CYTOFLOC

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

Statistical Analysis Plan

Version 1.0 – 22Apr2020

Based on Protocol version 3.0 – 20Feb2019

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1. INTRODUCTION

This document details the proposed data presentation and analysis for the main paper(s) and final study reports from the ***Evaluation of a Non-Endoscopic Immunocytological Device (Cytosponge™) for post chemo-radiotherapy surveillance in patients with oesophageal cancer – a feasibility study***. The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.

1.1 Key personnel

List of key people involved in the drafting and reviewing this SAP, together with their role in the trial and their contact details.

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1.2 Changes from previous version of SAP

A summary of key changes from earlier versions of SAP, with particular relevance to protocol changes that have an impact on the design, definition, sample size, data quality/collection and analysis of the outcomes will be provided. Include protocol version number and date.

Version number Issue date	Author of this issue	Protocol Version & Issue date	Significant changes from previous version together with reasons
V1.0_22Apr2020	HO	Protocol_3.0_20Feb2019	Not applicable as this is the 1st issue

2. BACKGROUND AND OBJECTIVES

2.1 Background and rationale

There are around 8900 new cases of oesophageal cancer in the UK every year and the UK incidence rate is the second highest in Europe for males and the highest for females (CRUK Cancer Statistics, 2013-2015). Surgery is often challenging in oesophageal cancer patients and a non-surgical approach, i.e. definitive chemoradiotherapy (dCRT), may be the preferred treatment, reserving surgery for salvage. Although overall survival in patients treated with dCRT remains comparable to those treated with surgical-based therapy (Stahl et al, 2005), the higher incidence of loco-regional recurrence and the lack of an effective surveillance strategy have deterred many clinicians from adopting dCRT plus salvage surgery as the standard approach. Regular endoscopic surveillance is invasive; this study will investigate the feasibility of a less invasive technique, Cytosponge™, in post CRT surveillance in oesophageal cancer. If found to be feasible, it will lead to larger studies evaluating the role of Cytosponge™ in surveillance post-dCRT. 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.

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 Cytosponge™ is supplied by Cambridge University Hospital, the legal manufacturer of the device. 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.

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, 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.

2.2 Objectives

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

3. STUDY METHODS

3.1 Trial Design/framework

CYTOFLOC is a feasibility study testing the use of the Cytosponge™ to determine completion rate, safety and acceptability of the procedure. 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 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. Three to five minutes after swallowing (once dissolved in the stomach) the spherical mesh can be retrieved by pulling on the cord.

Fifty patients will be recruited from approximately 10 sites in the UK.

Patients will receive one Cytosponge™ test at one time-point 4 to 16 weeks after completion of CRT (either dCRT or naCRT). Patients will be in the study for about a month in total, from entry until the last protocol visit.

Trial Open (start of recruitment):	13Apr2018
End of recruitment:	31Jan2020
Date expected end follow-up/start of data cleaning:	28Feb2020
Expected start of final analysis:	16Mar2020

3.2 Randomisation and Blinding

Not applicable: this is not a randomised study.

3.3 Sample Size

The trial aims to recruit 50 evaluable patients in 24 months from approximately 10 UK centres to give a completion rate estimate to +/-13% for feasibility (asymptotic 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.

3.4 Statistical Interim Analysis, Data Review and Stopping guidelines

There is no independent Data and Safety Monitoring Committee (DSMC) established for this study, a study oversight and trial conduct review will be provided by the independent Radiotherapy and Imaging Trial Oversight Committee (RIOC). The RIOC has three independent members and meets every six months, and its role includes providing oversight, monitoring completeness of data, and monitoring evidence for treatment harm. Further details are given in the RIOC Charter (which is held by the trial management team).

No formal interim analysis is planned. There is a stopping rule: 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). Within the remit of the Trial Management Group (TMG) is a discussion of any safety issues. The TMG will report any such complications to the RIOC.

3.5 Timing of Final Analysis

All primary and secondary feasibility outcomes will be collectively assessed i.e. assessed at the same time point. Tertiary outcomes will be analysed at the same time, if available or separately at a later date.

3.6 Blinded analysis

Not applicable.

3.7 Statistical Analysis Outline (as in Protocol)

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.

Inclusion in analysis

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

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).

Interim Analyses

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

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.

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 study testing Cytosponge™ in post-CRT follow-up.

4. STATISTICAL PRINCIPLES

4.1 Statistical Significance and Multiple Testing

A 5% significance level (corresponding to two-sided hypothesis tests) and 95% confidence intervals will be reported. No adjustments will be made for multiple testing as this is a feasibility study.

4.2 Definition of Analysis Populations

Population for assessment of completion rate: all patients who have provided consent and have attempted to swallow the Cytosponge™.

Population for calculation of consent rate: all patients who consented, out of all those patients who were eligible.

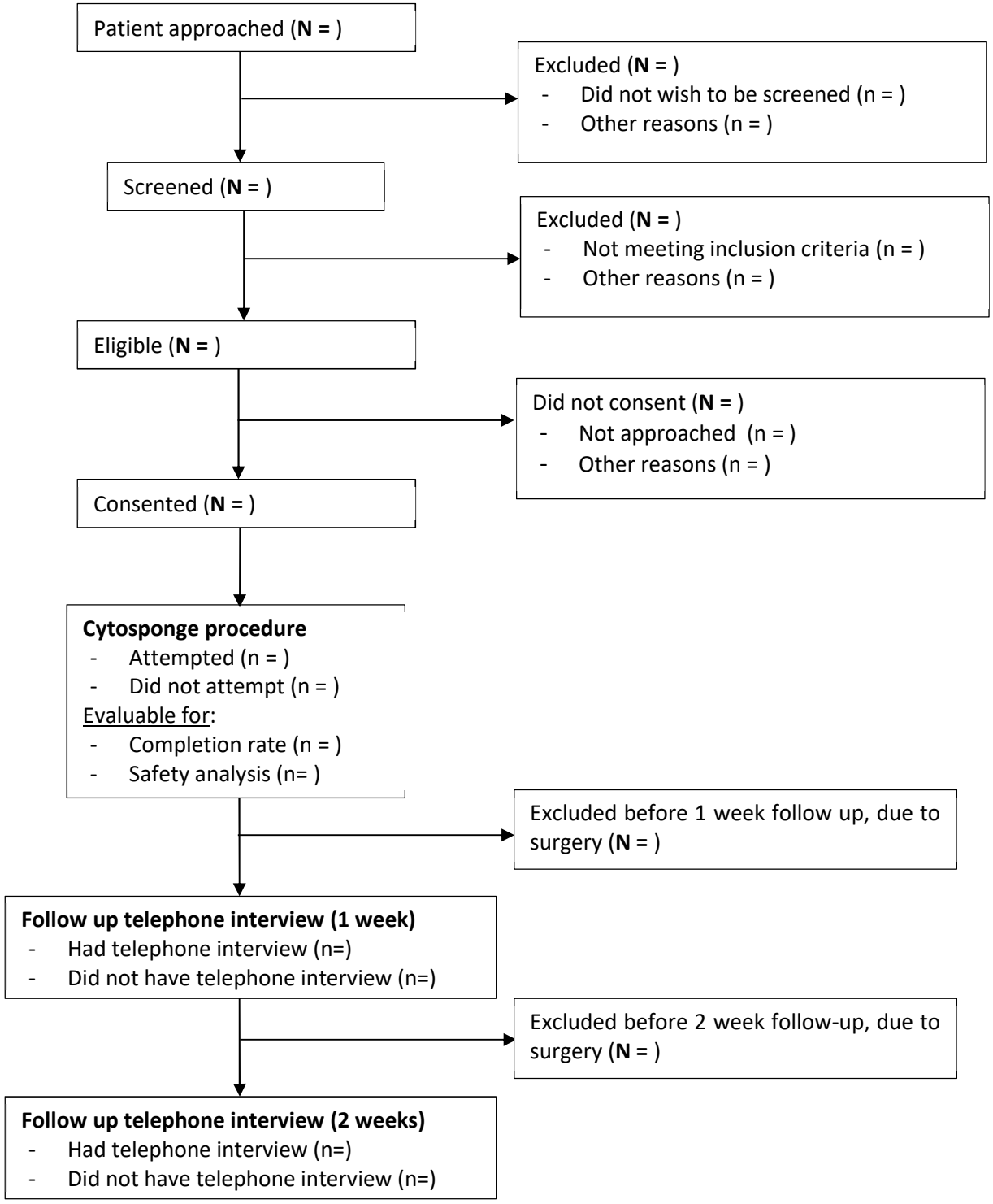
Population for assessment of acceptance rate: all patients who successfully undergo the Cytosponge™ procedure.

Population for assessment of suitability of sample: all samples (taken during the Cytosponge™ procedure) sent to Cambridge for analysis.

Safety population: all participants who attempted to swallow the Cytosponge™.

5. TRIAL POPULATION AND DESCRIPTIVE ANALYSES

5.1 Representativeness of Study Sample and Patient Throughput



5.2 Withdrawal from treatment and/or follow-up

Withdrawals/losses to follow-up together with reasons will be reported. Any occurrences of participants withdrawing before receiving study intervention and being replaced in study with a new participant will be reported.

5.3 Patient Demographic and Clinical Characteristics

There is no randomisation so "baseline comparability of randomised groups" is not applicable. Demographic and clinical characteristics of consenting patients will be summarised.

Table 1: Baseline characteristics of all participants

	N	Mean (SD)
Age	**xx**	**xx.xx (x.xx)**
	N	**%**
Gender	**xx**	**xxx**
Female	xx	xx.xx
Male	xx	xx.xx
Site	**xx**	**xxx**
1	xx	xx.xx
2	xx	xx.xx
3	xx	xx.xx
4	xx	xx.xx
5	xx	xx.xx
6	xx	xx.xx
7	xx	xx.xx
8	xx	xx.xx
9	xx	xx.xx
10	xx	xx.xx
11	xx	xx.xx

Table 2: Clinical characteristics of all participants

	N	%
Dysphagia level	**xx**	**xxx**
Able to eat normal diet / no dysphagia	xx	xx.xx
Able to swallow some solid foods	xx	xx.xx
Able to swallow only semi-solid foods	xx	xx.xx
Chemoradiotherary	**xx**	**xxx**

	N	%
Definitive chemoradiotherapy	xx	xx.xx
Neo-adjuvant chemoradiotherapy	xx	xx.xx

Table 3: Tumour characteristics of all participants

	N	%
Tumour site	**xx**	**xxx**
Lower thoracic oesophagus	xx	xx.xx
Middle thoracic oesophagus	xx	xx.xx
Oesophagogastric junction	xx	xx.xx
Upper thoracic oesophagus	xx	xx.xx
Tumour type	**xx**	**xxx**
Adenocarcinoma	xx	xx.xx
Squamous cell carcinoma	xx	xx.xx
T stage	**xx**	**xxx**
T1	xx	xx.xx
T2	xx	xx.xx
T3	xx	xx.xx
T4	xx	xx.xx
N stage	**xx**	**xxx**
N0	xx	xx.xx
N1	xx	xx.xx
N2	xx	xx.xx
M stage	**xx**	**xxx**
M0	xx	xx.xx
M1	xx	xx.xx

5.4 Unblinding

Not applicable.

5.5 Description of Compliance with Intervention

The number of eligible, consented patients who complete the Cytosponge™ procedure overall and in each subgroup (having received dCRT and naCRT) will be reported. The number of eligible patients who return the questionnaire and have a telephone interview at one and two weeks (i.e. those who do not have surgery in the two weeks after the Cytosponge™ procedure) will also be reported. Deviations from intended treatment (non-adherence to protocol) including losses to follow-up and withdrawals will be summarised.

5.6 Reliability

Any calculations performed using the computer will be checked by hand for the smallest of 5% or 20 observations within the dataset, where appropriate. It is not expected that any data derivation / manipulation will be required. If any should need to take place, the reasons for these will be given and the validity of the derived data will be checked.

6. ANALYSIS

6.1 Definitions of feasibility outcomes

6.1.1 Primary feasibility outcome

The primary outcome measure is the completion rate, defined as the proportion of consented, evaluable participants who are able to successfully undergo the Cytosponge™ procedure. i.e. patients who are able to swallow the sponge and have the sponge retrieved without additional intervention. Evaluable participants are those who have attempted to swallow the Cytosponge™.

6.1.2 Secondary feasibility outcomes

Safety: defined as all serious adverse events (including bleeding requiring transfusion and perforation) that occur between from the time of Cytosponge™ administration (D1) and when the patient completes the study at the two week telephone follow-up. If a participant undergoes surgery prior to their two week follow up visit, then their safety reporting period continues until the time of surgery. Adverse events will also be reported. Severity rating of adverse effects will be as per the Common Terminology Criteria for Adverse Events (CTCAE) V4.03.

Consent rate: defined as the proportion of eligible patients who consent to take part in the study.

Acceptance rate: defined as the proportion of patients who have successfully undergone the Cytosponge™ procedure and would be prepared to accept the procedure repeatedly if it was to be used for follow-up.

Suitability of sample for biomarker analysis: defined as the percentage of samples from the Cytosponge™ test with the presence of cytological atypia and/or p53 abnormality (also called “positive samples”).

6.2 Analysis Methods

6.2.1 Analysis of primary feasibility outcome

The **completion rate** (proportion of patients who swallow the sponge and have it retrieved without additional intervention) will be presented together with the corresponding 95% confidence interval. Participants who have attempted to swallow the Cytosponge™ are evaluable for the primary endpoint. Those who are not evaluable may be replaced at the Chief Investigator’s discretion.

6.2.2 Analysis of secondary feasibility outcomes

Safety

All Adverse Events (AEs) and Serious Adverse Events (SAEs) will be summarised using the safety population**.** As the Cytosponge™ is an Investigational Medical Device, additional adverse event categorisation – of Adverse Device Effect (ADE), Serious Adverse Device Effect (SADE), and Unanticipated Serious Adverse Device Effect (USADE) – is required. Serious Adverse Events related to the procedure and any events recorded as ADE, SADE, or USADE will be summarised over the follow-up period i.e. from the time of Cytosponge™ administration (D1) until the patient completes the study at the two week telephone follow-up, or until the time of surgery if the patient is operated on within the follow-up period.

Telephone follow-up interviews will not be undertaken after surgery. If an Investigator reports any study intervention related SADEs following the two week follow up period (as noted in Protocol V3.0), these will also be included in the summaries. Summaries will be in tabular form. It is intended that the number and percentage of patients experiencing SAEs overall, will be presented by treatment group (naCRT/dCRT). SAEs, SADEs, and USADEs will be will be grouped by system organ class (SOC) and according to whether they occurred on the day of the procedure or later.

In addition, all AEs and ADEs will be summarised in tabular form. It is intended that the number and percentage of patients experiencing AEs and ADEs will be presented overall and according to causality (treatment-relatedness). The AEs may be split by grade (1-2 and 3+) and presented by SOC and according to whether they occurred on the day of the procedure or later. These descriptive summaries will be based on the safety population.

Consent rate (agreement to undergo procedure), defined as the percentage of eligible patients who consent to participate in the study. Any patients who consent but withdraw their consent prior to the Cytosponge™ procedure will be considered not to have consented. The percentage will be presented together with the corresponding 95% confidence interval.

Acceptance rate (acceptance to repeat procedure), defined as the percentage of patients who have successfully undergone the Cytosponge™ procedure and would be prepared to accept the procedure repeatedly if it was to be used for follow-up. A questionnaire will be given to each patient, to be completed after the procedure. The questionnaire will either be completed just after the procedure or, if the patient cannot stay, completed later and returned by post.

Suitability of sample for biomarker analysis, defined as the percentage of samples from the Cytosponge™ test with the presence of cytological atypia and/or p53 abnormality (also called “positive samples”). Quality of material obtained from the Cytosponge™ test will be centrally analysed at Cambridge. Cellularity, yield and quality of extracted DNA will be used as measure of quality. Data for these analyses are not expected to be available with the data for the primary outcome and the acceptance- and safety-related secondary outcomes; biomarker analyses will therefore be conducted and reported separately.

6.3 Missing Data

Any missing data related to the data needed for the primary and secondary aims will be described in the statistical report. Patients who undergo surgery in the two weeks following the Cytosponge procedure will not have data on serious adverse effects after the time of surgery, and will not have follow-up interviews if surgery occurred prior to the planned follow-up times (one week and two weeks). No statistical methods are intended for imputation of missing data.

6.4 Pre-specified Subgroup Analysis

The primary endpoint, the completion rate, will be calculated overall and separately for patients recruited post definitive chemoradiotherapy (dCRT) and those recruited post neo-adjuvant chemoradiotherapy (naCRT). If patients receiving naCRT have their surgery within the two week follow-up period they will not provide follow-up data.

6.5 Tertiary Outcomes and Analyses

Tertiary outcomes of interest will be analysed by researchers at Cambridge and are:

- Level of ctDNA in responders vs non-responders
- Level of residual cancer, p53 mutations and other identifiable markers in Cytosponge™. The proportion of patients with "positive" Cytosponge will be compared against post- treatment biopsies (gold standard)

Data for tertiary outcomes analyses are not expected to be available with the data for the primary outcome and the acceptance- and safety-related secondary outcomes; these will therefore be conducted and reported separately.

7. SPECIFICATION OF STATISTICAL PACKAGES

All analysis will be carried out using appropriate validated statistical software such as STATA, SAS, SPLUS or R. The relevant package and version number will be recorded in the Statistical report.

8. REFERENCES

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

APPENDIX: GLOSSARY OF ABBREVIATIONS

ADE	Adverse Device Effect
AE	Adverse Event
CI	Chief Investigator
CRT	chemoradiotherapy
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumour DNA
dCRT	definitive chemoradiotherapy
naCRT	neo adjuvant chemoradiotherapy
RIOC	Radiotherapy and Imaging Oversight Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
USADE	Unanticipated Serious Adverse Device Effect