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PLATO

Person**a**L**L**ising **A**nal cancer radio**T**herapy d**O**se
– Incorporating ACT3, ACT4 and ACT5

Sponsor: University of Leeds

Funded by: Cancer Research UK [A19121]

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ACT 3, ACT 4 & ACT 5
PLATO
PersonaLising Anal cancer radioTherapy dOse

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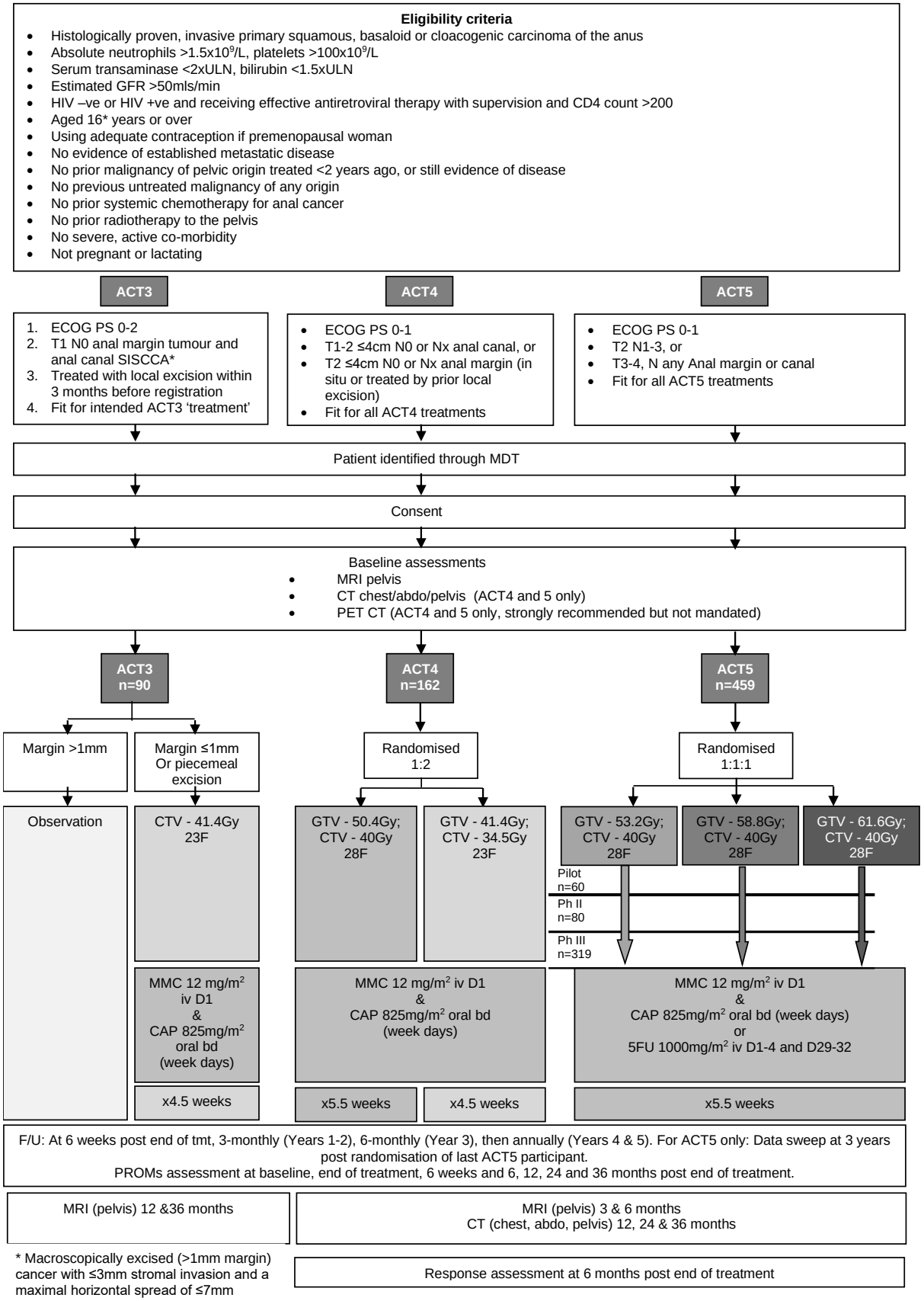
2 Table of Contents

3 Trial Summary

Title	**P**erson**a**L**i**sing **A**nal cancer radio**T**herapy d**O**se – Incorporating ACT3, ACT4 and ACT5		
Acronym	PLATO		
Background	Anal cancer is a rare disease, but its incidence is rising rapidly. Approximately 1000 cases in the UK and 5,000 in the USA are diagnosed each year. Standard treatment for anal cancer includes concurrent Mitomycin C, 5-Fluorouracil and radiotherapy, and more recently, concurrent Mitomycin C, capecitabine and radiotherapy. A new generation of clinical trials is now required that optimises radiotherapy dose based on stratified risk assessment. PLATO is an integrated protocol, comprising 3 separate trials (ACT3, ACT4 and ACT5) in which the most relevant clinical research questions are asked within three distinct risk strata.		
	ACT3	**ACT4**	**ACT5**
Population	Invasive primary squamous cell carcinoma of the of the anus:		
	T1 N0 or Nx anal margin tumour and anal canal SISCCA treated with local excision	T1-2 ≤4cm N0 anal canal, or T1-2 ≤4cm Nx anal canal, or T2 ≤4cm N0 anal margin, or T2 ≤4cm Nx anal margin	T2 N1-3, or T3-4 N any Anal margin or canal
Design	Non-randomised phase II trial.	Randomised phase II trial	A randomised seamless pilot / phase II (3-arm) / phase III (2-arm) trial
Objectives	To determine whether, in patients with anal margin tumours, a strategy of local excision, where selected patients receive additional lower-dose radiotherapy with chemotherapy, results in acceptably low rates of locoregional failure.	To determine whether, in early stage anal cancer, radiotherapy dose de-escalation in combination with chemotherapy results in acceptably low rates of locoregional failure and reduced acute and late toxicity.	To determine whether, in locally advanced disease, radiotherapy dose escalation in combination with chemotherapy reduces the proportion of locoregional failures with acceptable acute and late toxicity.
Intervention	Observation (margin >1mm) *or* 41.4Gy 23F & Mitomycin C and capecitabine (margin ≤1mm) *Non-randomised*	50.4Gy 28F & Mitomycin C and capecitabine *vs* 41.4Gy 23F & Mitomycin C and capecitabine *1:2 randomisation ratio*	53.2Gy 28F & Mitomycin C and 5FU or capecitabine *vs* 58.8Gy 28F & Mitomycin C and 5FU or capecitabine *vs* 61.6Gy 28F & Mitomycin C and 5FU or capecitabine *1:1:1 randomisation ratio*
	No elective nodal irradiation	With elective nodal irradiation	With elective nodal irradiation
Sample size	90 patients over 3 years	162 patients over 2 years	459 patients over 5 years Pilot phase n=60 Phase II n= 80 Phase III n= 319
Follow-up	All participants will be followed up at 6 weeks post-end of treatment, 3-monthly in Years 1-2, 6-monthly in Year 3, then annually for Year 4 and Year 5 (or until death) and via a data sweep at 3 years post randomisation of the last participant (for ACT5 only).		
Primary endpoints	• Locoregional failure (LRF)-free rate (ACT3 and 4) • Locoregional failure-free survival (ACT5)		
Secondary endpoints	• Acute and late toxicities • Treatment compliance • Clinical response rate (cRR) • Disease-free survival (DFS)	• Colostomy-free survival (CFS) • Progression-free survival (PFS) • Overall survival (OS) • Patient Reported Outcome Measures (PROMs)	

Figure 1: Trial Schema



4 Abbreviations

Abbreviation	Definition
5FU	5-Fluorouracil
ACT	Anal cancer trial
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
APER	Abdomino perineal excision of the rectum
AR	Adverse reaction
AST	Aspartate aminotransferase
BD	Twice a day
BNF	British National Formulary
cfDNA	Cell-free DNA
CFS	Colostomy-free survival
CI	Chief investigator or Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CR	Complete response
CRF	Case report form
CRP	C-reactive protein
cRR	Clinical response rate
CRt	Complete response with thickening
CRT	Chemoradiotherapy
CRUK	Cancer Research UK
CTCAE	Common Terminology Criteria for Adverse Events
CTRU	Clinical Trials Research Unit
CT	Computerised tomography
CTV	Clinical target volume
CV	Curriculum vitae
DFS	Disease-free survival
DICOM	Digital Imaging and Communications in Medicine
DMEC	Data monitoring and ethics committee
DNA	Deoxyribonucleic acid

ECOG	The Eastern Cooperative Oncology Group
ECG	Electrocardiogram
EORTC	The European Organisation for Research and Treatment of Cancer
EPCT	Early Phase Clinical Trials
ESMO	European Society for Medical Oncology
ESSO	European Society of Surgical Oncology
ESTRO	European Society for Radiotherapy and Oncology
FFPE	Formalin fixed paraffin embedded
FQ	Facility questionnaire
GCP	Good clinical practice
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
GTV	Gross tumour volume
Gy	Gray (unit of ionising radiation)
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HR	Hazard ratio
HRA	Health Research Authority
ICR	Individual case review
IMRT	Intensity modulated radiotherapy
ISF	Investigator site file
ITT	Intention-to-treat
KM	Kaplan-Meier
LE	Local excision
LLN	Lower limit of normal
LRF	Locoregional failure
MDT	Multidisciplinary team
MF-CRT	Mitocycin C, 5-fluorouracil and radiotherapy
MMC	Mitomycin C
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NCDR	National Cancer Data Registry
NCI	National Cancer Institute

NCRI	National Cancer Research Institute
Non-CTIMP	Not a trial of an investigational medicinal product
OAR	Organs at risk
OS	Overall survival
PAF	Plan assessment form
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PH	Proportional hazards
PI	Principal investigator
PIS	Patient information sheet
PPI	Patient and public involvement
PR	Partial response
PLATO	PersonaLising rAdioTherapy dOse in anal cancer
PPE	Palmar plantar erythema
PROMs	Patient reported outcome measures
PTV	Planning target volume
QA	Quality assurance
QoL	Quality of life
RCR	Royal College of Radiologists
REC	Research ethics committee
RGF	Research Governance Framework
RNA	Ribonucleic acid
RTOG	Radiation Therapy Oncology Group
RTTQA	Radiotherapy Trials Quality Assurance
RUSAE	Related unexpected serious adverse event
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SD	Stable disease
SIB	Synchronous integrated boost
SISSCA	Superficially invasive squamous cell carcinoma
SOP	Standard operating procedure

SUSAR	Suspected unexpected serious adverse reaction
TMG	Trial management group
TPN	Total parenteral nutrition
TRG	Tumour regression grading
TSC	Trial steering committee
UCSF	University of California, San Francisco
ULN	Upper limit of normal
WHO	World Health Organisation

5 Introduction

5.1 Background

Anal cancer is a rare disease, but its incidence is rising rapidly. Approximately 1000 new cases are currently diagnosed in the UK (1) and 5000 in the USA per year (2). 80% of tumours are squamous cell carcinoma, including basaloid and cloacogenic variants. Metastatic disease is present in less than 10% of patients at presentation. Surgery with a permanent colostomy was standard treatment prior to the 1980s, but is now used as a salvage procedure for local failure after chemoradiotherapy (CRT).

Three trials performed between 1987 and 1994 determined concurrent Mitomycin C (MMC), 5-Fluorouracil (5FU) and radiotherapy (MF-CRT) as the standard of care (3-5). ACT1 was performed in the UK and randomised 585 patients to radiotherapy alone or MF-CRT, and significantly reduced locoregional failure (LRF) from 59% to 36% (3). This benefit was maintained after a median follow up of 13 years (6), with a significant reduction in the risk of relapse or death. A recent publication demonstrates that ACT1 led to a change in routine clinical practice (7).

A parallel EORTC trial (4) of 110 patients with locally advanced disease also showed a significant reduction in the risk of LRF. A RTOG trial (5) (n=310) demonstrated a significant reduction in LRF colostomy-free survival with MF-CRT compared with 5-Fluorouracil and radiotherapy (F-CRT). All trials used crude radiotherapy schedules and a significant treatment gap between the first phase of radiotherapy and a subsequent boost.

Three further phase III trials were performed between 1998 and 2008 (8-10). The RTOG 9811 trial (8-9) of 641 patients found that neoadjuvant and concomitant cisplatin 5FU resulted in inferior LRF, colostomy-free survival (CFS) and overall survival (OS) when compared with MF-CRT. In the UK the largest trial, ACT2 (10) enrolled 940 patients and used a two phase radiotherapy schedule. There was no evidence of a difference in the complete response rate for concurrent cisplatin compared with mitomycin C, and no improvement in progression-free survival (PFS) with the addition of two cycles of maintenance 5FU cisplatin. Subset analysis did not show any benefit in subsets based on established prognostic factors. However the cancer outcomes were improved compared to ACT1 and the continuous radiotherapy schedule may have contributed to this finding. The ACCORD 03 (11) trial enrolled 307 patients and showed no benefit from the addition of neoadjuvant cisplatin 5FU chemotherapy. It also compared two doses of boost (15 vs 20-25Gy) after whole pelvic irradiation. A non-significant 5% improvement in local control was seen, although one third of patients had early stage disease. These three trials did not demonstrate any benefit from the use of cisplatin concurrently, neoadjuvantly or as adjuvant therapy.

5.2 Radiotherapy

The radiotherapy techniques used in previous phase III trials were relatively simple and resulted in substantial irradiation of the surrounding normal tissues. ACT2 used a shrinking field technique, treating the whole pelvis to 30.6Gy, followed by a reduced treatment volume to all sites of macroscopic disease, giving 19.8Gy using a generous margin and without a planned gap in treatment (10). Significant improvement in radiotherapy treatment can be achieved with the use of Intensity Modulated Radiotherapy (IMRT). This also allows the use of

altered doses to the gross tumour volume and sparing normal tissues. The use of IMRT in future trials is supported by the RTOG 0529 single arm phase II trial led by Kachnic et al (12). In the UK we have developed guidance for the multicentre introduction of IMRT (13) www.analimrtguidance.co.uk (14) and many UK centres have now implemented IMRT in routine clinical practice. We have recently completed a Royal College of Radiologists Audit of anal cancer in the UK including IMRT.

5.3 Concurrent chemotherapy

ACT1 and 2 have used a single dose of MMC and 5FU given continuously in the first and fifth week of radiotherapy. We have also demonstrated acceptable acute toxicity and compliance with the use of MMC and Capecitabine (using 825mg/m^2 bd on the days of radiotherapy) (15). Both regimens are currently used in the UK and internationally and their use is supported by ESMO ESSO ESTRO and NCCN guidelines (16-17).

5.4 Rationale for evaluating radiotherapy dose in anal cancer

Previous phase III trials have failed to improve anal cancer outcomes with intensification or alteration in the chemotherapy regimen before, during or after concurrent radiotherapy. Internationally there is a wide range in the radiotherapy dose fractionation that is used, with doses of 60Gy or more used in several countries. This is likely to result in substantial overtreatment of early stage disease. There is also a need to determine whether radiotherapy dose escalation above ACT2 doses improves anal cancer outcomes in locally advanced disease.

The PLATO (**P**erson**a**l**L**ising R**A**dio**T**herapy D**O**se in Anal cancer) trial is designed to address these questions with defined studies for the spectrum of locoregional disease:-

5.5 ACT3 – Local excision with selective use of lower-dose post-operative chemoradiotherapy

A small minority of patients present with T1 anal margin tumours without lymph node involvement. A local excision is performed. Pelvic staging performed either before or after local excision excludes lymph node involvement. To our knowledge there is no prospective multicentre data that describes the outcome for this patient group. We have determined, by expert consensus, a treatment strategy comprising local excision alone when the resection margins are >1mm. Reduced-dose 3D conformal radiotherapy (41.4Gy in 23 fractions) with chemotherapy is used for patients with margins ≤1mm.

The efficacy of this approach is supported by Leichman et al (18) who published a phase II single institution study of 45 patients using lower-dose CRT. Hatfield et al (19) reported a series of 21 patients who received lower-dose CRT after local excision with close or involved margins.

ACT3 will evaluate the strategy of local excision where patients with microscopic margins >1mm have local excision only and those with ≤1mm margins receive additional lower-dose CRT using 41.4Gy in 23 fractions. This is a prospective phase II trial.

5.6 ACT4 – Standard-dose IMRT with chemotherapy versus lower-dose IMRT with chemotherapy

Patients with T1-2 (≤4cm) tumours without lymph node involvement involving the anal canal and T2 (≤4cm) anal margin tumours have a low rate of locoregional failure of approximately 10% after standard-dose CRT. However even with IMRT, patients experience acute toxicity and late toxicity. Late toxicity is significantly linked to irradiation to the vagina, penile bulb, perianal skin and normal ano-rectal sphincter complex, due to the dose fractionation applied to the gross tumour and the close proximity of these normal tissues. To reduce the late toxicity from standard-dose IMRT would require a reduction in the dose delivered to the high-dose volume. Recent ESMO guidelines noted that sequential phase II studies with CRT have shown the efficacy of relatively low total radiation doses (30-50Gy) in combination with 5FU and MMC (20).

We plan to compare, in a randomised phase II trial, a lower-dose of IMRT applied to the macroscopic disease (41.4Gy in 23 fractions) plus chemotherapy, with standard-dose IMRT (50.4Gy in 28 fractions) plus chemotherapy, where elective nodal irradiation is used in both arms. The possibility of continuing ACT4 into a phase III trial is discussed later (Section 17.6).

Retrospective published series have described the increasing incidence of human papilloma virus (HPV) association with anal cancer (21-22). They have also described improved outcome in patients with p16+ disease (approximately 90% of patients). There is therefore evidence of the prognostic role of p16 status, but this requires further validation in prospective studies. We plan to study the phase II outcomes for ACT4 for the modified intention-to-treat population and a planned secondary analysis for the large p16+ subset that can assess the predictive value of p16 status for dose de-escalation.

5.7 ACT5 - Standard-dose IMRT with chemotherapy versus dose-escalated synchronous integrated boost (SIB) IMRT with chemotherapy

Patients with T3-4 N any and T2 N1-3 tumours have a substantial risk of locoregional failure of approximately 30%. The ACT2 trial used a dose of 50.4Gy in 28 fractions across the whole disease spectrum. However many clinicians outside the UK use substantially higher doses or variably apply a sequential radiotherapy boost (15-25Gy) after a gap in treatment following 45-50Gy. There is no randomised clinical trial evidence to determine the benefits of radiotherapy dose intensification.

Geltzeiler, (23) found that use of salvage abdomino perineal excision of the rectum (APER) was significantly reduced in the 57% of patients who received a sequential boost using the US National Cancer database of 1,025 patients. There is therefore a strong rationale to evaluate radiotherapy dose intensification through IMRT and a SIB, avoiding any extension in the overall treatment time. Deenan et al (24) have demonstrated in a phase I study, the feasibility and acceptable toxicity of a SIB IMRT approach using 59.4Gy to the gross tumour volume (GTV) and 49.5Gy to the clinical target volume (CTV) in 33 fractions, with MMC 10mg/m^2 day 1 and capecitabine 825mg/m^2 bd 5 days/week. We have recently published a tumour control probability model for anal squamous cell carcinomas that supports the rationale for dose escalation (25).

The supporting evidence for dose escalation is based on 1] the pattern of failure; 2] overall treatment time 3] IMRT providing the technological solution to dose intensify radiotherapy 4] radiobiological modelling.

1] Pattern of failure - Relapse/failure at the primary site is reported in over 70% of patients with anal cancer in single centre series comprising 675 patients (26-28) and the 940 patients in ACT2 (Sebag-Montefiore personal communication).

2] Overall treatment time - The importance of the overall treatment time in patients with squamous cell carcinoma treated with radiotherapy is supported by evidence from a number of tumour sites. A review (29) of 18 randomised radiotherapy trials of head and neck cancer demonstrated a significant improvement in local control with shortening of total treatment time, and a meta-analysis (30) demonstrated that this resulted in a significant improvement in overall survival (OS). Similar observations have been made in the treatment of cervical cancer (31-33). In anal cancer, Ben-Josef (34) performed a pooled data analysis of RTOG 87-04 and 98-11 to investigate whether the length of radiation or the total treatment time were associated with outcomes. On univariate analysis, colostomy failure was correlated with total treatment time; for each increase in duration beyond 14 days, there was a 9.4% increase in hazard for colostomy failure (HR 1.593, 95% CI 1.080-2.350, P = .02). On multivariate analysis, a trend was maintained for total treatment time and the risk for colostomy failure (HR 1.588, 95% CI 0.993-2.539, P= .053). A series from UCSF (35) reported that local control was enhanced when patients received 54Gy in 60 days with a local progression-free probability of 89% versus 42% in patients who did not (P = .01). Data from the ACT2 trial demonstrate improved cancer outcome when the overall treatment time is not prolonged (36).

3] Dose escalation with IMRT - IMRT provides the technical solution to dose intensify the gross tumour volume at the primary sites without prolonging overall treatment time. The accompanying radiotherapy planning document demonstrates that the planned dose escalation can be achieved without significantly altering the dose and volume delivered to the organs at risk. A small planning study has demonstrated the feasibility of two SIB schedules.

4] The use of SIB can reduce overall treatment time both by employing larger fraction sizes to the gross tumour and by causing fewer interruptions due to reduced toxicity. We performed a systematic review of PubMed and Embase databases to identify thirteen appropriate papers, including 625 patients. Predefined data fields were collected. A standard linear quadratic TCP model, which included repopulation, was fit by least squares minimization. The fitted TCP curve demonstrated a dose-response relationship with α=0.196 Gy(-1). The curve suggests: in early stage tumours, a dose reduction from 50Gy to 45Gy reduces 2 year local control from 98% to 95%; in late stage tumours, a dose escalation from 50Gy to 55Gy improves the 2 year local control rate from approximately 50% to 80% (37).

We plan a step-wise evaluation with a pilot study followed by a phase II evaluation prior to selecting which trial design will be taken forward in the phase III component of ACT5.

5.8 Late toxicity

Although IMRT is able to reduce the acute toxicity of CRT, patients still experience late toxicity. There is very little published data with mature follow-up following IMRT. Gilbert et al (38) have reviewed the choice of patient reported outcome measures for patients with anal cancer in clinical trials. Consequently, the EORTC QLQ-C30 generic cancer module in combination with

the new EORTC anal cancer-specific module, QLQ-ANL27, have been selected for use in the trial as these questionnaires have the best coverage of items. The dose escalation arms in ACT5 are modelled to result in acceptable late toxicity using normal tissue constraints as part of IMRT planning to organs at risk. Many European countries currently deliver doses up to at least 60Gy to the primary tumour and prospective studies are required to assess the balance between benefit and late toxicity. The treatment-related colostomy rate in ACT2 was less than 2%.

5.9 Primary endpoint

Anal cancer is a predominantly locoregional disease. Less than 10% of patients present with metastatic disease and the majority of patients relapse at the primary site with fewer patients having pelvic or nodal inguinal failure. The primary endpoints for all three studies relates to Locoregional failure (LRF) within the pelvis and are defined further in Sections 12.8 and 15.1.

5.10 Translational research

Key translational questions within the current treatment of anal cancer are whether dose escalation can make up for poor biology and conversely, which are the tumours that can be treated with lower radiotherapy doses. Equally, although the vast majority of relapses are loco-regional, these are often detected late (distinguishing between post-treatment change and recurrence in large tumours is challenging) and outcomes from salvage surgery are poor. The allied translational approach addresses both of these challenges. Specifically we will collect:

- Formalin fixed paraffin embedded (FFPE) archival tissue samples collected during routine procedures incorporating:
    - Baseline / pre-treatment tumour biopsy and
    - Biopsies / resections of recurrent disease.

  These will be collected in batches retrospectively for participants recruited to ACT3, ACT4 and ACT5. FFPE blocks will be used for immunohistochemical analyses and to extract DNA and RNA. Tumours will be characterised for p16 (immunohistochemistry), HPV status (PCR) and tumour infiltrating lymphocyte scores, and these correlated with outcome.

- Longitudinal blood (plasma) samples (ACT 5). These will be used for longitudinal analysis of cell free DNA (cfDNA) and characterisation and changes in levels of circulating tumour cells and comparison of these with conventional markers of response (clinical examination and imaging based follow up).

Additional biological insights into local immune response and potential factors relating to long term (bowel) toxicity might also be gained from understanding the gut microbiome and how this changes in patients undergoing radical treatment for anal cancer. To this end, in a cohort of patients enrolled in ACT5 we will collect:

- Faecal sample prior to commencing chemoradiotherapy and a paired sample 6-12 weeks (+/- 4 weeks) after the end of treatment. The microbiome will be characterized in these samples and compared pair-wise to understand the changes effected by chemoradiotherapy, and then with toxicity outcome measures and oncological outcomes to generate hypotheses

that might be tested in future prospective studies. A mid-stream urine sample will be collected alongside faecal samples to facilitate a metabolomic comparison.

6 Aims and Objectives

6.1 Aims

6.1.1 ACT3

The ACT3 trial aims to establish in patients with T1 N0 anal margin tumours requiring local excision (LE), whether a strategy of LE, with selective use of lower-dose CRT for patients with ≤1mm margins, results in acceptably low rates of locoregional failure (LRF).

6.1.2 ACT4

The ACT4 trial aims to establish in patients with early stage anal cancer whether IMRT dose de-escalation in combination with chemotherapy results in acceptably low rates of locoregional failure (LRF), and reduced acute and late toxicity, compared with standard-dose IMRT with chemotherapy.

6.1.3 ACT5

The ACT5 trial aims to establish in patients with locally advanced anal cancer whether IMRT dose escalation (using SIB) in combination with chemotherapy improves locoregional failure (LRF) free survival (evaluated using a time-to-event analysis), with acceptable acute and late toxicity, when compared with standard-dose IMRT with chemotherapy.

6.2 Primary objective

6.2.1 ACT3

To assess the 3-year locoregional failure-free rate in participants with anal margin tumours who have been treated by LE with selective lower-dose CRT.

6.2.2 ACT4

To assess the 3-year locoregional failure-free rates in participants with early stage disease who have received reduced-dose IMRT with chemotherapy.

6.2.3 ACT5

Pilot phase: To assess the acute toxicity rates and treatment compliance within each of the treatment arms.

Phase II: To assess acute toxicity data to determine which arms will be taken forward to phase III (3 arm/ 2 arm). (see section 17.3.2).

Phase III: To assess the 3-year locoregional failure-free rates and locoregional failure free survival at 3 years post-randomisation, in participants with locally

advanced disease, and compare between the experimental arms and the standard-dose arm.

6.3 Secondary objectives

To assess overall (all ACT trials) and between individual trial treatment arms (ACT4 and 5 only):

- Acute and late toxicities
- Treatment compliance
- Clinical response rate (cRR) (ACT4 and 5)
- Disease-free survival (DFS)
- Colostomy-free survival (CFS)
- Progression-free survival (PFS) (ACT4 and 5)
- Overall survival (OS)
- Patient Reported Outcome Measures (PROMs)

To describe:

- Pattern of failures
- Proportion of participants undergoing salvage surgery

See Section 15 for full definitions of the primary and secondary endpoints.

6.4 Translational objectives

To assess the prognostic utility of p16/HPV status and tumour infiltrating lymphocyte scores with respect to outcomes after chemoradiotherapy in ACT3, ACT4 and ACT5.

To investigate the nature and performance characteristics of circulating markers of disease (circulating tumour cells and cell free DNA) in response/relapse in high risk cases (ACT5 patients only).

To describe the changes in the gut microbiome caused by chemoradiotherapy and develop hypotheses around any potential relationship between this and patients experiencing late (gut) toxicities.

7.0 Design

7.1 ACT3

ACT3 is a non-randomised, phase II, multi-centre, open-labelled trial in patients with T1N0 anal margin tumours, to assess the overall treatment strategy of local excision (LE), with selective lower-dose CRT, for patients with ≤1mm margins.

Ninety eligible patients, who have undergone a LE no more than 3 months prior to registration, will be recruited to the trial. Patients with anal tumour margins >1mm (deep and lateral) will undergo observation as per local practice (approx. 75-80% of patients); patients with anal tumour margins ≤1mm (close or involved) will receive CRT (approx. 20-25% of patients).

Piecemeal excisions are eligible provided there is source data documentation prior to excision that the lesion was <2cm. These are considered to be margin positive and are selected for lower-dose CRT.

An A'Hern single-stage design (39) will be used to assess whether the overall treatment strategy results in acceptable rates of efficacy, as determined by 3-year locoregional failure-free rates. An interim analysis of the initial failure rate data will be conducted on the first 30 participants, see Section 17.3.1 for further details.

A non-randomised trial is appropriate given the very small number of patients in this trial population. The aim is to assess the overall treatment strategy as a way of treating this cohort of anal cancer patients.

7.2 ACT4

ACT4 is a randomised-controlled, phase II, multi-centre, open-labelled trial in patients with early stage anal cancer, to assess the efficacy rates of reduced-dose IMRT plus chemotherapy.

A total of 162 eligible patients will be recruited to the trial. Patients will be randomised on a 1:2 basis (standard-dose:reduced-dose) to receive either standard-dose IMRT in combination with chemotherapy, or reduced-dose IMRT in combination with chemotherapy.

An A'Hern single-stage design (39) will be used to assess whether the reduced-dose IMRT arm results in acceptable rates of efficacy, as determined by 3-year locoregional failure-free rates. The standard-dose IMRT arm will act as a calibration arm to ensure the desired efficacy rates are plausible. A subgroup analysis will be conducted on the subset of patients with a p16+ genotype for the primary endpoint. Such patients are shown to have significantly improved outcomes in terms of relapse-free, disease-specific and overall survival (21-22).

An interim analysis of the initial failure rate data will be conducted on the first 30 patients to be recruited to the reduced dose experimental arm (after 45 patients are recruited in total). See Section 17.3.1 for further details.

7.3 ACT5

ACT5 is a randomised-controlled, seamless pilot (3-arm) / phase II (3-arm) / phase III (2-arm/ 3-arm dependant on outcome of interim analysis), multi-centre, open-labelled trial in patients with locally advanced anal cancer. The trial aims to compare standard-dose IMRT in combination with chemotherapy with at least one increased-dose experimental arm of IMRT with SIB in combination with chemotherapy.

A total of 459 eligible patients will be recruited to the trial and patients will be initially randomised on a 1:1:1 basis to receive either standard-dose IMRT in combination with chemotherapy or one of two increased-dose experimental arms of IMRT with SIB in combination with chemotherapy. Following the Phase II component, the interim analysis will determine which arms will be taken forward to Phase III (Section 17.3.2).

The pilot stage of ACT5 will recruit a maximum of 60 patients; an exploratory analysis of the acute toxicity and treatment compliance data will be conducted in a limited number of sites to determine whether any modifications to the protocol are required before commencing Phase II.

Note that the exploratory analysis of the pilot stage was conducted and presented to the independent Data Monitoring and Ethics Committee (DMEC) in October 2018. The Committee determined that the data supported the continuation of ACT5 and recommended extending the follow-up of clinician reported toxicities post-end of treatment.

The Phase II component will recruit an additional 80 patients (140 in total); a formal interim analysis of the acute toxicity data will be conducted to determine which arms, and consequently which trial design, to take forward to Phase III (Section 17.3.2). Should the interim analysis show that both experimental arms are safe to be taken forward i.e. both arms pass the pre-specified acute toxicity threshold, recruitment will continue to both experimental arms in phase III. If one arm does not pass the pre-specified threshold the trial will continue with 2 arms (standard-dose arm and 1 experimental arm passing the threshold).

Note that the interim analysis of the phase II component was conducted and presented to the independent Data Monitoring and Ethics Committee (DMEC) in March 2021. The Committee determined that the data supported the continuation of both experimental arms in ACT5 and recommended that the phase III component continue as a 3 arm trial

The Phase III trial will assess whether the increased-dose experimental arms of IMRT with SIB in combination with chemotherapy are superior to the standard-dose IMRT in combination with chemotherapy in terms locoregional failure-free survival, assessed at 3 years post-randomisation of the final participant. Each experimental arm will be compared against the standard-dose arm.

Towards the end of the Phase III component, 3 years post end of treatment of the last participant, the LRF event will be assessed. The reasoning behind this is explained in Section 16.1.3. If the target event rate will not be reached, the experimental arms will be combined into a single dose-escalation arm for the primary analysis. This would still allow the primary objective to be tested, of assessing the efficacy of dose-escalation.

In both scenarios, no formal statistical comparisons for efficacy between the SIB dose arms will be performed. However, exploratory comparisons between the experimental arms will be performed to evaluate toxicity and patient reported outcomes, to enable practice definition based on the totality of information from this study.

An additional 319 patients will be recruited in Phase III, in order to achieve the required total planned sample size of 459 patients.

8 Participating Sites and Investigators

8.1 Participating sites

Each participating site must be able to comply with the following, as applicable to the trial activities taking place at the site:

- Trial treatments, imaging, clinical care, follow-up schedules and all requirements of the trial protocol
- Requirements of the Research Governance Framework and amendments
- Data collection requirements, including adherence to CRF submission timelines as per Section 12
- Collection, preparation and shipment of biological samples for translational research as per Section 12
- Monitoring requirements as outlined in Section 18

Participating sites will be required to complete a trial-specific feasibility questionnaire to confirm that they have adequate resources and experience to conduct the study. Whilst it is hoped that most sites will participate in all three trials within this protocol, sites can choose to opt out of one or more trials.

8.2 Principal Investigators and co-investigators

Sites must have an appropriate Principal Investigator (PI) authorised by the site and ethics committee to lead and coordinate the work of the trial on behalf of the site. Other investigators at site wishing to participate in the trial must be trained and approved by the PI. Investigators involved in the treatment and care of patients must be medical doctors and have experience of treating anal cancer.

8.3 Training requirements for site staff

All site staff must be appropriately qualified by education, training and experience to perform the trial related duties allocated to them, which must be recorded on the site authorised personnel log.

CVs for all staff must be kept up-to-date, signed and dated and copies (or statement of their location) held in the Investigator Site File (ISF) held at site. An up-to-date, signed copy of the CV for the PI must be forwarded to the CTRU prior to site activation.

GCP training is required for all staff responsible for trial activities. The frequency of repeat training may be dictated by the requirements of their employing institution, or 2 yearly where

the institution has no policy, and more frequently when there have been updates to the legal or regulatory requirements for the conduct of clinical trials. Evidence of current GCP training for the PI must be forwarded to the CTRU prior to site activation.

8.4 Radiotherapy quality assurance

The radiotherapy quality assurance (QA) programme will be implemented by the NCRI RTTQA group to ensure treatment is planned and delivered according to the trial protocol. A summary of the RTTQA requirements are described in Section 11.2.9 and further details in the PLATO Radiotherapy Guidelines.

8.5 Site initiation

Before a site is activated, the CTRU trial team will arrange a site initiation with the site which, as a minimum the PI, radiotherapy planner and research nurse must attend. The site will be trained in the day-to-day management of the trial. Essential documentation required for trial activation will be checked. Site initiation will normally be performed for each site by teleconference. On-site initiation visits will be conducted if deemed appropriate.

8.6 Essential documentation

The following documentation must be submitted by the site to the CTRU prior to site activation:

- Trial specific site feasibility questionnaire (identifying relevant local staff)
- All relevant institutional approvals (e.g. local NHS permission)
- A completed authorised personnel log that is initialled and dated by the PI (with all tasks and responsibilities delegated appropriately)
- Completed Site Contacts Form (with contact information for the PI, co-investigators, research/trial, pharmacy, radiography and pathology staff)
- A copy of the PI's current CV that is signed and dated
- A copy of PI's current GCP training certificate
- Signed PI declaration
- Radiotherapy Quality Assurance approval
- A signed Clinical Trial Site Agreement (model Non-commercial Agreement for UK sites) between the Sponsor and the relevant institution

Sites must inform the CTRU of any additional sites involved in the patient pathway. Recruiting sites which will be referring patients to a different site, for all or some of the trial activities, will not be activated until the relevant site involved is ready to be activated.

8.7 Site activation

Once the CTRU trial team has received all the required essential documentation, the site has received their investigator site file and the site has been initiated, a site activation email will

be issued to the PI and other research staff by CTRU. **Sites must not approach any potential patients until they have received an activation email from CTRU.**

9 Patient Eligibility

Patients meeting all of the inclusion criteria for the respective trial they are being considered for, and none of the exclusion criteria, will be considered for participation in the trial. Eligibility waivers to any of the inclusion and exclusion criteria are not permitted.

9.1 Inclusion criteria

Inclusion criteria		
ACT3	**ACT4**	**ACT5**
Informed consent	Informed consent	Informed consent
Histologically-proven, invasive primary squamous cell carcinoma of the anus (including basaloid, or cloacogenic carcinoma)[8]	Histologically-proven, invasive primary squamous cell carcinoma of the anus (including basaloid, or cloacogenic carcinoma)[1, 2]	Histologically-proven, invasive primary squamous cell carcinoma of the anus (including basaloid, or cloacogenic carcinoma)[1, 2]
ECOG performance status 0-2[3]	ECOG performance status 0-1[3]	ECOG performance status 0-1[3]
Absolute neutrophil count >1.5 x 10^9/L; platelets >100 x 10^9/L [3]	Absolute neutrophil count >1.5 x 10^9/L; platelets >100 x 10^9/L [3]	Absolute neutrophil count >1.5 x 10^9/L; platelets >100 x 10^9/L [3]
Serum transaminase <2x ULN [3]	Serum transaminase <2x ULN [3]	Serum transaminase <2x ULN [3]
Estimated GFR >50mls/min [3,5]	Estimated GFR >50mls/min [3, 5]	Estimated GFR >50mls/min [3, 5]
Bilirubin <1.5 x ULN [3, 4] (patients with a confirmed diagnosis of Gilbert's syndrome and Bilirubin levels up to x 3 ULN will be permitted entry as long as the patient is considered fit and healthy)	Bilirubin <1.5 x ULN [3, 4] (patients with a confirmed diagnosis of Gilbert's syndrome and Bilirubin levels up to x 3 ULN will be permitted entry as long as the patient is considered fit and healthy)	Bilirubin <1.5 x ULN [3, 4] (patients with a confirmed diagnosis of Gilbert's syndrome and Bilirubin levels up to x 3 ULN will be permitted entry as long as the patient is considered fit and healthy)
HIV negative or HIV positive and receiving effective antiretroviral therapy with supervision and CD4 count >200 [6,11]	HIV negative or HIV positive and receiving effective antiretroviral therapy with supervision and CD4 count >200 [6,11]	HIV negative or HIV positive and receiving effective antiretroviral therapy with supervision and CD4 count >200 [6,11]
T1 N0 or Nx[6] anal margin tumour and anal canal SISCCA[7] treated with local excision within 3 months before registration[7, 9, 10]	T1-2 ≤4cm N0 or Nx[7] anal canal or T2 ≤4cm N0 or Nx[7] anal margin (in situ or treated by prior local excision within 3 months before randomisation)	T2 N1-3 or T3-4 N any Includes anal margin and canal **Please note:** v7.0 of the AJCC (American Joint Committee on Cancer) anal cancer staging must be used for baseline staging assessments. Please refer to Appendix A.
Age 16 or over	Age 16 or over	Age 16 or over
Patient considered fit for either the protocol-defined follow-up, or reduced dose CRT.	Patient considered fit for all ACT4 protocol defined treatments	Patient considered fit for all ACT5 protocol defined treatments

Inclusion criteria		
ACT3	**ACT4**	**ACT5**
(There is no requirement to be fit for CRT if the patient is indicated for observation only).		
Prepared to practice methods of contraception of proven efficacy during treatment and until 6 months post end of treatment (if female participant of childbearing potential, or male participant who is sexually active with a female of childbearing potential)	Prepared to practice methods of contraception of proven efficacy during treatment and until 6 months post end of treatment (if female participant of childbearing potential, or male participant who is sexually active with a female of childbearing potential)	Prepared to practice methods of contraception of proven efficacy during treatment and until 6 months post end of treatment (if female participant of childbearing potential, or male participant who is sexually active with a female of childbearing potential)
Able to undergo all mandated staging and follow-up investigations, including MRI	Able to undergo all mandated staging and follow-up investigations, including MRI	Able to undergo all mandated staging and follow-up investigations, including MRI

Notes:

[1] biopsy "suspicious" of invasion are eligible if anal cancer MDT agree that all imaging and clinical findings support the diagnosis and proceed with treatment

[2] patients whose squamous cell carcinoma's lower limit is >1cm above the superior aspect of the anal canal are excluded

[3] within 14 days prior to recruitment

[4] patients with a confirmed diagnosis of Gilberts and Bilirubin levels up to x 3 ULN will be permitted entry as long as the patient is considered fit for protocol defined treatment

[5]estimated using a validated creatinine clearance calculation (e.g. Cockroft & Gault (Appendix E) or Wright formula

[6] supervision by an Infectious disease/genitourinary medicine team - depending upon drug interactions these patients may require 5FU rather than capecitabine)

[7] patients with equivocal findings regarding nodal involvement (considered Nx) for ACT 3 and 4 are eligible if supported by the MDT

[8] macroscopically excised cancer with ≤3mm stromal invasion and a maximal horizontal spread of ≤7mm

[9] patients with previous AIN grade 1-3 are eligible for registration. Patients with concurrent AIN in the excised specimen, including AIN 1-3 at the resection margins, are eligible for recruitment.

[10] piecemeal local excisions are permitted provided there is source data documentation prior to excision that the lesion was <2cm

[11] within 28 days prior to recruitment

9.2 Exclusion criteria

Participants meeting any of the exclusion criteria are not eligible to be enrolled.

Exclusion criteria		
ACT3	**ACT4**	**ACT5**
Definite evidence of metastatic disease – patients with equivocal lesions are eligible (determined by MDT)	Definite evidence of metastatic disease - patients with equivocal lesions are eligible (determined by MDT)	Definite evidence of metastatic disease - patients with equivocal lesions are eligible (determined by MDT)

Exclusion criteria		
ACT3	**ACT4**	**ACT5**
Previous malignancy of pelvic origin where treatment was completed less than 2 years before registration or there is still evidence of disease, or previous untreated malignancy of any origin. (Patients with previously treated malignancy not of pelvic origin are eligible if treatment of that malignancy was completed at least 2 years before registration and there is no evidence of disease).	Previous malignancy of pelvic origin where treatment was completed less than 2 years before randomisation or there is still evidence of disease, or previous untreated malignancy of any origin. (Patients with previously treated malignancy not of pelvic origin are eligible if treatment of that malignancy was completed at least 2 years before randomisation and there is no evidence of disease).	Previous malignancy of pelvic origin where treatment was completed less than 2 years before randomisation or there is still evidence of disease, or previous untreated malignancy of any origin. (Patients with previously treated malignancy not of pelvic origin are eligible if treatment of that malignancy was completed at least 2 years before randomisation and there is no evidence of disease).
Prior systemic chemotherapy for anal cancer	Prior systemic chemotherapy for anal cancer	Prior systemic chemotherapy for anal cancer
Prior radiotherapy to the pelvis	Prior radiotherapy to the pelvis	Prior radiotherapy to the pelvis
Uncontrolled cardiorespiratory comorbidity (includes patients with inadequately controlled angina or myocardial infarction within 6 months prior to registration)	Uncontrolled cardiorespiratory comorbidity (includes patients with inadequately controlled angina or myocardial infarction within 6 months prior to randomisation)	Uncontrolled cardiorespiratory comorbidity (includes patients with inadequately controlled angina or myocardial infarction within 6 months prior to randomisation)
Pregnant or lactating	Pregnant or lactating	Pregnant or lactating
Immuncompromised (organ transplant)	Immuncompromised (organ transplant)	Immuncompromised (organ transplant)
Requiring ongoing treatment with a contraindicated concomitant medication (please see section 11.3.4)	Requiring ongoing treatment with a contraindicated concomitant medication (please see section 11.3.4)	Requiring ongoing treatment with a contraindicated concomitant medication (please see section 11.3.4)
>1 local excision performed, at different times, for the same lesion		

9.3 Birth control

Female patients of childbearing potential should be advised to avoid becoming pregnant while receiving chemoradiotherapy. Male patients who are sexually active with a woman of childbearing potential should be advised to use barrier contraception during chemoradiotherapy and until 6 months after finishing treatment.

9.4 Prior and concurrent participation in other clinical trials

Participant eligibility for ACT3, 4 or 5 based on previous or concurrent participation in other clinical trials will be determined on a case by case basis and must be discussed with the CTRU prior to registration (ACT3) / randomisation (ACT4 and ACT5).

9.5 Eligibility and baseline assessments

Informed consent must be obtained (Section 10.3) prior to undertaking any trial-specific procedures, including non-routine screening investigations and assessments.

10 Consent, Recruitment and Registration / Randomisation

10.1 Recruitment setting

Participants will be recruited from NHS centres throughout the UK, and selected international sites. Research sites will be required to have obtained local management approval, completed and passed all the required quality assurance checks and undertaken a site initiation with the CTRU prior to the start of recruitment. Whilst it is hoped that most sites will participate in all three trials within this protocol, sites can choose to opt out of one or more trials.

ACT3 aims to recruit 90 patients over 3 years.

ACT4 aims to recruit 162 patients over 2 years.

ACT5 aims to recruit 459 patients over 5 years (pilot phase: n=60; phase II: n=80; phase III n= 319).

Patients recruited into the phase II & III components of ACT5 in the UK will be eligible to be enrolled in the serial plasma biomarker sub-study.

10.2 Eligibility screening

In order to determine the generalisability of the trial results, and for Consolidated Standards of Reporting Trials (CONSORT) requirements, participating research sites will be required to complete a Screening log for all patients presenting with anal cancer and screened for eligibility for the ACT trials who do not go on to be randomised. Documented reasons for ineligibility or declining participation will be closely monitored by the CTRU as part of a regular review of recruitment progress. Anonymised information will be collected including:

- date screened
- age
- gender
- the reason for non-randomisation:
  - the reason not approached, or
  - the reason not eligible for trial participation, or
  - the reason declined if eligible

However, the right of the patient to refuse consent without giving reasons will be respected. This information will be requested from participating sites on a regular basis (at least 3 monthly) by the CTRU.

10.3 Recruitment and informed consent

Patients will be approached for possible recruitment following MDT diagnosis and decision to treat. Suitability for inclusion into ACT3, ACT4 or ACT5 will be assessed according to the eligibility criteria for the respective trial (see Section 12.1 for list of eligibility assessments). A verbal explanation of the trial and the appropriate Patient Information Sheet (PIS) will be provided by the attending medical staff (and/or the trial Clinical Research Nurse) for the patient to consider. This will include detailed information about the rationale, design and personal implications of the trial.

Following information provision, patients will have as long as they need to consider participation, normally a minimum of 24 hours, and will be given the opportunity to discuss the trial with their family and healthcare professionals before they are asked whether they would be willing to take part in the trial.

Assenting patients will then be formally assessed for eligibility and invited to provide informed, written consent. The formal assessment of eligibility and informed consent may only be obtained by the Principal Investigator (PI) or an appropriate medically qualified healthcare professional. The healthcare professional must have knowledge of the trial interventions and have received training in the principles of GCP and the Declaration of Helsinki 2013. He/she must be fully trained in the trial according to the ethically approved protocol and be authorised and approved by the PI to take informed consent as documented in the trial Authorised Personnel Log. The PI retains overall responsibility for the informed consent of participants at their research site.

Informed consent must be obtained prior to the participant undergoing procedures specifically for the purposes of the trial which are out-with standard routine care at the participating site.

Site staff are responsible for:

- Checking that the correct (current approved) versions of the PIS and Consent Form are used
- Checking that information on the Consent Form is complete and legible
- Checking that the patient has completed/initialled all relevant sections and signed and dated the form
- Checking that an appropriate member of staff has countersigned and dated the Consent Form to confirm that they provided information to the patient
- Checking that an appropriate member of staff has made dated entries in the patient's medical notes relating to the informed consent process (i.e. information given, consent signed, etc.)
- Following registration/randomisation:

- Adding the patient trial number to the consent form and making sufficient copies and filing the original consent form in the investigator site file, and filing a copy in the patient's medical notes.
- Giving the patient a copy of their signed Consent Form and PIS
- Faxing a copy of the signed consent form to CTRU in line with the terms of the ethically approved consent form

The participant will be provided with a local contact point where he/she may obtain further information about the trial.

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki 2013.

The right of the patient to refuse consent without giving reasons will be respected. Consenting participants will remain free to withdraw from the trial at any time without giving reasons and without prejudicing any further treatment (see Section 11.6).

Where a participant is required to re-consent, or new information is required to be provided to a participant, it is the responsibility of the PI to ensure this is done in a timely manner and according to any timelines requested by the CTRU.

The responsibility for treatment with chemoradiotherapy and the prescription of chemotherapy and radiotherapy ultimately remains with the PI.

After the participant has entered the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the participant will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which he/she has been allocated.

10.4 Informed consent for the ACT5 phase II biological serial plasma biomarker sub-study

Please note: the serial plasma biomarker sub-study closed to new participants in March 2022.

Patients in the UK who are approached for participation in ACT5 phase II/III will be provided with a PIS for the ACT5 circulating biomarkers sub-study, in addition to the PIS for the main trial. Following information provision, patients will have as long as they need to consider participation, normally a minimum of 24 hours, and will be given the opportunity to discuss the sub-study with their family and healthcare professionals before they are asked whether they would be willing to take part in the sub-study. Participation in the sub-study is optional.

Patients who consent to participate in the main ACT5 phase II/III trial will then be invited to provide informed, written consent for the sub-study, prior to randomisation. HIV+ve patients enrolled in ACT5 should not be recruited to the serial plasma sub-study as samples cannot be

processed. Please refer to the separate translational sample handling manual for instructions regarding the criteria for acceptance of blood samples at the CRUK Manchester Institute laboratory.

Informed consent for the sub-study must be obtained before randomisation and before any research blood samples are taken. Blood samples must be taken from the patient after randomisation, as they will need to be labelled with the participant's trial number.

10.5 Informed consent for the ACT 5 gut microbiome sub-study

Patients in the UK who are approached for participation in ACT5 phase III will be provided with a PIS for the ACT5 gut microbiome sub-study, in addition to the PIS for the main trial. Following information provision, patients will have as long as they need to consider participation, normally a minimum of 24 hours, and will be given the opportunity to discuss the sub-study with their family and healthcare professionals before they are asked whether they would be willing to take part in the sub-study. Participation in the sub-study is optional.

Patients who consent to participate in the main ACT5 phase III trial will then be invited to provide informed, written consent for the sub-study, prior to randomisation. Patients will not be eligible for the gut microbiome sub-study if they have been defunctioned and have a stoma. Please refer to the separate translational sample handling manual for instructions

10.6 Loss of capacity following informed consent

Loss of mental capacity of a participant after giving informed consent for the trial is expected to be a rare occurrence. Should this eventuality occur, this should reported to CTRU via a withdrawal form with no further trial procedures or data collection occurring from this point. Any data collected up to the point of withdrawal will be kept on record and used in the trial analysis. This is explicit in the written information that the participant will receive.

10.7 Registration/randomisation

Written informed consent for entry into the trial must be obtained and eligibility must be confirmed prior to registration / randomisation.

10.8 Timing of registration/randomisation

Registration/randomisation should take place as soon as possible after informed consent has been obtained and eligibility confirmed. Randomisation must only occur after the results of all the baseline scans have been reported. The participant-completed baseline **Quality of Life (QoL) questionnaires (QLQ-C30 & QLQ-ANL27) must be completed after consent has been obtained and should where possible be completed prior to registration/randomisation, but must be prior to informing the participant of their treatment allocation in ACT4 and ACT5.**

10.8.1 Registration/randomisation process

Following confirmation of written informed consent and eligibility, participants will be registered (ACT3) or randomised (ACT4/5) into the trial by an authorised member of staff at the trial site.

Registration/randomisation will be performed centrally using the CTRU automated 24-hour randomisation system which can be accessed via the web or telephone. Authorisation codes and personal identification numbers (PINs), provided by the CTRU, will be required to access the registration/randomisation system.

The Registration Form (ACT3) or Randomisation Form (ACT4/5) will be completed prior to accessing the 24-hour registration/randomisation system. The following information will be required at registration/randomisation:

- Site code (assigned by CTRU) of the research site
- Name of person making the registration/randomisation
- Confirmation of which trial the participant is being enrolled in
- Participant details, including initials, date of birth and gender
- Confirmation of eligibility
- Confirmation of written informed consent for the trial
- Confirmation that the baseline QoL questionnaire has been completed (for participants who provided consent for this)
- Stratification factors (see Sections 10.9.2 and 10.9.3) for ACT4 and ACT5
- Confirmation of written informed consent for the serial plasma biomarker sub-study (ACT5 phase II/III, UK only) – consent for this is optional.

Once registration/randomisation is complete, the system will allocate participants a unique 5 digit trial number and, in ACT4 and ACT5, inform the randomised radiotherapy dose for that participant.

24hour registration / randomisation:

Telephone: 0113 343 2290
or
Web: *https://lictr.leeds.ac.uk/webrand/*

Please ensure that you have completed the Eligibility Checklist and Registration or Randomisation Forms and that patients have completed the baseline questionnaires before telephoning the registration/randomisation line or accessing the web registration/randomisation

10.9 Treatment allocation

10.9.1 ACT3

ACT3 is a non-randomised trial. Patients with anal tumour margins >1mm (deep and lateral) will undergo observation; patients with anal tumour margins ≤1mm (close or involved) including piecemeal excisions will receive lower-dose CRT.

10.9.2 ACT4

Patients who fulfil the eligibility criteria, and have given written informed consent will be randomised on a 1:2 basis (standard-dose:reduced-dose) to receive either standard-dose IMRT in combination with chemotherapy or reduced-dose IMRT in combination with chemotherapy, and will be allocated a trial number. A computer-generated minimisation program that incorporates a random element will be used to ensure the treatment groups are well-balanced for the following participant characteristics, details of which will be required at randomisation:

- T-stage (T1, T2)
- N-stage (N0, NX)
- Gender (M, F)
- HIV status (positive, negative)
- Randomising centre

10.9.3 ACT5

For the pilot study, Phase II and Phase III trial, patients who fulfil the eligibility criteria, and have given written informed consent will be randomised on a 1:1:1 basis to receive either standard-dose IMRT in combination with chemotherapy, or one of two increased-dose experimental arms of IMRT with SIB in combination with chemotherapy, and will be allocated a trial number. A computer-generated minimisation program that incorporates a random element will be used to ensure the treatment groups are well-balanced for the following participant characteristics, details of which will be required at randomisation:

- T-stage (T2/3, T4)
- N-stage (NX/0/1, N2/3)
- Gender (M, F)
- HIV status (positive, negative)
- Chemotherapy regimen (5FU, Capecitabine)
- Randomising centre

Please note: v7.0 of the AJCC (American Joint Committee on Cancer) anal cancer staging must be used for baseline staging assessments and not the current v8.0, which may be in use in standard practice. Please refer to Appendix A. If a patient's N staging is recorded as N1 with a suffix of a-c, this indicates that v8.0 has been used and the N staging must be changed in line with v7.0 prior to randomisation.

This is required as N stage is a minimisation factor at randomisation in ACT5.

11 Treatment Details

ACT3, ACT4 and ACT5 have been classified by the Medicines and Healthcare products Regulatory Agency (MHRA) as not Clinical Trials of and Investigational Medicinal Product (non-CTIMP). Therefore there are no Investigational Medicinal Products (IMPs) in this protocol.

Note that in the treatment schemas below, treatment is depicted as starting on a Monday. Although this is preferred, it is not mandated and **treatment may start on any day of the week.**

Before treatment commences, blood tests must be performed within 10 days before the start of treatment, as per Section 12.3. In the event that any results have fallen outside the eligibility thresholds in Section 9.1, refer to Appendix D – Dose modifications for guidance. If further guidance is needed, contact CTRU.

11.1 Treatment summary schedule

ACT3 treatment summary
Patients with anal tumour margins >1mm (deep and lateral) will undergo observation; patients with anal tumour margins ≤1mm (close or involved) will receive lower-dose CRT.

11.1.1 ACT3 observation arm (if margin >1mm)

Patients in this arm will be under observation as per local practice, but must attend trial follow-up assessments as per Section 12.

11.1.2 ACT3 – Radiotherapy plus MMC capecitabine (if margin ≤1mm)

Treatment	Week 1					2					3					4					5		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-31		
Mitomycin C 12mg/m^2 iv	•																						
Capecitabine 825mg/m^2 orally bd Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Radiotherapy CTV 41.4Gy 23F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

ACT4 treatment summary

Patients will be randomised on a 1:2 basis (standard-dose:reduced-dose) to receive either standard-dose IMRT in combination with chemotherapy or reduced-dose IMRT in combination with chemotherapy.

11.1.3 ACT4 - Standard-dose IMRT plus MMC capecitabine

Treatment	Week																											
	1					2					3					4					5					6		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-33					Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
Capecitabine 825mg/m^2 orally bd Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Radiotherapy GTV 50.4Gy CTV 40Gy 28F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

11.1.4 ACT4 - Reduced-dose IMRT plus MMC capecitabine

Treatment	Week																							
	1					2					3					4					5		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-31		
Mitomycin C 12mg/m^2 iv	•																						
Capecitabine 825mg/m^2 orally bd Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Radiotherapy GTV 41.4Gy CTV 34.5Gy 23F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

ACT5 treatment summary

Patients will receive either standard-dose IMRT or one of two increased-dose experimental arms of IMRT with SIB, both in combination with chemotherapy. ACT5 will use Mitomycin C combined with *either* Capecitabine *or* 5 Fluorouracil (5FU).

11.1.5 ACT5 - Standard-dose IMRT plus MMC capecitabine

Treatment	Week 1 Days 1-5					Week 2 Days 8-12					Week 3 Days 15-19					Week 4 Days 22-26					Week 5 Days 29-33					Week 6 Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
Capecitabine 825mg/m^2 orally bd Mon-Fri*	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •
Radiotherapy GTV 53.2Gy CTV 40Gy 28F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

11.1.6 ACT5 – SIB1 IMRT plus MMC capecitabine

Treatment	Week 1 Days 1-5					Week 2 Days 8-12					Week 3 Days 15-19					Week 4 Days 22-26					Week 5 Days 29-33					Week 6 Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
Capecitabine 825mg/m^2 orally bd Mon-Fri*	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •
Radiotherapy GTV 58.8Gy CTV 40Gy 28F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

11.1.7 ACT5 – SIB2 IMRT plus MMC capecitabine

Treatment	Week 1 Days 1-5					Week 2 Days 8-12					Week 3 Days 15-19					Week 4 Days 22-26					Week 5 Days 29-33					Week 6 Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
Capecitabine 825mg/m^2 orally bd Mon-Fri*	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •	• •
Radiotherapy GTV 61.6Gy CTV 40Gy 28F	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

*recommended, but treatment may start on any day of the week.

11.1.8 ACT5 - Standard-dose IMRT plus MMC 5FU

Treatment	Week																											
	1					2					3					4					5					6		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-33					Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
5FU 1000mg/m^2/24h iv	•	•	•	•																	•	•	•	•				
Radiotherapy GTV 53.2Gy CTV 40Gy 28F Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

11.1.9 ACT5 - SIB1 IMRT plus MMC 5FU

Treatment	Week																											
	1					2					3					4					5					6		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-33					Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
5FU 1000mg/m^2/24h iv	•	•	•	•																	•	•	•	•				
Radiotherapy GTV 58.8Gy CTV 40Gy 28F Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

11.1.10 ACT5 – SIB2 IMRT plus MMC 5FU

Treatment	Week																											
	1					2					3					4					5					6		
	Days 1-5					Days 8-12					Days 15-19					Days 22-26					Days 29-33					Days 36-38		
Mitomycin C 12mg/m^2 iv	•																											
5FU 1000mg/m^2/24h iv	•	•	•	•																	•	•	•	•				
Radiotherapy GTV 61.6Gy CTV 40Gy 28F Mon-Fri*	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

***recommended, but radiotherapy may start on any of the week day. Regardless of when radiotherapy starts, MMC and 5FU should start on the first Monday or Tuesday of radiotherapy.**

11.2 Radiotherapy

The protocol for PLATO study has been developed though a UK anal cancer radiotherapy working group (13).

The key elements of the radiotherapy protocol will be outlined in this section, but detailed radiotherapy guidelines are provided in a separate document: PLATO Radiotherapy Planning Guidance Document and can be accessed via the website www.rttrialsqa.org.uk.Investigators are required to follow the detailed instructions in the guidance document specific to the trial (ACT3, ACT4 or ACT5) to which the patients are recruited. Any centres wishing to participate in this study will comply with the defined Radiotherapy Quality Assurance (RTTQA) guidelines summarised below.

11.2.1 Radiotherapy treatment planning

A contrast enhanced radiotherapy planning CT scan is acquired using a CT slice thickness no greater than 3mm. It is recommended that patients are scanned in the supine position with immobilisation of the lower limbs according to local policy.

Prior to pre-treatment scan, the clinician will assess the diagnostic imaging and patient characteristics to ascertain if in supine position any anal verge extension of the tumour is adequately bolused by the surrounding buttocks. If the buttocks do not provide sufficient bolus, the patient may require to be positioned lying supine with additional bolus material.

Oral contrast should be used according to local protocol.

Patients should be scanned with a comfortably full bladder using local bladder filling protocol.

11.2.2 Target volume definition

Gross Tumour Volume (GTV) – ACT3, ACT4 and ACT 5 patients

The GTV should be determined by the treating clinician using the planning CT, and information from clinical data and diagnostic imaging.

- GTV_A = Site of primary tumour and scar (ACT3)
- GTV_A = Includes the gross primary anal tumour volume (ACT4 and ACT5)
- GTV_N = Includes all involved nodes ≤3cm (ACT5)
- GTV_N3 = Includes all involved nodes >3cm (ACT5)

Clinical Target Volume (CTV) – ACT3, ACT4 and ACT5 patients

All the expansions are in 3 dimensions unless stated otherwise.

- CTV_A = GTV_A + 10mm (ACT3)
- CTV_A = GTV_A + 10mm (ACT4)
- CTV_A = GTV_A +15mm (ACT5)

  This is manually enlarged to ensure coverage of the anal canal, anal verge, internal and external anal sphincters. In the absence of bony involvement this is then edited to exclude bone.
- CTV_N = GTV_N + 5mm. This is also edited off bone and muscles in the absence of bony involvement (ACT5)
- CTV_E = Elective nodal regions (defined in the radiotherapy planning document) (ACT4 and ACT5).

Planning Target Volume (PTV) – ACT3, ACT4 and ACT5 patients

All the expansions are in 3 dimensions unless stated otherwise.

ACT3 patients:-

- PTV_A= CTV_A + 10mm

ACT4 patients:-

- PTV_A= CTV_A + 10mm
- PTV_E = CTV_E + 5mm

ACT5 patients:-

- PTV_A= CTV_A + 10mm
- PTV_N = CTV_N + 5mm
- PTV_E = CTV_E + 5mm

Organs at risk

The following organs at risk (OAR) must be delineated by the radiographer/dosimetrist/physicist/consultant and this is described in detail in the Radiotherapy Planning Document. This includes the small bowel, external genitalia, bladder, right and left femoral heads.

11.2.3 ACT3 technique and dose prescription

For ACT3 patients selected for CRT, either a 3D conformal plan or a single phase inverse-planned IMRT treatment plan delivered with multiple fields, or arc techniques can be used. Choice of delivery technique is at the discretion of the treating clinician.

Dose prescription:- (PTV_A) = 41.4Gy in 23F (1.8Gy per F) in 4.5 weeks

ACT 3
GTV_A - Site of primary Tumour and scar
CTV_A = GTV_A + 10mm, enlarged to encompass anal verge, canal, internal and external sphincters.
PTV_A = CTV_A + 10mm

11.2.4 ACT4 technique and dose prescription

For ACT4, all patients will receive IMRT where different dose fractionations are delivered to the elective nodal region (PTV_E) and to the areas of gross tumour (PTV_A). A single phase inverse-planned IMRT treatment plan should be produced and delivered with multiple fields or arc techniques.

Dose prescription:-

Standard-dose arm

PTV_A	50.4Gy in 28F in 5.5 weeks
PTV_E	40.0Gy in 28F in 5.5 weeks

Reduced-dose (experimental) arm

PTV_A	41.4Gy in 23F in 4.5 weeks
PTV_E	34.5Gy in 23F in 4.5 weeks

ACT 4	
GTV_A = Primary Tumour	
CTV_A = GTV_A + 10mm, enlarged to encompass the anal verge, canal, internal and external sphincters.	**CTV_E** = at risk nodal and mesorectal regions as defined below
PTV_A = CTV_A + 10mm	**PTV_E** = CTV_E + 5mm

11.2.5 ACT5 technique and dose prescription

For ACT5, all patients will receive IMRT where different dose fractionations are delivered to the elective nodal region (PTV_E) and to the areas of gross tumour (PTV_A and PTV_N). A single phase inverse-planned IMRT treatment plan should be produced and delivered with multiple fields or arc techniques.

Dose prescription

Standard-dose arm

PTV_A	53.2.Gy in 28F in 5.5 weeks
PTV_N	50.4Gy in 28F in 5.5 weeks (involved nodes ≤3cm)
	53.2Gy in 28F in 5.5 weeks (involved nodes >3cm)
PTV_E	40.0Gy in 28F in 5.5 weeks

Dose escalation arm 1

PTV_A	53.2Gy in 28F in 5.5 weeks
PTV_Boost	58.8Gy in 28F in 5.5 weeks
PTV_N	53.2Gy in 28F in 5.5 weeks (involved nodes ≤3cm)
	53.2Gy in 28F in 5.5 weeks (involved nodes >3cm)
PTV_E	40.0Gy in 28F in 5.5 weeks

Dose escalation arm 2

PTV_A	53.2Gy in 28F in 5.5 weeks
PTV_Boost	61.6Gy in 28F in 5.5 weeks
PTV_N	53.2Gy in 28F in 5.5 weeks (involved nodes ≤3cm)
	53.2Gy in 28F in 5.5 weeks (involved nodes >3cm)
PTV_E	40.0Gy in 28F in 5.5 weeks

<table>
<tr><th colspan="3">ACT 5</th><th>Dose escalation arms</th></tr>
<tr><td>GTV_A = Primary Tumour</td><td>GTV_N =
Involved Nodes ≤3cm
GTV_N3 = involved nodes >3cm</td><td></td><td>GTV_Boost = GTV_A + GTV_N+ GTV_N3</td></tr>
<tr><td>CTV_A = GTV_A + 15mm, enlarged to encompass anal verge, canal, internal and external sphincters.</td><td>CTV_N =
GTV_N + 5mm</td><td>CTV_E</td><td></td></tr>
<tr><td>PTV_A = CTV_A + 10mm</td><td>PTV_N =
CTV_N + 5mm</td><td>PTV_E =
CTV_E + 5 mm</td><td>PTV_Boost = GTV_Boost + 5mm</td></tr>
</table>

11.2.6 Unplanned interruptions in radiotherapy treatment

When an unplanned break in radiotherapy occurs (toxicity, machine breakdown), patients receiving capecitabine should interrupt capecitabine treatment for that day and resume on the next planned day of radiotherapy.

ACT 3/4: using the remaining days between the interruption and the end of treatment it is preferable to treat over the weekend (preferable) or twice daily treatment on some of the other days (preferably Friday)

ACT 5: using the remaining days between the interruption and the end of treatment we strongly advise treating over the weekend (Saturday). Twice daily radiation is not permitted. If acceleration is not possible, and ≤5 days of radiotherapy have been missed, no biological allowance or increased total dose should be performed and missed fractions should be added on at the end of treatment, extending overall treatment time. If >5 days have been missed, please contact the trial office.

11.2.7 Treatment plan optimization (ACT 4 and 5)

Inverse plan using simultaneous integrated boost technique delivered with coplanar beams or arc delivery. An advanced convolution superposition algorithm should be used for calculation eg. AAA (Eclipse) CCCS (Pinnacle), CC (Oncentra).

11.2.8 Treatment verification

It is recommended that the best available positional verification methods should be used to ensure correct delivery. The recommendations for image guidance are provided in the radiotherapy planning document.

11.2.9 Radiotherapy quality assurance

The radiotherapy quality assurance (RT QA) programme for the study will be designed and implemented by the National Radiotherapy Trials QA (RTTQA) Group. The full details of the programme will be made available on the RTTQA group website www.rttrialsqa.org.uk and in the latest version of the PLATO Radiotherapy Document. The RT QA programme for the PLATO trial will include both pre-trial and on trial components. Attempts will be made to streamline the RT QA processes, where appropriate, with previously completed QA for other clinical trials. All centres using IMRT delivery must successfully complete the IMRT credentialing programme through the National RTTQA group or equivalent.

ACT3
Minimal QA. Outlining benchmark case.

ACT4 and ACT5

Pre-trial QA:

- Facility questionnaire (FQ) - General and trial specific questions on equipment, software and techniques to be used for the trial
- Process document – To include information on all aspects of the patient pathway from pre-treatment imaging to treatment and data collection, and QA processes for the treating centre.

- Benchmark cases - QA of the outlining and planning technique will be performed by each centre participating in the study completing test patient cases.
    - Outlining benchmark - Those centres recruiting to both ACT4 and ACT5 will complete the dose-escalated case only. Those centres recruiting to ACT4 only will complete the standard-dose case.
    - Planning Benchmark - Those centres recruiting to both ACT4 and ACT5 will complete the dose-escalated case only. Those centres recruiting to ACT4 only will complete the standard-dose case.
- Dosimetry audit – For IMRT delivery appropriate independent dosimetry audit evidence from centres will be required. Please contact the RTTQA group to discuss.

On trial QA

- Individual case review (ICR) - QA of the outlining and planning (including plan assessment form (PAF)) will be performed by the trial QA team.
- ACT3 – Prospective review (prior to the patient starting radiotherapy) on at least the first 10 cases registered to receive chemoradiotherapy.
- ACT4 and ACT5 - Prospective review for at least the first case from each centre.
- Timely retrospective review will be performed within the first 5 fractions for
    - ACT4 – 10% random sample of cases per site
    - ACT5 – All cases randomised in the pilot phase; 25% random sample of cases per site in phase II and at least 20% random sample of cases per site in phase III.

Universal data collection

Data collection for all patients - Anonymised data, in DICOM (Digital Imaging and Communications in Medicine) format, will be collected by the QA team for all patients treated in the trial. This will include a brief clinical history, diagnostic imaging, full planning data including; CT images, structure set, plan and dose cube and completed plan assessment form (PAF).

11.3 Chemotherapy

ACT3 and ACT4 will use a combination of Mitomycin C and Capecitabine.

ACT 5 will use Mitomycin C combined with either Capecitabine or 5 Fluorouracil (5FU) (see below)

ACT 3

Mitomycin C	12mg/m^2 iv bolus Day 1 only (max 20mg)
Capecitabine	825mg/m^2 bd orally 5 days per week (on days of radiotherapy) for **23** days.

ACT4
Standard-dose arm (50.4Gy)

Mitomycin C	12mg/m^2 iv bolus Day 1 only (max 20mg)
Capecitabine	825mg/m^2 bd orally 5 days per week (on days of radiotherapy) for **28** days.

Reduced-dose arm (41.4Gy)

Mitomycin C	12mg/m^2 iv bolus Day 1 only (max 20mg)
Capecitabine	825mg/m^2 bd orally 5 days per week (on days of radiotherapy) for **23** days.

ACT5

One of the two regimens below will be used for all treatment arms in ACT5

Mitomycin C	12mg/m^2 iv bolus Day 1 only (max 20mg)
Capecitabine	825mg/m^2 bd orally 5 days per week (on days of radiotherapy) for **28** days

OR

Mitomycin C	12mg/m^2 iv bolus 1st day of 5FU only (max 20mg)
5 FU	1000mg/m^2 per 24 hours for four days by continuous intravenous infusion - delivered on radiotherapy treatment days during the first and last full weeks of radiotherapy (eg Days **1-4** and Days **29-32 if radiotherapy commences on a Monday)**

11.3.1 Administration of Mitomycin C

- If given with capecitabine, Mitomycin C is given as an intravenous bolus on Day 1 only (i.e. the first radiotherapy treatment day) – 12mg/m^2 (maximum dose 20mg).

- If given with 5FU, Mitomycin C is given as an intravenous bolus on the first day of 5FU only – 12mg/m^2 (maximum dose 20mg).

11.3.2 Administration of capecitabine (if applicable)

- Capecitabine is taken orally twice a day for 5 days per week (normally Monday – Friday), on the days of radiotherapy administration only. If radiotherapy is not given (eg due to machine maintenance or bank holiday), then capecitabine must not be taken on that day either (see Section 11.2.6 - unplanned interruptions in radiotherapy treatment).
- Patients should be advised to take capecitabine with a glass of water within 30 minutes after food, approximately 12 hourly eg 8am and 8pm).
- For patients who have difficulty swallowing capecitabine, the tablets can be dissolved in approximately 200ml of water. By agitating the tablets for approximately 15 minutes, the tablets should dissolve. There is no stability data for any form of suspension, so the tablets should be dissolved immediately before use and the solution swallowed immediately, rinsing to ensure all of the suspension has been ingested. The solution has a very bitter taste and a fruit juice can be added to make the solution more palatable, but capecitabine should not be mixed with grapefruit juice.
- If a patient vomits after taking a dose of capecitabine, the dose should not be taken again.
- Capecitabine can be dose banded according to local policy.
- It is recommended that patients keep a diary of the number of capecitabine tablets taken, which should be checked on a weekly basis (or as per local practice).

It is recommended that the first fraction of radiotherapy should be given at least 2 hours after the commencement of the first dose of capecitabine.

11.3.3 Administration of 5FU (if applicable)

- 5FU is given for four days as a continuous intravenous 24 hour infusion – 1000mg/m^2/24 hours delivered on radiotherapy treatment days during the first and last full weeks of radiotherapy (eg Days 1-4 and Days 29-32 if radiotherapy commences on a Monday).

11.3.4 Contraindicated concomitant medications with capecitabine or 5FU

Concomitant administration of the following medications with capecitabine or 5FU is contraindicated:

- Sorivudine (or sorivudine analogues e.g. brivudine) - there must be at least a 4-week period between the end of treatment with sorivudine or its chemically related analogues and the start of CRT.
- Clozapine
- Warfarin – discontinue at least 7 days prior to the start of CRT (see section 11.3.6)

11.3.5 Medications to be avoided with capecitabine or 5FU

- Dipyridamol and allopurinol – concomitant use with capecitabine or 5FU should be avoided

- Phenytoin - patients receiving phenytoin concomitantly with capecitabine or 5FU should be regularly monitored for increase phenytoin plasma concentrations and associated symptoms
- Metronidazole –
    - metronidazole in combination with 5FU can increase plasma concentrations of 5FU, so should be used with extreme caution
    - there are no reports of interaction between capecitabine and metronidazole, however, caution is advised in its use for patients in combination therapy arm due to known interaction between 5FU and metronidazole

11.3.6 Use of anticoagulants with capecitabine or 5FU

Altered coagulation parameters and/or bleeding have been reported in patients taking capecitabine or 5FU concomitantly with anticoagulants such as warfarin and phenprocoumon. The mechanism of interaction is unclear. These events occur within several days and up to several months after initiating capecitabine/5FU therapy and, in a few cases after stopping capecitabine/5FU.

Patients receiving oral warfarin are only eligible for this study if one of the two options listed below can be used according to clinical judgement that is used in routine clinical practice:

- Discontinuation of warfarin at least 7 days prior to commencement of treatment and for the duration of CRT (this may be reasonable when given as prophylaxis for patients with atrial fibrillation – this is a local clinician decision).

or

- Conversion from oral warfarin to low molecular weight heparin where local clinical opinion considers this an acceptable option – the change to low molecular weight heparin with discontinuation of warfarin should be made at least 7 days prior to the commencement of treatment.

Warfarin must not be commenced during CRT.

11.3.7 Fluoropyrimidines and DPYD genotyping

Significant toxicity relating to Fluoropyrimidine (capecitabine or 5FU) treatment may be a result of reduced activity of the key metabolic enzyme, dihydropyrimidine dehydrogenase (DPD), in particular relating to genetic variants in the gene encoding DPD (*DPYD*). Where this information is available prior to commencement of treatment, appropriate dose reductions should be considered based on local guidelines and recorded on the treatment CRF.

11.4 Management of toxicity

Patients should be reviewed at least weekly during CRT, and assessed for acute toxicity using CTCAE. Clinicians will have considerable experience of delivering CRT for anal cancer. It is expected skin reactions to radiotherapy, diarrhoea and haematological toxicities will be most commonly observed. Prompt management of ongoing grade 2 or grade 3 diarrhoea is important in order to maximise chemotherapy and radiotherapy compliance.

Appendix D contains guidelines for dose modification in response to organ function and toxicity. These should be adhered to wherever possible, however, it is

acknowledged that trial investigators and treating consultants are likely to have significant experience with the trial drugs. Deviations from these guidelines and any dose reductions considered in the patients' best interest are therefore permitted and should be recorded in the Case Report Forms (CRFs) and will not constitute a protocol violation.

11.4.1 Recommendations for the management of diarrhoea

It is particularly important to assess and monitor patients who experience diarrhoea during CRT. If admission is required, it is recommended that this is to the radiotherapy centre. If circumstances prevent this, then this guidance must be rapidly shared with the local treating team and regular contact maintained. The option of subsequent transfer to the centre should be discussed.

The site team should document a baseline assessment of stool frequency/stoma output and this should be repeated once weekly at the same time as toxicity assessment (distinguishing from tenesmus/mucous discharge/wet wind).

The following guidance is recommended for patients who experience grade 3 diarrhoea during concurrent chemo-radiotherapy:

- Consider admission of the patient
- Commence loperamide
- Send stool for culture and C. difficile toxin
- Commence iv fluids with regular appropriate volumetric and electrolyte assessment
- Suspend chemotherapy
- If neutropenic, commence iv antibiotics and consider G-CSF

If grade 3 diarrhoea is not controlled to ≤ grade 1 by regular loperamide within 24 hours and patient not neutropenic:

- Commence iv broad spectrum antibiotics (including patients who are not pyrexial). The regimen used should be determined locally (an example option includes an intravenous second or third generation cephalosporin and metronidazole). The regimen used should cover likely enteric pathogens.

If grade 3 diarrhoea not controlled ≤ grade 1 by iv antibiotics and iv fluids and regular loperamide within 48 hours:

- Commence s/c octreotide – the recommended starting dose is 300µg per 24 hours by either s/c continuous infusion or s/c tds injections. The dose can be increased in accordance with BNF guidance and should be reviewed daily
- Closely monitor serum CRP, renal function and albumin. The role of total parenteral nutrition should be discussed with the multi-disciplinary team who are responsible for this therapy and may play an important role for patients not responding well to the supportive treatments described above.

If Grade 4 diarrhoea:

- By definition grade 4 diarrhoea is life-threatening. Patients developing grade 4 diarrhoea at any stage must be admitted urgently and treated with full supportive measures including fluid replacement, iv antibiotics and iv octreotide in addition to any other immediate resuscitative measures that might be deemed necessary.

Loperamide is recommended as the initial anti-diarrhoeal medication. Codeine phosphate up to 30 mg four times a day can be added if diarrhoea is not controlled with 16 mg loperamide per day.

Guidance regarding continuation of radiotherapy is given in Appendix D. **Radiotherapy should be withheld in the presence of lower abdominal peritonism (rebound tenderness in clinical examination).**

11.5 Further post protocol defined anti-cancer treatment

No specific recommendations are made regarding further post protocol defined anti-cancer treatment. Treatment should be as per local policy.

11.6 Withdrawal of treatment

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of attending clinicians or the participants themselves. All participants withdrawn from treatment or prescribed alternative treatment will still attend for follow-up assessments unless unwilling to do so and CRFs will continue to be completed.

The PI or delegate should make every effort to ensure that the specific wishes of any participant who wishes to withdraw consent for further involvement in the trial are defined and documented using the Withdrawal CRF, in order that the correct processes are followed by the CTRU and site following the withdrawal of consent. **It should be made clear to any participant specifically withdrawing consent for further data collection that further data pertaining to safety will continue to be collected, for example the outcome of an event that was reported prior to withdrawal, and will be included in any safety analysis. In addition it is suggested that the participant is made aware of the fact that if any significant new information becomes available in regard to the treatment they have received in the trial it may be necessary to contact them in the future.**

12 Trial assessments, data collection and translational sample collection

Participating sites will record trial participant data on trial-specific paper CRFs and submit them to the CTRU. Missing and discrepant data will be flagged and additional data validations raised as appropriate from the CTRU data management team.

Participating sites will be expected to maintain a file of essential trial documentation (Investigator Site File, ISF), which will be provided by the CTRU, and keep copies of all completed Case Report Forms (CRFs) for the trial. The CRFs and participant-completed Quality of Life questionnaires will contain the participant's unique trial number, date of birth, and initials.

The timing of assessments and serial plasma sample collection (ACT5 biomarkers sub-study) are summarised in the following tables and paragraphs.

General comments about translational sample collection

- Stored biopsy material from diagnosis and relapse will be requested periodically by CTRU. **Do not send any tissue blocks to the central laboratory until requested to do so.**
- Streck tubes and SafeBoxes will be provided for the collection and shipping of blood samples.
- Filled Streck tubes must be inverted 8 times after the blood draw to mix the contents.
- Blood samples must not be frozen or refrigerated, as ambient temperature is optimum. Blood samples must be sent immediately to central laboratory.
- Blood samples taken on a Friday should be posted the same day to ensure processing by the lab within 96 hours of blood draw. If possible, sample collection on a Friday before a bank holiday Monday should be avoided.
- **Refer to separate translational sample manual, for full details, including labelling and postage requirements.**

Table 12.1 - ACT3 assessment schedule

ACT3	Baseline			Treatment					Follow-up from end of treatment (or date of registration for observational arm)													
	Eligibility assessments	Pre reg	Pre tx	Week 1	Week 2	Week 3	Week 4	Week 5	6 weeks	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months	30 months	36 months	48 months	60 months	Relapse
Medical history	•																					
ECOG PS, Vital Signs, ht/wt	•[1]																					
Physical exam	•								•*	•	•	•	•	•	•	•	•	•	•	•	•	
Tumour biopsy	•																					
Pregnancy test	•*[1]																					
Full Blood Count:	•[1]		•*[4]	•*	•*	•*	•*	•*														
U&E	•[1]		•*[4]	•*	•*	•*	•*	•*														
LFTs	•[1]		•*[4]	•*	•*	•*	•*	•*														
HIV	•*[2]	•**[2]																				
CD4 count if HIV +ve	•*[2]	•**[2]																				
ECG	•*[1]																					
MRI scan	•[3]												•						•			
Informed consent		•																				
Registration		•																				
QoL		•						•*	• CTRU to administer		• CTRU to administer		• CTRU to administer				• CTRU to administer		• CTRU to administer			
Radiotherapy planning scan		•*																				
Data collection	•	•	•*	•*	•*	•*	•*	•*		•	•	•	•	•	•	•	•	•	•	•	•	
CTCAE acute toxicity monitoring				•*	•*	•*	•*	•*														
SAR monitoring and reporting				Monitor during weeks of treatment*																		
Translational research samples (explicit consent required)																						
Tumour Biopsy	•[5]																					•[5,6]

*RT arm only

** observation arm only

[1]within 14 days prior to registration; [2]within 28 days prior to registration

[3]within 63 days prior to registration

[4]within 10 days prior to start of treatment

[5]if consented to retreival of stored material for future cancer research [6]and where clinically indicated

Table 12.2 - ACT4 assessment schedule

ACT4	Baseline			Treatment						Follow-up from end of treatment													
	Eligibility assessments	Pre rand	Pre tx	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	6 weeks	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months	30 months	36 months	48 months	60 months	Relapse
Medical history	•																						
ECOG PS, Vital Signs, ht/wt	•[1]																						
Physical exam	•									•	•	•	•	•	•	•	•	•	•	•	•	•	
Tumour Biopsy	•																						
Pregnancy test	•[1]																						
Full Blood Count	•[1]		•[5]	•	•	•	•	•	•*														
U&E	•[1]		•[5]	•	•	•	•	•	•*														
LFTs	•[1]		•[5]	•	•	•	•	•	•*														
HIV	•[2]																						
CD4 count if HIV +ve	•[2]																						
ECG	•[1]																						
CT scan	•[3]													•				•		•			
MRI scan	•[4]										•	•											
PET scan	•[4] Strongly recommended																						
Informed consent		•																					
Randomisation		•																					
QoL		•						• 41.4Gy arm only	• 50.4Gy arm only	• CTRU to administer		• CTRU to administer		• CTRU to administer				• CTRU to administer		• CTRU to administer			
Radiotherapy planning scan		•																					
Data collection	•	•	•	•	•	•	•	•	•*		•	•	•	•	•	•	•	•	•	•	•	•	
CTCAE acute toxicity monitoring				•	•	•	•	•	•*														
SAR monitoring and reporting				Monitor during weeks of treatment																			
Response assessment											•	•											
Translational research samples (explicit consent required)																							
Tumour Biopsy	•6																						•[6,7]

*50.4Gy arm only

[1]within 14 days prior to randomisation; [2]within 28 days prior to randomisation

[3]within 63 days prior to randomisation. If the CT scan falls outside this timeframe but a PET CT has been carried out, there is no requirement to repeat the CT scan as long as the PET CT is a whole body scan.

[4]as a minimum, 1 of the MRI or PET scan (if done) for pelvic staging must be within 42 days before randomisation

[5]within 10 days prior to start of treatment

[6]if consented to retreival of stored material for future cancer research [7]and where clinically indicated

Table 12.3 - ACT5 assessment schedule

ACT5	Baseline			Treatment						Follow-up from end of treatment														
	Eligibility assessments	Pre rand	Pre tx	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	6 weeks	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months	30 months	36 months	48 months	60 months	Data sweep	Relapse
Medical history	•																							
ECOG PS, Vital Signs, ht/wt	•[1]																							
Physical exam	•									•	•	•	•	•	•	•	•	•	•	•	•	•		
Tumour Biopsy	•																							
Pregnancy test	•[1]																							
Full Blood Count	•[1]		•[5]	•	•	•	•	•	•															
U&E	•[1]		•[5]	•	•	•	•	•	•															
LFTs	•[1]		•[5]	•	•	•	•	•	•															
HIV	•[2]																							
CD4 count if HIV +ve	•[2]																							
ECG	•[1]																							
CT scan	•[3]													•				•		•				
MRI scan	•[4]										•	•												
PET scan	•[4] Strongly recommended																							
Informed consent		•																						
Randomisation		•																						
QoL		•							•	• CTRU to administer		• CTRU to administer		• CTRU to administer				• CTRU to administer		• CTRU to administer				
Radiotherapy planning scan		•																						
Data collection	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•[6]	
CTCAE acute toxicity monitoring				•	•	•	•	•	•	•	•	•												
SAR monitoring and reporting				Monitor during weeks of treatment and until 6 months post end of treatment																				
Response assessment											•	•												
Translational research samples (explicit consent required)																								
Tumour Biopsy	•[7]																							•[7,8]
2 x Streck Tubes Blood (only for participants currently enrolled in the sub-study)	**Blood sample sub-study closed to new patients in March 2022. Sample collections only applicable for patients already enrolled.**										•[9]	•[9]		•[9]										•[9]
Consent for micobiome sub-study (separate PIS/ICF)		•																						
Stool sample collection			•[10]								•[10]													
Midstream urine collection			•[10]								•[10]													

[1]within 14 days prior to randomisation; [2]within 28 days prior to randomisation
[3]within 63 days prior to randomisation. If the CT scan falls outside this timeframe but a PET CT has been carried out within 42 days prior to randomisation, then there is no requirement to repeat the CT scan as long as the PET CT is a whole body scan.
[4]as a minimum, 1 of the MRI or PET scan (if done) for pelvic staging must be within 42 days before randomisation
[5]within 10 days prior to start of treatment
[6]data sweep at 3 years post randomisation of last ACT5 participant
[7]if consented to retreival of stored material for future cancer research [8]and where clinically indicated
[9]if consented to biomarkers sub-study and after randomisation (ACT5 only)
[10]if consented to gut microbiome sub-study and after randomisation (ACT5 only)

12.1 Eligibility assessments

Participants must have provided written informed consent before being formally assessed for eligibility for any study. The following investigations and assessments must be carried out prior to registration (ACT3) / randomisation (ACT4 and 5) in order to establish eligibility (see Table 12.1 (ACT3), Table 12.2 (ACT4) or Table 12.3 (ACT5) – Section 12):

- Diagnostic biopsy
- Medical history
- Physical examination

Within 14 days prior to registration / randomisation:

- ECOG Performance status
- Vital signs, height, weight
- Pregnancy test (if woman of child bearing potential) as per local practice (not required for ACT3 observation arm)
- Full blood count, U&Es, LFTs
- ECG (not required for ACT3 observation arm)

Within 28 days prior to registration / randomisation:

- HIV status (not an eligibility criteria for ACT3 observation arm, but needed for baseline demographics on all patients)
- CD4 count (if HIV positive)

Prior to registration / randomisation:

- MRI scan (pelvis)[$]*
- CT scan (chest/abdomen/pelvis) (ACT4 and 5) – within 63 days before randomisation. If the CT scan falls outside this timeframe but a PET CT of the whole body (within 42 days) has been carried out as per local imaging protocols, then there is no requirement to repeat the CT scan.
- PET scan (ACT4 and 5)* – is strongly recommended but not mandated to enhance nodal status assessment

[$]**ACT3:** MRI must be within 63 days before registration
***ACT4 and ACT5:** as a minimum, 1 of the MRI or PET scan (if done) for pelvic staging must be within 42 days before randomisation.

If the participant has consented to complete QoL questionnaires, the baseline questionnaires should where possible be completed before registration / randomisation, but must be prior to informing the participant of their treatment allocation.

12.2 Pre-registration/randomisation / Baseline Assessments

Once eligibility has been confirmed, participants can then be registered/randomised (Section 10.8.1 Registration/Randomisation Process).

In addition to the above eligibility assessments, data collected on the pre-registration/randomisation CRFs (Eligibility Checklist, Baseline, Participant Contact Details and Registration/Randomisation Forms) will also include (but will not be limited to):

- Personal details and demographics including gender, date of birth, ECOG performance status, NHS number, postal or email address (if consented to QoL) and smoking use
- Confirmation of written informed consent
- Presence of pre-treatment stoma
- Planned start date of CRT.

12.3 Pre-Treatment Assessments and Sample Collection

Data collected following registration/randomisation and **within 10 days prior to the treatment start date** on the Pre-Treatment Form will include:

- Full blood count, U&Es, LFTs
- Baseline assessment of stool frequency/stoma output (distinguishing from tenesmus / mucous discharge / wet wind)

The results from eligibility assessments may be used if they are within this timeframe prior to the start of treatment).

Translational research blood samples

Please note: the serial plasma biomarker sub-study closed to new participants in March 2022.

- 2 x Streck Tubes of blood (ACT5 phase II/III, after randomisation, if consented to biomarkers sub-study). Invert each tube 8 times after the blood draw.

Send immediately to central lab (refer to separate translational sample manual, for labelling and postage requirements).

Please note: blood samples taken on a Friday should be posted the same day to ensure processing by the lab within 96 hours of blood draw. If possible, sample collection on a Friday before a bank holiday Monday should be avoided.

Translational research stool samples

- 1 x stool sample and 1 x mid-stream urine sample in ACT5 patients consenting to the microbiome sub-study prior to commencement of chemoradiotherapy

Store samples immediately at -80 prior to courier to central lab at St Mary's Hospital (refer to separate translational sample manual, for labelling and postage requirements).

12.4 Weekly CRT Treatment Assessments

Participants will be assessed clinically for symptoms and toxicity each week of CRT treatment, including full blood count and biochemistry.

Details of chemoradiotherapy treatment will be collected on a weekly basis by completing the Chemoradiotherapy Treatment CRF. Data collected will include (but will not be limited to):

- Date treatment started and ended
- Weekly number of fractions and weekly dose of radiotherapy given
- Details of any interruptions to radiotherapy, including reason
- Chemotherapy details (type of chemotherapy (ACT5 only) and whether any dose delays or dose reductions occurred and reason for these
- Acute toxicity scores for adverse events related to CRT using CTCAE

12.5 End of Treatment

If the participant has consented to complete QoL questionnaires, they must be completed at the end of CRT treatment. Research staff will provide the participant with the QoL questionnaire pack in clinic if they have chosen to complete them on paper (see section 14.2.2), or will remind the participant to complete the questionnaires online (see section 14.2.1) and provide a user guide with instructions on using the online system.

When a participant reaches the end of their CRT treatment, an End of Treatment CRF must be faxed to the CTRU within 2 working days of the end of treatment. This is to enable the CTRU to monitor outstanding online QoL questionnaires.

Translational research blood samples

Please note: the serial plasma biomarker sub-study closed to new participants in March 2022.

- 2 x Streck Tubes of blood (ACT5 phase II/III, if consented to biomarkers sub-study). Invert each tube 8 times after the blood draw.

Send immediately to central lab (refer to separate translational sample manual, for labelling and postage requirements).

Please note: blood samples taken on a Friday should be posted the same day to ensure processing by the lab within 96 hours of blood draw. If possible, sample collection on a Friday before a bank holiday Monday should be avoided.

12.6 Follow-up Assessments and Sample Collection

Follow-up visits will be as follows:

ACT3 observation arm	**ACT3 CRT arm** **ACT4 all arms** **ACT5 all arms**
All timings are from **date of registration** • 3-monthly (Years 1-2), then • 6-monthly (Year 3), then • Annually (Years 4 and 5)[+]	All timings are from the **end of treatment**: • 6 weeks* • 3-monthly (Years 1-2), then • 6-monthly (Year 3), then • Annually (Years 4 and 5) [+] • **For ACT5 only**: Data sweep at 3 years post randomisation of the last ACT5 participant

Follow-up data will be collected at these time points by completing the relevant CRF. *At the 6 week time point in the ACT3 CRT arm and all ACT4 arms, only Quality of Life data will be collected, which will be administered by CTRU. [+] As patients are usually discharged after 5 years in routine practice, there will be no annual follow-up past 5 years. In ACT5 only, a data sweep will be conducted at 3 years post-randomisation of the last randomised participant to collect any final event data (e.g. patient status, recurrence details) that will contribute to the primary endpoint.

Data collection during follow-up will include (but will not be limited to):

- Patient status
- Tumour response (3 and 6 months, not applicable for ACT3)
- Presence of stoma, including details of stoma reversal
- Details of any recurrence, including: date and site of recurrence and method of diagnosis.
- Details of any new primary cancer diagnoses including: date, site and method of diagnosis.
- Details of any salvage surgery for pelvic failure
- Late toxicity monitoring for ACT5 (at 6 weeks, 3 months and 6 months)
- Biopsy at suspected relapse (where clinically indicated and if consented to retrieval of archival material)

Where follow up visits cannot be conducted face to face, these assessments may be conducted by telephone, provided that clinical review is organised if telephone follow-up indicates symptoms that may be due to recurrence.

Patient-reported late toxicity data will be collected via QoL questionnaires (see Section 14).

Translational research blood samples

Please note: the serial plasma biomarker sub-study closed to new participants in March 2022.

At 3, 6 and 12 months and suspected relapse:

- 2 x Streck Tubes of blood (ACT5 phase II/III, if consented to biomarkers sub-study). Invert each tube 8 times after the blood draw.

Send immediately to central lab (refer to separate translational sample manual, for labelling and postage requirements).

Please note: blood samples taken on a Friday should be posted the same day to ensure processing by the lab within 96 hours of blood draw. If possible, sample collection on a Friday before a bank holiday Monday should be avoided.

Translational research stool samples

- 1 x stool sample and 1 x mid-stream urine sample in ACT5 patients consenting to the microbiome sub-study at the 3-month follow-up visit. Samples should be taken as close to the 3 month follow up assessment as possible but there is some flexibility to allow for variations in scanning times and patient visits. (Samples must be taken at 12 weeks +/- 4 weeks from the last day of treatment).

Store samples immediately at -80 prior to courier to central lab at St Mary's Hospital (refer to separate translational sample manual, for labelling and postage requirements).

12.7 Follow-up imaging

12.7.1 ACT3

Follow-up MRI (pelvis) will be carried out at 12 and 36 months post completion of CRT (or date of registration for observation arm).

12.7.2 ACT4 and ACT5

Follow-up MRI (pelvis) will be carried out at 3 and 6 months. Follow-up CT (chest/abdo/pelvis) will be carried out at 12, 24 and 36 months. All time points are from the completion of CRT.

12.8 Assessment of efficacy

All patients will be followed up to detect recurrence that may occur in the pelvis or as distant metastases. These will be recorded on the relevant CRF and completed at the time of each follow-up visit. For ACT5, after the last follow up visit at 5 years post-end of treatment, these will be collected through a data sweep at the end of the trial (3 years post randomisation of the last ACT5 participant randomised).

Locoregional failure (LRF) - All failures that occur within the pelvis up to the level of the sacral promontory are considered loco-regional. When LRF is suspected patients will be investigated according to standard local practice. Most cases of LRF will occur at the primary site and can be confirmed histologically by biopsy. For patients where histological confirmation of failure is not possible, LRF can be confirmed by the MDT on review of the imaging data and clinical assessment. The date of failure will be taken as the date of the biopsy, or where this is not available, the data of the first imaging assessment that confirmed the LRF.

Distant metastases – the presence of distant metastases is determined by cross sectional imaging. This will require confirmation by the MDT if there is any uncertainty regarding the imaging findings.

12.9 Response to treatment

ACT4 and 5 patients will be assessed for response to treatment at 3 and 6 months post-end of treatment. Response will be assessed via MRI imaging in accordance with the Tumour Regression Grading (TRG) System (Appendix F) as well as via local clinical assessment. **Assessment of response is reported completely separately to the assessment of LRF.**

12.10 Deaths

All deaths occurring from the date of registration/randomisation to the end of follow-up must be recorded on the Notification of Death CRF and sent to the CTRU within 7 days of the site team becoming aware of the death. It is important that the CRF is sent promptly so that any QoL questionnaire reminders sent by CTRU are stopped promptly. Data collected will include (but may not be limited to):

- Date of death
- Cause of death

12.11 Pregnancies

All pregnancies and suspected pregnancies in a trial participant, or their partner, occurring from the date of registration/randomisation to six months after completion of CRT treatment must be reported to the CTRU within 24 hours of the site becoming aware. All protocol treatment must be stopped immediately if a pregnancy in a female participant occurs or is suspected.

The CTRU will report all pregnancies occurring during treatment to the Sponsor along with any follow-up information.

12.12 End of Trial

The end of the trial is defined as the date of the collection of the last participant's data item. All evaluable trial participants will be followed up until this point. Follow-up data will be collected from sites until three years post end of treatment of the final participant in each trial.

13 Safety reporting

13.1 General definitions

13.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial subject which does not necessarily have a causal relationship with the treatment.

13.1.2 Adverse Reaction (AR)

Adverse reactions (ARs) are all untoward and unintended responses to the trial treatment. This definition implies a reasonable possibility of a causal relationship which is supported by facts, evidence or arguments to suggest a causal relationship. This definition includes medication errors and uses outside what is foreseen in the protocol (i.e. if an AR occurs as a result of a medication error).

13.1.3 Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that:

- results in death.
- is life-threatening.
- requires inpatient hospitalisation or prolongation of existing hospitalisation.
- results in persistent or significant disability or incapacity.
- consists of a congenital anomaly or birth defect.
- is otherwise considered medically significant by the Investigator.

13.1.4 Serious Adverse Reaction (SAR)

A Serious Adverse Reaction (SAR) is an SAE deemed to have been related to the trial treatment. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

Medical and scientific judgement must be exercised in deciding whether an event is serious (see protocol Section 13.5 for Responsibilities). These characteristics / consequences must be considered at the time of the event and do not refer to an event which hypothetically may have caused one of the above.

13.2 Related Unexpected Serious Adverse Event (RUSAE)

A serious adverse reaction which is related and unexpected (termed Related Unexpected Serious Adverse Event, or RUSAE) will require expedited reporting (see Section 13.4.4) to enable reporting to the main Research Ethics Committee (REC) and Sponsor.

The Health Research Authority (HRA) defines the terms related and unexpected as:

- Related: that is, it resulted from administration of any research procedures.
- Unexpected: that is, the type of event that in the opinion of the investigator is not considered expected.

When determining whether an SAR is expected or not, please refer to the relevant version of the Summary of Product Characteristics that is used locally.

13.3 Reporting requirements for ARs

13.3.1 ACT3

Non-serious Adverse Events (AEs) which have no causal relationship with trial treatment and grade 1 or 2 ARs will not be collected in this trial, but must still be recorded in the participant's medical notes.

In ACT3 (CRT arm), grade 3 and grade 4 ARs will be collected during each week of treatment on the Chemoradiotherapy CRF. The decision to only collect grade 3 and 4 ARs is based on the trial using lower dose radiotherapy in combination with a chemotherapy regime with a well understood safety profile.

Information about grade 3 and 4 ARs, whether volunteered by the participant, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded on the CRF and will be evaluated for duration and intensity according to the current National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) – see Appendix C.

13.3.2 ACT4

Non-serious AEs which have no causal relationship with trial treatment and grade 1 ARs will not be collected in this trial, but must still be recorded in the participant's medical notes.

In ACT4, all grade 2 and above ARs will be collected during each week of treatment on the Chemoradiotherapy CRF. The decision to collect grade 2 and above ARs in ACT4 is based on the trial using standard or reduced dose radiotherapy in combination with chemotherapy regimes with well understood safety profiles. It will ensure that a full spectrum of toxicities is collected for these radiotherapy doses in order for any differences in ARs between the two doses to be observed.

Information about all grade 2 and above ARs, whether volunteered by the participant, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded on the CRF and will be evaluated for duration and intensity according to the current National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) – see Appendix F.

13.3.3 ACT5

Non-serious AEs which have no causal relationship with trial treatment will not be collected in this trial, but must still be recorded in the participant's medical notes.

In ACT5, all ARs will be collected during each week of treatment on the Chemoradiotherapy CRF and at 6 weeks, 3 months and 6 months on a Clinician Reported Toxicity Assessment CRF. The decision to collect all ARs for ACT5 was based on the use of IMRT dose escalation in combination with chemotherapy, for which currently there is no randomised clinical trial evidence for. The acute toxicity data from the first 60 participants has been reviewed at the end of the pilot phase and has led to the decision to collect ARs post-end of treatment.

Information about all ARs, whether volunteered by the participant, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded on the CRF and will be evaluated for duration and intensity according to the current National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)– see Appendix F.

13.4 Recording and reporting SARs and RUSAEs

13.4.1 Events classed as expected SARs

Examples of events which will be classed as expected SARs within this trial and therefore will not be reportable as RUSAEs are given below. This is not intended to be an exhaustive list, therefore when determining whether an SAR is expected or not, please always refer to the relevant SPC that is used locally.

13.4.2 Examples of expected SARs related to radiotherapy:

Blood/Bone Marrow
Anaemia
Neutropenia
Thrombocytopenia
Infection

Dermatology/Skin
Dermatitis radiation
Skin erythema/ulceration

Gastrointestinal
Constipation
Diarrhoea
Nausea
Proctalgia
Proctitis
Rectal bleeding
Rectal stricture
Rectal urgency

Musculoskeletal
Bone fractures

Renal
Dysuria
Haematuria
Urinary urgency
Incontinence

Constitutional Symptoms
Fatigue

13.4.3 Examples of expected SARs related to Mitomycin C / Capecitabine / 5FU:

	Capecitabine	5-Fluorouracil	Mitomycin C
Blood & Lymphatic System			
Anaemia	✓	✓	✓
Leucopenia	✓	✓	✓
Neutropenia	✓	✓	
Febrile neutropenia	✓		
Pancytopenia		✓	
Thrombocytopenia	✓	✓	✓
Cardiac			
Chest pain	✓	✓	
Tachycardia	✓	✓	
ECG changes	✓	✓	
Dermatology/Skin			
Alopecia	✓	✓	✓
Rash			✓
Hand-foot syndrome or palmar-plantar erythrodysesthaesia	✓	✓	
Nail changes	✓		
Gastrointestinal			
Abdominal pain	✓		
Anorexia	✓		✓
Constipation	✓		
Diarrhoea	✓	✓	✓
Dyspepsia	✓		
Nausea	✓	✓	✓
Stomatitis	✓	✓	✓
Mucositis		✓	
Vomiting	✓	✓	✓
Infections/Infestations			
Infections		✓	
Metabolic/Laboratory			
Creatinine			✓
Musculoskeletal			
Arthralgia	✓		
Myalgia	✓		
Ataxia		✓	
Dysgeusia	✓		
Dysaesthesia	✓		
Headache	✓		
Paraesthesia	✓		
Peripheral neuropathy	✓		
Ocular/Visual			
Conjunctivitis		✓	
Watery eye (epiphora, tearing)	✓		
Vascular			
Lower limb oedema	✓		
Hypertension	✓		
Embolism and thrombosis	✓		
General Symptoms			
Asthenia	✓		
Fever		✓	✓

Fatigue	✓	✓	✓
Lethargy	✓		
Other effects			
Interstitial pneumonitis			✓
Pulmonary fibrosis			✓

13.4.4 Reporting and recording requirements for SARs and RUSAEs

Reporting timeframes for SARs and RUSAEs are as follows:

ACT3 CRT arm and ACT4:

All SARs and RUSAEs occurring during the weeks of CRT treatment.

ACT5:

All SARs and RUSAEs occurring during the weeks of CRT treatment and up to 6 months post-end of treatment.

SARs and RUSAEs must be recorded on the SAR or RUSAE CRF and faxed to the CTRU **within 24 hours** of the trial site team becoming aware of the event.

For each SAR and RUSAE the following information will be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- whether the event would be considered expected or unexpected.

Any change of condition or other follow-up information should be faxed to the CTRU 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.

Assessment of expectedness must be made by an authorised medically qualified person. If such as person is unavailable, initial reports without expectedness assessment should be faxed to the CTRU by a healthcare professional within 24 hours but must be followed up by medical assessment as soon as possible thereafter.

Once all resulting queries have been resolved, the CTRU will request the original form should also be posted to the CTRU and a copy to be retained on site.

All SARs assigned by the PI or delegate (or following Chief Investigator review) as unexpected will be classified as RUSAE and will be subject to expedited reporting to the Sponsor and the REC by the CTRU on behalf of the Chief Investigator in accordance with current HRA guidance, CTRU Standard Operating Procedures (SOPs) and Sponsor requirements.

13.4.5 Serious Adverse Events of Interest (SAEoI)

The following serious events occurring during the weeks of CRT treatment must be reported to the CTRU **within 24 hours** of the trial site team becoming aware of the event. They should be reported in the same way as SARs as detailed in Section 13.4.4..

1. Angina / myocardial infarction
2. Pulmonary embolism

13.5 Responsibilities

Principal Investigator (PI):

1. Checking for ARs when participants attend for treatment.
2. Using medical judgement in assigning seriousness and expectedness using the relevant Summary of Product Characteristics used locally.
3. Ensuring that all SARs (including RUSAEs) are recorded and reported to the CTRU within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SARs (including RUSAEs) are chased with CTRU if a record of receipt is not received within 2 working days of initial reporting.
4. Ensuring that ARs are recorded and reported to the CTRU in line with the requirements of the protocol.

Chief Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning seriousness and expectedness of SARs where it has not been possible to obtain local medical assessment.
3. Immediate review of all RUSAEs.
4. Review of specific SARs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.

CTRU:

1. Central data collection and verification of ARs, SARs and RUSAEs according to the trial protocol onto a MACRO database.
2. Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
3. Reporting safety information to the independent oversight committees identified for the trial (Data Monitoring & Ethics Committee (DMEC) and Trial Steering Committee (TSC)) according to the Trial Monitoring Plan.

4. Expedited reporting of RUSAEs to the REC and Sponsor within required timelines.
5. Notifying Investigators of RUSAEs that occur within the trial which compromise participant safety.
7. Preparing annual safety reports for the REC.

Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing blinded safety data and liaising with the DMEC regarding safety issues.

Data Monitoring & Ethics Committee (DMEC):

In accordance with the Trial Terms of Reference for the DMEC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

14 Quality of Life

Assessment of participants' Quality-of-life (QoL) will take place at baseline, end of treatment, 6 weeks, 6, 12, 24 and 36 months post-end of treatment. ACT3 participants in the observation arm who are not receiving CRT treatment will complete QoL questionnaires at 6 weeks, 6, 12, 24 and 36 months post-date of registration. **See Table 12.1 (ACT3), Table 12.2 (ACT4) or Table 12.3 (ACT5) – Section 12.** Questionnaires should be completed independently by the participant.

Questionnaires to be completed include the EORTC QLQ-C30 and QLQ-ANL27. The EORTC QLQ-C30 is a validated multi-dimensional tool for assessing QoL in cancer patients, formed of 30 questions addressing aspects of patients' physical and mental functioning and symptoms as well as their overall health and quality of life. The EORTC QLQ-ANL27 is a validated anal specific module.

14.1 Questionnaires at Baseline

Research staff will provide the participant with the baseline QoL questionnaire pack in clinic, prior to registration/randomisation. Participants will be asked to complete the questionnaires in clinic, seal the completed questionnaires in an envelope and hand it to the research staff. Research staff will then send the sealed envelopes to the CTRU for entry into the database.

14.2 Format of future follow-up questionnaires

At their baseline visit, participants will be asked whether they are able and willing to complete future follow-up questionnaires using REDCap. Participants who are unable or unwilling to use REDCap will be able to complete paper questionnaires during follow-up.

14.2.1 REDCap

REDCap is a secure website that allows participants to complete their QoL questionnaires online. Participants who choose to use REDCap will require access to a computer and the internet and will be required to provide their email address to CTRU. Participants will access the REDCap online system via a unique link that will be emailed to them when a questionnaire is due. Participants will also be given a user guide with brief instructions on accessing and completing the online questionnaires and details of who to contact if more help is needed. CTRU will send an email to participants with a link to access a questionnaire when it is due to be completed. Should a questionnaire not be completed on REDCap by the required time point, CTRU will send a reminder email to the participant.

14.2.2 Paper

Participants who choose to complete paper questionnaires during follow-up will be required to provide their home address to CTRU. Participants will receive follow-up questionnaires by post from the CTRU. Participants will complete the questionnaires at home and return them to the CTRU using a pre-supplied stamped addressed envelope. A thank you letter will be sent to participants by CTRU upon receipt of a completed questionnaire. Should a completed questionnaire not be received at CTRU by the required time point, CTRU will send a reminder letter to the participant.

14.3 Questionnaires at the end of chemoradiotherapy treatment

For participants receiving CRT treatment, the second time point for completion of QoL questionnaires is at the end of CRT treatment.

Participants using REDCap - If the participant has chosen to complete the questionnaires online via REDCap, research staff will remind the participant to complete the questionnaires online and CTRU will send an email with a link to access the questionnaire.

Participants using paper - Research staff will provide the participant with the QoL questionnaire pack in clinic if they have chosen to complete them on paper. Participants will be asked to complete the questionnaires in clinic, seal the completed questionnaires in an envelope and hand it to the research staff, who will then post the sealed envelopes to the CTRU.

14.4 Questionnaires at all other follow-up times

CTRU will send an email (if using REDCap) or paper questionnaire pack by post (if using paper) to participants when a questionnaire is due to be completed.

15 Endpoints

15.1 Primary endpoint:

- **Locoregional failure (LRF) free survival**

LRF includes a failure at the primary site (local) and/or surrounding nodal sites (regional) i.e. any failure within the pelvis up to the level of the sacral promontory. New primary tumours or distant metastases are not included in this definition.

LRF is to be confirmed by the local clinical team. The majority of LRFs will occur at the primary site and can be confirmed histologically (by biopsy). For patients where histological confirmation is not possible, LRF can be confirmed by the MDT on review of the imaging data and clinical assessment. The date of failure will be taken as the date of the biopsy, or where this is not available, the date of the imaging assessment that determined LRF.

For ACT3 and ACT4, the primary endpoint will analyse LRF at 3 years post-recruitment/end of treatment (registration for ACT3, randomisation for ACT4). The assessment of LRF will be in terms of locoregional failure-free rate i.e. the number and proportion of patients without failure events at 3 years post registration/ randomisation.

For ACT5, the primary endpoint will analyse LRF-free survival at 3 years post randomisation of the last randomised participant. All instances of LRF occurring up to this time point will be included in the analysis, using a time to event analysis of locoregional failure-free survival.

15.2 Secondary endpoints:

- **Acute toxicities**

Assessment of acute toxicities will take place during each week of treatment (with the exception of the ACT3 observation arm). In ACT5 only, clinician reported acute toxicities will be captured at 6 weeks, 3 and 6 months post-end of treatment. They will be evaluated according to the current NCI-CTCAE criteria and include all ARs, SARs/SAEoIs and RUSAEs for ACT5, all grade 2 and above ARs, SARs/SAEoIs and RUSAEs for ACT4 and all grade 3 and above ARs, SARs/SAEoIs and RUSAEs for ACT3 (see section 13.3). Patient reported acute toxicities will be captured at the end of treatment, 6 weeks and 6 months post the end of treatment via the EORTC QLQ-C30 and QLQ-ANL27 questionnaires (see section 14)

- **Late toxicities**

Participant reported late toxicities will be captured via the EORTC QLQ-C30 and QLQ-ANL27 questionnaires (see section 14) at 12, 24 and 36 months post the end of treatment.

- **Treatment compliance**

Data on treatment received will be collected on a weekly basis (with the exception of the ACT3 observation arm). In order to evaluate this endpoint and assess compliance with the radiotherapy schedule, data will be collected on the total dose of radiotherapy received (i.e. dose and fractions), the overall treatment time (i.e. start and end date of treatment), details of any interruptions to the radiotherapy, and the reasons for these (i.e. toxicity or other).

Participants will be considered to have adhered to the radiotherapy schedule if they have completed their scheduled course of radiotherapy with no greater than 3 treatment days of delays due to toxicity.

In order to assess compliance with the chemotherapy schedule, data will be collected on the type of chemotherapy received (ACT5), and any modifications to the protocol defined schedule of chemotherapy i.e. any dose delays, omissions or reductions and reasons for these.

- **Clinical response rate (cRR)**

Participants will be assessed for response to treatment at 3 and 6 months post-end of treatment (ACT4 and 5 only). Response to treatment will be assessed via MRI imaging in accordance with the Tumour Regression Grading (TRG) System (Appendix F). Participants with a TRG score of 1 or 2 will be classed as having achieved a Complete Response (CR), 4 and 5 as non-responders, and 3 as an indeterminate group. In addition, response to treatment will be assessed via local clinical assessment.

- **Disease-free survival (DFS)**

Disease-free survival is defined as the time from registration (ACT3)/ randomisation (ACT4 and ACT5), to the first documented evidence of pelvic failure which includes: locoregional failures, distant metastases, new primary tumours or death. The majority of patients are expected to be disease-free at the 6 months post end of treatment scan.

- **Colostomy-free survival (CFS)**

The evaluation of this endpoint involves four distinct groups of participants:

- Participants who have a pre-treatment colostomy that is still present at 12 months
- Participants who have a pre-treatment colostomy that is reversed within 12 months
- Participants who have a post-treatment colostomy either for toxicity (i.e. treatment-related) or for local disease failure (including APER)
- Participants who never have a colostomy fitted
- Death from any cause

- **Progression-free survival (PFS) (ACT4 and ACT5 only)**

Progression-free survival is defined as the time from registration (ACT3)/ randomisation (ACT4 and ACT5) to the first documented evidence of disease progression or death (from any cause). Progression can be determined from the 3 month post end of treatment assessment onwards. Progression is reported variably within anal cancer, within the context of this trial, disease progression refers to progression of a patient's anal cancer. Progression events include locoregional failures and distant metastases.

- **Overall survival (OS)**

Overall survival is defined as the time from registration (ACT3)/ randomisation (ACT4 and ACT5) to the date of death from any cause. Survival data will be collected at standard follow-up visits and OS will be assessed at 3-years post-registration/randomisation of the final participant.

Patient Reported Outcome Measures (PROMs)

Questionnaires to be completed include the EORTC QLQ-C30 and QLQ-ANL27. See Section 14 for further details

15.3 Descriptive endpoints

- **Pattern of failure**

Failures includes locoregional failures (primary site or nodal), distant metastases or new primary tumours. Failures can occur at a single or multiple sites.

- **Salvage surgery and resection status**

Salvage surgery can be performed in patients with a local relapse (i.e. recurrence at the primary site) in all ACTs. Salvage surgery is usually an abdominoperinal excision. A local excision may sometimes be performed in ACT3 patients and a diverting stoma of inoperable pelvic recurrence. Resection status refers to the absence or presence of residual tumour, post-surgical resection.

16 Statistical Considerations

16.1 Sample size and planned recruitment rates

16.1.1 ACT3

Sample size: 90 patients are required

This study is powered to demonstrate that the overall treatment strategy of LE alone with or without additional lower-dose radiotherapy with chemotherapy, does not produce unacceptable rates of efficacy (i.e. below 80%), but can reach efficacy rates of up to 90% (i.e. a locoregional failure rate of 10%). A minimum target efficacy rate of 90% or greater is considered desirable based on data from the ACT2 trial (10) where 10% of T1-2 N0 patients had a pelvic failure at 3 years post-registration.

An exact A'Hern single-stage (39) design will be used to test the null hypothesis H_0: P <0.80, against the alternative hypothesis H_1: P≥0.90. Thus, if the lower bound of the one-sided 95% confidence interval (CI) around the efficacy rates (i.e. proportion of patients without locoregional failure) falls below 80%, the overall treatment strategy will be deemed unacceptable.

With 80% power and 5% 1-sided significance, i.e. type 1 error for incorrectly determining the efficacy rates are acceptable, 82 patients are required. Allowing for a drop-out rate of 10%, the target sample size is 90 patients. If we were to recruit the full 90 patients, should 11 or more 'failures' occur within 3 years post-registration, the overall treatment strategy would be deemed unacceptable.

The sample size calculations were performed using the Early Phase Clinical Trials (EPCT) program, V1.0 and exact CIs were derived using the exact binomial distribution.

16.1.2 ACT4

Sample size: 162 patients are required

This study is powered to demonstrate that the reduced-dose IMRT arm does not produce unacceptable rates of efficacy (i.e. below 80%), but can reach efficacy rates of up to 90% (i.e. locoregional failure rate of 10%). A minimum target efficacy rate of 90% or greater is considered desirable based on data from the ACT2 trial (10) where 10% of T1-2 N0 patients had a pelvic failure at 3 years post-randomisation.

An exact A'Hern single-stage (39) design will be used to test the null hypothesis H_0: P <0.80, against the alternative hypothesis H_1: P≥0.90. Thus, if the lower bound of the one-sided 95% confidence interval (CI) around the efficacy rates (i.e. proportion of patients without locoregional failure) in the reduced dose arm falls below 80%, this will be deemed unacceptable. The standard-dose IMRT arm will act as a calibration arm, to ensure the desired efficacy rate is plausible.

With 80% power and 5% 1-sided significance, i.e. type 1 error for incorrectly determining the efficacy rates are acceptable, 123 patients are required with a 1:2 randomisation (standard-dose:reduced-dose). In order to address the research hypothesis for the p16+ subset, for

which there is evidence of improved outcomes, the sample size has been inflated by 20% (as 90% of patients are expected to present with this genotype, and a further 90% have samples suitable for analysis). Allowing for a 10% drop-out rate, the target sample size is 162 patients (54:108). Of the 108 patients in the reduced-dose IMRT arm, should 14 or more 'failures' occur within 3 years post-randomisation, the reduced dose arm would be deemed unacceptable.

The sample size calculations were performed using the Early Phase Clinical Trials (EPCT) program, V1.0 and exact CIs were derived using the exact binomial distribution.

16.1.3 ACT5

Sample size: 459 patients are required

The pilot stage of ACT5 will recruit a maximum of 60 patients (20:20:20). A formal power calculation was not performed for the pilot study given the aim is to review the initial feasibility of dose escalation and concomitant chemotherapy, rather than make any decisions on study continuation. A cohort of 60 patients was deemed sufficient to be able to assess this. Recruitment will be suspended whilst the analysis takes place and any required modifications are made to the protocol.

Note that the exploratory analysis of the pilot stage was conducted and presented to the independent Data Monitoring and Ethics Committee (DMEC) in October 2018. The Committee determined that the data supported the continuation of ACT5 and recommended extending the follow-up of clinician reported toxicities post-end of treatment.

The Phase II trial will recruit an additional 80 patients. The phase II analysis will focus on the acute toxicity data including grade 3 and above neutropenias from the first 140 patients randomised (overall in the three arms). An A'Hern single-stage design (39) has been applied to each of the three arms to derive the sample size for phase II. The formal analysis of the phase II data will determine which trial design will be taken forward to phase III (see section 17.3.2). If both experimental arms pass the pre-specified threshold for toxicity then all three arms will be taken forwards (trial design 2). If an arm does not pass the pre-specified threshold then it will close to recruitment and 2 arms (control and 1 experimental arm) will continue into phase III (trial design 1). With 80% power and 5% 1-sided significance, an acceptable rate of grade 3/4 neutropenias of <40% and an unacceptable rate of ≥60% yields a sample size of 126 patients (42:42:42), 140 with 10% drop-out. The desired rate of 40% is based on data from the RTOG 9811, RTOG 0529 and ACT2 trials where rates of grade 3/4 neutropenias were 45%, 58% and 24% respectively.

The overall trial is powered on detecting an absolute difference of 10% in 3-year LRF rates between the control and experimental arm(s). This difference is equivalent to a hazard ratio of 0.63 and assumes a LRF rate of 30% in the control arm based on the ACT2 data (10).

Trial design option 1- Phase III trial with one experimental arm, dropping one after phase II

This design drops an arm after phase II. If an arm does not pass the pre-specified boundaries for acceptable toxicity at interim analysis. With 80% power and 5% 2-sided significance, 386 patients are required (193:193) (including a 10% drop out rate) for the primary endpoint

analysis. The target event rate is 143 based on assuming 6 years of recruitment and a minimum of 3 years follow up for all patients.

An additional 293 patients will be recruited during Phase III, in order to achieve the required total planned sample size of 433 (total from the pilot, phase II and III). After Phase II, approximately 47 patients from the 'dropped' experimental arm will not continue through to Phase III, leaving 386 patients required for the primary endpoint analysis.

Trial design option 2- Phase III trial with two experimental arms

If both experimental arms pass the pre-specified boundaries for acceptable toxicity at interim analysis, all three arms will continue into the phase III trial.

This would compare each experimental arm against the standard dose in terms of 3 year LRF-free survival. With 80% power and 10% 2-sided significance 459 (153:153:153) patients are required (including 10% drop out) for the primary endpoint analysis. The target event rate is 113 per comparison based on assuming 6 years of recruitment and a minimum of 3 years follow up for all patients.

An additional 319 patients will be recruited during Phase III, in order to achieve the required total planned sample size of 459 (total from the pilot, phase II and III). The sample size calculations were derived using nQuery Advisor® and assumed exponential survival times.

The increased type I error associated with this 3-arm design, increasing the error rate due to multiple comparisons with the control arm, is acknowledged, and error rates will be published. This pragmatic approach was taken to deliver an essential evidence base given the current treatment landscape in anal cancer, providing rich clinically relevant evidence enabling practice definition based on the totality of information from the study.

The trial will continue to recruit whilst the Phase II analysis takes place. A 10% drop-out rate has been included to account for losses to follow-up for the primary endpoint within the recruitment and follow-up periods which span 9 years in total. The drop out rate also accounts for the fact that there will be a small group of patients recruited between phase II and phase III whilst the interim analysis is conducted to an experimental arm which may not continue to phase III.

Note that the interim analysis of the phase II component was conducted and presented to the independent Data Monitoring and Ethics Committee (DMEC) in March 2021. The Committee determined that the data supported the continuation of both experimental arms in ACT5 and recommended that the phase III component continue as a 3 arm trial (trial design option 2).

Following discussion with the TMG, DMEC and TSC (TSC meeting 21st April 2023), it was agreed that annual follow up would not continue beyond 5 years post end of treatment. Primary endpoint data for participants who have passed the 5 year follow up mark would be obtained by a data sweep carried out at 3 years post randomisation of the final participant. The decision was based on patients being discharged by sites at 5 years in routine practice and ACTII[10] trial data where 93% of all pelvic recurrences were detected within the first 3 years. In order to manage this, the event rate will be

assessed 3 years post end of treatment of the last participant using both the information collected during the data sweep and forms collected as planned for patients that have not passed the 5 year follow up mark. If the target event rate will not be reached, the two experimental dose-escalation arms will be combined for a single comparison with the standard arm during the primary analysis.

Gut microbiome sub-study

Our primary outcome measure in the sub-study is the feasibility of collecting and analysing the faecal microbiome in patients enrolled in ACT5, defined as the ability to collect and analyse paired samples. Assuming a target rate of paired, evaluable samples in at least 80% of patients, we require at least 55 patients to be recruited to the sub-study to demonstrate an acceptable 'feasible' rate of at least 65%, with 80% power at the 1-sided 5% significance level. If we observe at least 42/55 patients with paired evaluable samples, this would mean the lower limit of the 1-sided 95% confidence interval would be above 65%.

17 Statistical Analysis

17.1 General considerations

Statistical analysis is the responsibility of the CTRU Statisticians. The analysis plan detailed below provides an overview of the analyses to be performed. A separate and fully detailed statistical analysis plan (SAP) will be written before any analyses are undertaken (separate DMEC and final SAPs) and in accordance with CTRU standard operating procedures.

All efficacy analyses for ACT3 and ACT5 will be performed on an intention-to-treat (ITT) basis, where participants will be included according to the treatment arm they were recruited/randomised to. For ACT4 a modified intention-to-treat population will be used whereby participants are included if they receive at least one dose of trial treatment. Per-protocol analyses will be performed should there be a sufficient number of major protocol violators. Safety and toxicity analyses will be performed on the safety population, where participants will be included according to the treatment they received.

17.2 Frequency of analysis

The final analysis of the primary endpoint LRF-free rate (ACT3 and 4)/survival (ACT5), all time-to-event secondary endpoints, late toxicities and PROMs will take place 3 years post-registration (observation arm of ACT3 only)/ end of treatment of the final participant.

Formal interim analyses will be carried out in ACT3 and 4 on the first 30 participants to be recruited (30 in the reduced dose IMRT arm in ACT4).

For ACT5 only, event rate monitoring will take place3 years post end of treatment of the final participant, to determine whether the target event rate will be reached in order for the primary endpoint analysis to continue as planned, or whether the dose escalation arms will be combined for the primary analysis.

Analysis of all other secondary endpoints will take place once the relevant data has been received for all patients.

An exploratory analysis of the pilot data in ACT5 will be carried out on the first 60 participants to be randomised. The analysis of the phase II data will be conducted once the required 140 participants have been recruited and followed-up until the end of treatment.

17.3 Interim analyses

17.3.1 ACT3 and ACT4

The DMEC will meet to review the initial failure rate (LRF) data once the first 30 patients in ACT3, and 45 patients in ACT4 (30 patients in the reduced-dose IMRT arm) have been recruited. A cohort of 30 patients it felt to be appropriate, any less and there would be insufficient data for the DMEC to make a reasonable assessment of failure rates, and increase the possibility of seeing extreme results by chance; anymore and we potentially compromise

the ability of the DMEC to stop the trials sufficiently early during the recruitment stages, should they have any major concerns.

The following are guidelines which the DMEC should consider in their review and in reaching any decisions on study continuation:

The anticipated failure rate in the ACT3 and ACT4 trials is 10% and between 10-15%, respectively (based on the ACT2 data). The number of locoregional failures and one-sided 95% confidence intervals around these rates will be reported, however no formal statistical testing will be carried out and there are no formal stopping rules in place. *As a guide*, in ACT3, in the first 30 patients, should 8 or more participants have a locoregional failure this would be a clinically significant cause for concern. The exact one-sided 95% confidence interval around this rate would exclude the anticipated failure rate of 10% and would in fact comprise the total expectation of events from the whole trial population. In ACT4, in the first 30 patients in the IMRT dose de-escalation arm, should 10 or more participants relapse this would be a cause for concern. The exact one-sided 95% confidence intervals around this rate would exclude the anticipated failure rate of 15% and would again comprise the total expectation of events from the whole trial population.

With the permission of the independent DMEC and TSC, we plan to publish our experience with the initial 30 patients recruited into ACT3, and initial 45 patients recruited into ACT4 (30 patients in the reduced-dose IMRT arm), if this is not deemed to be contraindicated or affect the scientific integrity of the trial or future recruitment. Specifically in ACT3 we would aim to publish (but not limited to) descriptive analyses of local excision specimens, and proportion of patients with margins ≤1mm vs >1mm. For both trials we would also aim to publish the acute toxicity and treatment compliance data.

17.3.2 ACT5

An exploratory analysis of the first 60 patients randomised into ACT5 will be conducted (i.e. end of the pilot study). This should equate to 20 patients in each of the three arms. The aim of the pilot study is to determine whether any modifications to the protocol (in particular to the chemotherapy dose) are required before commencement of the phase II trial. This exploratory analysis will focus on a review of the acute toxicity and treatment compliance data (radiotherapy and chemotherapy). Recruitment will be suspended during this time.

A formal interim analysis will take place at the end of the phase II trial after 140 participants have been recruited. The DMEC will meet to review this interim data and to determine which trial design will be taken forward into phase III. The interim analysis will be focused on acute toxicity data (see section 16.1.31). If the interim analysis shows that both experimental arms are safe to be taken forward, i.e. both arms pass the pre-specified toxicity threshold, then all 3 arms will continue into phase III. If an arm does not pass the pre-specified threshold, then it will close to recruitment and the trial will continue with 2 arms (control and 1 experimental arm). The decision as to which trial design will be taken forward will be primarily based on the acute toxicity data. 'Acceptable' rates of CTCAE grade 3 and 4 neutropenias must be observed for an SIB arm to be considered for Phase III where 40% is the target rate and ≥60% would be deemed unacceptable.

With the permission of the independent DMEC and TSC, we plan to publish the results of the pilot study and phase II trial, if this is not deemed to be contraindicated or affect the scientific integrity of the trial or future recruitment. Specifically (but not limited to this) we would aim to publish at the end of the pilot study, descriptive summaries of the acute toxicity and treatment compliance data, and report on any amendments to the protocol e.g. change to chemotherapy dose. At the end of phase II, we would aim to publish the acute toxicity and treatment compliance data.

17.4 Primary endpoint analysis

17.4.1 ACT3 and ACT4

In ACT3 and 4, the analysis of LRF will be in terms of proportions i.e. the number and proportion of patients without an LRF event occurring from registration (ACT3) /randomisation (ACT4) up until 3 years post-registration/randomisation.

In ACT3, the analysis of the primary endpoint will be based on the whole trial cohort i.e. all 90 patients. The analysis of primacy will assess the proportion of participants without a LRF at 3 year post-registration, and the one-sided 95% CI (corresponding to a two-sided 90% CI) around this rate. Assessment of the efficacy of the overall treatment strategy will focus on the lower bound of the CI, and whether or not it falls below the 'unacceptable' rate of 80%.

In the ACT4 trial, the analysis of primacy will look at the proportion of participants without an LRF event in the reduced-dose IMRT arm at 3 years post-randomisation. One-sided 95% CIs (two-sided 90% CIs) will be presented for this rate, and the assessment of the efficacy of the reduced dose strategy will focus on the lower bound of this CI, and whether or not it falls below the 'unacceptable' rate of 80%. The proportion of participants without a LRF in the standard-dose IMRT arm with corresponding CIs will be presented to act as a calibration arm, and to establish whether the desired efficacy rate of 90% is plausible. In addition, the analysis will be performed on the subgroup of patients with a p16+ genotype.

17.4.2 ACT5

In ACT5, the assessment of LRF will be based on a time-to-event analysis i.e. locoregional failure-free survival from randomisation.

LRF-free survival for each experimental arm will be compared against the control, i.e. two separate comparisons, using Cox's proportional hazards (PH) modelling, adjusting for the minimisation factors.

No formal statistical comparisons will be made between the experimental arms for the primary endpoint. The hazard ratio (HR) for the experimental arm versus the control arm (where a HR < 1 would indicate the experimental arm is better than the control) will be presented along with two-sided 90% confidence intervals (CIs) and associated p-value testing for the difference between the arms (10% significance level as per the design outlined in Section 16.1.31). This will be the analysis of primacy. A secondary analysis will be undertaken comparing the combined dose escalation arms with the control to test the hypothesis that dose escalation generally provides improved LRF compared to control.

Following an assessment of event rate 3 years post end of treatment of the final participant, the dose-escalation experimental arms may be combined if the target event rate will not be reached. In this case, the combined dose escalation arm will be compared to the control using Cox's proportional hazards (PH) modelling, adjusting for the minimisation factors, as the analysis of primacy. In this instance, the secondary analysis will then compare each experimental arm against the control, in two separate analyses.

Participants who are failure-free at 3 years post-randomisation of the final participant, or who have come off the trial prior to having an event (e.g. withdrawals, losses to follow-up or death), will be censored at the last date they were known to be alive and failure-free.

The assumptions of the Cox PH model will be tested.

LRF-free survival will be presented via Kaplan-Meier (KM) curves. The median time to LRF, 95% CIs will be presented along with the log-rank test statistic (and associated p-value) which tests for a difference in the median time to LRF.

17.5 Secondary endpoint analysis

Time-to-event endpoints will be presented using KM curves. For ACT5 Cox's PH model will be used to compare each experimental arm with the control. There will be no formal comparison between the arms for ACT3 and ACT4. The relevant parameter estimate and test statistics will be presented as per the primary analysis. The proportion of participants experiencing each event will be presented by treatment group (where applicable) and overall. For DFS, patients who are not 'disease-free' at 6 months post-end of treatment (around 10% of patients) will be classed as having 'failed' at the date of randomisation.

Participants who are event-free at 3 years post-registration/randomisation of the final participant or who come off trial prior to observing an event (e.g. withdrawals, losses to follow-up, or death), will be censored at the last date they were known to be alive and event-free.

The proportion of participants experiencing each CTCAE grade of acute toxicities will be summarised for each treatment arm, for the overall treatment period and at each follow-up assessment. The number and proportion of participants experiencing grade 3 and above acute toxicities will be reported along with 95% CIs. These will be compared between each treatment arm and the control using a logistic regression model adjusted for the minimisation factors, for ACT5 only. Exploratory comparisons of acute toxicity may be performed between the experimental arms. Summaries will be presented for the overall treatment period at each follow-up assessment.

Summary statistics will be presented for the total dose of radiotherapy received in each treatment arm and duration of treatment. The proportion of participants adhering to the radiotherapy schedule will be reported and compared between each treatment arm and the control ACT5 only) using a logistic regression model, adjusted for the minimisation factors. Reasons for interruption to radiotherapy schedule will be summarised. The proportion of patients receiving chemotherapy as planned, and those who experienced dose reductions will be reported including summary statistics for the level of dose reduction. The effect of radiotherapy dose on chemotherapy compliance will be explored.

The proportion of participants within each clinical response status, as assessed by the TRG grading system, at 3 and 6 months post-end of treatment, will be reported. The proportion of participants achieving a Complete Response (CR) at 3 and 6 months will be presented along with 95% CIs. A logistic regression model, adjusted for the minimisation factors will be used to compare each treatment arm with the control (ACT 5 only) for the proportion of participants who have and have not achieved a CR at 6 months post-end of treatment.

Mean scores with corresponding 95% CIs will be calculated for all domains of the EORTC QLQ-C30 and ANL27 for each treatment group and overall, at each follow-up time point. Change in mean score from baseline with 95% CIs will be reported at each follow-up time point. Treatment groups (ACT5 only) will be compared using a mixed effects linear regression model, adjusted for the minimisation factors, baseline QoL scores, time and treatment time interaction as fixed effects. In order to evaluate the clinical significance of any observed differences between the treatment arms, the proportions of patients showing a minimally clinically important improvement/deterioration will be calculated, as per the published guidelines. Exploratory comparisons of patient reported outcomes may be performed between the experimental arms (ACT5).

Descriptive summaries will be presented by treatment arm for the pattern of failure and the proportion of participants with local relapse undergoing salvage surgery. Data will be presented for the type (single or multi-site) and site of recurrence e.g. nodal and or primary tumour site, and if nodal, which lymph nodes showed evidence of relapse. Summary statistics will be reported for the timing of salvage surgery post-randomisation, and the number and proportion of participants with a resection status R0 vs R1/2.

Final analysis of the short-term secondary endpoints will be performed once the final participant has been followed up for at least 6 months. This will include acute toxicities, treatment compliance and clinical response rate (ACT4 and ACT5 only). With the permission of the independent DMEC and TSC, we plan to publish the results of the short term endpoint analysis if this is not deemed to be contraindicated or affect the scientific integrity of the trial.

17.6 Additional considerations

The ACT4 trial was designed as a randomised phase II trial as it was considered too premature to propose a phase III trial given the lack of late toxicity data for the standard-dose IMRT arm. The phase II trial is designed to ensure that desired efficacy rates are plausible with the reduced-dose IMRT arm, using the standard-dose arm as a calibration arm. Ultimately the aim would be to demonstrate non-inferiority of the reduced-dose arm in terms of 3 year locoregional failure and reduced late toxicity with significant patient benefit.

17.7 Translational statistical analysis

Analysis of p16, HPV and loco-regional failure;

The sample size calculations in ACT4 are inflated to allow for a pre-planned analysis of p16/HPV. With 108 in the dose de-escalation arm of ACT4, we will have around 88% power to the test the dose de-escalation strategy and demonstrate efficacy rates of up to 90%. Assuming only 90% of these patients will have samples suitable for analysis, and 85% will be

p16+, we will have 80% power to demonstrate efficacy rates of up to 90% within the p16+ve sub-group.

With respect to the ACT5 question around dose escalation, 140 patients in the pilot and phase II stages of ACT5 give approximately 94 patients in the dose escalated arms. Assuming a relapse rate of 30%, where 85% are p16+ve (n=80) and 15% p16-ve (n=14), there would be 94% power to detect a HR of 4.5 between the p16 +ve/-ve groups. With a lower relapse rate of 20% in the dose escalated arms (as targeted within ACT5), the power would reduce to 85%. With 90% of samples suitable for analysis (so 126 patients with samples at the end of phase II, 84 in the dose escalated arm) and assuming a relapse rate of 30%, 85% p16+ve (n=71), and 15% p16-ve (n=13), there would be 90% power to detect a HR of 4.5 between the p16 +ve/-ve groups. If we assumed a relapse rate of 20% in the dose escalated arms (which is what were are targeting in ACT5), the power would reduce to 78%. The power to demonstrate a relationship between TIL score and relapse within p16 +ve patients is about 80% for a relapse rate of 30% and just over 50% for a relapse rate of 20%.

p16/HPV and TILs will be analysed as part of the main trial analysis (primary endpoints of 3 year loco-regional failure) and as such, results of the biomarker analysis from ACT4 and the pilot/phase II stages of ACT5 will be made available to the DMEC for PLATO with a plan to validate the predictive value in later stages of the platform and inform subsequent biomarker stratification in later stages of the trial (i.e. potentially to inform entry criteria for a phase III expansion of ACT4, or to enrich the phase III extension of ACT5).

Analysis of cfDNA;

Serial data on CTCs and cfDNA (presence/absence and enumeration/ quantification) will be analysed for the cohort of patients in ACT5 (phase II/III) with an estimated 25% risk of recurrence at 1 year. Performance characteristics (including ROC) will be determined for these data (CTC and cfDNA) in relation to complete response and 1 year disease free survival. Comparisons will also be available with the performance of MRI scans at 3 and 6 months (complete response).

For such longitudinal monitoring power calculation methodology is less clearly developed, but with 6 marker values throughout the follow-up period on 80 patients we will only be powered to detect relatively large effects. We will evaluate whether detection of relapse relates to the actual marker level or change over time in these levels.

Analysis of changes in gut microbiome;

The faecal microbiome in patients undergoing radical treatment for anal cancer has not previously been investigated and as such all analyses and comparisons with e.g. long term toxicity outcome measures are exploratory. Our primary outcome measure is the feasibility of collecting and analysing the faecal microbiome in patients enrolled in ACT5 in >65% cases, i.e. obtaining paired, evaluable samples in at least 65% of patients. If we recruit at least 55 patients into the faecal microbiome sub-study, evaluable paired samples in 42 of these will give us 80% power (at the 1-sided 5% significance level) to achieve this, assuming a true paired, evaluable rate of 80%.
Descriptive summary statistics on sample collection and evaluability will be produced. A separate translational analysis plan will be produced describing the associated sample analysis.

18 Trial monitoring

Participating sites and PIs must agree to allow trial-related on-site monitoring, Sponsor audits and regulatory inspections by providing direct access to source data/documents as required. Patients are informed of this in the patient information sheet and are asked to consent to their medical notes being reviewed by appropriate individuals on the Consent Form.

CTRU will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.

18.1 Trial Steering Committee and Data Monitoring and Ethics Committee

The trial will be overseen by an independent Trial Steering Committee (TSC) and Data Monitoring and Ethics Committee (DMEC).

The DMEC will monitor the trial data, safety including SARs, RUSAEs, treatment related mortalities and the associated ethics of the trial. Listings of SARs and RUSAEs will be provided to the DMEC on a regular basis, frequency to be determined at the first DMEC meeting. The DMEC will be provided with detailed un-blinded reports containing the information agreed in the data monitoring analysis plan, by the CTRU, at approximately 12-monthly intervals.

After each review, the DMEC will make their recommendations to the TSC about the continuation of the trial. The DMEC will also be responsible for monitoring the early failure rate data in ACT3 and 4 and acute toxicity data in ACT5 at each of the pre-planned interim analysis time points (Section 17.31)

18.2 Data Monitoring

A Trial Monitoring Plan will be developed and agreed by the Trial Management Group (TMG) and TSC based on the trial risk assessment.

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until it is received, confirmed as not available or the trial is at analysis. However, missing data items will not be chased from participants. The CTRU/Sponsor will reserve the right to intermittently conduct source data verification exercises on a sample of participants, which will be carried out by staff from the CTRU/Sponsor. Source data verification will involve direct access to patient notes at the participating hospital sites and the ongoing central collection of copies of consent forms and other relevant investigation reports.

18.3 Clinical Governance Issues

To ensure responsibility and accountability for the overall quality of care received by participants during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the TSC, Sponsor and, where applicable, to individual NHS Trusts.

19 Quality Assurance Processes

19.1 Quality assurance

The trial will be conducted in accordance with the principles of Good Clinical Practice (GCP) in clinical trials, as applicable under UK regulations, the NHS Research Governance Framework (RGF) and Scottish Executive Health Department Research Governance Framework for Health and Social Care 2006, and through adherence to CTRU Standard Operating Procedures (SOPs).

19.2 Serious breaches

CTRU and Sponsor have systems in place to ensure that serious breaches of GCP or the trial protocol are picked up and reported. Investigators are required to promptly notify the CTRU of a serious breach (as defined in the latest version of the HRA SOP) that they become aware of. A 'serious breach' is a breach which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial.

In the event of doubt or for further information, the Investigator should contact the Senior Trial Co-ordinator at the CTRU.

20 Ethical Considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. Informed written consent will be obtained from the participants prior to randomisation into the study. The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment.

20.1 Ethical approval

The trial will be submitted to and approved by a REC and the appropriate Site Specific Assessor for each participating centre prior to entering participants into the trial. The CTRU will provide the REC with a copy of the final protocol, patient information sheets, consent forms and all other relevant study documentation.

21 Confidentiality

All information collected during the course of the trial will be kept strictly confidential. Information will be held securely on paper and electronically at the CTRU. The CTRU will comply with all aspects of the 2018 Data Protection Act and operationally this will include:

- consent from participants to record personal details including name, address/ email address (if consented to QoL), date of birth, NHS number, hospital number.
- appropriate storage, restricted access and disposal arrangements for participant personal and clinical details.
- consent from participants for access to their medical records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to trial participation.
- consent from participants for the data collected for the trial to be used to evaluate safety and develop new research.
- copies of participant consent forms, which will include participant names, will be sent to the CTRU when a participant is registered/randomised into the trial. Participant NHS number (and name and address/email, if participating in the QoL component) will be collected at baseline, but all other data collection forms that are transferred to or from the CTRU will be coded with a trial number and will include two participant identifiers, usually the participant's initials and date of birth.
- where central monitoring of source documents by CTRU (or copies of source documents) is required (such as scans or local blood results), the participant's name must be obliterated by site before sending.
- where anonymisation of documentation is required, sites are responsible for ensuring only the instructed identifiers are present before sending to CTRU.

If a participant withdraws consent from further trial treatment and / or further collection of data their data will remain on file and will be included in the final trial analysis.

22 Archiving

22.1 Trial data and documents held by CTRU

At the end of the trial, data and the Trial Master File will be securely archived by CTRU in line with the Sponsor's procedures for a minimum of 15 years.

22.2 Trial data and documents held by research sites

Site data and documents will be archived at the participating research sites. Following authorisation from the Sponsor, arrangements for confidential destruction will then be made.

22.3 Participant medical records held by research sites

Research sites are responsible for archiving trial participant medical records in accordance with the site's policy and procedures for archiving medical records of patients who have participated in a clinical trial. However, participant medical records must be retained until authorisation is received from the Sponsor for confidential destruction of trial documentation.

23 Statement of indemnity

The University of Leeds is able to provide insurance to cover for liabilities and prospective liabilities arising from negligent harm. Clinical negligence indemnification will rest with the participating NHS Trust or Trusts under standard NHS arrangements.

24 Trial Organisational Structure

24.1 Responsibilities

24.1.1 Individuals and individual organisations

Chief Investigator (CI) – The CI is involved in the design, conduct, co-ordination and management of the trial. The CI will have overall responsibility for the design and set-up of the trial, and pharmacovigilance within the trial.

Trial Sponsor (University of Leeds) – The Sponsor is responsible for trial initiation management and financing of the trial as defined by Directive 2001/20/EC. These responsibilities are delegated to the CTRU as detailed in the trial contract.

Clinical Trials Research Unit – The CTRU will have responsibility for conduct of the trial as delegated by the Sponsor in accordance with the NHS Research Governance Framework (RGF) and CTRU SOPs. The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs, and the RGF including, randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, monitoring schedule and statistical analysis for the trial. In addition the CTRU will support ethical approval submissions, any other site-specific approvals and clinical set-up, ongoing management including training, monitoring reports and promotion of the trial. The CTRU will be responsible for the day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting and all statistical analyses.

24.1.2 Oversight and trial monitoring groups

Trial Management Group (TMG) – The TMG, comprising the CI, CTRU team, other key external members of staff involved in the trial and a nursing representative will be assigned responsibility for the clinical set-up, on-going management, promotion of the trial, and for the interpretation and publishing of the results. Specifically the TMG will be responsible for (i) protocol completion, (ii) CRF development, (iii) obtaining approval from the REC and supporting applications for Site Specific Assessments, (iv) completing cost estimates and project initiation, (v) nominating members and facilitating the TSC and DMEC, (vi) reporting of serious adverse events, (vii) monitoring of screening, recruitment, treatment and follow-up procedures, (viii) auditing consent procedures, data collection, trial end-point validation and database development.

Trial Steering Committee (TSC) – The TSC, with an independent Chair, will provide overall supervision of the trial, in particular trial progress, adherence to protocol, participant safety and consideration of new information. It will include an Independent Chair, not less than two other independent members and a PPI representative. The CI and other members of the TMG may attend the TSC meetings and present and report progress. The Sponsor will be invited to TSC meetings. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee (DMEC) – The DMEC will include independent membership and will review the safety and ethics of the trial by reviewing interim data during recruitment and the follow-up period. The Committee will meet annually as a minimum.

25 Publication policy

The trial will be registered with an authorised registry, according to the International Committee of Medical Journal Editors (ICMJE) Guidelines, prior the start of recruitment.

The success of the trial depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contributorship. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data,
- drafting the article or revising it critically for important intellectual content,
- and final approval of the version to be published,
- and that all these conditions must be met (www.icmje.org).

In light of this, the Chief Investigator, trial leads and relevant senior CTRU staff will be named as authors in any publication. In addition, all collaborators will be listed as contributors for the main trial publication, giving details of roles in planning, conducting and reporting the trial.

To maintain the scientific integrity of the trial, data will not be released prior to the first publication of the analysis of the primary endpoint, either for trial publication or oral presentation purposes, without the permission of the DMEC and TSC. Plans to publish data following pilot and interim analysis are outlined in Section 17.31. In addition, individual collaborators must not publish data concerning their participants which is directly relevant to the questions posed in the trial until the first publication of the analysis of the primary endpoint.

An electronic copy of peer-reviewed, published papers arising from this research will be deposited in the Europe PubMed Central database.

Authors will acknowledge the support of CRUK using the format, “This work was supported by CRUK [C19942/A19121/CRUK/15/007].and, where possible, display the ‘funded by CRUK’ logo.

26 Dissemination plan

The trial results will be published in peer reviewed medical journals and will be presented at national and international medical conferences. The project team will work with the University of Leeds media office on a press release of trial results.

In addition to this, Investigator meetings will be arranged for research teams at the participating sites and the trial results will be presented. A separate forum will be held for participants. Also, the trial results will be summarised in a lay language which can be provided to participants or next of kin by the treating site. The trial Patient and Public Representatives will be involved in the writing and review of the summary information.

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Appendix A - TNM Staging for Anal Cancer (AJCC v7.0)

Please note: v7.0 of the AJCC (American Joint Committee on Cancer) anal cancer staging listed below must be used for baseline staging assessments.

Primary tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (Bowen disease, high-grade squamous intraepithelial lesion [HSIL], anal intraepithelial neoplasia II-III (AIN II-III)
T1	Tumour 2 cm or less in greatest dimension
T2	Tumour more than 2 cm but not more than 5 cm in greatest dimension
T3	Tumour more than 5 cm in greatest dimension
T4	Tumour of any size invades adjacent organ(s) (eg, vagina, urethra, bladder); *direct invasion of the rectal wall, perirectal skin, subcutaneous tissue, or the sphincter muscle(s) is not classified as T4*
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in perirectal lymph node(s)
N2	Metastasis in unilateral internal iliac and/or inguinal lymph node(s)
N3	Metastasis in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or inguinal lymph nodes
Distant metastasis (M)	
MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Appendix B – ECOG Performance Status

GRADE	ECOG PERFORMANCE STATUS
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 selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair

Appendix C – National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)

Toxicities will be assessed based on the latest National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)
A copy of NCI-CTCAE is provided in the Investigator Site File and may be obtained at:
http://evs.nci.nih.gov/ftp1/CTCAE/About.html

Appendix D – Dose modifications

Note – the guidance in this appendix should be followed wherever possible; deviation is permitted, but should be in line with local practice.
Where information is available on Fluoropyrimidines and DPYD genotyping local guidance on dose adjustments should be followed.

Supportive therapy for all toxicities should be in line with local practice.

DOSE MODIFICATIONS FOR CHEMOTHERAPY TOXICITIES

IMPAIRED RENAL FUNCTION	
GFR **Calculated as per Cockroft and Gault calculation (**Appendix E**)**	**Capecitabine or 5FU**
≥50 mL/min	Full dose (100%)
30 – 49 mL/min	75% dose
<30 mL/min	Stop permanently

IMPAIRED LIVER FUNCTION		
CTCAE Grade	**Description**	**Capecitabine or 5FU**
2	Elevated bilirubin* >1.5 – 3.0 x ULN	75% dose
3	Elevated bilirubin >3.0 – 10 x ULN	Stop permanently
≥2	ALT or AST > 3 x ULN	Interrupt until Grade 1 then restart at 75% dose

*Participants with a diagnosis of Gilberts and elevated Bilirubin up to 3.0 x ULN should adjust dose as per local guidance.

PALMAR PLANTAR ERYTHEMA				
CTCAE Grade	**Description**	**Capecitabine or 5 FU**		
		1st appearance	**2nd appearance**	**3rd appearance**
1	**CLINICAL DOMAIN** Numbness, dysaesthesia/parasthesia, tingling, painless, swelling or erythema. **FUNCTIONAL DOMAIN** Discomfort which does not disrupt normal activities.	Full dose (100%)	Full dose (100%)	Full dose (100%)
2	**CLINICAL DOMAIN** Painful erythema, with swelling. **FUNCTIONAL DOMAIN** Discomfort which affects activities of daily living.	Interrupt treatment until resolved to Grade 0-1, then continue at full dose (100%)	Interrupt treatment until resolved to Grade 0-1, then continue at full dose (75%)	Stop permanently
3	**CLINICAL DOMAIN** Moist desquamation, ulceration, blistering, severe pain. **FUNCTIONAL DOMAIN** Severe discomfort, unable to work or perform activities of daily living.	Interrupt treatment until resolved to Grade 0-1, then restart at 50% dose	Stop permanently	

Supportive therapy can be used according to local policy

ORAL MUCOSITIS			
CTCAE Grade	**Description**	**Capecitabine or 5 FU**	
		1st appearance	**2nd appearance**
1	Asymptomatic or mild symptoms; intervention not indicated	Full dose (100%)	Full dose (100%)
2	Moderate pain; not interfering with oral intake; modified diet indicated	Interrupt until Grade 0 – 1, then resume at 75% dose	Stop permanently
3	Severe pain; interfering with oral intake	Interrupt until Grade 0 – 1, then resume at 50% dose	Stop permanently
4	Life-threatening consequences; urgent intervention indicated	Stop permanently	

Management of chest pain whilst receiving capecitabine or 5FU

- Fluoropyrimidines are known to rarely cause a syndrome of angina like chest pain, which is thought to relate to coronary artery spasm.
- If patients develop angina like pain whilst receiving capecitabine or 5FU, then treatment should be discontinued immediately pending further assessment.
- An ECG must be performed and serum cardiac enzymes (including troponin) measured.
- Patients should be admitted overnight if significant pain has occurred within the previous 24 hours (with repeat ECGs and serum cardiac enzymes).
- If abnormalities are found on ECG or serum cardiac enzyme levels, then a cardiology opinion should be considered.
- If chest pain is deemed to be capecitabine or 5FU related, patients should not recommence this treatment. If the patient is fit to continue treatment once the episode resolves and the treating consultant agrees it is appropriate to do so, please discuss with the Trial Management Group to consider whether raltitrexed should be used as an alternative to the fluropyrimidine

DOSE MODIFICATIONS FOR CHEMOTHERAPY AND RADIOTHERAPY TOXICITIES

DIARRHOEA			
CTCAE Grade	**Description**	**Capecitabine or 5FU**	**Radiotherapy**
1	Increase of < 4 stools per day **over baseline**; mild increase in ostomy output compared to baseline	Full dose (100%)	Continue
2	Increase of 4 – 6 stools per day **over baseline**; moderate increase in ostomy output compared to baseline; Moderate cramping	Continue as long as patient considered fit for treatment.	Continue Manage as clinically indicated (eg. Loperamide, ensure oral hydration maintained)
3	Increase of > 7 stools per day **over baseline**; severe increase in ostomy output compared to baseline; limiting self-care ADL; Severe cramping or peritonism (localised guarding on abdominal examination)	Interrupt until Grade 0 – 1, ≤ 6 mg loperamide per 24 hours required, and patient considered fit, then recommence at 75% dose.	For incontinence - continue. Management as per clinically indicated (eg. loperamide, codeine, iv hydration, monitor renal function), consider inpatient management for treatment and support. Check that stoma is avoided from radiotherapy portals. **Do not treat if localised peritonism**
4	Life threatening consequences; urgent intervention indicated	Stop permanently.	Interrupt until resolved to Grade 2. Reassess daily

VOMITING			
CTCAE Grade	**Description**	**Capecitabine or 5FU**	**Radiotherapy**
3	≥ 6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalisation indicated	Interrupt until resolved to Grade 0 or 1, then restart with additional antiemetic and at 75% dose	Continue if haemodynamically stable. Manage as per clinically indicated (eg. s/c antiemetics, IV hydration, consider TPN). Rule out alternative causes of vomiting (obstruction, ischaemic bowel etc).
4	Life-threatening consequences; urgent intervention indicated	Discontinue permanently	Interrupt until resolved to Grade 0 - 2. Manage as per clinically indicated (eg. s/c antiemetics, iv hydration, consider TPN). Rule out alternative causes of vomiting (obstruction, ischaemic bowel etc).

HAEMATOLOGICAL			
CTCAE Grade	**Description**	**Capecitabine or 5FU**	**Radiotherapy**
1	Haemoglobin ≥10.0g/dL – LLN	Full dose (100%)	Continue
	Neutrophils ≥1.5 x 10^9/L – LLN	Full dose (100%)	Continue
	Platelets ≥75 x 10^9/L - LLN	Full dose (100%)	Continue
2	Haemoglobin <10.0 - 8.0g/dL	Full dose (100%)	Continue
	Neutrophils <1.5 – 1.0 x 10^9/L	Full dose (100%)	Continue
	Platelets <75 – 50 x 10^9/L	Interrupt until resolved to Grade 0 or 1 then continue at full dose (100%)	Continue
3*	Haemoglobin <8.0g/dL transfusion indicated.	Interrupt until resolved to Grade 0 or 1 then restart at 75% of starting dose	Continue. Transfuse in the next 24-48 hours.
	Neutrophils <1.0 – 0.5 x 10^9/L.	Interrupt until resolved to Grade 0 or 1 then restart at 75% dose	Continue. Prophylactic Antibiotics (eg. Ciprofloxacin 500mg BD)
	Platelets <50 – 25 x 10^9/L	Interrupt until Grade 0 or 1, then resume at 75% dose	Continue. Consider platelet transfusion if clinically indicated (eg. bleeding).
If patient is neutropenic and has sepsis,		Stop permanently	Continue, provided patient haemodynamically stable and considered fit for treatment.
4*	Haemoglobin - Life threatening consequences; urgent intervention indicated	Discuss with the TMG	Interrupt until Grade 2. Emergency transfusion, consider other causes of falling Hb (eg. bleeding).
	Neutrophils < 0.5 x 10^9/L	Stop permanently	Continue. Prophylactic Antibiotics (eg. Ciprofloxacin 500mg BD)
	Platelets < 25 x 10^9/L	Stop permanently	Interrupt until Grade 2. Consider platelet transfusion. Consider other causes of thrombocytopenia.

***Frequent blood tests must be performed in the presence of grade 3 or 4 haematological toxicity. This will range from a minimum of twice per week to daily depending on clinical circumstances**

RADIATION DERMATITIS			
CTCAE Grade	**Description**	**Capecitabine or 5FU**	**Radiotherapy**
1	Follicular, faint or dull erythema/epilation/dry desquamation/ decreased sweating.	Full dose (100%)	Continue
2	Tender or bright erythema, patchy moist desquamation/moderate oedema.	Full dose (100%)	Continue. Manage skin toxicity as clinically indicated (eg. aqueous cream or hydrocortisone on intact skin, hydrogel and non-adhesive / silicone based dressings as appropriate on areas of desquamation).
3	Confluent, moist desquamation other than skin folds, pitting oedema.	Full dose (100%)	Continue. Manage skin toxicity as per local protocol (eg. aqueous cream on intact skin, hydrogel and non-adhesive / silicone based dressings as appropriate on areas of desquamation). Manage pain with paracetamol, weak analgesics using WHO pain control ladder
4	Ulceration, haemorrhage, necrosis.	Stop permanently	Interrupt until Grade 3.

OTHER NON-HAEMATOLOGICAL TOXICITY		
CTCAE Grade	**1st appearance**	**2nd appearance**
1	- Full dose (100%) chemotherapy with supportive treatment - Continue radiotherapy	- Full dose (100%) chemotherapy with supportive treatment - Continue radiotherapy
2	- Interrupt chemotherapy treatment until resolved to Grade 0-1, then continue at full dose (100%) with prophylaxis where possible - Continue radiotherapy	- Interrupt chemotherapy treatment until resolved to Grade 0-1, then restart at 75% dose - Continue radiotherapy
3	- Interrupt chemotherapy treatment until resolved to Grade 0-1, then consider restart at 75% dose if deemed suitable by treating clinician - Please contact trial team for advice on radiotherapy interruptions if ≥G3 toxicity excluding PPE, diarrhoea, mucositis and deranged liver function tests, haematological, radiation dermatitis or vomiting.	- Discontinue chemotherapy permanently
4	- Discontinue chemotherapy permanently - Please contact trial team for advice on radiotherapy interruptions if ≥G3 toxicity excluding PPE, diarrhoea, mucositis and deranged liver function tests, haematological, radiation dermatitis or vomiting.	

UNPLANNED INTERRUPTIONS IN RADIOTHERAPY TREATMENT

See Section 11.2.6

Appendix E - Cockroft & Gault formula

Males

GFR= $\frac{1.2 \times [140\text{-age}] \times \text{wt (kg)}}{\text{serum Creatinine (µmol/l)}}$

Females

GFR= $\frac{1.2 \times [140\text{-age}] \times \text{wt (kg)} \mathbf{\times 0.85}}{\text{serum creatinine (µmol/l)}}$

- A patient with a Cockcroft & Gault estimate of GFR ≥ 50 ml/min using the above formula is eligible for entry into the trial.
- See Appendix D for dose modifications in the event that the calculated GFR falls below 50ml/min

Appendix F - Tumour regression grading (TRG) for anal cancer post treatment MRI

GRADE	Description
1	Complete response with no evidence of tumour and normal appearances of the anus
2	Excellent response with only low signal post treatment fibrotic change and no evidence of tumour
3	Moderate response with reduction in size but evidence of intermediate tumour signal in keeping with residual disease
4	Minimal response with reduction in size but evidence of intermediate tumour signal in keeping with residual disease
5	No response of the primary tumour or frank tumour progression

Kochhar R, Renehan A, Mullan D, Chakrabarty B, Saunders M, Carrington B. The assessment of local response using magnetic resonance imaging at 3- and 6- month post chemoradiotherapy in patients with anal cancer. *European Radiology* (in press).