CYTOFLOC

Full title: Evaluation of a Non-Endoscopic Immunocytological Device (Cytosponge™) for post chemo-radiotherapy surveillance in patients with oesophageal cancer – a feasibility study

Short title: Cytosponge™ for post-chemoradiation surveillance of oesophageal cancer

Protocol Version & date	Version 3.0 20Feb2019
Sponsor Protocol Number	OCTO_068
Ethics Number	17/SC/0661
NIHR Portfolio Number	36659
ClinicalTrials.gov	NCT03529669
IRAS Project ID	227234

Sponsor:	University of Oxford
Funder:	CRUK Population Research Committee Ref: C28958/A22173
Conflict of Interest statement	None of the protocol authors have declared a potential conflict of interest
Confidentiality Statement	This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Trial Office, the Investigator Team, host NHS Trust(s), Regulatory Authorities and members of the Research Ethics Committee unless authorised to do so.

OXFORD INSTITUTE FOR RADIATION ONCOLOGY

General contact information

Chief Investigator

Prof Somnath Mukherjee
Consultant Clinical Oncologist
Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology
University of Oxford
Old Road Campus Research Building
Off Roosevelt Drive
Oxford
OX3 7DQ
Tel: 01865 235207
Email: somnath.mukherjee@oncology.ox.ac.uk

Clinical Co-Investigator

Prof Rebecca Fitzgerald
Programme Leader MRC Cancer Cell Unit Hutchison-MRC Research Centre and Hon. Consultant Gastroenterologist
Addenbrooke's Hospital
Cambridge
CB2 OXZ
Email: pa-fitzgerald@mrc-cu.cam.ac.uk

Clinical Co-Investigator

Dr Adam Bailey
Consultant Gastroenterologist
John Radcliffe Hospital
Headley Way
Headington
Oxford
OX3 9DU

Clinical Co-Investigator

Prof Maria Hawkins
Consultant Clinical Oncologist
CRUK MRC Oxford Institute for Radiation Oncology
Gray Laboratories, University of Oxford
Old Road Campus Research Building
Oxford OX3 7DQ

Clinical Co-Investigator

Dr Tom Crosby
Consultant Clinical Oncologist
Velindre Hospital
Velindre Road
Cardiff CF14 2TL

Co-Investigator (Pathology)

Dr Maria O'Donovan
Consultant Histopathologist/ Cytopathologist
Addenbrooke's Hospital and MRC Cancer Cell Unit
Hutchison-MRC Research Centre
Cambridge

Trial Office (OCTO)

CYTOFLOC Trial Office
Oncology Clinical Trials Office (OCTO)
Department of Oncology, The University of Oxford
Old Road Campus Research Building
Oxford OX3 7DQ, UK
Email: octo-cytofloc@oncology.ox.ac.uk
Website: http://www.oncology.ox.ac.uk/research/oncology-clinical-trials-office-octo

Radiotherapy and Imaging Portfolio Lead	Claire Brooks Tel: 01865 617082 Email: claire.brooks@oncology.ox.ac.uk
Trial Manager	Stephanie Levy Tel: 01865 617084 Email: stephanie.levy@oncology.ox.ac.uk
Sponsor	Ms Heather House The University of Oxford Clinical Trials and Research Governance Team Joint Research Office Block 60 Churchill Hospital Oxford OX3 7LE Tel: 01865 572224 Fax: 01865 572242 E-mail: ctrg@admin.ox.ac.uk
Lead NHS Trust	Ms Heather House R&D Department, Oxford University Hospitals NHS Trust Joint Research Department, Block 60, Churchill Hospital Oxford OX3 7LE Tel: 01865 572224 E-mail: ouhtma@ouh.nhs.uk
Statistician	Dr Joanna Moschandreas Tel: 01865 223449 Email: joanna.moschandreas@ndorms.ox.ac.uk
Central Laboratory	Dr Shona MacRae Senior Laboratory Scientist MRC Cancer Cell Unit Hutchison-MRC Research Centre Cambridge

Trial oversight is provided by the Radiotherapy and Imaging Oversight Committee (RIOC)

RIOC Chairman	Prof Peter Hoskin

During office hours, clinical queries should be directed to the CYTOFLOC Trial Office. If appropriate, the query will be passed on to the Chief Investigator, or an appropriate member of the Trial Management Group.

For urgent out of hours clinical queries site staff may contact: Oxford University NHS Trust switchboard 01865 741166 and ask for the Oncology on-call doctor.

Patient Registration: Email the completed Registration Form to the CYTOFLOC Trial Office octo-CYTOFLOC@oncology.ox.ac.uk

SAEs: All reportable SAEs & SADEs must be notified on the study-specific SAE form and emailed to the Trial Office Pharmacovigilance Email: octo-safety@oncology.ox.ac.uk within 24 hours of becoming aware of the event.

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Full Title of study	Evaluation of a Non-Endoscopic Immunocytological Device (Cytosponge™) for post chemo-radiotherapy surveillance in patients with oesophageal cancer – a feasibility study
Short Title	Cytosponge™ for post-chemoradiation surveillance of oesophageal cancer
Study Acronym	CYTOFLOC
Objectives	1. Assessment of completion rate of Cytosponge™ as a diagnostic test post chemo-radiotherapy 2. Assessment of safety of Cytosponge™ as a diagnostic test post chemo-radiotherapy 3. Assessment of patient acceptability of Cytosponge™ as a diagnostic test post chemo-radiotherapy 4. Assessment of suitability of Cytosponge™ sample for biomarker analysis 5. Evaluation of ctDNA and other circulatory markers in assessment of residual disease post CRT 6. Evaluation of the comparative efficacy of Cytosponge™ and post-treatment biopsy in identifying residual cancer, p53 mutations & other identifiable markers in pre-treatment biopsy sample
Clinical rationale	Although definitive chemoradiotherapy (dCRT) is a less invasive treatment option in oesophageal cancer compared to surgery, there is higher incidence of local recurrence. If detected early, local recurrence can be salvaged through surgery. Regular endoscopic surveillance is invasive; this study will investigate the feasibility of a less invasive technique, Cytosponge™, in post CRT surveillance in oesophageal cancer.
Primary Endpoint	Completion Rate: The proportion of consented, evaluable patients successfully undergoing Cytosponge™ will be presented, with the corresponding 95% confidence interval. The proportion will be calculated overall and separately for those having definitive chemoradiotherapy (dCRT) and neo-adjuvant chemoradiotherapy (naCRT).
Secondary Endpoints	Safety: All serious adverse effects related to the procedure, including bleeding (requiring transfusion) and perforation will be assessed. Acceptance Rate: a. Proportion of eligible patients approached who consent b. Proportion of patients who have successfully undergone the procedure & would be prepared to accept the procedure repeatedly if it was to be used for follow-up (data will be captured through questionnaire after procedure) Suitability of Sample for Biomarker analysis: Quality of material obtained from Cytosponge™ test will be centrally analysed at Cambridge. A positive Cytosponge™ result will be defined as presence of cytological atypia and/or p53 abnormality.
Tertiary Endpoints	Level of ctDNA in responders vs non-responders Level of residual cancer, p53 mutations & other identifiable markers in Cytosponge™, pre & post- treatment biopsies.
Study Design	This is a feasibility study involving patients with known oesophageal cancer treated with pre-operative or definitive chemoradiation. All participants will undertake the Cytosponge™ test. The Cytosponge™ will be processed for evidence of residual cancer through analysis of cellular atypia and molecular biomarkers. Where available, the results will be compared with histology.
Patient Numbers	50 evaluable participants
Target Population	Patients who have undergone CRT for oesophageal cancer
Inclusion criteria	1. Male or female, age $\geq$ 16 years who a. have undergone pre-operative CRT as treatment for oesophageal cancer and are due to undergo oesophagectomy or b. have undergone definitive CRT as treatment for oesophageal cancer 2. 4-16 weeks post completion of CRT 3. Dysphagia score 0-2 (Mellow Scale) 4. Able to swallow tablets 5. Physiologically fit for endoscopy 6. Written (signed and dated) informed consent

	7. The patient is willing and able to comply with the protocol for the duration of the study, and scheduled follow-up visits and examinations.
Exclusion criteria	1. Known to have oesophageal varices or stricture requiring dilatation of the oesophagus 2. Unable to temporarily discontinue anticoagulation therapy/medication prior to their procedure* 3. Oesophageal stent 4. Other psychological, social or medical condition, physical examination finding or a laboratory abnormality that the Investigator considers would make the patient a poor study candidate or could interfere with protocol compliance or the interpretation of study results. * Patients on anti-coagulation therapy are eligible for the study as long as they are considered suitable candidates for endoscopic biopsy (follow local hospital procedures for management of patients on anticoagulation due to undergo endoscopy). If temporary discontinuation of anticoagulation is required, this should be after consultation with the patients clinical care team.
Study intervention and administration	Cytosponge™ test kit, is a Class I, single-use, non-sterile, non CE-marked device that consists of an expandable spherical 3cm diameter mesh, encapsulated in a gelatine capsule, which is attached to a cord. The capsule is swallowed and allowed to reach the stomach while remaining attached to the cord. All patients will undergo Cytosponge™ test at one time-point 4-16 weeks after completion of CRT.
Duration on study	Approximately 1 month
Patient care post-study	Following the end of study visit, patients will receive standard care.
No. of Study Site(s)	UK multicentre trial; Approximately 10 sites.
End of study	Last Patient Last Visit (including telephone follow up).

SUMMARY OF EVENTS

	During or post routine CRT	**Pre–study intervention**	**Cyotosponge™ procedure**	**Telephone follow up[5]**	**Telephone follow up[5]**
	At least 24 hours before consent	Ideally on D1 where possible, otherwise within 7 day period preceding procedure	D1	1 week (+/-2days) post Cytosponge™ procedure	2 weeks (+/-2 days) post Cytosponge™ procedure
PIS provided to potential participant	X				
Inclusion / exclusion criteria confirmation [1]		X			
Written informed consent		X			
Study Registration		X			
Demographics		X			
Medical history: • Clinically significant disease history • Prior treatment • Comorbidities		X			
Concomitant medications		X		X	X
Physical examination[2] including vital signs		X			
WHO Performance Status		X			
Dysphagia score		X	X	X	X
Cytosponge™ procedure*			X		
Safety monitoring[3]			X	X	X
Questionnaire[4]			X		
Research Bloods (optional)		X			

[1] Refer to re-screening section 4.4 [2] See section 5.2 [3] Week 1 and Week 2 safety monitoring will be undertaken by telephone call

[4]Questionniare administered post procedure to those who successfully complete the procedure. This can be returned in the post if the participant is unable to stay.

[5] Only applicable for participants that have received definitive CRT, or for participants who have received neo-adjuvant CRT, if these times are prior to surgery.

*Cytosponge™ procedure will be performed 4-16 weeks after completion of CRT. Refer to section 5.3 & 8.1

-If patient has received definitive CRT, and endoscopy and biopsy is being performed to assess response to CRT (as part of routine procedure for centre), aim to perform Cytosponge™ ideally on the same day or within a week preceding endoscopy to allow collection of matched samples for analysis.

-If patient has received neo-adjuvant CRT, aim to perform Cytosponge™ on the same day or within a week prior to surgery, to allow collection of matched samples for analysis.

ABBREVIATIONS

AE	Adverse Event	MHRA	Medicines and Healthcare products Regulatory Authority
ADE	Adverse Device Effect	medDRA	Medical Dictionary for Regulatory Activities
BE	Barrett's oEsophagus	naCRT	Neo-adjuvant Chemoradiotherapy
BP	Blood Pressure	NIHR	National Institute for Health Research
CI	Chief Investigator	OCTO	Oncology Clinical Trials Office
CTA	Clinical Trials Agreement	OS	Overall Survival
CTAAC	Clinical Trials Awards and Advisory Committee	pCR	Complete pathological response
CRT	Chemoradiotherapy	PI	Principle Investigator
dCRT	Definitive Chemoradiotherapy	PIS	Patient Information Sheet
DSMC	Data and Safety Monitoring Committee	REC	Research Ethics Committee
eCRF	Electronic Case Report Form	RSI	Reference Safety Information
GCP	Good Clinical Practice	SADE	Serious Adverse Device Effect
GI	Gastrointestinal	SAE	Serious Adverse Event
GLP	Good Laboratory Practice	SCC	Squamous Cell Cancer
GP	General Practitioner	SOP	Standard Operating Procedure
HRA	Health Research Authority	TFF3	TreFoil Factor 3
HTA	Human Tissue Act	TMG	Trial Management Group
IMP	Investigational Medicinal Product	USADE	Unanticipated Serious Adverse Device Effect
ISRCTN	International Standard Randomised Controlled Trials Number	WHO	World Health Organisation
LPLV	Last Patient Last Visit		

1 INTRODUCTION

1.1 Background

Around 8900 cases of oesophageal cancer are diagnosed in the UK each year, and incidence has been rising since the mid-1970s (CRUK Cancer Stats, 2014). Sixty percent present at age >70 years, and many - particularly those with squamous cell cancer (SCC) - have associated co-morbidities, making surgery challenging. Ivor-Lewis oesophagectomy still remains a huge undertaking for most patients, many of whom do not regain their prior quality of life during their life time [1]. A non-surgical approach, i.e. definitive chemoradiation (dCRT), may be the preferred treatment, reserving surgery for salvage. Ten-year survival is only 12% (http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer) and although overall survival (OS) in patients treated with dCRT remains comparable to those treated with surgical-based therapy [2], the higher risk of loco-regional recurrence and the lack of effective surveillance strategy have deterred many clinicians from adopting dCRT plus salvage surgery as the standard approach.

Chemoradiotherapy has been shown to achieve complete pathological response (pCR) in 25-49% of cases [3] and the current genre of oesophageal trials approved by CTAAC (SCOPE2 CRUK/14/022 investigating RT dose escalation and early evaluation with PET scan) and New Agents Committee (CHARIOT: ATR inhibitor in combination with conventional dCRT, CRUK grant number C43735/A20874) focuses on maximizing tumour eradication through intensification of local therapy. If an effective surveillance strategy could be coupled with an effective dCRT regimen, this may allow us to shift the treatment paradigm by offering dCRT as the primary therapy for all patients with localised oesophageal cancer, reserving surgery as salvage option; this approach may allow organ preservation in many patients currently subject to oesophagectomy, however effective local surveillance strategies is imperative to make this approach successful.

Endoscopy-based surveillance is invasive and onerous and relies on macroscopically visible abnormalities to trigger suspicion of local recurrence. One study using endoscopic surveillance showed that about 25% of first relapses are local only, 90% of which occur within 2 years and about one third could be salvaged through surgery [4]. The proportion with salvageable cancer in the study was low possibly because many patients were likely to have been pre-selected for dCRT due to factors precluding surgery. Also, endoscopy may not have been frequent enough to detect

early recurrence (done 6 monthly first year, yearly in years 2-3) and lesions beyond the resolution of the endoscopy may have been missed. The availability of a less invasive screening tool which could be done more frequently, coupled with a 'triggered' endoscopy and imaging would make post-CRT surveillance a more attractive option in this patient group.

1.2 Research intervention

The Cytosponge™ is a Class I, non-CE marked device that consists of an expandable, spherical 3cm diameter reticulated polyester foam compressed and encapsulated in a standard soluble gelatine capsule (size 00). The sponge is attached to a cord (Astralen, braided synthetic non-absorbable suture) which passes out through the capsule. The capsule is swallowed and allowed to reach the stomach while remaining attached to the cord, which is held onto by the patient or a qualified member of the trial team. 3-5 minutes after swallowing (once dissolved in the stomach) the spherical mesh can be retrieved by pulling on the cord. Cells are collected by the mesh scraping against the oesophageal mucosa. The sponge sample is then placed into a preservative fluid and the specimen can be processed for molecular indicators. The Cytosponge™ samples the entire oesophagus and therefore removes the sampling bias inherent in endoscopic biopsies, it is not operator dependent and does not rely on lesions to be macroscopically visible to sample.

Cytosponge™ has previously received a letter of no objection from the Medical Healthcare products Regulatory Agency (MHRA Reference CI/2007/0053) to screen for Barrett's Oesophagus (BE).

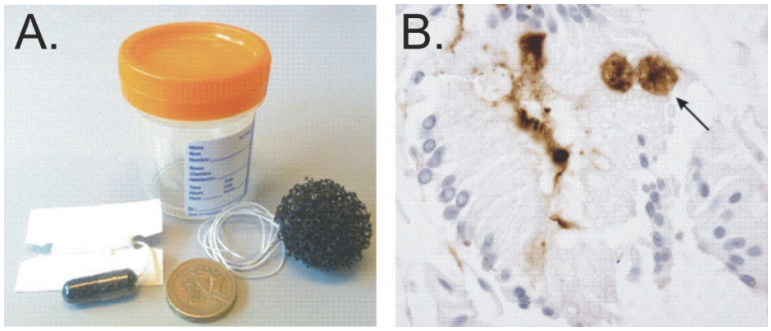

Figure 1: Cytosponge™ within the capsule and expanded (A) and representative picture of positive TFF3 staining in a sample from a patient with BE (B)

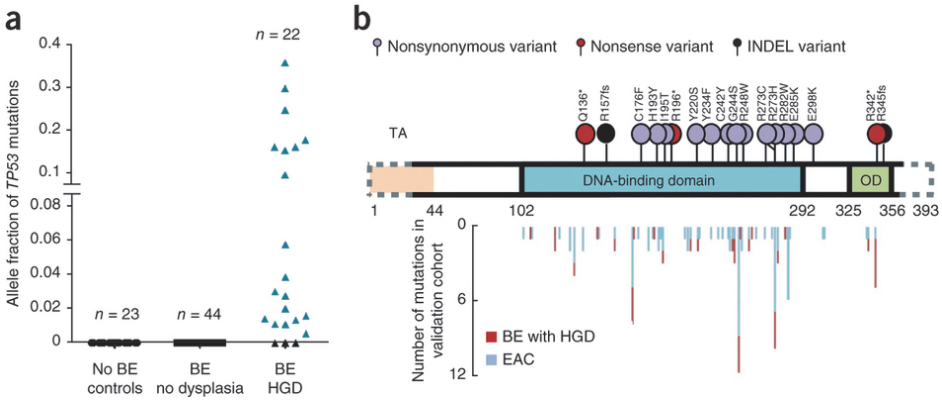

Figure 2: (a) The allele fraction of TP53 mutations identified in Cytosponge™ samples is shown for the three case groups: no Barrett's esophagus (n = 23), Barrett's esophagus with no dysplasia (n = 44) and Barrett's esophagus with HGD (n = 22). (b) The positions of the TP53 mutations identified for the Cytosponge™ samples are shown above the gene diagram compared with those found in the EAC and Barrett's esophagus HGD biopsy cohorts. The dashed line on the gene outline denotes the two small areas not covered by the multiplex PCR assay (amino acids 1–27 and 361–393). TA, transcription activation domain; OD, oligomerization domain (taken from Nature Genetics 2014; 46:837-843)

1.3 Rationale for the study

Clinical

It has been demonstrated recently that the entire clonal architecture of a dysplastic oesophagus delineated from multiple biopsies was captured in a single Cytosponge™ sample [5]. The feasibility and acceptability of Cytosponge™ as a tool for detection of Barrett's esophagus (in non-cancer patients) has been tested in over 2000 patients including a primary care study of 504 patients and a secondary care case control study of 1,100 patients [6, 7] . The procedure is very safe. There has been temporary ooze from the Cytosponge™ noted at endoscopy but this did not require any intervention apart from one patient with varices and this is an exclusion criterion for this study. Detachments occurred in 1:1000 patients but the sponge was easily removed at endoscopy and there were no consequences for the patient. The device is not CE-marked. The only Cytosponge™-related adverse effects noted were a temporary sore throat.

In the BEST1 and BEST2 studies, Cytosponge™ test detected Barrett's Esophagus (BE), by assaying for a biomarker TFF3, with a sensitivity and specificity of 73%-90% and 92-94% respectively compared with endoscopy [6, 7]. In addition to cellular atypia, it is possible to detect p53 mutation by immunostaining and by sequencing [8, 9] the most prevalent mutation in both squamous and adenocarcinoma of the oesophagus. Cellular atypia and a surrogate measure of abnormal ploidy using immunohistochemistry for Aurora Kinase A were also good predictors of cancer in BE [9]. For squamous cell dysplasia, procedure completion rate of 96.5%, sensitivity and specificity of 100% and 97% have been reported, and when combined with p53 staining, the accuracy rate of the procedure was 100% [10] (Fig 2). Therefore, the detection of new onset atypia or p53 mutation within an oesophagus previously cleared of these abnormalities through dCRT could be potentially used for early identification of disease recurrence. Detection of these abnormalities can thereafter trigger more detailed investigations like endoscopy, EUS and cross-sectional imaging. Although the Cytosponge™ has been well studied in the context of Barrett's there is no safety or efficacy data for the use of this device in carcinoma patients following dCRT.

For the Cytosponge™ to have a wider application in oesophageal cancer, including treatment surveillance, a feasibility study is required. One of the important differences from patients with BE is that these patients already have dysphagia which could make swallowing of the capsule or retrieval of Cytosponge™ more difficult. Secondly, radiotherapy would have resulted in inflammation of the surrounding tissue. Sample analysis should be robust enough to differentiate molecular markers of inflammation from that of residual cancer. Finally, although a molecular marker for BE and dysplasia that can be detected by Cytosponge™ is well defined and assessed through immunohistochemistry Trefoil Factor 3 (TFF3) and p53 sequencing, markers for invasive cancer detectable with this device need to be developed and validated.

Benefits

This study will test the acceptability and feasibility of using Cytosponge™ post chemo-radiation. The participants will not benefit directly. However, if this study shows feasibility/acceptability, it will lead to larger studies evaluating its role in surveillance post-dCRT, using the Cytosponge™ at multiple time-points during follow-up. An effective surveillance programme may detect a greater proportion of patients with local-only recurrences treatable by curative salvage resection. It may also lead to a shift in standard care where patients could be offered dCRT as first choice, keeping surgery in reserve for patients who fail dCRT.

Risks

The risk for Cytosponge™ in patients with oesophageal cancer has not been assessed previously. It has however, been tested in >1000 patients with Barrett's esophagus. The most common side-effect is a sore throat. Rare complications could be bleeding, perforation, effects on the airway, the Cytosponge™ getting stuck beyond the tumour or detachment of the Cytosponge™ from the string requiring endoscopy to recover the Cytosponge™.

2 STUDY DESIGN

This is a feasibility study testing the use of the Cytosponge™ to determine completion rate, safety and acceptability of the procedure. Fifty patients will be recruited from approximately 10 sites in the UK. Patients will receive one Cytosponge™ test at one time-point within 4-16 weeks after completion of CRT. Refer to the summary of events for details of the study visits and procedures.

2.1 Duration of patient participation

Participants will be in the study for approximately 1 month from entry on study to last protocol visit.

2.2 Post-study care and follow-up

Following each patients end of study visit, they will receive standard care.

3 OBJECTIVES AND ENDPOINTS

Primary Objective	**Endpoint**
Completion rate	The proportion of consented, evaluable, patients successfully undergoing Cytosponge™ will be presented, with the corresponding 95% confidence interval. The proportion will be calculated overall and separately for those having dCRT and naCRT.
Secondary Objectives	**Endpoints**
Safety	All serious adverse effects related to the procedure, including bleeding (requiring transfusion) and perforation*
Suitability of sample for biomarker analysis	Quality of material obtained from Cytosponge™ test will be centrally analysed at Cambridge (cellularity, yield and quality of extracted DNA will be used as measure of quality). A positive Cytosponge™ result will be defined as presence of cytological atypia and/or p53 mutation
Acceptance rate	1. Proportion of eligible patients approached who consent 2. Proportion of patients who have successfully undergone the procedure & would be prepared to accept the procedure repeatedly if it was to be used for follow-up (data will be captured through questionnaire after procedure)
Tertiary Objectives	**Endpoints**
Residual disease markers	Level of ctDNA in responders vs non-responders
Cytosponge™ comparative efficacy to biopsy	Level of residual cancer, p53 mutations & other identifiable markers in Cytosponge™, pre & post- treatment biopsies

* A TMG meeting will be called if there are >3 cases of procedure related bleeding requiring a blood transfusion.

4 PATIENT SELECTION

Written informed consent must be obtained before any study specific procedures are performed. The Investigator will determine patient eligibility based on the following criteria.

4.1 Inclusion criteria:

A patient will be eligible for inclusion in this study if all of the following criteria apply.

1. Male or female, Age ≥ 16 years who
    a. have undergone pre-operative CRT as treatment for oesophageal cancer and due to undergo oesophagectomy or
    b. have undergone definitive CRT as treatment for oesophageal cancer
2. 4-16 weeks post completion of CRT
3. Dysphagia score 0-2 (Mellow Scale)*
4. Able to swallow tablets
5. Physiologically fit for endoscopy
6. Written (signed and dated) informed consent
7. The patient is willing and able to comply with the protocol for the duration of the study, and scheduled follow-up visits and examinations.

*Rescreening: For patients with a dysphagia score >2 (and those unable to swallow tablets due to cancer related dysphagia), please refer to re-screening section 4.4.

4.2 Exclusion criteria:

A patient will not be eligible for the study if any of the following apply:

1. Known to have oesophageal varices or stricture requiring dilatation of the oesophagus
2. Unable to temporarily discontinue anticoagulation therapy/medication prior to their procedure*
3. Oesophageal stent
4. Other psychological, social or medical condition, physical examination finding or a laboratory abnormality that the Investigator considers would make the patient a poor study candidate or could interfere with protocol compliance or the interpretation of study results

* Patients on anti-coagulation therapy are eligible for the study as long as they are considered suitable candidates for endoscopic biopsy (follow local hospital procedures for management of patients on anticoagulation due to undergo endoscopy). If temporary discontinuation of anticoagulation is required, this should be after consultation with the patients clinical care team.

4.3 Protocol deviations and waivers to entry criteria

Protocol adherence is a fundamental part of the conduct of a clinical study. Changes to the approved protocol need prior approval unless for urgent safety reasons. Investigators should not deviate from the protocol for the management of enrolled subjects unless essential to protect the rights or safety of the individual. All deviations should be fully documented, justified and reported to the Trial Office without delay. It may be necessary to withdraw the patient from further study.

Investigators must contact the CYTOFLOC Office to obtain guidance and/or clarification as necessary if unsure whether the patient satisfies all the entry criteria and to clarify matters of clinical discretion. OCTO will contact the Chief Investigator or clinical coordinators as necessary.

Investigators must not request a protocol waiver to enter a patient who does not satisfy the selection criteria.

4.4 Re-screening if patient does not meet inclusion/exclusion criteria first time round

Patients with dysphagia grade >2undergoing CRT, who are otherwise potentially eligible, can be given a PIS (with the anticipation that their swallowing will improve after treatment) to allow patients sufficient time to think about the study, however, they will only be recruited at a later time point when they satisfy the full inclusion/exclusion criteria.

4.5 Patient registration procedure

Potential participants will be identified & approached by their clinical care team while the patients are undergoing CRT, or during post-treatment follow-up. Appropriate patients may also be identified from MDT or endoscopy lists.

A screening log must be kept of all patients given a Patient Information Sheet, including any that are subsequently excluded. The reason for exclusion must be recorded on this form. A copy of the screening log will be sent to the Trial Office on request.

Before entering a patient onto the study the Principal Investigator or designee will confirm eligibility.

Completed patient Registration Forms must be sent to:

CYTOFLOC email address: octo-cytofloc@oncology.ox.ac.uk

OCTO will confirm eligibility and register the patient by assigning a study number.

5 STUDY PROCEDURES AND ASSESSMENTS

Please refer to the summary of events given at the front of this protocol. Details of all protocol evaluations and investigations must be recorded in the patient's medical record for extraction onto the eCRF.

5.1 Informed consent

Potential participants will be given a current, approved version of the Patient Information Sheet. They will also receive clear verbal information about the study detailing no less than: the nature of the study; the implications and

constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the study at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal. They will have at least 24 hours to consider the information provided and the opportunity to question the Investigator, their GP or other independent parties before deciding whether to participate.

Willing participants will be consented during a separate visit, and will have the opportunity to talk to the PI or a nominated health professional, should they have any further questions. As these patients are attending daily for CRT or post CRT follow up, study appointments can be worked around hospital visits and should not need additional visits.

If local practice requires temporary discontinuation of anticoagulation for endoscopy, this should only be advised after consultation with the patients clinical care team.

The Principal Investigator (or delegate) who obtains consent must be suitably qualified and experienced. All delegates working on their behalf must be authorised by the Principal Investigator. The Investigator is responsible for ensuring that the study consent procedures comply with principles of GCP. Informed consent discussions and outcomes must be well documented in the medical record. The Investigator (or delegate) must be satisfied that the patient has made an informed decision before taking consent. The patient and the Investigator (or delegate) must personally sign and date the current approved version of the informed consent form in each other's presence. A copy of the information and signed consent form will be given to the participant. The original signed form will be retained at the study site, with copies held in both the medical record and Investigator Site File (ideally the original if local policy permits).

5.2 Pre-study intervention evaluations

The following must be performed/ obtained within 1 week before the patient undergoes the Cytosponge™ procedure or on the day of the procedure.

- Written informed consent
- Demographic details: age, sex
- Medical History including prior diagnosis, prior treatment and concomitant diseases
- Concomitant medications
- Physical examination including vitals (pulse, BP, respiratory rate, O2 saturation), general appearance, respiratory, cardiovascular, gastrointestinal
- WHO performance status
- Dysphagia score (Mellow Scale)
- Research bloods (optional)

5.3 Evaluations during the study

Evaluations on day 1

On the day the intervention is given:

- Dysphagia score (Mellow Scale)
- Research bloods (optional) if not already collected at Pre-study intervention

Post procedure:

- Assessment of immediate complications (safety monitoring)
- Questionnaire (participants complete post procedure in clinic, or return by post to their recruiting hospital if this cannot be achieved)

Telephone Follow-up evaluations (End of study)

No face-to-face follow-up visit is planned beyond the Cytosponge™ procedure, however patients will have telephone appointments with a qualified member of the research team as per summary of events (week 1 and week 2) to review concomitant medications, dysphagia score and assess for any complications. This can be completed in person if deemed appropriate by the local study team. This is only applicable for participants that have received definitive CRT (not for surgery), or for participants who have received neo-adjuvant CRT, if these times are prior to surgery. This is because the trial is exploring the Cytosponge™ effect on the oesophagus, which will no longer be applicable after this has been removed in surgery.

6 EARLY PATIENT WITHDRAWAL

Study Withdrawal
During the course of the study, a patient may withdraw early. This is expected to be a rare event as the Cytosponge™ procedure is given at a single visit during the course of the study. However, a patient may withdraw for a number of reasons, including:

- Loss to follow-up
- Significant protocol deviation
- Clinical decision
- Patient decision

Consent Withdrawal
Consent withdrawal means that a patient has expressed a wish to withdraw from the study altogether. Under these circumstances, the site needs to document all relevant discussions in the patient notes and notify the Study Office, which will allow the office to mark all future eCRFs as not available. Under these conditions, research samples and study data (including imaging data) already collected will be retained for use in study analysis. In some cases further information about any unwanted effects of study participation may need to be collected by the study team.

6.1 Patient evaluability and replacement

Participants who have attempted to swallow the Cytosponge™ are evaluable for the primary endpoint. Those who are not evaluable will be replaced at the Chief Investigator's discretion.

7 SAMPLES FOR LABORATORY ANALYSIS

7.1 Samples to be analysed in local Trust's laboratories

Pathology
Any surplus tissue remaining after routine biopsies and local pathology diagnostics will be requested by the Trial Office.

An anonymised copy (study number only) of the diagnostic & any follow up histopathology/cytology report should be sent to the Trial Office when requested. If the participant goes on to receive surgery, an anonymised copy of the surgical report should also be sent to the Trial Office.

7.2 Samples to be sent to and analysed in a Central Laboratory

All Cytosponge™ specimens will be couriered to the Fitzgerald Laboratory, at the MRC Cancer Unit. Cytosponge™ specimens will be processed in conjunction with the Cambridge University Hospitals' NHS Foundation Trust tissue bank which is accredited to GLP standards [10]. Samples will be processed to a paraffin embedded cell clot, sectioned and stained with Haematoxylin and Eosin (2 levels) and for immunohistochemical markers (TFF3 if adenocarcinoma, p53 in all cases) using a DAKO autostainer according to GCP standards. An expert GI cytologist will verify any atypia and positive immunostaining. In addition, DNA will be extracted for p53 sequencing using a multiplex assay as previously described and for ploidy status. Although timely processing will ensure that the study completes on time the results of Cytosponge™ samples will not affect patient clinical care since it is not being used as a diagnostic tool in the trial, and therefore there is some flexibility in the timeline. The biomarkers will be scored according to previously optimised protocols [10, 13-15]. These data will be compared to the degree of dysplasia determined from endoscopic biopsies or surgical specimen where surplus material is available as a result of routine care procedures. For further details, please refer to the Device & Sample Handling Manual.

7.3 Labelling and confidentiality of samples sent

All samples sent to the laboratories will be labelled with the study code, study patient number, and date taken.
A unique Cytosponge™ identifier will be added by the manufacturer to aid device tracking and accountability. In line with regulatory requirements, labels will carry the following wording CYTOFLOC Clinical Trial — Chief Investigator Prof Somnath Mukherjee. This device is only to be used for the CYTOFLOC Clinical Trial.
Should a laboratory receive any samples carrying personal patient identifiers the recipient must immediately obliterate this information and re-label. The Trial Office will be informed of their error.

7.4 Study sample retention at end of study

The Chief Investigator has overall responsibility for custodianship of the study samples. Laboratories are instructed to retain any surplus samples pending instruction from the Chief Investigator on use, storage or destruction. It is possible that new or alternative assays may be of future scientific interest. At the end of the research study any surplus samples of participants who have consented may be retained for use in other projects, including whole genome sequencing, that have received ethical approval. These may include other organisations in the UK and overseas and the commercial sector. Hence, any surplus study samples may be transferred to a licensed tissue bank, or new research tissue bank, where they will be managed in accordance with applicable host institution policies and the Human Tissue Act (HTA) requirements. Where mandated, biopsy samples from endoscopy or surgery may be returned to sites.

8 INVESTIGATIONAL MEDICINAL DEVICE (CYTOSPONGE™)

8.1 Cytosponge™

The Cytosponge™ is a non-CE-marked, single-use, non-sterile, 3cm diameter, polyester, medical grade sphere on a string, compressed within a capsule. The capsule is bovine gelatine, certified as both Halal and Kosher. The device will not be provided sterile (Annex I Section 8.4 & 8.5). According to Annex IX of the Medical Devices Directive 93/42/EEC the device has been classified as Class 1.

- The use of the device is “transient” in duration, (Definition 1.1) with the whole procedure taking less than 10 minutes.
- The use of the device is “invasive”, but not “surgically invasive” in that the entrance of the device is via a “body orifice” , namely mouth and throat (Definition 1.2)
- According to Rule 5 (Annex IX, Section 3, Clause 2.1) all invasive devices with respect to body orifices, other than surgically invasive devices and which are not intended for connection to an active medical device: are in Class I if they are intended for transient use.

More than 2000 individuals have swallowed this device in a previous MHRA approved trial and detachment was reported in 1:1,000 procedures with no adverse impact on the patients.

8.2 Cytosponge™ administration

Participants will be asked to not eat or drink for 4 hours prior to the procedure. Participants will be given the option of receiving an anaesthetic throat spray (Lidocaine) before the Cytosponge™ is withdrawn, providing there is no contraindication to its use. Details on the administration of Lidocaine can be found in the Device & Sample Handling Manual.

The Cytosponge™ will be administered by a suitably qualified member of the study team. The capsule along with three quarters of the string (which is bunched together holding it close to the capsule to make it easy for swallowing) is swallowed by drinking a small glass of room temperature water continuously. The participant is asked to hold the end of the string with the Cytosponge™ in situ for 5 minutes. This is to allow the outer gelatine cover of the capsule to dissolve in the stomach acid. The sponge contained within expands and is then drawn back by a qualified member of the research team up the oesophagus by the attached string, collecting cells as it moves upwards.

Once the Cytosponge™ is withdrawn it is placed in Surepath preservative fluid and the excess string is cut and disposed of in the clinical waste. The Cytosponge™ sample can initially be kept at room temperature but should be stored in a fridge, at approx. 4^{o}C as soon as possible or at least within 24 hours of use. The samples will be sent to the laboratory at Cambridge (MRC/Hutchison Research Centre) to be analysed in batches. For details of labelling, storage, collection, transport and delivery of samples refer to the Device & Sample Handling Manual.

8.3 Special precautions

The Cytosponge™ is not CE marked and not authorised for use in the UK. The trial will be conducted under a Notification of No Objection from the MHRA.

If the Cytosponge™ detaches from the string or if the Cytosponge™ cannot be removed, the recommended approach is to remove the Cytosponge™ by endoscopy ideally within 3 hours of the ingestion. Therefore, where patients are

planned to have re-assessment endoscopy for detection of response to dCRT, the Cytosponge™ procedure should be undertaken prior to endoscopy for logistic reasons. Similarly, for patients who have undergone naCRT, the procedure should be performed on the day of surgery prior to the operation (but this is not mandatory).

Pregnancy is not a contraindication to the Cytosponge™, however, endoscopy is generally avoided in pregnant women. Sites should ensure that participants are able to receive endoscopy in accordance with their local practices in the event this is required. This may include excluding those who are pregnant depending on local endoscopy guidelines. Many of the patients enrolled in this study will be required to undergo endoscopy in standard care, and when applicable, will be informed for their routine care procedure and via the CYTOFLOC PIS, that endoscopy is not advised for pregnant women.

9 INVESTIGATIONAL MEDICINAL DEVICE MANAGEMENT

9.1 Cytosponge™ manufacturer

The Cytosponge™ is supplied by Cambridge University Hospital, the legal manufacturer of the device. The Cytosponge™ manufacturing activities are subcontracted by the legal manufacturer to Europlaz Technologies Ltd, 1-9 The Maltings Industrial Estate , Southminster , Essex CM0 7EQ. This company worked with Cambridge University Hospitals to produce the non-CE marked device for research study, BEST2, BEST3 and other international studies.

The legally responsible manufacturer for the trial is Cambridge University Hospitals NHS Foundation Trust (CUH), Box 277, Addenbrookes Hospital, Cambridge Biomedical Campus, CB2 0QQ.

9.2 Cytosponge™ ordering

Cytosponge™ devices will be sent from Cambridge University Hospital to trial sites once they have been informed by the Trial Office that all approvals are in place. Upon receipt, the study team will log the devices on an accountability log. Devices will be logged to facilitate stock management. Subsequent supplies will be ordered by the Trial Office.

Each device will have a unique identifier to further facilitate accountability, and safety-related measures. Expired devices will be placed in the clinical waste and logged using paper and electronic records.

Lidocaine throat spray should be sourced by sites & may be reimbursed by the trial centre (OCTO).

9.3 Receipt

A copy of the 'Acknowledgement of Receipt Form' should be sent to the CYTOFLOC Trial Office on receipt of the device.

9.4 Handling and storage

Devices should be stored in a secure area at room temperature. The device has a shelf life of 6 months. After use, the Cytosponge™ sample can initially be kept at room temperature but should be stored in a fridge, at approx. 4^{o}C as soon as possible or at least within 24 hours of use.

Lidocaine storage should be per local site guidelines.

9.5 Labelling

A unique identifier will be added by the manufacturer to aid device tracking and accountability. In line with regulatory requirements, labels will carry the following wording: "CYTOFLOC Clinical Trial — Chief Investigator Prof Somnath Mukherjee. This device is only to be used for the CYTOFLOC Clinical Trial".

10 OTHER MEDICATIONS

10.1 Support medication

Symptomatic management of any side effects experienced during or after the Cytosponge™ procedure should be managed according to local hospital guidance (i.e. paracetamol as required).

10.2 Concomitant medication

Details (including indication, doses, frequency and start / stop dates) of concomitant medication taken prior to Cytosponge™ procedure (to ensure able to receive endoscopy if required) & during the study until the completion of the off-study visit must be recorded in the medical record and the appropriate CRF.

Concomitant medication may be given as medically indicated.

10.3 Prohibited therapies

Patients on anti-coagulation are eligible for the study as long as they are considered suitable candidates for endoscopic biopsy (follow local hospital procedures for management of patients on anti-coagulation due to undergo endoscopy). This may require a temporary discontinuation of the medication prior to the procedure. If temporary discontinuation of anticoagulation is required, this should be after consultation with the patients clinical care team.

11 ASSESSMENT OF SAFETY

There are no Investigational Medicinal Products (IMPs) in this trial. The only study-specific interventional procedure is the investigational Cytosponge™ procedure, which will comply with the MHRA Medical Device Directive.

The Investigator will monitor each patient for clinical evidence of Adverse Events (AEs) & Serious Adverse Events (SAEs) on a routine basis throughout the study. The Cytosponge™ is an Investigational Medical Device & therefore additional categorisation of Adverse Device Effect (ADE) and Serious Adverse Device Effect (SADE) is required. Adverse Event monitoring starts from the time of Cytosponge™ administration until the participant completes the study at the Two Week Telephone Follow Up appointment. If a participant receives surgery prior to their two week follow up visit, then their safety reporting period will be up until they receive surgery (see section 5.3, Telephone Follow-Up Evaluations (End of Study). Should an Investigator become aware of any study intervention related SADEs following the Two Week Telephone Follow Up, these must also be reported as stated below. All reportable AEs will be followed to a satisfactory conclusion.

All AEs reported to the Trial Office will be processed according to internal SOPs. The Trial Office may request additional information for any AE as judged necessary.

The study team will keep in close communication with the manufacturer Cambridge University Hospitals including in the case of a serious adverse device effect, device deficiencies and other quality control aspects. Main quality and safety issues will be reported via email to Cambridge University Hospital within 48 hours of the sponsor becoming aware. No patient identifiable information will be disclosed to the manufacturer at any time.

11.1 Adverse Event Definitions

An **Adverse Event or experience (AE)** is any untoward medical occurrence in a study participant temporally associated with the administration of a medical device, whether or not considered caused by or related to the medical device. An AE can therefore be any unfavourable and unintended sign, symptom, disease (new or exacerbated) and /or significant abnormal laboratory or physiological observation temporally associated with the use of a medical device. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

An **Adverse Device Effect (ADE)**, as it relates to the use of the Cytosponge™ procedure, is defined as including an untoward medical occurrence resulting from: insufficiencies or inadequacies in the instructions for use, deployment, implantation, installation, operation, or any malfunction, a user error or intentional misuse.

A **Serious Adverse Event (SAE)** is any AE, regardless of causality or expectedness, that:

• **Led to death**	
• **Led to a serious deterioration in health that either:**	
○ **Resulted in a life-threatening illness or injury.**	This refers to an event in which the subject 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 resulting in a permanent impairment of a body structure or a body function.
○ **Resulted in permanent impairment of a body structure or a body function.**	
○ **Requires in-patient hospitalisation or prolongation of existing hospitalisation.**	In general, hospitalisation signifies that the subject has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether hospitalisation occurred or was necessary, the AE should be considered serious. Any hospitalisation that was planned before trial entry, or a planned hospitalisation for a pre-existing condition, without serious deterioration in health, will not meet SADE criteria.
○ **Resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function.**	This means a substantial disruption of a person's ability to conduct normal life functions. It does not include experiences of relatively minor medical significance or accidental trauma (e.g. sprained ankle), which do not constitute a substantial disruption.
• **Led to foetal distress, foetal death or a congenital abnormality or birth defect.**	

A **Serious Adverse Device Effect (SADE)**, as it relates to the use of the Cytosponge™ procedure, is defined as any Adverse Device Effect (ADE) that has resulted in any of the characteristics of a Serious Adverse Event (SAE).

This includes device deficiencies that might have led to a serious adverse event if:

- suitable action had not been taken
- intervention had not been made
- circumstances had been less fortunate

For the purposes of this Trial, device deficiency is defined as: inadequacy of a medical device with respect to its identity, quality, durability, safety or performance.

An **Unanticipated Serious Adverse Device Effect (USADE)** is defined as any SADE which is unexpected. In the trial, a USADE, as it relates to the use of the Cytosponge™ procedure, is defined as a SADE that, by its nature, incidence, severity of outcome, has not been identified in the current version of the protocol, investigator's brochure or risk assessment. A list of anticipated SADEs are listed below.

11.2 Determining adverse event causality

Each AE must be assessed for causality, seriousness, severity and expectedness by the site PI or delegate, including association with the Investigational Medical Device.

A Serious Adverse Device Effect (SADE) is a SAE that may be related to the investigational device. The assessment of "relatedness" must be determined by a medically qualified individual and is primarily the responsibility of the PI at site or agreed designee. SAEs that will be considered related will include any SAE that is documented as possibly, probably or definitely related to protocol device. The assessment of relatedness is made using the following:

Classification	Relationship	Definition
Device related	**Definitely**	• Starts within a time related to the study intervention *and* • No obvious alternative medical explanation.
	Probably	• Starts within a time related to the study intervention administration *and* • Cannot be reasonably explained by known characteristics of the patient's clinical state.
	Possibly	• Starts within a time related to the study intervention *and* • A causal relationship between the intervention and the adverse event is at least a reasonable possibility.
Not device related	**Probably not**	• The time association or the patient's clinical state is such that the study intervention is not likely to have had an association with the observed effect.
	Definitely not	• The AE is definitely not associated with the study intervention.

The Investigator must try to obtain sufficient information to confirm the causality of the adverse event (i.e. relation to intervention, background treatment, other illness, progressive malignancy etc.) and give their opinion of the causal relationship between each AE and the study intervention. This may require taking supplementary investigations of significant AEs based on their clinical judgement of the likely causative factors and/or include seeking a further specialist opinion.

11.3 Reference Safety Information (RSI) for assessment of expectedness

The Reference Safety Information (RSI) for the Cytosponge™ device is in Section 9 of the Cytosponge™ Investigator Brochure and lists all the expected side effects associated with the use of the Cytosponge™ device.

11.4 Expected adverse events that do not require reporting to REC

Effects relating to the Cytosponge™ could include:

- Cytosponge™ detached from the string while in the patient's oesophagus/stomach (SADE)
- Inability or difficulty to remove the Cytosponge™ (SADE)
- Laceration at the back of the throat (ADE or SADE depending on Bleeding and size of the laceration)
- Perforation or tear of the oesophagus (SADE)
- Bleeding from biopsy site or Cytosponge™ abrasion (SADE)
- Obstruction on breathing or airway as a result of the Cytosponge™ (SADE)
- Mild sore throat (ADE)

11.5 Reporting of SAEs to the Trial Office

All SAEs & SADEs must be reported on the study-specific SAE Form and sent to:

Pharmacovigilance Office, OCTO
Email: octo-safety@oncology.ox.ac.uk
Tel no: +44 (0) 01865 227181

SAE forms must be completed and submitted within **24hrs** of becoming aware of the event. If the SAE has not been reported within the specified timeframe, a reason for lateness must be provided when sending the SAE Report Form. For the initial report the following elements must be completed:

- Overall diagnosis (CTCAE grading)
- Reason for seriousness
- Causality (must be assessed by a medically qualified person)
- Name and signature of the reporting person

Any change of condition or other follow-up information should be sent by sites to the Pharmacovigilance office, as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

Each separate AE episode must be recorded. For example, if an AE resolves completely or resolves to baseline and then recurs or worsens again, this must be recorded as a separate AE. For AEs to be considered intermittent, the events must be of similar nature and severity.

11.6 Follow-up of Serious Adverse Events

A follow-up report must be completed when the SAE resolves, is unlikely to change, or when additional information becomes available. If the SADE is a suspected USADE then follow up information must be provided as requested by the Trial Office.

If new or amended information on a reported SAE becomes available, the Investigator should report this on a new SAE form using the completion guidelines. If this is not possible and any changes are made to the original completed form, you must initial and date all new or amended information so that all changes are clearly identified.

SAEs that are considered to be definitely unrelated to the trial intervention will not be followed up and monitored.

11.7 Reporting AEs to the MHRA and REC

Reporting to the MHRA: All SAEs, will be reported **immediately** to the MHRA (devices) by the Sponsor or delegate (see further timings under Urgent Safety Reporting). This is irrelevant of whether the event is believed to be device related or not.

Urgent safety reporting:
Any SAE, SADE or USADE which indicate an imminent risk and require prompt remedial action, or new finding related to such an event, will be reported to the MHRA immediately, but no later than 2 calendar days following awareness by the sponsor or delegate.

Any other SAEs, SADEs or USADEs, or new finding/update, will be reported immediately to the MHRA, but no later than 7 calendar days after awareness by the sponsor or delegate.

Any urgent safety measures will be reported to the MHRA and REC within 3 days of the measures being implemented.

Reporting to the REC: All SADEs & USADEs, other than those listed in section 11.4, will be reported to the REC by the Chief Investigator in conjunction with the Sponsor within 15 days of the CYTOFLOC Trial Office & CI becoming aware. This will follow the current HRA guidance: http://www.hra.nhs.uk/resources/during-and-after-your-study/progress-and-safety-reporting/ Safety aspects will be addressed in the Annual Progress Report to the REC by the Chief Investigator. Please see further guidance under Urgent Safety Reporting.

Anonymised data may be shared with the manufacturer and their associates for safety and product development purposes.

11.8 Reporting Adverse Events on the CRF

All AEs, including Serious AEs must be recorded on the case report forms (CRF) for that patient (unless otherwise specified in section 11.9. The information provided will include date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome and relationship of the AE to device. Any concomitant medications or any other therapy used to treat the event must be listed. The Investigator will provide an "other" cause for serious AEs considered to be unrelated to the study device. Sites should ensure data entered into the CRF is consistent with the SAE report information where applicable.

Each separate AE episode must be recorded. For example, if an AE resolves completely or resolves to baseline and then recurs or worsens again, this must be recorded as a separate AE. For AEs to be considered intermittent, the events must be of similar nature and severity.

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

AEs which are serious must be reported to OCTO from the time of Cytosponge™ administration until the participant completes the study at the Two Week Telephone Follow Up appointment. If a participant receives surgery prior to this point, the safety reporting period will be up until the participant receives surgery (see section 5.3, Telephone Follow-Up Evaluations (End of Study). Should an Investigator become aware of any study intervention related Serious Adverse Device Effects (SADEs) following the Two Week Telephone Follow Up, these must also be reported to OCTO.

Terms and Grading of Events
The NCI CTCAE Version 4.0 (currently up to Version 4.03) must be used by the site for grades; the events will be coded in-house by the Pharmacovigilance team using MedDRA and where possible using the Lowest Level Terms provided. Where indicated on the SAE form, provide the severity grade, and the worst grade recorded.

11.9 Events exempt from being reported as AE/SAE

Progression of underlying disease
Disease progression and resultant death will be captured on the CRF. Adverse events including hospitalisation that are clearly consistent with disease progression will not be reported as individual AE/SAEs. Clinical symptoms of progression will only be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Every effort should be made to document the objective progression of underlying malignancy. In some cases, the determination of clinical progression may be based on symptomatic deterioration. For example, progression may be evident from clinical symptoms, but is not supported by tumour measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments.

Elective admissions and supportive care
Planned hospitalisation for a pre-existing condition, or a procedure required by the CIP [Clinical Investigation Plan], without serious deterioration in health, is not considered a serious adverse event, and do not require SAE reporting.

12 DEFINING THE END OF STUDY

For this study the end of the study is defined as “The last visit of the last patient undergoing the study (LPLV)”. Telephone follow ups are included as visits.

Recruitment to the study will be stopped when:

- The stated number of patients to be recruited is reached.
- The stated primary and secondary objectives of the study are achieved.

The Sponsor and the Chief Investigator reserve the right to terminate the study earlier at any time. In terminating the study, they must ensure that adequate consideration is given to the protection of the participants’ best interests.

13 STATISTICAL CONSIDERATIONS

13.1 Sample size and power

The trial aims is to recruit 50 evaluable patients in 24 months from approximately 10 UK centres to give a completion rate estimate to +/-13% for feasibility (95% CI, estimated 70% rate) and enough samples to consider markers. Participants that do not receive the intervention will be withdrawn and replaced at the Chief Investigator’s discretion.

14 STATISTICAL ANALYSIS PLAN

The completion rate (swallowing of the sponge, retrieval of the sponge without additional intervention) with corresponding 95% confidence interval will be calculated at the end of the trial. Results will be given for all patients and also separately for (1) patients recruited post dCRT and (2) patients recruited post naCRT.

Stopping rules: The study will be stopped if there is more than one serious life-threatening complication from the Cytosponge™ (e.g. oesophageal perforation or life-threatening gastro-intestinal bleeding).

A statistical analysis plan will be finalised before release of the final data set.

14.1 Inclusion in analysis

To be evaluable for assessment of the study primary endpoint, participants must have attempted to swallow the Cytosponge™.

14.2 Subgroup analysis

The primary endpoint, the completion rate, will be calculated overall and separately for those having Definitive Chemoradiotherapy (dCRT) and Neo-adjuvant Chemoradiotherapy (naCRT).

14.3 Interim Analyses

No formal interim analysis of results is planned for this study.

14.4 Procedures for reporting any deviation(s) from the original statistical plan

Any deviations from the original statistical plan will be described and justified in the final report.

14.5 Final analysis

Final analysis will be after end of study evaluations have been recorded and data has been checked to be complete and accurate. Decision to proceed to a formal phase II will be determined by whether the primary and secondary objectives of this study have been met. If the completion rate is >70% then the trial outcome will be to develop a randomised phase II/ III testing Cytosponge™ in post-CRT follow-up.

15 TRANSLATIONAL SAMPLE COLLECTION (OPTIONAL)

CYTOFLOC is an excellent opportunity to prospectively collect biopsy and blood samples for translational research. Translational studies would look at a number of key areas which could include (but not restricted to):

- Co-relate Cytosponge™ sample with endoscopic biopsy
- Baseline diagnostic biopsy
- Blood biomarkers of tumour response

Biopsy sample collection

Participants may consent to their surplus biopsy samples being used by the research team. For patients with baseline diagnostic biopsies, and/or undergoing endoscopic re-assessment of tumour response at around the time of Cytosponge™ testing, optional paraffin tissue blocks of tumour samples should be requested from pathology. These should be posted as instructed in the Device & Sample Handling Manual. All samples should be labelled using the labels provided by OCTO.

Below is one example of standard practice biopsy collection:

Sample	Visible tumour	No visible tumour	Comments
Biopsy in formalin	x2	x2 from previous tumour site	Sample to histopathology.

Blood sample collection

An optional blood sample will be taken if agreed by the participant and will be processed and stored using the procedures in the Device & Sample Handling Manual provided in the Investigator Site File. It is recommended that these samples are taken at the same time as any routine bloods if possible so that participants do not have to undergo any additional venepunctures.

16 STUDY COMMITTEES

16.1 Trial Management Group (TMG)

The TMG will oversee the running of the study. Members of the TMG will include the Chief Investigator, Co-Investigators, Trial Manager, Trial Statistician and others as required. The TMG will meet as necessary.

16.2 Data and Safety Monitoring

There is no independent Data and Safety Monitoring Committee (DSMC) established for this study.

16.3 Trial Steering Committee

Study Oversight will be provided by an independent Radiotherapy & Imaging Trial Oversight Committee (RIOC).

17 DATA MANAGEMENT

17.1 Database considerations

Data management will be performed via a web-based, bespoke study database (OpenClinica). OpenClinica is a dedicated and validated clinical study database designed for electronic data capture. http://www.openclinica.org.

A guide explaining how to use OpenClinica will be provided to every site. Relevant OCTO staff will have overview of all entered data.

17.2 Electronic Case Report Forms (eCRFs)

It is important that the investigator makes sure that:

- The relevant eCRFs are completed.
- All eCRF data are verifiable in the source documentation and any discrepancies are explained.
- eCRF events are completed in a timely fashion, as close to the visit or event being recorded as possible.
- Data queries are resolved and documented by authorised study staff, giving a reason for the change or correction where appropriate.

The above considerations also apply to patients who are withdrawn early. If a patient is withdrawn from the study, the reason must be noted on the appropriate form.

17.3 Accounting for missing, unused, or spurious data.

Missing data will be chased up and supplemented where possible after consultation with the investigator. The completeness and correctness of the data will be monitored as per the monitoring plan.

18 CLINICAL STUDY REPORT

All clinical data will be presented at the end of the study as data listings. These will be checked to confirm the lists accurately represents the data collected during the course of the study. The study data will then be locked and a final data listing produced. The clinical study report will be based on the final data listings. The locked study data may then be used for analysis and publication.

19 STUDY SITE MANAGEMENT

19.1 Study site responsibilities

The Principal Investigator (the PI or lead clinician for the study site) has overall responsibility for conduct of the study, but may delegate responsibility where appropriate to suitably experienced and trained members of the study site team. All members of the study site team must complete the Staff Contact and Responsibilities Sheet provided prior to undertaking any study duties. The PI must counter sign and date each entry in a timely manner, authorising staff to take on the delegated responsibilities.

19.2 Study site set up and activation

A Principal Investigator should lead the study at each site, providing the local study office with all core documentation and attend the 'Site Initiation Visit' organised between the site and the Trial Office before the site becomes activated (usually carried out as a telephone conference call or personal visit). The Trial Office will call to check that the site has all the required study information/documentation and is ready to recruit. The site will then be notified once they are activated on the OCTO-CYTOFLOC database and able to register.

19.3 Study documentation

The Trial Office will provide an Investigator Site File to each investigational site containing the documents needed to initiate and conduct the study. The Trial Office must review and approve any local changes made to any study documentation including Patient Information and Consent Forms prior to use. Additional documentation generated during the course of the study, including relevant communications must be retained in the site files as necessary to reconstruct the conduct of the study.

20 REGULATORY & ETHICAL CONSIDERATIONS

Declaration of Helsinki: The investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for Good Clinical Practice: The investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice (GCP), and the applicable policies of the sponsoring Institution and host Trusts.

Participants and their clinical teams will not receive any results from their Cytosponge™ samples. This is because at this feasibility stage we are unclear on the material/data that will be obtained from the Cytosponge™ or how reliable this is. Therefore, the Cytosponge™ should not influence clinical care.

20.1 Ethical conduct of the study and ethics approval

The Protocol, Patient Information Sheet, Consent Form and any other information that will be presented to potential study patients (e.g. advertisements or information that supports or supplements the informed consent) will be reviewed and approved by an appropriately constituted, independent Research Ethics Committee (REC). Principal Investigators will be approved by the REC.

20.2 Regulatory Authority approval

Appropriate approvals will be sought to receive a Notification of No Objection from the MHRA to use the device in the Trial. All reporting to the MHRA will follow the Trial Office's Sponsors' Standard Operating Procedures.

20.3 NHS Research Governance

Investigators are responsible for ensuring they obtain confirmation of capability and capacity to conduct the study in accordance with HRA approval, local arrangements and policies. This confirmation will take the form of fully executed contract or written agreement of Statement of Activities and must be sent to the Trial Office.

20.4 Protocol amendments

Amendments are changes made to the research following initial approval. A 'substantial amendment' is an amendment to the terms of the REC application, MHRA approval or to the protocol or any other supporting documentation that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects of the study;
- The scientific value of the study;
- The conduct or management of the study; or
- The quality or safety of the NIMP/intervention used in the study.

Non-substantial amendments are those where the change(s) involve only minor logistical or administrative aspects of the study.

All amendments will be generated and managed according to OCTRU SOPs to ensure compliance with applicable requirements. Written confirmation of the REC, MHRA and HRA approval must be in place prior to implementation by Investigators. The only exceptions are for non-substantial amendments or any amendments that do not require REC

or MHRA review which will be submitted to HRA, and amendments that are necessary to eliminate an immediate hazard to study patients (see below).

It is the Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the study. The Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented if appropriate.

20.5 Urgent safety measures

The Sponsor or Investigator may take appropriate urgent safety measures to protect study participants from any immediate hazard to their health or safety. Urgent safety measures may be taken without prior authorisation. The study may continue with the urgent safety measures in place. **The Investigator must inform the Trial Office IMMEDIATELY if the study site initiates an urgent safety measure:**
The notification must include:

- Date of the urgent safety measure;
- Who took the decision; and
- Why the action was taken.

The Investigator will provide any other information that may be required to enable the Trial Office to report and manage the urgent safety measure in accordance with the current ethical requirements for expedited reporting. The Trial Office will follow written procedures to implement the changes accordingly.

20.6 Temporary halt

The Sponsor and Investigators reserve the right to place recruitment to this protocol on hold for short periods for administrative reasons **or** to declare a temporary halt. A temporary halt is defined as a formal decision to:

- Interrupt the treatment of subjects already in the study for safety reasons;
- Stop recruitment on safety grounds; or
- Stop recruitment for any other reason(s) considered to meet the substantial amendment criteria, including possible impact on the feasibility of completing the study in a timely manner.

The Trial Office will report the temporary halt via an expedited substantial amendment procedure to the REC. The study may not restart after a temporary halt until a further substantial amendment to re-open is in place. If it is decided not to restart the study this will be reported as an early termination.

20.7 Serious Breaches

Investigators must notify the Trial Office at once if any serious breach of GCP is suspected. A serious breach is defined as "A breach of GCP or the study protocol which is likely to effect to a significant degree:

- The safety or physical or mental integrity of the subjects of the study; or
- The scientific value of the study"

The Trial Office will review the event and, if appropriate a report will be submitted to the Sponsor office and to the REC, where appropriate within 7 days of the Trial Office becoming aware of the breach, as per OCTRU SOPs.

20.8 Trial Reports

This protocol will comply with all current applicable Research Ethics Committee, Health Research Authority, Medicines and Healthcare products Regulatory Agency and Sponsor requirements for the provision of periodic study safety and progress reports. Any additional reports will be provided on request. Reporting will be managed by the Trial Office according to internal SOPs. Sites will be urged to return as much data as possible before each database lock point.

The Trial Office will determine which reports need to be circulated to Principal Investigators and other interested parties according to internal SOPs. Study teams at sites are responsible for forwarding study reports they receive to their local Trust as required.

21 EXPENSES AND BENEFITS

The study sites may provide reasonable patient travel expenses as per local practice. There is no specific funding within the budget of this study for expenses.

22 QUALITY ASSURANCE

22.1 Risk assessment

A risk assessment and a monitoring plan will be prepared before the study opens. The risk assessment will be repeated if necessary in the light of changes while the study is ongoing or in response to monitoring reports. Monitoring plans will be amended as appropriate.

22.2 Monitoring

Monitoring will be performed according to the monitoring plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The investigator and institutions involved in the study will permit study-related monitoring and provide direct access to all study records and facilities. They will provide adequate time and space for the completion of monitoring activities.

The study site will be monitored centrally by checking incoming data for compliance with the protocol, consistency, completeness and timing. The case report data will be validated using appropriate set criteria, range and verification checks. The study site must resolve all data queries in a timely manner. All queries relating to key outcome and safety data and any requiring further clarification will be referred back to the study site for resolution. For other non-critical data items, OCTO staff may resolve data queries centrally providing the correct answer is clear. Such changes will be clearly identified in the eCRF and the study site informed.

The study site will also be monitored by site visit as necessary to ensure their proper conduct of the study. OCTO staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have.

All patient personal identifiers must be obliterated from the information except where explicit consent for release of personal information has been obtained from the patient. The study site will provide copies of the following participant information to the Trial Office on request for remote monitoring purposes:

- Participant screening log

22.3 Audits

All aspects of the study conduct may be subject to internal or external quality assurance audit to ensure compliance with the protocol, GCP requirements and other applicable regulation or standards. It may also be subject to a regulatory inspection. Such audits may occur at any time during or after the completion of the study. Investigators and their host Institution(s) should understand that it is necessary to allow auditors direct access to all relevant documents, study facilities and to allocate their time and the time of their staff to facilitate the audit or inspection visit. Anyone receiving notification of a Regulatory Inspection that will (or is likely to) involve this trial must inform the Trial Office without delay.

23 RECORDS RETENTION & ARCHIVING

During the clinical study and after study closure the Investigator must maintain adequate and accurate records to enable the conduct of a clinical study and the quality of the research data to be evaluated and verified. All essential documents must be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed. It is the University of Oxford's policy to store data for a minimum of 5 years. Investigators may not archive or destroy study essential documents or samples without written instruction from the Trial Office. The medical files of study participants shall be retained in accordance with national legislation and in accordance with the host institution policy.

Retention and storage of laboratory records for clinical study samples must also follow these guidelines.

Retention and storage of central laboratory records supporting the endpoints of the study and the disposition of samples donated via the study must also comply with applicable legislation and Sponsor requirements.

24 PATIENT CONFIDENTIALITY

Personal data recorded on all documents will be regarded as confidential, and to preserve each patient's anonymity, only their month and year of birth will be recorded on the eCRF. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

The Investigator site must maintain the patient's anonymity in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled patients' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally.

Anonymised data may be used in future research, including by other organisations in the UK and overseas and the commercial sector.

The Sponsor or delegate will act as Data Custodian for the trial.

25 STUDY FUNDING

This study is funded by Cancer Research UK (Population Research Committee ref A22173) and supported by the CRUK Oxford Centre (formerly the Oxford Cancer Research Centre) and the CRUK/MRC Oxford Institute for Radiation Oncology. The Cytosponge™ devices are kindly supplied by Cambridge University Hospitals. The trial will be NIHR adopted. There will be no funding available to the study sites for NHS excess treatment costs.

26 SPONSORSHIP AND INDEMNITY

26.1 Sponsorship

The Sponsor will provide written confirmation of Sponsorship and authorise the study commencement once satisfied that all arrangements and approvals for the proper conduct of the study are in place. A separate study delegation agreement, setting out the responsibilities of the Chief Investigator and Sponsor will be put in place between the parties.

26.2 Indemnity

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London, policy numbered: WD1200463). Product liability insurance will be provided by the legal manufacturer of the Cytosponge™ device.

NHS indemnity operates in respect of the clinical treatment, which is provided.

26.3 Contracts/Agreements

This study is subject to the Sponsor's policy requiring that written contracts/agreements are agreed formally by the participating bodies as appropriate. A Clinical Trial Agreement (CTA) will be placed between the Sponsor and participating NHS Trusts prior to site activation.
The Sponsor will also set up written agreements with any other external third parties involved in the conduct of the study as appropriate.

27 PUBLICATION POLICY

The intention is to publish this research in a specialist peer reviewed scientific journal on completion of the study. The results may also be presented at scientific meetings and/or used for a thesis. No study results may be published or presented without the prior approval of the TMG. The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. Authors will ensure the current Department of Oncology publications acknowledgement statement is inserted. Authors will acknowledge that the study was sponsored by the University of Oxford.

28 REFERENCES

1 Lagergren, P., Avery, K. N. L., Hughes, R., Barham, C. P., Alderson, D., Falk, S. J. and Blazeby, J. M.**Health-related quality of life among patients cured by surgery for esophageal cancer**. *Cancer* 2007, **110**: 686–693. doi:10.1002/cncr.22833

2 Stahl, M., Stuschke, M., Lehmann, N., Meyer, H., Walz, M., Seeber, S., Klump, B., Budach, W., Teichmann, R., Schmitt, M., Schmitt, G., Franke, C., and Wilke, H. **Chemoradiation With and Without Surgery in Patients With Locally Advanced Squamous Cell Carcinoma of the Esophagus**. *Journal of Clinical Oncology* 2005, **23** (10): 2310-2317

3 Van Hagen P, Hulshof MCCM, van Lanschot JJB, Steyerberg EW, Henegouwen MIB, Wijnhoven BPL, et al. **Preoperative chemoradiotherapy for esophageal or junctional cancer**. *N Engl J Med*. 2012, **366**(22):2074–84. doi: 10.1056/NEJMoa1112088.

4 Sudo K, Xiao L, Wadhwa R, Shiozaki H, Elimova E, Taketa T, Blum MA, Lee JH, Bhutani MS, Weston B et al: **Importance of surveillance and success of salvage strategies after definitive chemoradiation in patients with esophageal cancer.** *J Clin Oncol* 2014, **32**(30):3400-3405.

5 Ross-Innes CS, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M3, Malhotra S, di Pietro M, Ivakhno S, He M, Weaver JM, Lynch AG, Kingsbury Z, Ross M, Humphray S, Bentley D, Fitzgerald RC; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Study Group; Oesophageal Cancer Clinical and Molecular Stratification OCCAMS Study Group. **Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma.** Nat Genet. 2015 Sep;47(9):1038-46. doi: 10.1038/ng.3357. Epub 2015 Jul 20

6 Kadri SR, Lao-Sirieix P, O'Donovan M, Debiram I, Das M, Blazeby JM, Emery J, Boussioutas A, Morris H, Walter FM, Pharoah P, Hardwick RH, Fitzgerald RC. **Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study**. BMJ. 2010 Sep 10;341:c4372. doi: 10.1136/bmj.c4372

7 Ross-Innes CS, Debiram-Beecham I, O'Donovan M, Walker E, Varghese S, Lao-Sirieix P, Lovat L, Griffin M, Ragunath K, Haidry R, Sami SS, Kaye P, Novelli M, Disep B, Ostler R, Aigret B, North BV, Bhandari P, Haycock A, Morris D9, Attwood S, Dhar A, Rees C, Rutter MD, Sasieni PD, Fitzgerald RC; BEST2 Study Group. **Evaluation of a minimally invasive cell sampling device coupled with assessment of trefoil factor 3 expression for diagnosing Barrett's esophagus: a multi-center case-control study.** PLoS Med. 2015 Jan 29;12(1):e1001780. doi: 10.1371/journal.pmed.1001780. eCollection 2015 Jan

8 Weaver JM, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, Barbera M, Murtaza M, Ong CA, Lao-Sirieix P, Dunning MJ, Smith L, Smith ML, Anderson CL, Carvalho B, O'Donovan M, Underwood TJ, May AP, Grehan N, Hardwick R, Davies J, Oloumi A, Aparicio S, Caldas C, Eldridge MD, Edwards PA, Rosenfeld N, Tavaré S, Fitzgerald RC; OCCAMS Consortium. **Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis.** Nat Genet. 2014 Aug;46(8):837-43. doi: 10.1038/ng.3013. Epub 2014 Jun 22.

9 Ross-Innes CS, Chettouh H, Achilleos A, Galeano-Dalmau N, Debiram-Beecham I, MacRae S, Fessas P, Walker E, Varghese S, Evan T, Lao-Sirieix PS, O'Donovan M, Malhotra S, Novelli M, Disep B, Kaye PV, Lovat LB, Haidry R, Griffin M, Ragunath K, Bhandari P, Haycock A, Morris D, Attwood S, Dhar A, Rees C, Rutter MD, Ostler R, Aigret B, Sasieni PD, Fitzgerald RC; BEST2 study group. **Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: a cohort study.** Lancet Gastroenterol Hepatol. 2017 Jan;2(1):23-31. doi: 10.1016/S2468-1253(16)30118-2. Epub 2016 Nov 11.

10 Roshandel G, Merat S, Sotoudeh M, Khoshnia M, Poustchi H, Lao-Sirieix P, Malhotra S, O'Donovan M, Etemadi A, Nickmanesh A, Pourshams A, Norouzi A, Debiram I, Semnani S, Abnet CC, Dawsey SM, Fitzgerald RC, Malekzadeh R. **Pilot study of cytological testing for oesophageal squamous cell dysplasia in a high-risk area in Northern Iran.** Br J Cancer. 2014 Dec 9;111(12):2235-41. doi: 10.1038/bjc.2014.506. Epub 2014 Sep 23.

29 WHO PERFORMANCE STATUS

Grade	Explanation of activity
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair

30 APPENDIX 1: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
001	2.0	22Jan2018	Stephanie Levy	Section 24 Patient Confidentiality: - Data collection on CRF changed from initials and date of birth to month and year of birth at request of REC. - Dr Maria O'Donovan role changed. Approved by the REC during their initial approval. Changes only submitted to the MHRA as an amendment.
002	3.0	20Feb2019	Stephanie Levy	Clarification of a minor typographical error in Protocol exclusion criteria number 1 listed in Protocol Synopsis & Section 4.2