown stage. This composite endpoint will be referred to here as "advanced OAC". For estimation of the cumulative risk of death from OAC (inc. GOJ), the event of interest will be death from OAC (inc. GOJ) of any stage.

For the primary (ITT) analyses, we will test for a difference between the trial arms (Kaplan-Meier curves) using a log-rank test. For the secondary (ITT) analyses, hazard ratios (with 95% confidence intervals) will be obtained by fitting Cox regression models, with and without adjustment for covariates that may influence risk of OAC such as age, sex, waist-to-hip ratio, family history and duration of GERD. For the CACE analysis, the effect of the intervention will be estimated in the latent population who would comply with capsule sponge screening, if invited. Compliers, in the intervention arm, will be defined as those who accepted the invitation to be screened and attended for screening. The CACE will be estimated using a latent class approach under exclusion restriction and monotonicity assumptions. (24)

ITT and CACE estimates of the intervention effect on risk death from oesophago-gastric cancer will also be calculated as above, but with the event of interest defined to be death from oesophageal or gastric cancer.

The positive predictive value of screening will be calculated as the proportion of screened individuals (i.e. participants in the intervention arm who attended for screening and swallowed the capsule sponge successfully) with dysplastic BO or IM OAC diagnosed via

endoscopy triggered by a TFF3 positive screen divided by the number of screened individuals with dysplastic BO or IM OAC.

Analyses will be instigated using data at 4.5, 6.0, 9.0 and 12 years after the first participant is randomised (mean follow-up of approximately 3.25, 4.75, 7.75 years and 10.75 years) using the following conditional strategy:

At 4.5 years test for a significant (alpha = 1.0%) difference in advanced OAC.
If significant, at 6.0 years, test for a significant (alpha = 1.0%) difference in OAC mortality.

If no significant reduction in advanced OAC at 4.5 years, at 6.0 years test for a significant (alpha = 2.5%) difference in advanced OAC. If this is significant, test for a significant (alpha = 2.5%) difference in OAC mortality at 7.5 years.
If there was no significant difference in advanced OAC at 6.0 years, at 9.0 years test for a significant (alpha = 1.5%) difference in advanced OAC. If there was a significant difference in advanced OAC at 4.5 years, at 9.0 years look at trends in the hazard ratio for advanced OAC by time from randomisation. Formal inference will focus on data that has accrued since the 4.5-year analysis.

At 12.0 years test for a significant (alpha = 1.5%) difference in OAC mortality.

It is noted that it is likely to take at least 6 months after diagnosis for cancer registry data to be reasonably complete and the database will only be "locked" for each interim analysis once sufficient time has elapsed for registrations to be reasonably complete.

Overall, alpha for the primary outcome is 5% (1% +2.5% +1.5%). The results of each analysis will be published independently, as soon as it has been completed. A detailed statistical analysis plan (SAP) will be produced before the final data base lock for the first interim analysis (4.5 years after the first participant is randomised).

12.1.1. Analysis populations

The ITT analysis set will comprise all randomised participants according to their randomised trial arm. This analysis set will also be used for the CACE analysis.

12.2. Viability Analysis

A viability analysis will take place 12 months after the first participant is randomised with results discussed by the Trial Steering Committee, using the following traffic lights:

Number randomised over the previous 6 months:

o Green: ≥24,000

o Amber: 15,000-23,999

o Red: <15,000

Uptake of capsule sponge test amongst those recruited in the first 10 months:

o Green: ≥67%

o Amber: 55%-67%

o Red: <55%

Detection of Barrett's oesophagus amongst participants swallowing a sponge:

o Green: ≥7%

o Amber: 5%-7%

o Red: <5%

Detection of dysplastic Barrett's oesophagus or intramucosal cancer amongst participants swallowing a sponge:

o Green: ≥0.5%

o Amber: 0.3%-0.5%

o Red: <0.3%

The TSC will discuss stopping the trial if there are two or more red categories. If there is one red category or two or more that are red or amber, the TSC will re-consider the viability of the trial at subsequent meetings after recommending changes to improve the situation.

12.3. Interim Analyses

At 4.5 years, the first interim analysis will take place using the endpoint of advanced OAC (stage II+ or fatal OAC) using a two-sided alpha of 1.0%. The results of this analysis will be published in a peer-reviewed journal. If there is a significant reduction in this endpoint associated with screening: (i) the analysis of advanced OAC at 6.0 years will not take place but there will be an analysis at 9.0 years that will focus on the new data since the 4.5 year analysis and will look at the hazard ratio as a function of time from randomisation; (ii) there will be an analysis of OAC mortality at 6.0 years also using a two-sided alpha of 1.0% and this analysis will be published. If the mortality analysis at

6.0 years is not significant, mortality will also be studied at 7.5 years (using two-sided alpha of 2.5%).

If there is no significant reduction at 4.5 years, there will be no mortality analysis at 6.0 years, but there will be a second advanced OAC analysis at 6.0 years (using two-sided alpha of 2.5%). If this analysis shows a significant reduction associated with screening, then there will be a OAC mortality analysis at 7.5 years.

The final primary analysis of advanced OAC will be at 9.0 years with a two-sided alpha of 1.5%. The final analysis of OAC mortality will be at 12.0 years regardless of the result of the 9.0-year advanced OAC analysis. At 12.0 years, trends in the hazard ratios of advanced OAC and of OAC mortality over time since randomisation will also be analysed.

Note that each interim analysis conducted will be published regardless of its result. If at any time point, there is a significant reduction in either advanced OAC or in OAC mortality that will be considered significant at the 5% level.

12.4. Sample Size Calculation/Justification

We have calculated the sample size required for a group-sequential analysis of the co-primary endpoints using a multistate disease progression model (full details are given in BEST4 Technical Supplement 1 v1.0.docx). In this model (Figure 4), participants enter the trial in one of 5 latent disease states (GERD, NDBE, DBE, IM OAC1, Stage II+ OAC), which are listed in order of severity and represent what we believe to be the key milestones for the development of OAC. During the trial, each year, participants either remain in their previous disease state (i.e. do not progress), move to the next disease state (i.e. experience disease progression) or die of other causes (a competing risk). Progression from stage II+ disease can only be to one of two absorbing states; either death from OAC or death from other causes (Note: an absorbing state is one that, once entered, cannot be left).

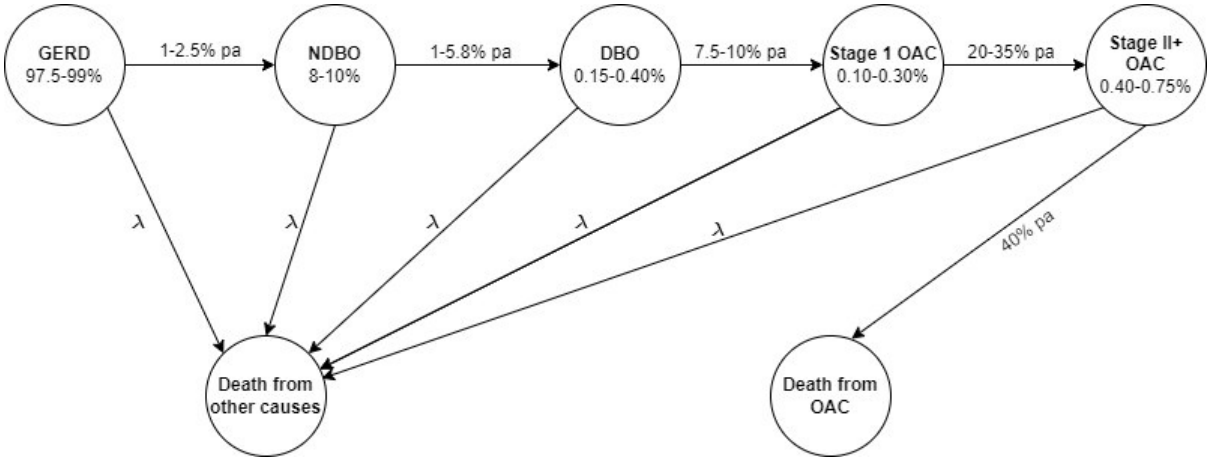

Figure 4 *Multi state model representing progression from GERD to stage II+ OAC, with death from other causes as a competing risk.*

Legend: At entry into the BEST4 Screening Trial a participant might be in any of five (live) states. The range of proportions assumed to be in each state at entry in different scenarios is shown within the state circle. λ represents all-cause mortality, which increases annually from 1.8% (year 1) to 6.5% (year 12).

The numbers remaining in the same state, progressing to the next disease state or to the absorbing states are calculated each year for a specific set of assumptions (i.e. prevalence and transition rates) and estimates of uptake, screening sensitivity and screening, surveillance and treatment effectiveness are used to model the effect of the intervention in the screened arm. The number in each group are then adjusted to reflect the planned sample size (120,000) and randomisation ratio (2:1, control vs intervention) so that the anticipated power under each scenario (set of assumptions) may be calculated. Overall type II error (α) is controlled using an alpha spending function (so-called because at each analysis some of the overall α is "spent") to allow for sequential (multiple) testing. Specifically, our group-sequential design spends 1% at the first analysis, 2.5% at the second analysis and 1.5% at the final analysis. Note that because the mortality analyses at 6 and 7.5 years are contingent on a significant reduction in advanced OAC (at 4.5 or 6.0 years, respectively) this does not add to the Type I error.

There is considerable uncertainty (and disagreement among experts) regarding what values should be assumed for the prevalence, transition rates and efficacy. Therefore, we have estimated power under a range of plausible assumptions (scenarios 1-16, Appendix - 2). All scenarios assume that 40% of those (alive) with Stage II+ oesophageal cancer die from OAC each year.

Our results show that in many scenarios there will be 80% power for the analysis of the first coprimary endpoint (advanced OAC) at 6.0 years and that in some scenarios there will be sufficient power to undertake this analysis at 4.5 years. For the analysis of the second coprimary endpoint (OAC mortality), the power is generally poor at 6 years but exceeds 80% for many scenarios at 7.5 years and 12 years. Furthermore, it should be

noted that in some scenarios, for both endpoints, the sequential design leads to insufficient power at 9.0/12 years, which is unsurprising as we are setting the threshold for significance very high (there is little alpha left to spend). Thus, in some scenarios, it would be beneficial to drop the proposed analyses at 4.5/6 years (and maybe also at 6.0/7.5 years) to preserve alpha for the final timepoint. We therefore need our sequential trial design to also be adaptive (i.e. to allow, in certain circumstances, the proposed analyses at 4.5/6 years and 6.0/7.5 years to be postponed or dropped). We may for instance, postpone the first interim analysis by 9 months if the total number of events (advanced OAC) is between 350 and 399, and cancel it and "move the alpha" to the final time point (i.e. allowing alpha at that time to increase from 1.5% to 2.5%) if there are fewer than 350 advanced OAC events at 4.5 years. This will require a data-based decision rule that doesn't adversely affect overall power. We will therefore develop a microsimulation model to explore the impact of any proposed adaptations/decision rules on overall power and help identify an appropriate adaptation strategy.

12.5. Criteria for the Premature Termination of Trial

The viability analysis (12.2) will take place 12 months after the first participant is randomised and the TSC will discuss stopping the trial if there are two or more red categories or recommend changes to improve the situation if there is one red category or two or more that are red or amber. As all participants will have been recruited prior to the first interim analysis there are no other pre-determined stopping criteria. However, premature termination would be triggered by concerns raised by the investigators and/or the Data Monitoring Committee (DMC) and/or the Trial Steering Committee (TSC) regarding safety and ethical implications of continuing the trial.

If the trial shows a significant effect on OAC mortality at 6.0 or 7.5 years, there is no plan to offer screening to those in the control arm of the trial. However, it may be that such a result will trigger the roll-out of a national screening programme, in which case some participants in the control arm may be offered screening. Note also that if, for instance, there is a significant reduction in advanced OAC at 4.5 years, there may well be planning and piloting of screening within the NHS whilst awaiting the mortality result.

12.6. Procedure to Account for Missing or Spurious Data

Procedures for handling missing data will be detailed in the SAP. We do not anticipate missing data (other than censoring) for the randomisation or the for primary outcomes. (Note that stage missing will be treated as stage II+).

12.7. Economic Evaluation

The aim is to evaluate the incremental cost (positive or negative) and cost-effectiveness (in terms of the incremental cost per quality-adjusted life year (QALY) gained; a cost-utility analysis) of screening using the capsule sponge-TFF3 test versus no screening. We will also undertake a budget impact analysis to evaluate the total national budget impact of conducting one round of Cytosponge-TFF3 screening in the UK. For both analyses we will take an NHS and personal social services perspective. Costs and benefits will be discounted at the recommended rates at the time of analysis.

Our analysis will adapt the economic model that we developed for the BEST3 study, which included Markov model with a one-year cycle-length and a lifetime time horizon, modelling movement between health states (NDBO, LGD, HGD, OAC, death).[25] To reflect current practice and uncertainty, the model will be adapted/updated to account for different models of triage and surveillance among individuals with a positive screening test result. The model will also be updated to include the interim findings at 4.5, 6, 9 and 12 years after the first participant is randomised, thus producing four versions of the health economic analysis, updating over the duration of the trial.

We will produce a detailed Health Economic Analysis Plan before the first interim analysis point at 4.5 years, which will be reviewed by the Trial Steering Committee (TSC).

12.7.1. Measuring costs and outcomes

To measure costs, we will include the cost of the capsule sponge-TFF3 test (including that multiple tests may be needed), endoscopies, and biopsies, which will be recorded directly in the trial. We will also include the costs of treating LGD, HGD and OAC for those who require such treatment during their lifetime, including the use of PPI drugs, H2 antagonist drugs, RFA, EMR and treatment of invasive cancer and palliative care. We will measure outcomes in terms of QALYs; initial health states will be ascertained directly during the trial and the utility values for these health states will be taken from published sources. Transition probabilities for the movement between health states will be measured directly during the trial, updated at each analysis point.

12.7.2. Measuring cost-effectiveness

The primary economic endpoint will be the incremental cost per QALY gained of screening using capsule sponge-TFF3 testing versus usual care. We will also report incremental costs, QALYs gained, incremental net monetary benefits and outputs relating to different cost components and number of endoscopies. We will also measure national budget impact, utilising different assumptions around uptake of screening. We will undertake sensitivity analyses to explore the impact of uncertainty on our findings at each analysis

point, including one-way deterministic sensitivity analysis, and probabilistic sensitivity analysis, also producing cost-effectiveness acceptability curves and calculating the probability that screening will be cost-effective.

13. Data Handling and Record Keeping

13.1. Case Report Form (CRF)

Data for this trial collected at participating mobile unit sites will be captured in electronic CRFs and general clinical notes held in the BEST4 Clinical Data Management Application in REDCap (as outlined in section 13.1.1). All clinical trial data in the CRF must be extracted from and be consistent with the relevant source documents, where provided by particpants, or accurately recorded when patient reported. Source documentation will only apply to medication history data, where prescription slip is provided. The CRFs must be completed by the investigator or designee in a timely manner. REDCap automatical creates an audit trail of the user completing the CRF, date/time of change and changes made. It remains the responsibility of the investigator for the timing, completeness, legibility and accuracy of the CRF pages.

Paper CRFs will be provided to mobile unit sites should electronic CRFs or access to the database is not available. Completed pCRF data should be entered into the CDMA as soon as possible i.e. within 7 days of the pages being completed/or participant visit being completed. The investigator will retain all copies of the pCRF(s) in the relevant sections of their ISF as required. All pCRF pages must be clear, legible and completed in black ink. The pCRF should be dated and signed by the investigator or designee. Any errors should be crossed with a single stroke so that the original entry can still be seen. Corrections should be inserted, and the change dated and initialled by the investigator or designee. If it is not clear why the change has been made, an explanation should be written next to the change. Typing correction fluid must not be used.

It is planned for data requested from the National Cancer Registration and Analysis Service (NCRAS) to be stored in the Data Safe Haven (DSH) provided for Queen Mary University of London by Arrow Business Communications Limited trading as ARO. The DSH is further described in section 13.1.1 (CDMA).

13.1.1. Clinical Data Management Application (CDMA)

Queen Mary University of London will be responsible for securely collecting and storing trial data. The BEST4 CDMA will be built in REDCap. The DSH is provided by Arrow Business Communications Limited trading as ARO, a GDPR compliant third-party storage provider within the UK.

Queen Mary University of London will be the data processor.

The DSH is a controlled and secure environment for undertaking research using sensitive data. The service provides robust controls and safeguards to enable the secure transfer of sensitive data into a highly secure environment where it can be stored, manipulated, and analysed by approved members of the research team. Only designated members of the CPTU and BEST4 trial team will have access to the trial CDMA (and DSH).

Furthermore, REDCap supports data management practices such as encryption protocols, data locking mechanisms, data verification, and validation methods. With these quality assurance measures in place ensures data quality and consistency.

Full details will be provided in the BEST4 Screening Data Management Plan.

13.2. Source Data

- To enable peer review, monitoring, audit and/or inspection the investigator must agree to keep records of all participants (sufficient information to link records e.g. CRFs, hospital records and samples), all original signed ICFs and copies of the pCRF pages.
  For this trial, the majority of eCRFs within the REDCap CDMA are considered as the only source for the data collected. These include:

- eCRFs for all participant reported trial data collected at MDUs

- Electronically signed ICFs

Additional electronic free textnotes within REDCap (outside of trial specific eCRF data)Exceptions to these, where alternative sources of data are expected, include:

- Visit and medical records (including endoscopy and histopathology reports) held via electronic or paper patient notes at Secondary Care sites as appropiate Cyted Laboratory records and pathology reports (held electronically)

- Long-term follow-up (LTFU) datasets provided from NCRAS

- Prescription slips provided by participants at baseline appointment

13.3. Data Protection & Participant Confidentiality

All investigators and site staff involved in this trial must comply with the requirements of the Data Protection Act 2018, the UK General Data Protection Regulation (GDPR) and Trust Policy with regards to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles.

Data for the trial will be shared via secure NHS email or a secure data sharing platform. Robust data sharing agreements will be put in place with all organisations as necessary to ensure the confidentiality and appropriate data handling.

Access to Personally Identifiable Data in the BEST4 CDMA will be managed by the CPTU and will be limited to specific individuals who require access to fulfil their role i.e. for monitoring purposes.

14. Trial Committees

14.1. Independent Data Monitoring Committee

The aim of the Independent Data Monitoring Committee (IDMC) will be to review the safety and design of the trial and advise the Trial Steering Committee (TSC) on the conduct of the trial. The role, function and meeting frequency will be in a charter written by CPTU which will be signed by all members.

14.2. Trial Steering Committee

The aim of the Trial Steering Committee (TSC) is to provide overall supervision of the Trial and will include members who are independent of the investigators and their organisations, funders and Sponsors. The TSC will monitor trial progress and conduct and advise on scientific credibility. The role, function and frequency of TSC meetings will be described in a charter written by the CPTU and signed by all members.

14.3. BEST4 Platform Management Group

The aim of the BEST4 Management Group (MG) is to manage and monitor all aspects of the set-up of the BEST4 Platform (BEST4 Surveillance Trial and BEST4 Screening Trial). Members will consist of the lead investigators of the BEST4 Platform, Chief Investigators, the BEST4 Trial managers at CPTU, Cambridge Clinical Team. The role, function, and frequency of MG meetings during the set-up of the BEST4 Platform is described in a charter written by the CPTU and signed by all members.

14.4. Trial Management Group

The BEST4 Screening Trial Management Group (TMG) will be formed once the trial begins active recruitment. The TMG will meet regularly to oversee the day-to-day conduct of the trial, review safety events and oversee recruitment and the proportion of participants recruited into each risk group. Members will consist of the lead investigators of the BEST4 Platform and the Chief Investigator of the BEST4 Screening Trial, the BEST4 trial Team

at CPTU and Clinical Team at UoC. The role, function and frequency of TMG will be described in a charter written by the CPTU and signed by all members.

14.5. Coordinating Centre

The central coordinating centre (non-clinical) is The Cancer Research UK Cancer Prevention Trials Unit (CPTU) at the Queen Mary University of London.

The central coordinating centre (clinical) is based at the Early Cancer Institute at University of Cambridge and will be responsible for any relevant clinical oversight activities e.g. Sponge capsule test training and referrals.

14.6. Patient and Public Involvement (PPI)

The BEST4 PPI Panel and other PPI contributors from the Cancer Prevention Group PPI Pool will advise on the trial design and participant information. The BEST4 PPI Panel is comprised of people with reflux, Barrett's oesophagus and/or oesophageal cancer in the trial age range. Any additional contributors will also meet these criteria. A PPI member will also sit on the TMG and TSC. This involvement will be coordinated by the CPTU.

15. Ethical & Regulatory Considerations

15.1. Declaration of Helsinki and Good Clinical Practice

The trial will be performed in accordance with the spirit and the letter of the Declaration of Helsinki, the conditions and principles of GCP, the Protocol and applicable local regulatory requirements and laws.

15.2. Medicines and Health Regulatory Agency (MHRA)

This trial is not considered to be under the remit of the MHRA and as such we will not receive a Notice of No objection from the MHRA. The device will be used within its licenced intended use and the results generated will be shared with participant's healthcare professionals for clinical assessment i.e. the capsule sponge is not making the clinical management decision.

15.3. Health Research Authority Review

Before the start of the trial or implementation of any amendment, we will obtain approval of the Protocol, Protocol amendments, ICFs and other relevant documents e.g., advertisements and General Practitioner (GP) information letters if applicable from the HRA. All correspondence with the HRA will be retained in the Trial Master File (TMF) and Investigator Site File (ISF).

15.4. Ethics Committee Review

Before the start of the Trial or implementation of any substantial amendment we will obtain approval of the Protocol, Protocol amendments, ICFs and other relevant documents e.g. advertisements and GP information letters if applicable from the REC. All correspondence with the REC will be retained in the TMF/ISF.

Annual reports will be submitted to the REC in accordance with national requirements. It is the CI's responsibility to produce the annual reports as required.

15.5. Confidentiality Advisory Group (section 251 support)

We will seek Confidentiality Advisory Group (CAG) Section 251 support because it is not practical to seek individual consent from a patient population of this size. Our aim is to identify eligible participants with a greater risk of GORD via centralised NHS Digitrials Recruitment Service Personal Demographic Service (PDS) dataset from the post code sector within a target location.

For efficient identification and invitation, we will collaborate with NHS Digitrials and iPLATO. NHS Digitrials will formally specify the data type and its intended purpose in an application to NHS England. Once access to PDS data is granted, data extracts will be made available to iPLATO, who will send invitations to potential participants.

The identification and invitation process will encompass the following stages:

1. **PDS data extraction:** NHS Digitrials will tailor an application to NHSE for PDS access to identify and securely extract patient identifiable data of eligible individuals from the postcode sector within a target location.
2. **Secure Data transfer:** NHS Digitrials will deposit data extracts into a secure data repository accessible by iPLATO.
3. **SMS invitation:** iPLATO will utilise extracted data to send SMS text messages to eligible participants. Recipients will be invited to join the programme and directed to detailed information through provided links.

Data retention will not exceed 30 days. After this period, data for non-responders and those who decline participation will be promptly deleted. iPLATO will transmit patient details (name, date of birth, sex, NHS number, postcode, mobile number, and email address) to QMUL's DSH only following electronic consent.

15.6. Protocol Amendments

Protocol amendments must be reviewed and agreement received from the Sponsor for all proposed amendments prior to submission to the HRA and REC.

The only circumstance in which an amendment may be initiated prior to HRA or REC approval is where the change is necessary to eliminate apparent, immediate risks to the participants (i.e. urgent safety measures). In this case, accrual of new participants will be halted until the HRA and REC approval has been obtained.

In the event of an urgent safety measure, the CPTU will be responsible for notifying each participating site as agreed with the Joint Sponsors.

15.7. Peer Review

The Trial design was peer reviewed as part of the competitive project grant application with CRUK.

15.8. GCP Training

All trial staff must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this trial. This training should be updated every 2 years or in accordance with the sponsors policy.

15.9. Financial and other competing interests

Rebecca Fitzgerald is a named inventor of the Cytosponge technology and associated assays and holds shares in Cyted Ltd.

All BEST4 Committee members will be required to disclose any competing interests prior to joining the BEST4, MG, TMG, TSC, IDMC.

16. Sponsorship, Financial and Insurance

This trial is jointly sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge. The University of Cambridge will arrange insurance for negligent harm caused as a result of Protocol design and for non-negligent harm arising through participation in the trial.

CPTU are delegated trial activities and oversight functions for this trial by the joint sponsors as outlined in the research collaboration agreement. QMUL will be responsible for the provision of liability for the trial design of this trial.

The trial is funded by Cancer Research UK (SEBSTF-2021\100036) and the NIHR HTA (NIHR135565).

17. Monitoring, Audit & Inspection

The Principal Investigator must make all trial documentation and related records available should an inspection or audit occur. Should a monitoring visit or audit be requested, the Prinicipal Investigator must make the trial documentation and source data available to the Sponsor's representative. All participant data must be handled and treated confidentially.

The Joint Sponsors' monitoring frequency will be determined by an initial risk assessment performed prior to the start of the trial. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the trial. Throughout the course of the trial, the risk assessment will be reviewed and the monitoring frequency adjusted as necessary.

Central monitoring will be conducted for all vendors. The scope and frequency of both central and on-site monitoring will be determined by the risk assessment and detailed in the Monitoring Plan developed by CPTU.

Central monitoring will be conducted for all mobile units and supporting secondary care sites. The scope and frequency of both central and on-site monitoring will be determined by the risk assessment and detailed in the Monitoring Plan developed by CPTU. Triggered monitoring visits based on data queries, safety report and deviations will be detailed in the Monitoring Plan.

The following areas may be reviewed as part of trial monitoring:

- Participant enrolment, consent, and eligibility
- Adherence to trial interventions and policies to protect participants, including reporting of harm
- Completeness, accuracy, and timeliness of data collection

18. Protocol Compliance and Breaches of GCP

Prospective, planned deviations or waivers to the Protocol are not allowed under UK Clinical Trials regulations and must not be used.

Protocol deviations, non-compliances, or breaches are departures from the approved Protocol. They can happen at any time, but are not planned. They must be adequately documented on the deviation log and reported to CPTU immediately. CPTU will be responisble for reviewing and escalating deviations to the CI and Joint Sponsors where required according to the BEST4 Deviations SOP.

Deviations from the Protocol which are found to occur repeatedly will not be accepted and will require immediate action and could potentially be classified as a serious breach. A "serious breach" is a breach which is likely to affect to a significant degree the safety or physical or mental integrity of the subjects of the trial, or the scientific value of the trial. The Sponsors will be notified immediately by CPTU of any case where the above definition may apply during the trial conduct phase.

Any potential/suspected serious breaches of GCP must be reported immediately to the Sponsor and CI without any delay.

Any complaints about this research will be managed by the CPTU and escalated as required to the Sponsors.

19. Vendors / Contractors

Contracts will be executed for all vendors and will document specification for the work to be undertaken.

19.1. Medical device manufacture and distribution

Cyted Ltd are responsible for manufacturing devices and for distributing devices to EMS Healthcare headquarters for this trial.

19.2. Patient Identification and Survey service

iPLATO will be responsible for providing a healthcare technology service which includes; access to participant demographic and contact information, sending invitation text messages including an eligibility survey and an appointment booking schedule system. iPLATO will only share participant data with the CPTU for individuals who have provided explicit informed consent to the Heartburn Health Programme.

19.3. Mobile Diagnostic Unit and nurse provision

EMS Healthcare are responsible for the provision and maintenance of the mobile diagnostic units for the trial, including necessary facilities and consumables. EMS Healthcare will be responsible for the provision of qualified nursing staff on the units to carry out the procedures outlined in the schedule of events table (see Section 9).

EMS Healthcare will internally identify an oversight Trial lead Principal Investigator who will delegate to a nurse lead (per mobile unit site). The delegation log will outline responsibilities as per each role.

19.4. Sample shipment

Cyted is responsible for the shipment of Capsule Sponge samples from Mobile Diagnostic Unit locations to Cyted Ltd laboratory.

University of Cambridge is responsible for the shipment of residual capsule sponge material from Cyted back to University of Cambridge for long term sample storage.

University of Cambridge is responsible for the shipment of blood and saliva samples from Secondary Care sites to the Early Cancer Institute, Cambridge.

19.5. Laboratory sample analysis

Cyted Ltd is responsible for the processing, analysis and evaluation of capsule sponge samples. All capsule sponge samples will be sent to a central laboratory for processing after collection. The cells are processed into an FFPE block from which slides are cut for analysis and pathology reporting for signs of early cancer and Barrett's oesophagus with the TFF3 and p53 biomarkers respectively. Cyted have established a proprietary, quality-controlled process for this laboratory processing and pathology reporting, with a team of consultant pathologists that are specially trained on this cell type and test. Details of capsule sponge analysis and result reporting to the Cambridge Clinical Team will be documented in the BEST4 Laboratory Manual.

20. Publications Policy

Data gathered from the trial will be collated and analysed by researchers at QMUL and the University of Cambridge. Findings will be published in peer reviewed scientific journals. Summary reports of the main trial findings will be written and shared with the participating sites. Any reported serious breaches will be detailed in all publications in line with regulatory requirements.

A Dissemination Plan will be developed by the BEST4 Screening Trial Management Group for the research results.

The trial will be registered on the ISRCTN registry.

20.1. Authorship guidelines

The final trial report and related publications will be authored in line with the arrangement set out in the research collaboration agreement dated 05 July 2022. All publications relating to the Project must be approved for publication by the Lead Grant Applicants. Authorship will follow criteria established by the International Committee of Medical Journal Editors.

Heartburn Health programme final publications will jointly be authored by the University of Cambridge on behalf of the BEST4 platform and/or BEST4 collaborators, in line with the arrangements set out in the material and data sharing agreement.

21. Data Transparency

21.1. Informing Participants of the Results of the Trial

Results of the trial will be made available to participants via the BEST4 website. The Participant Information Sheet will describe when and how the information will be provided to participants. CPTU and Cambridge will collaborate with PPI contributors and relevant organisations to identify the best ways of communicating the trial results to participants and wider audience to whom the results are relevant.

21.2. Informing Participants of the results of the Heartburn Heath Programme studies

Results of programme studies will be made available to participants via the Heartburn Health Programme website. The Participant Information Sheet will describe when and how the information will be provided to participants. CPTU and Cambridge will collaborate with PPI contributors and relevant organisations to identify the best ways of communicating the trial results to participants and wider audience to whom the results are relevant.

22. Data and Sample Sharing

22.1. Heartburn Health Programme Data Sharing

Data collected for the Heartburn Health Programme will be suitable for sharing upon quality control checked. De-identified data, collected during the programme invitiation process, will be stored in conventional formats for data sharing, and will be shared in line with the policies of the Joint Sponsors, the Trial Funders and the current regulatory

requirements. Participant contact details will only be shared for the purpose of future research, upon patient consent to be contacted to participate in a new programme.

22.2. Screening Trial Data Sharing

The data collected during the trial will be suitable for data sharing once all the data collected has been quality control checked. De-identified data will be stored in conventional formats for data sharing, and will be shared in line with the policies of the Joint Sponsors, the Trial Funders and the current regulatory requirements. No participant identifiable data will be shared.

Only fully de-identified data will be passed to the public domain (i.e. on an open access data repository/journal) once sufficient validation has been conducted, and meaningful analysis and publication is complete.

22.3. Sample Sharing

Consent will be sought from participants for any residual capsule sponge, blood and saliva sample to be stored at the University of Cambridge in a long-term HTA licenced facility. Residual capsule samples will form the BEST4 Biobank and will be used in future ethically approved research and may be shared with external collaborators and organisations as part of the Heartburn Health Programme. This may include overseas and commercial organisations.

The University of Cambridge will be the sample custodians of any samples collected as part of the BEST4 Platform and will comply with the requirements as set out in the Human Tissue Act 2004. Release of residual sample material during the trial to external investigators will only be permitted to inform the primary or secondary endpoints of the trial or after all planned analysis of the trial data as outlined in this Protocol have been performed to ensure that materials are retained for any quality control.

During the trial, the BEST4 Trial Committees will be responsible for authorising any sample analysis requests (not outlined in this Protocol) involving the residual capsule sponge material.

After the trial, all requests for samples and data via the Heartburn Health Programme will be reviewed by a BEST4 committee that will be established once this trial (and the BEST4 Surveillance trial) ends, to ensure that the scientific rationale is clear.

The joint sponsors will be responsible for implementing Material Transfer Agreements with approved requests outside of the collaborating BEST4 partners.

23. Archiving

As per current regulations, once the trial has come to an end, essential trial documentation as part of the TMF will be archived in keeping with the Sponsor's policy and applicable regulations for a period of 5 years.

CPTU will be responsible for developing an Archiving Plan with the Joint Sponsors which will detail how the trial will be archived, including vendor archiving instructions.

All trial-related documentation and data as part of the ISF will be archived in accordance with participating site's standard operating procedures and the Sponsor's timelines. These procedures state suitable locations to be specified at the time of archiving with limited access to named members of the research team only.

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25. Appendices

25.1. Appendix 1 – Amendment History

Table 3 *– Protocol Amendment History*

Version Number	Date	Description of Amendment
1.0		*N/A*
2.0		• Update Chief Investigator to Professor Rebecca Fitzgerald • Update statistician details • Updated acronyms to be spelled out where used in the first instance
3.0	03/07/24	• Update to the reporting of Negative capsule sponge results which will now be communicated to participants via text message as opposed to communications via participant's GP • Update to the conditions for capsule sponge results that triggers an endoscopy referrals and addition of flow diagram illustrating result reporting procedures • Changes on the Capsule sponge results criteria for participants where blood and or saliva will be collected for • Update in wording to include a larger number of Secondary Care sites • Change in wording to clarify eligibility criteria will be patient self reported as opposed to taken from patient record
4.0	05/08/24	• Update to endoscopy referral process for participants with a positive capsule sponge result • Update to Heartburn Heath Programme eligibility criteria • update NHS Digitrials patient identification process, • updated end of trial and passive follow-up wording • Minor protocol updates to clarify pseudonymisation of blood and saliva samples, •

5.0	• *13/09/24*	• Removal of wording in the protocol and study documentation pertaining to KCL being a data processor or having access to BEST4 screening trial data as CPTU has moved to QMUL. • Corrections of typos and formatting errors in the protocol. • SAEs reporting – clarifications added to specify that all SAEs, not just SARs, will be collected and assessed by the PI and CI or delegate for causality and expectedness. Also clarified that the CPTU QA Manager role is to receive the SAE forms but not to be involved in the assessment of causality or expectedness. •
6.0	• *16/10/24*	• Addition of Heartburn Health background questionnaire • Wording about third party vendor Twilio based outside of the UK • Addition of self enrolment mechanism • Corrections of typos and formatting errors in the protocol • Updated Protocol Exclusion criteria
7.0	• *10/02/25*	• Section 7.2: Clarification added that inclusion criteria for BEST4 Screening is based on information provided when participant completes Heartburn Health Invitation & Consent. • Clarification of exclusion criteria for participants with the inability to (or have not on the day of the capsule sponge test) temporarily discontinue anti-thrombotic medication prior to procedure as per the guidance in the PIS • Section 9.10.1: Clarification that participants taking anticoagulant medication will be provided with specific instructions available in the PIS. • Clarification added that site staff are required to confirm eligibility for the capsules sponge procedure on the day of the procedure.

		• Section 19.6: Reinstatement of previous wording about the storage of participant data,as changes have not been implemented.

25.2. **Appendix 2 –** Estimated power of the group-sequential analysis

Table 4 *Estimated Power*

	Prevalences per 100,000				Transition rates							Power (Advanced OAC)			Power (OAC mortality)		
	NDBE	DBE	IM OAC	Sym OAC	GERD2BE	NDBE2D	D2OAC	Ia2Inv	Efficacy	Sensitivity	Uptake	4.5 yrs	6 years	9 yrs	6 yrs	7.5 years	12 yrs
Scenario 1	10000	200	300	75	1.00%	2.50%	10.00%	30.00%	75.00%	80.00%	70.00%	92.53%	99.50%	99.97%	78.02%	97.47%	99.96%
Scenario 2	9500	300	100	50	2.50%	2.00%	9.00%	30.00%	75.00%	80.00%	70.00%	36.55%	75.16%	92.72%	22.96%	60.03%	91.65%
Scenario 3	8000	250	150	75	1.50%	1.50%	10.00%	35.00%	75.00%	80.00%	70.00%	57.12%	87.27%	95.98%	38.15%	75.09%	94.93%
Scenario 4	10000	300	300	75	1.50%	1.00%	8.00%	25.00%	65.00%	80.00%	70.00%	84.54%	97.83%	99.39%	65.66%	92.84%	98.97%
Scenario 5	9000	150	250	50	1.00%	2.00%	9.00%	35.00%	60.00%	80.00%	70.00%	90.51%	98.49%	99.27%	75.33%	95.04%	98.77%
Scenario 6	10000	200	100	50	1.20%	1.50%	8.00%	25.00%	65.00%	80.00%	70.00%	22.83%	54.25%	74.22%	14.08%	40.73%	72.90%
Scenario 7	12000	400	200	50	1.20%	2.00%	7.50%	25.00%	35.00%	75.00%	70.00%	49.85%	76.04%	78.04%	33.11%	63.46%	73.06%
Scenario 8	8500	200	200	70	1.50%	1.50%	8.00%	25.00%	50.00%	75.00%	70.00%	45.99%	75.68%	82.06%	29.66%	62.03%	77.86%
Scenario 9	12000	300	150	75	2.00%	2.50%	9.00%	20.00%	40.00%	75.00%	75.00%	24.52%	54.14%	65.97%	15.09%	40.97%	62.19%
Scenario 10	10000	250	250	75	1.00%	1.00%	10.00%	30.00%	70.00%	80.00%	60.00%	64.36%	89.22%	94.95%	44.87%	78.54%	93.42%
Scenario 11	8500	160	160	40	1.50%	2.00%	10.00%	25.00%	70.00%	80.00%	67.00%	48.05%	80.71%	92.00%	31.49%	67.31%	90.37%
Scenario 12	9000	200	125	75	1.20%	1.50%	10.00%	30.00%	60.00%	80.00%	67.00%	29.54%	60.97%	75.59%	18.35%	47.07%	72.99%
Scenario 13	9000	400	150	40	1.20%	1.50%	10.00%	30.00%	50.00%	80.00%	65.00%	46.96%	75.08%	80.99%	31.12%	62.28%	77.00%
Scenario 14	9500	200	275	70	1.00%	1.00%	10.00%	30.00%	65.00%	80.00%	70.00%	87.46%	98.17%	99.34%	69.99%	93.89%	98.89%
Scenario 15	10000	300	250	50	1.00%	5.80%	10.00%	30.00%	65.00%	80.00%	70.00%	90.36%	99.36%	99.98%	75.01%	97.01%	99.97%
Scenario 16	10000	300	250	50	1.00%	4.50%	10.00%	30.00%	65.00%	80.00%	70.00%	89.69%	99.15%	99.95%	73.90%	96.41%	99.92%

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