CEDAR Protocol

Full title: A Phase 1 trial of the safety, tolerability and biological effects of intravenous Enadenotucirev, a novel oncolytic virus, in combination with chemoradiotherapy in locally advanced rectal cancer

Short title: **C**hemoradiation with **E**na**d**enotucirev as a radiosensitiser in locally **A**dvanced **R**ectal cancer

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Conflict of Interest statement Details of potential conflicts of interest are given on page 3

Confidentiality Statement This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Trial Office, the Investigator Team, host NHS Trust(s), regulatory authorities, and members of the Research Ethics Committee unless authorised to do so.

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Patient Registration: To register a patient on the trial please email the completed and scanned Registration Form to the CEDAR Trial Office: octo-CEDAR@oncology.ox.ac.uk

Protocol Authors and conflict of interest statement

Translational Research Collaborators Len Seymour and Kerry Fisher own equity in PsiOxus Therapeutics Limited – their role within the trial is limited to scientific advisors. They are not involved directly in the trial design, management or interpretation of trial results and have no decision making roles at any stage of the trial.

PsiOxus are providing enadenotucirev and contribute to funding for CEDAR.

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PROTOCOL SYNOPSIS

Full Title :	A Phase 1 trial of the safety, tolerability and biological effects of intravenous enadenotucirev, a novel oncolytic virus, in combination with chemoradiotherapy in locally advanced rectal cancer	
Short Title:	Chemoradiation with Enadenotucirev as a radiosensitiser in locally Advanced Rectal cancer	
Trial Acronym:	CEDAR	
Clinical Phase:	Phase 1	
Trial Design:	Interventional	
	Objectives	Endpoints
Primary Endpoint:	Determine the optimal dose and frequency of enadenotucirev that can be administered with chemoradiation	• Dose limiting toxicities • MRI tumour regression grade
Secondary Endpoints:	Ability to deliver enadenotucirev concurrently with chemoradiation	Treatment tolerance measured by proportion of patients completing at least 80% of the prescribed capecitabine dose and at least 20 fractions of radiotherapy
	To measure local response rate to combined therapy	• MRI tumour regression grade • Pathological complete response rate • Neoadjuvant rectal score
Investigational Medicinal Product(s)	Name of drug	Formulation, dose, route of administration
	- Enadenotucirev	- Solution, diluted and delivered intravenously There are 2 potential doses given over 4 different dosing levels: • 1 x 10^{12} vp (viral particles) • 3 x 10^{12} vp
	- Capecitabine	- Tablet, 900mg/m^2, oral administration
Other interventions:	Neoadjuvant radiotherapy	
Treatment Duration	9 weeks	
Follow-up duration	Trial follow-up visits continue until week 13. Following this, patients will receive surgery and on-going follow up as standard of care.	
End of Trial	Last Patient Last Visit (i.e. 4-6 weeks post-surgery visit)	

SUMMARY SCHEDULE OF EVENTS

Procedure	Screening / Baseline Day -14 - 1	Week 1/2			Week 3[3]		Week 4	Week 5	Week 6	Week 7	Week 8/9[5]			Week 13 (+/- 3 days) EOT VISIT	Week 14 +	4-6 weeks post-surgery
		Dosing day 1	Dosing day 2	Dosing day 3	Day 1	Day 5[6]					Dosing day 1	Dosing day 2	dosing day 3			
Informed consent	X															
Demographic & medical history	X															
Height	X															
Weight	X	X		X	X		X	X	X	X	X		X	X		
Vital signs [1]	X	X	X	X	X	X[6]	X	X	X	X	X	X	X	X		
Haematology	X	X[12]		X[12]	X	X[6, 12]	X	X	X	X	X[12]		X[7, 12]	X		
Biochemistry	X	X[12]		X[12]	X	X[6, 12]	X	X	X	X	X[12]		X[7, 12]	X		
Coagulation profile	X	X[12]		X[12]	X	X[6, 12]	X	X	X	X	X[12]		X[7, 12]	X		
Urine dipstick	X	X		X	X	X[6]	X	X	X	X	X		X[7]	X		
Pregnancy test (FCBP only)	X															
ECG	X															
DPYD deficiency test[14]	X															
Enadenotucirev		X[2]	X[2]	X[2]	X[3] (dose schedule 4 only)	X[3] (dose schedule 4 only)					X[5] (dose schedules 2,3 & 4)	X[5] (dose schedules 2,3 & 4)	X[5] (dose schedules 2,3 & 4)			
Radiotherapy					XXXXX		XXXXX	XXXXX	XXXXX	XXXXX						
Capecitabine					(X)XXX(X)		XXXXX	XXXXX	XXXXX	XXXXX						
Tumour Biopsy	X[9]						X									
Investigational Blood Sample	X[8]						X[8] DAY 10							X		X
Faecal sample & questionnaire[13] (Optional)	X			X			X			X				X		
AE assessments		X	X	X	X	X[6]	X	X	X	X	X	X	X	X		X
DLT assessments		X	X	X	X	X[6]	X	X	X	X	X	X	X	X		
Physical exam	X	X	X	X	X	X[6]	X	X	X	X	X	X	X	X		

Procedure	Screening / Baseline Day -14 - 1	Week 1/2			Week 3[3]		Week 4	Week 5	Week 6	Week 7	Week 8/9[5]			Week 13 (+/- 3 days) EOT VISIT	Week 14 +	4-6 weeks post-surgery
		Dosing day 1	Dosing day 2	Dosing day 3	Day 1	Day 5[6]					Dosing day 1	Dosing day 2	dosing day 3			
ECOG Performance Status	X	X	X	X	X	X[6]	X	X	X	X	X	X	X	X		X
Concomitant Meds	X	X	X	X	X	X[6]	X	X	X	X	X	X	X	X		
CT Chest/Abdo/ Pelvis	X[4]													X[10]		
Pelvic MRI	X[4]													X[11]		
Surgery															X	
Neoadjuvant rectal score (NAR)																X
Pathological resection specimen findings															X	

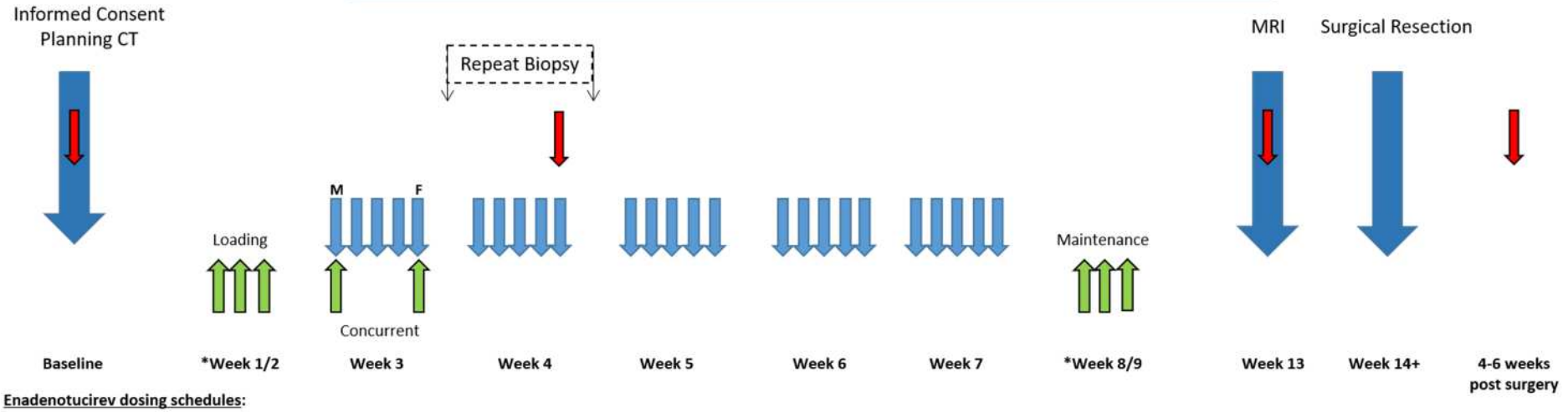
1 - Vital signs include: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature
2 - Virus loading administration given on at least alternate days for 3 doses.
3 - For dosing schedules 1, 2 & 3, no enadenotucirev dosing is required in week 3. A **single visit** should be completed to assess the patient
4 - Standard of care diagnostic CT and pelvic MRI are acceptable as baseline imaging if performed within 62 days (CT) & 42 days (MRI) of start of treatment. If > 62 (CT) or 42(MRI) days these should be repeated after patient has given informed consent (within 14 days of consent date). Whole body PET-CT is an acceptable alternative to CT.
5 - For dosing schedules 1 no enadenotucirev dosing is required in weeks 8-9. A single visit should be completed to assess the patient post completion of CRT with the 2nd & 3rd visits omitted
6 - These assessments apply to schedules 4 only
7- These assessments apply to groups 2, 3 & 4
8. Additional, investigational blood sample must be taken AFTER consent/ registration, for the week 4 blood sample aim to be on the same day of the biopsy
9. Archival tumour tissue sample
10. Only complete if local site policy
11. Pelvic MRI for week 13 visit can be +/- 7 days
12. Additional blood sample – (taken as standard at other time points)
13. Not applicable to all sites
14. Any time before trial enrolment (see Section 9.4).

Trial Flow chart

CEDAR: Chemoradiation with EnaDenotucirev as a radiosensitiser in locally Advanced Rectal cancer

Timescale of events

Chemoradiation 50 Gy in 25 fractions with concurrent capecitabine
Blue = Standard of care interventions



Enadenotucirev dosing schedules:

Dose schedule	Loading x 3 (pre CRT)	Concurrent		Maintenance x 3 (post CRT)
		Week 1 Day 1 CRT	Week 1 Day 5 CRT	
1 (start level)	Low-Low-Low[1*]			
2	Low-Low-Low[1*]			Low-Low-Low[1*]
3	Low-High-High[2*]			Low-High-High[2*]
4	Low-High-High[2*]	3 x 10^{12} vp	3 x 10^{12} vp	Low-High-High[2*]

Enadenotucirev – I.V

Research blood sample

[1] *Low-Low-Low = 3 x doses @ 1 x 10^{12}VP*
[2] *Low-High-High = 3 Doses, (1) 1 x 10^{12}VP, (2) 3 x 10^{12}VP, (3) 3 x 10^{12}VP*
** 3 doses administered on 3 non-consecutive days over 7 days*

ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
ALT/ AST	Alanine/ Aspartate Transaminase
ANC	Absolute Neutrophil Count
BP	Blood Pressure
BSA	Body Surface Area
CRF	Case Report Form
CRT	Chemoradiotherapy
CRM	Circumferential Resection Margin
CRUK	Cancer Research United Kingdom
CSM	Centre for Statistics in Medicine
CT	Computed Tomography
CTA	Clinical Trial Agreement/ Clinical Trial Authorisation
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
ECOG	Eastern Co-operative Oncology Group
ECG	Electrocardiogram
Enadenotucirev	Enadenotucirev
FBC	Full Blood Count
GCP	Good Clinical Practice
Gy	Gray
Hb	Haemoglobin
HCG	Human Chorionic Gonadotropin
HTA	Human Tissue Act
IB	Investigator Brochure
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
IMRT	Intensity Modulated Radiotherapy
IV	Intravenous
LARC	Locally advanced rectal cancer
LCCR	Long course chemo-radiotherapy
LDH	Lactate dehydrogenase
LPLV	last visit of the last patient undergoing the trial
MDT	Multi-disciplinary team
MHRA	Medicines and Healthcare products Regulatory Agency
MRF	Mesorectal Fascia
MRI	Magnetic Resonance Imaging
mrTRG	Magnetic resonance Tumour Regression Grade
MTD	Maximum Tolerated Dose
NCI CTCAE	National Cancer Institute - Common Terminology Criteria for Adverse Events
OAR	Organs At Risk
OCTO	Oncology Clinical Trials Office
OCTRU	Oxford Clinical Trials Research Unit
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
pCR	Pathological complete response
PT	Prothrombin Time
REC	Research Ethics Committee
RIOC	Radiotherapy & Imaging Oversight Committee
RSI	Reference safety information
RTTQA	Radiotherapy Trials Quality Assurance
SAR	Serious Adverse Reaction

SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Drug Reaction
TiTE CRM	Time To Event Continual Reassessment Method
TME	Total Mesorectal Excision
TMG	Trial Management Group
TNM	Tumour, Node and Metastasis Staging System
ULN	Upper Limit of Normal
Vp	Viral particles
WBC	White Blood Cells

1 INTRODUCTION

1.1 Background

The use of radiotherapy (RT), in combination with chemotherapy and/or surgery, is the main curative treatment modality and standard of care in locally advanced rectal cancer. Improvements in locoregional control and overall survival have been realised with small increments in each of the combined modality treatments (surgery, radiotherapy and chemotherapy). Precision radiotherapy is delivered as highly conformal image guided external beam radiotherapy and offers opportunities to combine novel anticancer agents due to reduced toxicity. Despite the promise of increased cure rates when pairing novel treatment modalities with precision radiotherapy, the majority of phase I clinical trials are generally conducted in non-curative, metastatic populations. An alternative approach of targeting tumours earlier in their history might increase cure rates especially when the additional agents augment local tumour control and /or ablate micro- or oligo metastatic disease. The strategy holds tremendous potential to increase survival for a significant number of patients who might otherwise develop metastases, and/or fail locally.

In 2010-12 11,567 people were diagnosed with rectal cancer in UK (1). Locally advanced rectal cancer (LARC) is defined by the presence of T3/4 disease and/or nodal involvement in the absence of distal metastatic disease. Patients with locally advanced disease requiring radiotherapy can represent up to 30% of this population and often have a poor outcome. The lack of a universally accepted standard radiotherapy treatment remains a challenge.

Optimal outcomes are achieved with a multidisciplinary approach, with the aim of achieving local control with successful sphincter preservation and reducing the risk of distant recurrence. For many years, the standard of care has been a multimodal approach incorporating neoadjuvant long-course chemoradiotherapy (CRT) with concurrent fluoropyrimidine followed by total mesorectal excision surgery. With this approach, local recurrence rates have fallen from 30%-45% to <10%. Attempts to add additional chemotherapy, such as oxaliplatin or irinotecan have not meaningfully improved outcomes, whilst concurrent biologic therapies (vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), Poly(ADP-ribose) polymerase (PARP) inhibitors, etc.) have shown, in part, promising results at the expense of increased toxicity but possibly did not enhance radiosensitisation as local control remains unchanged. This paradigm as intensification of local treatments to achieve better response have had limited impact due to increased toxicity, and more than one in 5 patients still develop metastatic disease (2).

An alternative approach is to offer short course radiotherapy, 25Gy over 5 fractions in one week, and proceed to surgery within a week. This approach is often coupled with neoadjuvant or adjuvant courses of chemotherapy. Multiple studies have failed to show the superiority of one approach over the other (3,4).

The current research paradigm includes two priority research areas: treatment de-intensification with the option of organ preservation (surgery deferral) and treatment intensification using total neoadjuvant treatment (TNT) approach where chemotherapy is used prior to chemoradiation, or short course radiotherapy, and surgery. These are on the highest priority list for cancer related questions on the Association of Coloproctology of Great Britain and Ireland (ACPGBI) which were designed to reach a consensus on prioritizing clinical research questions in colorectal disease (5). At present identifying novel radiosensitising agents in colorectal cancer is an area of high need for patients considering sphincter preserving surgery or needing downstaging to facilitate surgery, and neoadjuvant systemic treatment is thought to be required for patients at risk of developing metastases, especially as the use of adjuvant chemotherapy is controversial in terms of benefit. Radical surgery can be associated with significant morbidity and

mortality in elderly populations and these patients would benefit from improving radiation response. Rectal cancer also offers excellent translational opportunities as the majority of patients proceed to surgery.

Oncolytic viruses are novel anticancer agents that target, selectively replicate in, and kill cancer cells, spreading through the tumour microenvironment, generating local and systemic immune response both to themselves as well as the dying tumour. Radiotherapy elicits a multitude of biological responses and the evidence from experimental models suggests that the irradiated tumour is "converted" in an in situ vaccine (6,7). There is some evidence that higher dose per fraction radiotherapy may be more useful in eliciting an immune response, providing further rationale for the inclusion of patients receiving short course radiotherapy. RT can modulate the expression of a large number of cellular genes involved in cell cycle checkpoints, cellular stress, DNA repair and apoptosis (8). Combining radiotherapy with immunotherapy is emerging as a further opportunity to improve therapeutic results and is a seen as a priority for research. Enadenotucirev would address the combined requirements as the therapy would act as both a local (DDR inhibitor/ direct tumour kill) and systemic (immune response) agent.

1.2 Investigational Medicinal Product(s) used in the trial

Enadenotucirev
Enadenotucirev is a group B oncolytic adenovirus under development for the systemic treatment of metastatic or advanced epithelial tumours. Enadenotucirev is a chimeric adenovirus type 11p (Ad11p)/adenovirus type 3 (Ad3) virus, discovered through a process of bio-selection from a library of chimeric viruses produced from a pool of adenoviruses from seven different serotypes utilizing human HT-29 colorectal cancer (CRC) cells (9) .

Enadenotucirev shows selective and potent toxicity in human carcinoma cells with only very limited or no toxicity to normal (non-cancerous) human cells. Other than humans, there is no known permissive species for enadenotucirev. The principal advantage of oncolytic therapy is that the drug (the virus) replicates only in diseased cells meaning that the concentration of drug is amplified at the site of pathology so that it is higher in the tumour than in healthy tissue. Virus particles spread from cell to cell within a tumour nodule until they reach non-permissive normal tissues, in principle destroying all viable tumour cells they encounter (10,11)

While the overall understanding of the mechanism of action of enadenotucirev in humans is still under investigation, it is now well established from both non-clinical and clinical studies that the mechanism of anti-cancer efficacy of oncolytic viruses not only involves direct infection and lysis of tumour cells, but that immune responses stimulated via an increased release of tumour-associated antigens and immune-inflammatory activation signals also play a key role. The direct oncolytic efficacy of enadenotucirev has been demonstrated in a number of non-clinical studies with human tumour cells in vitro and in vivo. Replication of the enadenotucirev virus in carcinoma cells results in direct necrolytic killing of carcinoma cells by a non-apoptotic, immunogenic cell death mechanism which would be expected to trigger immune cells. However, it has not been possible to directly demonstrate this mechanism in non-clinical studies due to the human specificity of enadenotucirev. Full details of the enadenotucirev non-clinical development are provided in the Investigator's Brochure.

Two clinical studies of enadenotucirev given as a monotherapy are completed and two studies of enadenotucirev in combination regimens are on-going. The two completed studies were a first in human study that included dose escalation and dosing schedule evaluation in metastatic epithelial solid tumours (ColoAd1-1001) and a mechanism of action study (ColoAd1-1002). The on-going studies are dose finding studies of intraperitoneal administration or intravenous (IV) infusion of enadenotucirev in combination with paclitaxel and IV infusion of enadenotucirev in combination with nivolumab.

In the completed studies, a total of five subjects received intratumoural injections of enadenotucirev. These were well tolerated and no subjects had any treatment related adverse events. However, systemic exposure to enadenotucirev was much lower than with IV infusions. Seventy three subjects have received IV infusions of enadenotucirev at doses from 1×10^{10} vp to 1×10^{13} vp. Of these, 40 subjects received a single cycle of treatment and 33 subjects received repeat cycles of treatment up to a maximum of six cycles. Findings in these subjects can be summarised as follows:

- Dose-limiting toxicities of acute lung injury and dyspnoea with hypoxia were experienced after the first dose with 1×10^{13} vp
- The overall frequency and severity of adverse events including severe hypoxia and increased transaminases following doses of 6×10^{12} vp resulted in this dose being determined as not tolerated

- A dose of 3 x 10^{12} vp is considered the maximum tolerated dose (MTD)
- At the MTD the majority of subjects experience adverse events of an inflammatory nature particularly pyrexia and chills which have onset within 24 hours of dosing. The frequency of these type of events is lower after subsequent doses and appear to reflect the cytokine responses to treatment
- Adverse events relating to general disorders and gastrointestinal disorders were the most common type of events considered related to treatment with IV infusion of enadenotucirev
- Further experience with enadenotucirev is required to understand the frequency and severity of other common events such as nausea, vomiting and fatigue and other findings such as thrombocytopenia and proteinuria
- Serious adverse events (SAEs) were reported in about a third of subjects. The majority of the SAEs were single cases. However, there were seven reports of obstruction of the intestines which were considered unrelated to treatment in five subjects. The other two subjects had early onset after dosing but also had other predisposing conditions, such as a history of abdominal surgery, although the obstruction could also be explained by tumour flare. Other SAEs occurring in more than one subject were hypoxia, pyrexia and dyspnoea in three, three and two subjects, respectively
- There was no apparent difference in tolerability when 3 x 10^{12} vp were dosed with the 3-weekly or weekly schedule
- Viral kinetic data suggest enadenotucirev has a half-life of 17 minutes in keeping with other described iv adenoviruses. No infectious virus was detected later than 48 hours after dosing in blood samples
- Shedding data has indicated a positive signal by qualitative polymerase chain reaction (qPCR) analysis from rectal and buccal swabs and urine samples with little evidence for long-term shedding after dosing. However, the analysis cannot discriminate between infectious virus and inactive virus or fragments of viral deoxyribonucleic acid (DNA)
- Anti-enadenotucirev antibodies have been measured in the majority of subjects and levels increase until about Day 15 then plateau irrespective of whether a subject receives one or repeated cycles of treatment

A further DLT of Grade 4 acute renal injury has also been seen in a single subject with Stage IV colon cancer in the on-going study with enadenotucirev and nivolumab. The subject presented with clinical symptoms from around Day 21 with marked decline in renal function by Day 28 and required long term dialysis; the patient subsequently died from disease progression. Although the subject met the eligibility criteria for study entry (with adequate renal function) he had a past history of methotrexate-related renal injury and chronic kidney disease that was not declared at study entry.

Two similar events had also been reported in the completed study of enadenotucirev monotherapy (Study ColoAd1-1001). Review of the available data from the 61 patents treated in this study appears to indicate two separate time periods following enadenotucirev administration when there are discernible effects on renal function (although the majority of these are noted to not have been considered of clinical significance or reported as adverse events). The first in the acute period following dosing (on Days 1, 3, 5) where proteinuria, reduction in albumin, reduction in blood pressure and reduced renal function are likely to be related to cytokine-mediated vascular leakage, in turn recovering over time. In some subjects, similar findings occur around Day 21 onwards, again, in most cases, resolving over time. The latter appear not to be related to dose or schedule.

In the overall context of three patients out of 100 treated with intravenous enadenotucirev presenting with significant renal injury, the measures that have been and are to be implemented in the on-going studies of enadenotucirev are considered appropriate to managing the potential risk to patients in the context of treatment of their underlying disease. These measures, excluding those considered broadly to be at increased risk of renal injury, serve to identify any decline in renal function quickly with a view to implementing early intervention to any renal injury to in turn limit the damage of any recurrence of such an event

Low-High-High dosing regimen
Since the cytokine release is believed to be the most important mediator of the adverse effects of viral therapy, this tolerising effect allows subsequent doses to be delivered with a lower risk of high grade side effects. Splitting the dose may therefore allow a higher and more effective dose without dose limiting toxicities.

After the first dose has been administered, the cytokine response to subsequent doses of enadenotucirev is attenuated when delivered within a few days of the first dose.

Attenuation of this cytokine response by administration of a well-tolerated day 1 dose of enadenotucirev to desensitise the cytokine response would allow the potential for delivery of higher subsequent doses to be tolerated. In turn this may allow more virus to be delivered to the tumour and increase the potential of clinical benefit. This type of dosing schedule is referred to as a "low-high-high" dosing regimen and has been successfully implemented in clinical studies of other systemically administered oncolytic viruses (Laurie et al, 2006).

Dosing groups 1 & 2 remain unchanged and will use a 'low-low-low' dosing regimen where are all 3 doses pre and post chemoradiotherapy will be given at $1x10^{12}$VP.

The low-high-high dosing regimen will be used for the pre and post chemoradiotherapy doses of enadenotucirev in dosing groups 3 & 4. The low dose is 1×10^{12}VP and the high dose is 3×10^{12}VP. All pre and post chemoradiotherapy doses will continue to be given on non-consecutive days, over a 7 day period.

Capecitabine
Capecitabine is a chemotherapy drug licensed for use in rectal cancer, it is a non-cytotoxic pre-cursor of the cytotoxic 5-fluourouracil (see SPC for more details).

1.3 Rationale for the trial

Non-clinical
Although the proposed trial of enadenotucirev with radiation is novel, there is a wealth of evidence to support the rationale for combining this class of agent with radiation. Adenoviruses have developed a range of interactions with cellular DNA damage repair proteins to allow successful viral replication. This has implications for the initiation of a number of DNA repair pathways activated in response to radiation-induced damage, in particular all adenoviral serotypes tested appear to target NHEJ repair (12). The hypothesis that oncolytic adenovirus infection would work synergistically with radiotherapy has been tested by a number of groups. The combination of CG7870 with radiation resulted in a synergistic increase in cell killing, both in vitro and in vivo in the LNCaP xenograft model, than either agent alone (13). Toth et al studied 3 Ad5-based vectors and combined them with radiation in A549 lung cancer cells (14)[1]. Again, they found that in vivo and in vitro tumour cell kill was increased with the combination approach than was seen with either agent alone. Similar findings have been noted with a variety of different adenoviral vectors in other cell types including ovarian cancer cell lines (15) and glioma xenografts (16). Importantly however the effect of radiosensitization does not appear to extend to normal tissues. The combination of Ad5/CMV/p53 synergistically radiosensitised two non-small cell lung cancer cell lines (A549 and H322) in vitro and in xenograft models, in a synergistic fashion, but showed increased radiosensitization effect on normal lung fibroblasts (17).

Clinical
To date, clinical experience with virus/radiation combinations has been limited to local (most commonly intratumoural) administration. This mode of delivery facilitates direct infection ensuring correct dosing and avoids rapid hepatic uptake seen with systemic delivery (18). The downside is only tumour types that can be easily accessed with a needle, such as skin, head and neck cancers, prostate cancers, are considered suitable for clinical trials. Nevertheless, the results of these studies provide us with useful mechanistic indicators as well as guiding assessment of toxicity. Whilst the authors acknowledge there are other oncolytic viruses in clinical practice we will focus on the clinical experience with adenoviral agents.

A study of intraprostatic injection of an oncolytic Ad5 PSE/PBN E1A-AR (Ad5: adenovirus 5; PSE: prostate-specific enhancer; PBN: rat probasin promoter; E1A: early region 1A; AR: androgen receptor), combined with either low or high dose rate radiation therapy, showed few side effects (19). Although DNA damage, as assessed by γH2AX foci, viral replication and viral induced cell death all favoured the high dose radiation arm, the side effect profile was similar in both arms. This indicates that the therapeutic efficacy is separate from the toxicity, in contrast to traditional radiosensitisers where a higher dose often increases both efficacy and toxicity. These findings support the large body of preclinical data, along other prostate specific adenovirus constructs, that there is little additive toxicity to that seen with either agent alone (20).

A Phase I trial of intraprostatic injection of a replication competent adenovirus in combination with radical dose (74Gy delivered in daily 2 Gy fractions) of intensity modulated radiotherapy (IMRT) showed no significant differences in gastro-intestinal or genitourinary toxicity in comparison to the toxicity seen when administering the adenovirus as a

single agent (21). The investigational agent had already proven safe and efficacious as a single agent (22). These results were confirmed in a follow on randomized Phase II trial (23). There was a non-significant 42% reduction in biopsy positivity in the investigational arm suggesting improved efficacy and synergy with radiation. Clinical outcomes at 2 years show no difference, likely reflecting the excellent prognosis of both groups. A Phase II/III (ReCAP) open label adaptive trial of 280 men, randomized to combination treatment or radiation efficacy with biochemical failure free survival as the primary endpoint (24). Other groups have shown that administering a different type of adenovirus is safe, both concurrently and after radiation to the prostate (25,26) ,when all cells should be at maximal damage and repair rates. Again, the viral compound was administered intratumourally.

There is also evidence from early phase clinical trials that a combination approach of radical dose (76Gy delivered in 2.17 Gy daily fractions) with Ad5 replication defective adenoviral vector stimulates a systemic response (27). IMRT was commenced 48 hours after the second of three doses of the viral agent, therefore patients were effectively loaded and then treated concurrently. Again, drug was administered intraprostatically. Both HLA DR+ CD8+ and CD4+ T cells were increased in the combination arm compared to the radiation alone arm suggesting the potential development of a Th1 response.

In a mixed solid tumour cohort an adenovirus vector under the control of EGF-1 promoter was combined with radiation in 36 patients (28). 70% of subjects showed evidence of a partial response with the main side effects relating to intratumoural administration of the agent. Using the same agent in combination with chemoradiation for squamous cell carcinomas of the Head and Neck in a Phase I dose escalation trial was performed (29), again with intratumoural administration. The main dose limiting toxicity seen was thrombosis with no increase in acute radiation side effects incidence or intensity, underlining the safety of the combination approach. Loco regional response was 83.3%. Preclinical studies with this agent have shown impressive ability to suppress regional metastatic node formation highlighting its ability to influence intrinsic tumour biology (30). The same agent was also combined with incrementally increasing doses in combination with radiation in soft tissue extremity sarcoma (31). No dose limiting toxicity (DLT) was noted and the combination was well tolerated. 91% of patients undergoing surgery showed a pathological complete response to treatment highlighting significant potential synergy between both agents. The same adenovirus composite has been successfully combined with radical chemoradiotherapy (50.4Gy delivered in 1.8Gy daily fractions concurrently with fluoropyrimidine) for locally advanced pancreatic cancer in a non-randomized Phase I/II setting (32). The main DLTs were pancreatitis and cholangitis but no specific increases in observed acute radiotherapy or chemotherapy side effects, respectively, were seen. The adenovirus was administered intratumourally.

Combination of yet another adenovirus, designed to transfer p53 to malignant cells, in a radically treated non- small cell lung cancer population has shown impressive response data (33). This prospective Phase II trial of 60 Gy in combination showed no evidence of pathologically viable tumour in 63% of patients (12 out of 19) evaluable. The most common adverse events were virus related; fevers (79%) and chills (53%).

On-going studies in brain malignancies such as glioblastoma multiforme (GBM) are also encouraging. Intratumoural injection at the time of surgery of an adenoviral vector expressing herpes simplex virus (HSV) thymidine kinase gene, combined with radical chemoradiation post operatively (34), has been tested prospectively. 12 of 13 patients completed therapy, at varying dose levels in this Phase Ib trial, with no DLTs or significant toxicity. A phase II trial is on-going (NCT00589875). Further evidence of safety in GBM patients is provided by the small phase I study which used a conditionally replicating HSV, G207 (35). Following two prior safety studies with single agents use they showed, in 9 patients, that intratumoural injection followed by 5Gy of radiation twenty four hours later had no increased risk of toxicity. Preclinical data with G207 also points to efficacy in other tumour sites such as head and neck SCC and lung cancer (36,37).

Chemoradiotherapy induces DNA damage. The central hypothesis is that enadenotucirev will, selectively, downregulate DNA repair pathways in rectal cancer cells, making them more susceptible to DNA damage already incurred. The high selectivity of enadenotucirev means normal tissue sensitization should be negligible. Enadenotucirev also has the potential to induce an oncosis phenotype cell death in malignant cells adding a complimentary, cytotoxic mechanism of action.

The aim of the trial is to find the treatment schedule that has the optimal response-toxicity trade-off with no more than 35% probability of a DLT. This is on the basis that G3+ adverse event rate for CRT, historically, is approximately 30% (38,39). However, these radiotherapy techniques have been superseded and toxicity is expected to be lower from CRT. Recent studies with novel radiosensitisers such as oxaliplatin reported G3/G4 toxicity in the order of 25% (40).

2 TRIAL DESIGN

CEDAR is a dual endpoint, dose escalation phase I trial using a time to event continual reassessment method (TiTE CRM). Response and Toxicity endpoints will be combined via dose escalation rules to identify the optimal dose schedule. At most, 30 patients will be recruited to the trial.

Dose escalation will be achieved by first increasing the frequency of administration of enadenotucirev followed by increasing the dose of enadenotucirev as detailed in the trial flow chart. These dose schedules are considered ordered with increasing toxicity expected from one dose schedule to the next.

See section 8 and 17.2 for further details.

3 OBJECTIVES AND ENDPOINTS

Primary Objective	**Endpoints/ Outcome measures**	**Time point(s) of evaluation of this end point**
• Determine the optimal dose and frequency of enadenotucirev that can be administered with chemoradiation	• Dose limiting toxicity • MRI tumour regression grade	• From Day 1 to Week 13 • Week 13
Secondary Objectives	**Endpoints**	
• Ability to deliver enadenotucirev concurrently with chemoradiation	• Treatment tolerance measured by proportion of patients completing at least 80% of the intended Capecitabine dose and at least 20 fractions of radiotherapy	• Week 9
• To measure local response rate to combined therapy compared to pre-treatment status	• MRI tumour regression grade • Pathological complete response • Neoadjuvant Rectal (NAR) score	• Week 13 • Post resection • Post resection
Tertiary/Exploratory Objectives	**Endpoints**	
• To identify 'proof of concept' that enadenotucirev replicates in the tumour	• IHC staining of hexon protein coat, viral gDNA and viral mRNA gene expression in tumour cells	• Archival tumour tissue • Week 4 biopsy • Resection sample
• To analyse gene expression changes in rectal cancer in response to enadenotucirev	• Whole genome RNA expression via RNA sequencing/ Nanostring gene expression analysis	• Archival tumour tissue • Week 4 biopsy • Resection sample
• Analyse changes in circulating tumour DNA in response to chemoradiation and enad	• ctDNA analysis exploring clearance of ctDNA and any/or emerging changes in persisting ctDNA	• Baseline (Pre 1st loading dose) • Week 4 • Week 13 • 4-6 weeks post-surgery
• Assess the changes in microbiome taxa during therapy	• Extraction of DNA and 16S sequencing and meta-transcriptomics from faecal samples	• Baseline (pre-enad) • End of week 1 / 2 • End of week 4 • End of week 7 • Week 13
• Analyse the immune microenvironment as evidenced by immune cell infiltrates	• Multiplex Immunohistochemistry of immune cell markers	• Archival tumour tissue • Week 4 biopsy • Resection sample

4 PATIENT SELECTION

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

4.1 Eligibility criteria

Inclusion criteria:

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

1. Histologically confirmed invasive adenocarcinoma of the rectum.
2. Locally advance colorectal cancer as defined by pelvic MRI with a threatened circumferential resection margin (cT3mrf+ve), or inclusion of an adjacent organ, or low tumours at/below the level of the levators or enlarged pelvic side wall nodes or selected by the multidisciplinary team MDT for treatment with neoadjuvant (chemo)radiotherapy, regardless of TNM classification
3. Patients with oligometastatic disease suitable for radical treatment are permitted provided that the site specific MDT deems them suitable for chemoradiation
4. Male or female, Age $\geq$ 18 years.
5. ECOG performance score of 0 - 1
6. The patient is willing and able to comply with the protocol scheduled biopsy, follow-up visits and examinations for the duration of the trial.
7. Written (signed and dated) informed consent.
8. Adequate renal function demonstrated by:

   - Creatinine ≤1.5 ULN and estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m^2 (or measured creatinine clearance ≥60 mL/min)
     and
   - Urine dipstick for proteinuria at screening and baseline negative or trace. Patients may be included with results of 1+ if they have a spot urinary albumin creatinine ratio (ACR) of either:
     (i) ≤3 mg/mmol **or**
     (ii) >3 mg/mmol with a 24 hour urinary protein <1.0 g/24hours
     and
   - Serum complement components C3 and C4 within the normal range

9. Haematological and biochemical indices within the ranges shown below:

Lab Test	Value required
Haemoglobin (Hb)	≥90 g/L
Absolute neutrophil count	≥1.5x10^9/L
Platelet count	≥100x10^9/L
Bilirubin	< 1.5 upper limit of normal
Aspartate transaminase and/or alanine transaminase	≤3 x upper limit of normal
INR	≤1.5
aPTT	Within laboratory normal range

Exclusion criteria:

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

1. Pregnant or breast-feeding women, or women of childbearing potential unless effective methods of contraception are used.
2. Pulmonary lymphangitis (if metastatic disease present)
3. Past medical history:
   a. Known history or evidence of significant immunodeficiency due to underlying illness and/or medication (e.g. systemic corticosteroids, or other immunosuppressive medications including cyclosporine, azathioprine, interferons in the 4 weeks before the first dose of trial treatment)
   b. Splenectomy
   c. Prior allogeneic or autologous bone marrow or organ transplantation

d. Patients with a history of, or active, known or suspected auto-immune disease or a syndrome that requires systemic or immunosuppressive agents; patients with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune disease only requiring hormone replacement, psoriasis not requiring systemic treatment or conditions not expected to recur in the absence of an external trigger are permitted to enrol
e. History of idiopathic pulmonary fibrosis, drug-induced pneumonitis, or evidence of active pneumonia or pneumonitis on computed tomography scan
f. Active viral disease or known positive serology for HIV, hepatitis B or hepatitis C
g. Active infections requiring antibiotics, physician monitoring, or recurrent fevers >38.0˚C associated with a clinical diagnosis of active infection
h. Prior pelvic radiotherapy
i. Any other active malignancy, with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and non-melanoma skin lesions.
j. Uncontrolled cardiorespiratory comorbidity (e.g. severe pulmonary fibrosis, inadequately controlled angina or myocardial infarction in the last 6 months)
k. Major disturbance in bowel function (e.g. severe incontinence, Crohn's disease, >6 loperamide/day), risk of bowel obstruction due to tumour- exception defunctioning colostomy performed
l. Treatment with any COVID-19 vaccine in the 28 days before the first dose of enadenotucirev, unless the vaccine is known to not be based on an adenoviral vector (e.g. mRNA vaccines)
m. Treatment with any vaccine (including known non-adenoviral COVID-19 vaccines) in the 7 days before first dose of enadenotucirev

4. Use of the following antiviral agents: ribavirin, adefovir, lamivudine or cidofovir within 7 days prior to the first dose of trial treatment; or pegylated interferon in the 14 days before the first dose of trial treatment
5. Treatment with any other investigational agent, or participation in another interventional clinical trial within 28 days prior to enrolment. In follow up for an interventional trials and observational studies are allowed
6. History of DVT or pulmonary embolus in the 12 months before the first dose of study treatment
7. History of significant bleeding requiring hospitalisation in the 12 months before the first dose of study treatment
8. Patients receiving therapeutic or prophylactic anticoagulation therapy
9. Known dihydropyrimidine dehydrogenase (DPYD) deficiency
10. Prior chemotherapy is allowed as long as >28 days since the last administration and any toxicity has resolved to NCI CTCAE grade 1 or less
11. Other psychological, social or medical condition, physical examination finding or a laboratory abnormality that the Investigator considers would make the patient a poor trial candidate or could interfere with protocol compliance or the interpretation of trial results.

4.2 Protocol deviations and waivers to entry criteria

Protocol adherence is a fundamental part of the conduct of a clinical trial. Changes to the approved protocol need prior approval unless for urgent safety reasons.

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

Investigators should not request a protocol waiver to enter a patient who does not satisfy the inclusion and exclusion criteria.

The investigator must document and explain any deviations from the approved protocol. The investigator should promptly report any important deviations that might impact patient safety, data integrity or be a possible serious breach (see 23.7 below) to the trial office.

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

Screen failures are ineligible and will not be rescreened unless imaging is not within the required timeframe - this should be repeated and patient reconsidered for the trial.

4.4 Patient registration procedure

The site must contact OCTO to check the availability of a screening slot and if available reserve the slot prior to giving out a Patient Information Sheet. A screening number should be requested prior to screening the patient and the site should register the participant within 2 weeks of receiving the screening number or relinquish the slot unless an extension is agreed with the trial office.

Before entering a patient onto the trial, the Principal Investigator or designee will confirm eligibility. If in any doubt the Chief Investigator must be consulted before entering the patient. Details of the query and outcome of the decision must be documented on the registration/ eligibility checklist. **Protocol waivers should not be requested to enter patients who are not eligible.**

Site staff will complete the trial Registration Form and email the form to the Trial Office to further confirm the patient's eligibility. The original Registration Form should be retained at site.

A Screening Log must be kept of all patients considered for the trial including any that are subsequently excluded; the reason for exclusion must be recorded on this form. A copy of the Screening Log must be sent to the trial office on request, but without patient identifiers. The original log must be retained on site in the ISF.

To register a patient:

After checking patient eligibility site staff will complete the trial Registration Form. Scan the Registration Form and email to: **octo-CEDAR@oncology.ox.ac.uk**

The trial office will check the submitted form and register the patient on the trial. Confirmation of the patient's trial number will be emailed to the PI & other relevant site contacts.

Enadenotucirev dosing schedule assignment will be determined pre-registration and confirmed by email to the PI & other relevant site contacts. See sections 8.2 and 17.2 for information on dosing schedules and dosing group decisions.

5 TRIAL ASSESSMENTS AND PROCEDURES

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

5.1 Informed consent

Potential participants will be given a current, approved version of the Patient Information Sheet and Consent Form. They will also receive clear verbal information about the trial detailing no less than: the nature of the trial; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the trial at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal. They will have at least 24 hours to consider the information provided and the opportunity to question the Investigator, their GP or other independent parties before deciding whether to participate. Once registered onto the trial, the patients GP will be notified via a letter with specific consent for this process being obtained.

The Investigator who obtains consent must be suitably qualified and experienced. All delegates must be authorised by the Principal Investigator to obtain consent. The Investigator is responsible for ensuring that the trial consent procedures comply with current applicable GCP Regulatory and ethical requirements. Informed consent discussions and outcomes must be well documented in the medical record. The Investigator must be satisfied that the patient has made an informed decision before taking consent. The patient and the Investigator must personally sign and date the current approved version of the Informed Consent Form in each other's presence. A copy of the Patient Information Sheet and signed Consent Form will be given to the participant. The original signed form will be retained at the trial site, ideally in the Investigator Site File (if local policy permits) and one copy held in the medical records. When required, a copy will also be provided to the tissue bank to evidence consent for storage of samples.

Contraceptive/ Pregnancy counselling

All participants must be advised on the need to use reliable methods of contraception during the trial. The advice should include:

(1) The acceptable methods, including: male or female sterilization, implants, injectables, combined oral contraceptives, some intrauterine devices (IUDs), and abstinence.
(2) The recommendation that a barrier method should be used in addition to another form of contraception.
(3) Males should continue to take these precautions for a minimum 6 months after the last dose of trial drug.
(4) Females should continue to take these precautions a minimum of 6 months after the last dose of trial drug.
(5) That any pregnancy (also applies to females partners of male trial subjects) occurring within 6 months of the last administration of trial drug will be followed up and the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) will be reported and followed up even if participant is discontinued from the trial.

5.2 Screening/Baseline evaluations

Potential participants will be identified by their existing clinical care team at surgical clinics, oncological clinics or multidisciplinary team meetings. Local clinical teams can confirm they are potential eligible for the trial and can therefore be approached. After the patient has been approached and deemed potentially eligible, the following screening and eligibility assessments must be performed/ obtained within the 2 weeks before the patient receives the first trial dose (with the exceptions of CT & MRI which must be within 62 & 42 days of start of treatment respectively, and standard of care tests which have been done, before consent, but within 21 days before the first treatment day).

- Written informed consent
- Demographic details including age, gender
- Medical History to include cancer history, prior cancer therapies and procedures, reproductive status, and clinically significant disease history and concomitant diseases.
- Concomitant medications (including prescription drugs, over-the-counter drugs, herbal/ homeopathic remedies, nutritional supplements) used by the subject within 14 days before the start of trial treatment
- Complete physical examination to include lungs, abdomen, heart and symptom driven examination. Any abnormality identified at baseline should be recorded
- Height, weight and body surface area (BSA)
- ECOG performance status
- Vital signs: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature.
- Screening blood tests:
    - Haematology – Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets
    - Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (alk phos), AST and/or ALT and LDH
    - Coagulation screen (aPTT and INR)
- Pregnancy test (females of childbearing potential only): serum or urine Human Chorionic Gonadotropin (HCG) test to rule out pregnancy at trial entry; results must be obtained and reviewed before the first dose of IMP is administered.
- Electrocardiogram (ECG)
- Dihydropyrimidine dehydrogenase (DPYD) deficiency test (any time before trial enrolment)
- Urine dipstick for protein
- Confirm histological diagnosis from pathology report
- CT chest/ abdomen/ pelvis- diagnostic CT may be used if within 62 days of first trial dose. Repeat CT to be scheduled within 14 days of informed consent, if > 62 days. Whole body PET-CT is an acceptable alternative.
- Pelvic MRI- diagnostic MRI may be used if within 42 days of first trial dose. Repeat MRI to be scheduled within 14 days of informed consent if > 42 days.
- Investigational blood sample (after informed consent)
- Faecal sample collection & diet questionnaire (collected at patients' home after informed consent (optional) and before 1st dose of enadenotucirev – not applicable to all participating sites)

Results of standard-of-care tests or examinations performed before obtaining informed consent and within 21 days before the start of trial treatment may be used; such tests do not need to be repeated for screening.

All screening evaluations must be completed and reviewed to confirm that subjects meet all eligibility criteria before the first dose of trial treatment. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

The CRF will only be completed for those subjects who are eligible for the trial and then subsequently registered. At the time of enrolment, each eligible subject will be assigned a unique subject number and the next sequential number to be allocated.

Also, pre dose assessments (section 5.3.1) need to be taken within 72 hours prior to first loading dose at the start of week 1.

It is recommended that the investigational blood sample is taken during a regularly scheduled visit such as the planning CT appointment to minimise extra visits. However, it can be done at any stage prior to the first dose of enadenotucirev.

5.3 Pre-treatment evaluation

Evaluation schedule A applies to all patients prior to day 1 of any dose or sequence of treatment.

The table below summarises what pre dose evaluations (schedule A or B) should be undertaken for each patient on any given day, depending on which dosing group they are assigned to (Section 8.2 and 17.2 for more information). Loading and after loading doses of enadenotucirev should be given on 3 non-consecutive days over a 7-day period during weeks 1-2-and 8-9 (e.g. Mon, Wed, Fri **or** Fri, Mon, Wed **or** other convenient schedule). Doses given during the 1st week of chemoradiation should be given on day 1 and day 5. On each dosing day the following assessments are required:

Dosing group 1-4

	Dosing Day 1	Dosing Day 2	Dosing Day 3
Week 1/2	A	B	A

Dosing group 4

	Dosing Day 1 (Day 1 CRT)	Dosing Day 2 (Day 5 CRT)
Week 3	A	A

Dosing groups 2, 3 & 4

	Dosing Day 1	Dosing Day 2	Dosing Day 3
Week 8/9	A	B	A

5.3.1 Evaluation schedule A

Pre- Dose – *the following assessments can be completed within 72 hours prior to the dose.* ***Vital signs to be completed on day of dosing***

- Adverse events and DLTs since the previous assessment
- New or changes to existing medications
- Physical examination of major body systems
- Weight
- ECOG performance status

- Local laboratory assessments:
  - Urine dipstick – if 2+ or greater proteinuria enadenotucirev dosing should be interrupted pending completion of 24 hour urine collection to exclude significant proteinuria, see further guidance in section 9.3.

- Routine blood samples
  - Haematology – Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets
  - Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (alk phos), AST and/or ALT and LDH
  - Coagulation screen (aPTT and INR)

TO BE COMPLETED ON DAY OF DOSING:

- Vital sign measurements: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature

5.3.2 Evaluation schedule B

Pre- Dose

- Adverse events and DLTs since the previous assessment
- New or changes to existing medications
- Physical examination of major body systems
- Vital sign measurements: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature
- Weight
- ECOG performance status

It is recommended that subjects also receive antipyretic prophylaxis, steroid prophylaxis and hydration at the time of each injection of enadenotucirev (See section 8.3). The recommended regime is:

- 1 hour prior to administration
  - IV hydrocortisone 100mg
  - PO Paracetamol 1000mg

5.4 Post dose evaluations

Once the enadenotucirev has been administered to the patient the following post dose evaluations should be carried out:

- Vital sign measurements (systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature):

First Dose:
After the first dose of enadenotucirev the patient's vital signs must be monitored at the following time points:

- Immediately after the end of the infusion
- 30 minutes (±5 minutes) post end of infusion
- 1 hour (±15 minutes) post end of infusion
- 4 hour (±15 minutes) post end of infusion
- 6 hour (±15 minutes) post end of infusion

Subsequent doses:
On all subsequent doses the patient's vital signs must be monitored at the following time points:

- Immediately after the end of the infusion
- 30 minutes (±5 minutes) post end of infusion
- 1 hour (±15 minutes) post end of infusion
- 3 hour (±15 minutes) post end of infusion

They will also receive a further dose of Hydrocortisone (IV) and paracetamol (PO) at 3 hours [See section 8.3]. If they are clinically well and vital signs are normal they can be discharged. If not improving consider admission for further active management.

5.5 Weeks 1/2

The following assessments should be completed for all patients:

Dosing day 1:

- Pre dose – Evaluation Schedule **A** (5.3.1)
- Post dose evaluations (5.4)

Dosing day 2:

- Pre dose – Evaluation Schedule **B** (5.3.2)
- Post dose evaluations (5.4)

Dosing day 3:

- Pre dose – Evaluation Schedule **A** (5.3.1)
- Post dose evaluations (5.4)

Faecal sample (after last dose of enadenotucirev):
Home collection and postage of faecal sample & diet questionnaire (optional consent required and may not be applicable to all participating sites)

5.6 Week 3

5.6.1 Dosing Groups 1, 2 & 3

Day 1 – Evaluation Schedule **A** (5.3.1)

5.6.2 Dosing group 4

Dosing day 1 & 5:

- Pre dose – Evaluation Schedule **A** (5.3.1)
- Post dose evaluations (5.4)

5.7 Week 4, 5, 6, 7

5.7.1 Week 4 only

The following assessments should be completed for **all patients**, during week 4 only and in addition to the assessments in 5.7.2.

Tumour biopsy
During week 4 (second week of chemoradiation) a biopsy of the tumour is mandated as part of the trial. This should be performed at endoscopy by an experienced endoscopist. Under direct visualisation (proctoscopy) a maximum of 3 samples of tissue will be taken, each measuring less than 6mm.

Investigational blood sample
An investigational blood sample will also be taken on day 10 of chemoradiation. (See Laboratory manual)

Faecal sample (after week 4 treatment):
Home collection and postage of faecal sample & diet questionnaire (optional consent required and may not be applicable to all participating sites)

5.7.1 Week 4, 5, 6, 7 - Other assessments

To be completed, for **all patients**, as standard of care prior to chemoradiation:

– Evaluation Schedule **A** (5.3.1)

5.7.2 Week 7 only

Faecal sample (after week 7 treatment):
Home collection and postage of faecal sample & diet questionnaire (optional consent required and may not be applicable to all participating sites)

5.8 Week 8, 9

5.8.1 Dosing group 1

Day 1 Evaluation Schedule **A** (5.3.1)

5.8.2 Dosing groups 2, 3 & 4

Dosing day 1:
- Pre dose – Evaluation Schedule **A** (5.3.1)
- Post dose evaluations (5.4)

Dosing day 2:
- Pre dose – Evaluation Schedule **B** (5.3.2)
- Post dose evaluations (5.4)

Dosing day 3:
- Pre dose – Evaluation Schedule **A** (5.3.1)
- Post dose evaluations (5.4)

5.9 End of trial evaluations at 13 weeks (+/- 3 days)

The following assessments should be completed for **all patients**:

- Adverse events and DLTs since the previous assessment
- New or changes to existing medications
- Physical examination of major body systems
- Vital sign measurements: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature
- Weight
- ECOG performance status
- Local laboratory assessments:
    - Urine dipstick
    - Blood samples for laboratory safety tests:
        - Haematology – Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets
        - Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (alk phos), AST and/or ALT and LDH
        - Coagulation screen (aPTT and INR)

MRI:
Patients should also have their standard of care, post treatment MRI scan to assess for response. Can be up to +/- 7 days of week 13.

Investigational blood sample
An investigational blood sample will also be taken during week 13, at the same time as the other blood tests.

Faecal sample:
Home collection and postage of faecal sample & diet questionnaire (optional consent required and may not be applicable to all participating sites)

5.10 Follow-up evaluations (post 14+ weeks)

The patient will return to follow-up as per standard care in the NHS, with surgery completed as per standard care at week 14+. A further visit to the trial team 4-6 weeks after surgery should occur to formally record the following:

1. Pathological resection specimen findings as per the RCPath reporting guidelines recorded on CRF
2. Neoadjuvant rectal score details
3. Any surgical and other complications – AE assessments
4. ECOG performance status
5. Investigational Blood sample taken

If patient has not received surgery due to complete clinical response, they should be followed up as per standard of care. Confirmation of their complete clinical response will be obtained from their medical records if available.

6 EARLY PATIENT WITHDRAWAL

Treatment Withdrawal

During the course of the trial, a patient may withdraw early from treatment. This may happen for a number of reasons, including:

- Unacceptable toxicity
- AE/SAEs requiring discontinuation
- Loss to follow-up
- Significant protocol deviation or inability to comply with trial procedures
- Clinical decision
- Patient decision

When the patient stops treatment early, the 'End of Treatment' Form needs to be completed, and any other relevant CRFs (e.g. SAE Form). The reason for and date of withdrawing from treatment early should be clearly documented in the medical records. The following evaluations should be carried out on early withdrawal unless the patient has withdrawn consent also.

- Physical examination of major body systems
- Adverse events and DLTs since the previous assessment
- Vital sign measurements: systolic / diastolic blood pressure (BP), pulse rate, respiratory rate and temperature
- ECOG performance status
- Local laboratory assessments:
    - Blood samples for laboratory safety tests:
        - Haematology – Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets
        - Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (alk phos), AST and/or ALT and LDH
        - Coagulation screen (aPTT and INR)

Patients who have stopped treatment early should continue to be followed-up for toxicity, response and survival, unless patient consent for follow-up is withdrawn.

Consent Withdrawal

Consent withdrawal means that a patient has expressed a wish to withdraw from the trial altogether. Under these circumstances, the site needs to document all relevant discussions in the patient notes and notify the Trial Office, which will allow the office to mark all future CRFs as not applicable.

Under these conditions, investigators are still responsible to follow up any SAEs until resolution.

Patient evaluability and replacement

All patients enrolled in the trial and who received at least one dose of enadenotucirev will be considered evaluable. Patients who are not evaluable will be replaced. The TMG may decide to replace a patient if they withdraw from the trial for a reason other than a DLT. The dose of a patient replacing a non-evaluable patient will be assigned in the same way as all other patients and they will not necessarily be allocated the same dose as the non-evaluable patient they replace.

Patients who do not complete the response primary endpoint evaluation will be treated as non-responders.

7 SAMPLES FOR LABORATORY ANALYSIS

7.1 Samples to be analysed in local Trust's laboratories

Diagnostic Laboratories
Samples for haematology, biochemistry and urinalysis will be labelled with standard patient identifiers and sent to the local hospital diagnostic laboratory. Results will be processed in the standard way and entered into the routine hospital reporting system. Samples will be stored, held, reported and subsequently destroyed in accordance with standard local laboratory practice.

Pathology
The routine diagnostic pathology samples and additional research samples taken at biopsies and during surgery will also be labelled, processed and reported according to local hospital protocols.

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

The following samples are required to be collected for shipment to central laboratories:

Time Point	Screening/Baseline	Week 1 / 2	Week 4	Week 7	Week 13	Week 14 +	4-6 weeks post-surgery
Investigational blood sample	X		X		X		X
Tumour sample for viral load and gene expression	X (archived diagnostic sample)		X (biopsy)			X (resection)	
Faecal sample (optional consent required – may not be applicable to all sites)	X	X	X	X	X		

Assay/sample handling and storage will be managed according to separate written instructions. The following sections summarise the arrangements for collection, close to patient handling, timings, and analytical laboratories responsible.

7.3 Labelling and confidentiality of samples sent

All samples sent to analytical Laboratories will be labelled with the trial patient number, sampling time-point and date/time taken. Should a laboratory receive any samples carrying unique patient identifiers the recipient must immediately obliterate this information and re-label as well as informing the trial office.

7.4 Clinical reporting of exploratory research assay results

Although the results of the CEDAR trial research assays are exploratory, and the assays performed are not to current NHS standards, clinically relevant genetic information could be revealed. Relevant results will be confirmed by an appropriate expert before the patient and their clinical care team are informed and changes may be made to their clinical care if required. Due to the exploratory nature of the assays and the subsequent results, patients will be given the option to decide if they would like to be informed of any clinically relevant genetic information via the consent form. The local site Principal Investigator will be notified by the appropriate expert if any clinically relevant genetic information is revealed. At this stage, patients will only be referred to by their unique trial number with it only being possible for the site team to re-identify the patient so that they can be contacted if required and if they provided consent.

7.5 Trial sample retention at end of trial

The Chief Investigator has overall responsibility for custodianship of the trial samples. Laboratories are instructed to retain any surplus samples pending instruction from the Chief Investigator on use, storage or destruction. It is possible that new or alternative assays may be of future scientific interest. At the end of the research trial any surplus samples may be retained for use in other projects that have received ethical approval. Hence, any surplus trial samples may be analysed as part of a separate ethically approved project, transferred to a licensed tissue bank where they will be managed in accordance with applicable host institution policies and the Human Tissue Act (HTA) requirements.

7.6 Withdrawal of consent for sample collection and/or retention

A patient may withdraw consent to provide samples for research at any time without giving a reason. The Investigator must ensure that their wishes are recorded in the medical record and will inform the Trial Office accordingly. The investigator should discuss with patients the valuable use of samples that have already been provided and under circumstances where these samples have already been processed and anonymised, it would not be possible to destroy such samples.

8 INVESTIGATIONAL MEDICINAL PRODUCTS (IMPS)

8.1 Name of IMPs

The trial is investigating the unlicensed drug enadenotucirev in combination with chemoradiotherapy. The chemotherapy agent used is capecitabine. Enadenotucirev and capecitabine are both considered IMPs.

8.2 Treatment dose and duration

Enadenotucirev

All participants will receive 3 x loading doses in weeks 1-2, prior to initiation of chemoradiotherapy. Loading doses should be given on 3 non-consecutive days over a 7-day period (e.g. Mon, Wed, Fri **or** Fri, Mon, Wed **or** other convenient schedule).

There are 2 different dosing patterns for the 3 loading doses.

- Low-Low-Low, for dosing groups 1 & 2; 3 individual doses of $1x10^{12}$VP given
- Low-High-High, for dosing groups 3 & 4; 3 individual doses of (1) 1 x 10^{12}VP, (2) 3 x 10^{12}VP, (3) 3 x 10^{12}VP given

Further doses of enadenotucirev after the 3 loading doses are dependent on the dose schedule assigned, as per table below. See section 17.2 for information on dose decision making and allocation

Dose schedule	Loading x 3 (pre CRT) (3 doses given on 3 non-consecutive days over a 7 day period)	Concurrent		Maintenance x 3 (post CRT) (3 doses given on 3 non-consecutive days over a 7 day period)
		Week 1 Day 1 CRT (week 3 day 1)	Week 1 Day 5 CRT (week 3 day 5)	
1 (start level)	**Low-Low-Low[1*]**			
2	**Low-Low-Low[1*]**			**Low-Low-Low[1*]**
3	**Low-High-High[2*]**			**Low-High-High[2*]**
4	**Low-High-High[2*]**	**3 x 10^{12} vp**	**3 x 10^{12} vp**	**Low-High-High[2*]**

[1] Low-Low-Low = 3 x doses at $1x10^{12}$VP
[2] Low-High-High = 3 Doses, (1) 1 x 10^{12}VP, (2) 3 x 10^{12}VP, (3) 3 x 10^{12}VP
*** 3 doses administered on 3 non-consecutive days over 7 days**

Dose Schedules 2 & 3:

After loading doses and completion of CRT, 3 x maintenance doses outlined in the table above of enadenotucirev will be delivered in weeks 8-9 for all participants assigned to dose schedules 2 & 3. Maintenance doses should be given on 3 non-consecutive days over a 7 day period. As with the loading doses, the maintenance doses are given in 2 different dosing patterns dependant on dosing schedule:

- Low-Low-Low, for dosing group 2; 3 individual doses of $1x10^{12}$VP given
- Low-High-High, for dosing group 3; 3 individual doses of (1) 1 x 10^{12}VP, (2) 3 x 10^{12}VP, (3) 3 x 10^{12}VP given

Dose schedule 4:

After loading doses, participants assigned to dosing schedule 4 will receive enadenotucirev concurrently with CRT during their 1st week of CRT, as indicated in the table. This will consist of two doses delivered, one on the first and then last day of CRT in that week, post radiotherapy. Each dose will be given at $3x10^{12}$VP

NB **Morning and evening doses of capecitabine are to be omitted on enadenotucirev dosing days.**

Participants will also receive 3 x maintenance doses of enadenotucirev delivered in weeks 8-9 after completion of CRT. Maintenance doses should be given on 3 non-consecutive days over a 7 day period and will be given as the Low-High-High dosing pattern consisting of 3 individual doses of (1) 1 x 10^{12}VP, (2) 3 x 10^{12}VP, (3) 3 x 10^{12}VP

Capecitabine

Capecitabine must not be taken on enadenotucirev dosing days.

With the exception of enadenotucirev dosing days capecitabine should be taken twice daily (morning and evening) Monday to Friday for 5 weeks, concurrently with radiotherapy.

Capecitabine is taken daily at a dose of 900 mg/m^2 (the standard dose used for chemoradiotherapy treatment of locally advanced rectal cancer). Sites should use national dose banding tables for capecitabine.

CRT Week (trial week)		**1 (trial week 3)**					**2 (trial week 4)**					**3 (trial week 5)**					**4 (trial week 6)**					**5 (trial week 7)**				
Days		1-5					8-12					15-19					22-26					29-33				
Radiotherapy: 50 Gy/25#		#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
Oral capecitabine* 900 mg/m² orally bd Mon – Fri x 5 weeks	**Dose schedules 1, 2 & 3**	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Dose schedule 4	-	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		-	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

8.3 Management of drug administration

Enadenotucirev

Enadenotucirev is a live replicating oncolytic adenovirus; it is considered a BioSafety Level 1 (BSL-1) infectious substance. Appropriate precautions should be followed for a BSL-1 substance, as per section 8.4 and local site policy.

Subjects must receive antipyretic prophylaxis, steroid prophylaxis and hydration at the time of each injection of enadenotucirev. The recommended regime is:

- 1 hour prior to administration
    - IV hydrocortisone 100mg
    - PO Paracetamol 1000mg
- 3 hours following administration
    - IV hydrocortisone 100mg
    - PO Paracetamol 1000mg

It is also strongly recommended that all patients have loperamide prescribed prior to commencement of treatment, in case of development of diarrhoea (see section 9.3).

Patients should also be given a prescription for oral paracetamol 1000mg four times daily to be taken in the 24 hours after receiving enadenotucirev.

The enadenotucirev infusion solution should be made up as per the administration protocol. Once diluted in the infusion bag/syringe it must be administered to the patient within 6 hours of preparation. The diluted solution is stable at room temperature (20-26^{o}C) for up to 1 hour. If not administered within 1 hour it can be stored at +2°C to 8^{o}C for no longer than 6 hours until administration. The full infusion volume must be administered over a 15 minute (+/- 5minutes) period for all dosing groups.

NOTE: At the time of administration, the extension set used to administer enadenotucirev can be primed with saline. At the end of infusion, the line will also need to be flushed with saline. This will ensure that all 30 mL of diluted enadenotucirev are administered to the patient.

The patient should be observed after administration as per post-dosing assessments detailed in section 5.3. N.B. serious infusion reactions have been observed in previous trials of enadenotucirev, appearing to reflect cytokine responses to treatment. In the event of a serious infusion reaction the infusion should be discontinued and the patient monitored and treated as per standard local protocol for management of cytokine release syndrome. Further guidance on managing toxicities is given in section 9.2.

Capecitabine

Refer to the Summary of Product Characteristics (SPC) for full prescribing information and details of drug reconstitution, administration and stability (http://www.medicines.org.uk/emc/).

Patients will be instructed to take capecitabine tablets twice a day starting in the morning/evening, starting on the first day of radiotherapy. Tablets will be taken twice daily on every day that radiotherapy is given (therefore excluding the weekends). **Capecitabine must not be taken on enadenotucirev dosing days.**

It is recommended that patients are carefully monitored for ophthalmologic complications, such as keratitis and corneal disorders, especially if they have a prior history of eye disorders. Treatment of eye disorders should be initiated promptly. For patients with diabetes mellitus, caution must be exercised as it may be aggravated.

Patients should be instructed to take the drug twice daily as above **only** on the days radiotherapy is delivered. Doses should be omitted on enadenotucirev dosing days. Where the start of radiotherapy is delayed for scheduling reasons the start of concurrent chemotherapy should also be delayed.

Missed doses/dosing day will not be made up. The next dose must be taken as scheduled. Doses should NOT be doubled to make up for missed doses.

If a patient vomits after taking the tablets, they should not take another dose. The next doses should be taken as scheduled.

If a patient has taken their morning dose of capecitabine on an enadenotucirev dosing day, administration of enadenotucirev should be delayed to the next day of radiotherapy. Patients should be reminded to take their evening dose as normal and to not take capecitabine the following day. If delay of enadenotucirev dosing is not possible, enadenotucirev can still be administered and patients reminded to omit their evening capecitabine dose.

Any unused tablets should be returned to pharmacy (via research nurse). All patients are asked to keep a record of their capecitabine use in their site specific diary card. Diary cards will be reconciled by site staff and confirmation of capecitabine use to be recorded on the relevant CRF.

8.4 Special precautions

Enadenotucirev is a live replicating oncolytic adenovirus that has not been genetically modified, it is considered a BioSafety Level 1 (BSL-1) infectious substance. BSL-1 agents pose minimal potential hazard to personnel. Sites should follow local procedure for handling a BSL-1 biological agent, it is suggested that this includes:

- No eating and drinking in areas where enadenotucirev doses are prepared or administered
- Safe disposal of used vials and all equipment used to prepare and administer enadenotucirev

8.5 Dose modification

Enadenotucirev

No dose modifications may be made. **With the exception of renal injury, specifically proteinuria of 2+ where enadenotucirev must be delayed and or discontinued (see section 9.3),** treatment may be interrupted because of a non-DLT of Grade 3 or higher, at the discretion of the Investigator. Treatment may be resumed when all toxicities have returned to grade 2 or less, at the discretion of the Investigator. If two consecutive doses of enadenotucirev are missed due to enadenotucirev related toxicity, this constitutes a DLT, and no further enadenotucirev will be administered. If the subject misses a dose of enadenotucirev for any reason other than toxicity the dose should not be made up. The scheduling should continue as normal, from the next planned dose of enadenotucirev. If radiotherapy is

withheld for toxicity or other reasons, enadenotucirev should also be withheld and the dosing schedule should continue from the next planned dose once radiotherapy has resumed.

Capecitabine

In the event of haematological toxicity or other adverse events capecitabine dose may be modified as per standard of care (treatment interruption or dose reduction). Dose adjustments are to be made according to the greatest degree of toxicity and in accordance with the local hospital protocol. Once the dose has been reduced, it should not be increased at a later time.

8.6 Compliance

Enadenotucirev

All doses of enadenotucirev will be delivered intravenously at a research site. Site staff will maintain records of enadenotucirev administration.

Capecitabine

Patients will be instructed to keep a record of compliance with capecitabine treatment, by means of using a patient diary card provided to the patient by the site. Patients should be asked to bring completed diary cards or other records and all their unused / remaining capecitabine tablets (empty, open or unopened) with them to each clinic visit. The patient diary cards should not be sent to the Trial Office but kept by the centre to monitor patient drug compliance.

Accountability logs are required for capecitabine to determine that patients have received at least 80% of the prescribed treatment dose. Returns should be reconciled against the patient diary and the reason for any discrepancy documented. Site staff will collect and count patient returns which must be recorded on the drug accountability log.

8.7 Management of overdose

In the case of enadenotucirev overdose the patient should be monitored for evidence of toxicity and standard supportive treatment provided based on any signs or symptoms experienced.

In the case of capecitabine overdose refer to guidance provided in the SPC.

Administration of a dose of trial drug exceeding that permitted by the trial protocol should be notified to the trial office. Any toxicity resulting from administration of an overdose of trial drug should also be reported as an adverse event/ serious adverse event (as appropriate) as per reporting processes detailed in section 14.

9 DOSE LIMITING TOXICITY

9.1 Definition of a dose limiting toxicity (DLT)

All patients who have received at least one dose of enadenotucirev will be evaluable for DLTs. DLTs are defined as any of the following occurring between the start of trial treatment until the Week 13 visit and by the principal investigator (PI) or agreed designee assessed as possibly, probably or definitely related to enadenotucirev or the interaction between enadenotucirev and radiotherapy and/or capecitabine. Patients who experience a DLT will receive no further trial treatment.
Dose limiting toxicities must be reported within 24 hours of the site becoming aware using the SAE form and scan and email as a PDF attachment to octo-safety@oncology.ox.ac.uk and send an email notification to octo-CEDAR@oncology.ox.ac.uk.

DLTs will be defined as per NCI CTCAE v4.03, except for proteinuria, and include:

Renal:

- Development of proteinuria, 2+ as measured by urinalysis and confirmed with an albumin/creatinine ratio of >3g/mmol with a 24-hour urinary protein ≥1g/24h or if there is a decline in eGFR (where a decline in eGFR is defined as eGFR <60ml/min/1.73m^2 or a drop in eGFR by 20% from screening, baseline of previous visit), following administration of enadenotucirev, shall be classified as a DLT. No further doses of enadenotucirev will be administered to that patient.

Acute hematologic toxicity:

- Infection (documented clinically or microbiologically) with grade 3 or 4 neutropenia (absolute neutrophil count (ANC) <1.0 x 10^9/L)
- Neutropenia grade 4 (ANC < 0.5 x 10^9/L) lasting for ≥7 days

- Febrile neutropenia grade 4 (fever of unknown origin without clinically or microbiologically documented infection) (ANC <1000/mm3 with a single temperature of >38.3 degrees C or a sustained temperature of >=38 degrees C for more than one hour)
- Thrombocytopenia grade 3 (Platelet count < 50 x 10^9/L) in the presence of bleeding or requiring platelet transfusion
- Thrombocytopenia grade ≥ 4 (Platelet count < 25 x 10^9/L)
- Anemia grade 3 in the presence of blood transfusion dependency as judged by the PI
- Anemia grade ≥ 4
- Clinically significant bleeding attributed to grade 3 thrombocytopenia or requiring platelet transfusion or other grade ≥3 clotting disorder or occurring concurrently with Grade 2 or 3 aPTT prolongation, unless there is a clear explanation for the event, such as tumour-related bleeding in the presence of Lupus Anticoagulant.
- Any other grade ≥3 non-hematological toxicity with the exception of: activated partial thromboplastin time (aPTT) prolongation
- Clotting event (i.e. deep vein thrombosis [DVT], pulmonary embolism [PE]) occurring concurrently with Grade 2 or 3 aPTT prolongation

Acute non-hematologic toxicity:

- Any documented ≥ grade 4 non-hematologic toxicity in the presence of maximal support/active management
- Grade ≥3 cystitis or radiation dermatitis onset within 2 weeks of starting radiotherapy or lasting more than 2 weeks after the end of radiotherapy.
- Grade ≥3 proctitis or diarrhea onset within 2 weeks of starting radiotherapy
- Grade ≥3 nausea or vomiting not controlled by optimal outpatient anti-emetic treatment
- Grade ≥3 diarrhea despite optimal outpatient anti-diarrheal medication use
- Grade ≥3 hematuria or neuropathic pain
- Other grade 3 ≥ effects thought to be due to the combination of enadenotucirev with radiotherapy
- Missing 2 consecutive doses of enadenotucirev due to enad toxicity
- An elevation of ALT or AST >5 x ULN lasting 8 days or more
- A concurrent elevation of ALT or AST >3 × ULN and total bilirubin >2 × ULN in whom there is no evidence of biliary obstruction or other causes that can reasonably explain the concurrent elevation
- Death due to drug related complications
- Grade ≥3 cytokine release syndrome

General:

- Discontinuation of the active treatment due to toxicity definitely attributable to enadenotucirev, irrespective of the grade of toxicity
- Missing 3 consecutive fractions of radiotherapy, related to enadenotucirev, as judged by the PI or designee
- Any toxicity causing a delay of radiotherapy completion by greater than one week

This is not an exhaustive list. All suspected DLTs should be discussed with the Chief Investigator and the Trial Management Group.

9.2 Toxicity associated with Enadenotucirev

Below is a summary table of commonly reported treatment related adverse events (≥10% patients) by incidence and preferred term following IV infusion of enadenotucirev at the doses used in the trial. Further explanation can be found below the table.

Preferred Term	**Dose level (1 x10^{12})**	**Dose level (3 x10^{12})**
Pyrexia	39%	75%
Chills	33%	75%
Fatigue	6%	35%
Nausea	17%	40%
Asthenia	39%	25%
Vomiting	17%	30%
Decreased Appetite	17%	45%
Diarrhoea	11%	10%
Flu like illness	6%	30%
ALT increased	0 %	5%
Fibrin d-dimer increased	0%	20%

Hypertension	0 %	30%
Musculoskeletal pain	11%	10%
AST increased	0%	5%
Thrombocytopenia	6%	5%
Neutropenia	22%	0%
Platelet count decreased	6%	10%
Proteinuria	0%	20%
Headache	11%	10%

Inflammatory/influenza-like Symptoms
Pyrexia has been reported in the majority of patients at doses of 1 x 10^{12} vp. Chills are very common above 1 x 10^{12} vp. Additionally, influenza-like illness, the diagnostic term used when multiple symptoms including pyrexia and chills were reported, has been reported frequently at these doses. These symptoms usually have onset shortly after dosing and are manageable with antipyretic medication. Other symptoms typical of an influenza-like illness such as myalgia, arthralgia and musculoskeletal pain have been reported across the dose range but much less frequently. These events have not been reported as SAEs. General symptoms such as asthenia, fatigue and malaise may be attributed to an inflammatory response to viral particles but are also frequently seen in cancer patients. They were commonly reported in the studies with enadenotucirev but with no clear dose relationship. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea may also be attributed to an inflammatory response to viral particles. The incidence of diarrhoea was predominantly above the MTD. Nausea and vomiting appeared dose-related with an increase in frequency at doses of 3 x 10^{12} vp and above. There was one non-serious CTCAE Grade 2 infusion-related reaction after the first dose of 6 x 10^{12} vp. The details are unknown but no action was required and the patient continued to receive enadenotucirev.

9.3 Management of enadenotucirev toxicity

Nausea and vomiting should be treated with antiemetics and hydration as per institutional protocol.
Flu-like syndrome should be treated with supportive measures, antipyrexials (e.g. paracetamol) and hydration. If acute respiratory symptoms such as hypoxia develop then symptomatic treatment with supplemental oxygen and bronchodilators may be used if clinically indicated. Hospitalisation for observation during the dosing period may be considered according to Investigator judgement and circumstances of the patient.
Antivirals may be used to treat patients that exhibit prolonged and/or difficult to manage toxicity that is thought to be due to viral replication. High dose steroid therapy may be required to manage the associated inflammatory response.

Diarrhoea: It is strongly recommended that all patients have loperamide prescribed prior to commencement of treatment, in case of development of diarrhoea. Loperamide is recommended as the initial antidiarrhoeal medication. Patients should be instructed to contact the trial site for further guidance if diarrhoea is not controlled after 12 hours of loperamide treatment. Codeine phosphate up to 30 mg 4 times a day can be added if diarrhoea is not controlled with 16mg of loperamide per day.

Serious infusion reaction: These have been observed in a small number of participants in previous trials of enadenotucirev, appearing to reflect cytokine responses to treatment. Infusion reactions are more common with the first infusion, although reactions cannot be excluded on subsequent infusions, even if no reaction was seen during the first. In the event of a serious infusion reaction discontinue the infusion and monitor and treat the patient as per standard local protocol for management of cytokine release syndrome (e.g. vigilant supportive care, maintenance of adequate hydration and blood pressure, and corticosteroids and or other appropriate immunosuppression). Extensive comorbidities and older age should be closely monitored for cardiac function and immunosuppression should be instituted earlier. Cytokine release syndromes of grade 3 or higher are considered a DLT so no further drug should be administered.

Renal injury: Three patients out of 123 patients (2.4%) have developed significant renal impairment while receiving enadenotucirev. Whilst no confirmed pathophysiology has been identified it is felt to be due to vascular leakage due to cytokine release. Proteinuria of 2+ on a urine dipstick should therefore be further investigated, due to the potential risk of serious renal injury, with 24 hour urine collection for protein/creatinine ratio. If preferable locally, a urine protein- to-creatinine ratio or urine albumin – to – creatinine ratio are also acceptable in lieu of a 24 hour collection test. Additional blood tests should also be up to date including a serum albumin and serum creatinine. A consultation with nephrology is advised for specialist input. Specifically, early specialist intervention for investigation and treatment if decline in renal function is detected will include:

1. Full renal work-up, including examination of urine sediment, discussion with renal team and then commence high dose corticosteroids and then, if further decline in renal function;
2. Renal biopsy, followed by plasmapheresis should be considered with close involvement of the renal team.

No enadenotucirev is to be administered after the development of proteinuria as this constitutes a DLT.

Refer to section 8.5 for guidance on dose modification/ interruption due to toxicity.

aPTT Prolongation
If at any time a patient has Grade 2 or 3 aPTT prolongation, planned biopsies and IT treatment should not go ahead until the results of the following tests have been received:
1. aPTT 1:1 mixing studies (required for real-time treatment decisions)
2. Hexagonal phase phospholipid (Staclot Lupus Anticoagulant) assay (or local equivalent test for Lupus Anticoagulant) (required for real-time treatment decisions)
If Lupus Anticoagulant is not shown to be present, planned biopsies and IT treatment may not go ahead. If Lupus Anticoagulant is shown to be present, planned biopsies and IT treatment may go ahead (provided that the other clotting parameters such as platelet count and International Normalised Ratio and/or prothrombin time are within acceptable ranges).
In addition, the following analyses will be conducted:
1. Anti-cardiolipin antibodies by enzyme-linked immunosorbent assay (ELISA) (immunoglobulin G [IgG] and immunoglobulin M [IgM])
2. Anti-beta2-glycoprotein I antibodies by ELISA (IgG and IgM)
3. Additional laboratory testing for further characterisation of Lupus Anticoagulant
Patients with aPTT prolongation should be followed up every 2 months until aPTT has normalised to Grade 1 or less, or disease progression.

Once confirmed, tests for Lupus Anticoagulant do not need to be repeated.

9.4 Toxicity associated with capecitabine

Haematological toxicity
Myelosuppression is uncommonly observed with capecitabine. Neutropenia and thrombocytopenia should be monitored according to the recommended protocol and appropriate dose modifications made. Anaemia may occur cumulatively and should be corrected during radiotherapy to maintain the haemoglobin > 10g/dL. The FBC should be taken and reviewed (up to 3 days) prior to Day 1 of each cycle of chemotherapy.

Gastrointestinal toxicity
Nausea and vomiting is an overlapping toxicity of both capecitabine and enadenotucirev. Anorexia, nausea and occasional vomiting may persist. Nausea occurs less commonly with capecitabine. Diarrhoea occurs with capecitabine and patients should receive advice regarding discontinuation of therapy and use of loperamide or codeine phosphate (see section 9.3). Clinicians should be aware of infective causes of diarrhoea (e.g. Clostridium difficile), and patients should be tested in cases of concern. Antibiotic treatment is not recommended routinely but may be required in such circumstances. Stomatitis occurs with capecitabine and patients should receive advice regarding good oral care, and the use of mouthwash (e.g. Corsodyl™).

DPYD deficiency
If a patient has not received capecitabine in the past DPD testing should be undertaken as per Institutional protocol. Patients with partial or full DPD deficiency are not eligible for the study. Occasionally (approximately 1-3%) a patient may have a markedly exaggerated toxicity due to reduced 5FU catabolism. If this occurs, await full recovery of toxicities. Further treatment should be discussed with the Chief Investigator or one of the clinical co-investigators.

9.5 Management of capecitabine toxicity

Toxicity due to capecitabine administration may be managed by symptomatic treatment and/or modification of the dose as per standard of care. Further information on dose modification is provided in section 8.5. Patients taking capecitabine should be informed of the need to interrupt treatment immediately if moderate or worse toxicity occurs. Detailed information can be found in the product SPCs available from www.medicines.org.uk

10 OTHER TREATMENTS (NON-IMPS)

10.1 Support medication

Local anti-emetic and anti-diarrheal policy may be followed. Medications for the standard management of symptoms or supportive care for cancer or for the management of the effects of enadenotucirev may be administered at the Investigator's discretion; unless they are excluded concomitant medications.

Antipyretic prophylaxis: Subjects must receive antipyretic prophylaxis and hydration at the time of each injection of enadenotucirev (as per section 8.3) unless clinically excluded (e.g. allergic to paracetamol, when alternatives should be used such as NSAIDS).

10.2 Concomitant medication and non-drug therapies

Concomitant medication may be given as medically indicated. All patients will be asked to provide a complete list of prescription and over-the-counter medications that have been taken within the previous 4 weeks prior to the first treatment visit. They must also inform the Investigator about any new medication started while in the trial.

10.3 Prohibited therapies

Any COVID-19 vaccine in the 28 days before OR after any dose of enadenotucirev, unless the vaccine is known to not be based on an adenoviral vector.

- COVID-19 vaccines known to not be based on an adenoviral vector (e.g., the mRNA-1273 SARS-CoV-2 [Moderna Therapeutics] and BNT162b2 [BioNTech/Pfizer] mRNA COVID-19 vaccines or other non-adenoviral vaccines) are excluded in the 7 days before OR after any dose of enadenotucirev

Any other vaccine, in the 7 days before OR after any dose of enadenotucirev.

Medications inducing immune suppression (e.g. systemic corticosteroids or other immunosuppressive medications) in the 14 days after the last dose of enadenotucirev unless they are:

- Required as part of emergency therapy
- Required for the treatment of adverse events
- Used as an inhaled agent for bronchospasm or COPD

Anti-viral agents e.g. ribavirin, adefovir, lamivudine or cidofovir in the 7 days after each trial treatment; or PEG-IFN in the 14 days after each dose of trial treatment and only then if the benefit of their use will outweigh the possible risks.

Any systemic cancer therapy other than capecitabine and any local therapy to the primary tumour or its metastases during trial treatment.

10.4 (Potential) Drug Interactions

Enadenotucirev

Enadenotucirev is sensitive to the effects of cidofovir and likely to be sensitive to other agents with known anti-viral effects, therefore these medications are prohibited as detailed in the previous section. Systemic cancer therapy may have an inhibitory effect on enadenotucirev, hence capecitabine should not be administered on enadenotucirev dosing days. Refer to the current Investigator Brochure for further detail.

The inflammatory reactogenicity observed with COVID-19 vaccinations may overlap with the acute cytokine driven symptoms observed shortly after dosing with EnAd-based IMP (including chills, pyrexia and potential acute respiratory symptoms). To mitigate against this risk patients in this trial should not be treated with EnAd-based IMP within one week of receiving any vaccination (either first or second dose). Additionally, patients should not receive a vaccination within one week of their last dose of EnAd-based IMP.

In addition, several COVID-19 vaccines based on adenoviruses have now been approved. Due to the specific potential for overlap in immune responses between adenovirus-based vaccines and EnAd-based IMP, a default requirement that patients do not receive a COVID-19 vaccine in the 28 days before or after any dose of EnAd-based IMP has been added to the protocol. If the COVID-19 vaccine is known to be non-adenoviral (e.g. mRNA vaccines), then a 7-day exclusion before or after any dose of EnAd-based IMP is required.

Capecitabine - advisory
Capecitabine interacts with several medications and investigators should refer to the SPC for full guidance. For ease of reference the following are the main interactions:

- Coumarin-derivative anticoagulants e.g. warfarin- more frequent is monitoring required when administered with capecitabine due to altered coagulation parameters. Effects may occur up to several months after initiating capecitabine therapy.
- Phenytoin- increased phenytoin plasma concentrations resulting in symptoms of phenytoin intoxication in single cases have been reported during concomitant use of capecitabine. Patients should be regularly monitored for increased phenytoin plasma concentrations.
- Folinic/ folic acid has an effect on the pharmacodynamics of capecitabine and its toxicity may be enhanced.
- Capecitabine must not be administered concomitantly with sorivudine or its chemically related analogues (e.g. brivudine)
- Aluminium hydroxide or magnesium hydroxide containing antacids – cause a small increase in plasma concentrations of capecitabine and its metabolite 5DFCR (5'-deoxy-5-flurocytidine)
- Cytochrome p450 down regulation by capecitabine may affect the following class of drugs – angiotensin II blockers (losartan, valsartan); oral hypoglycaemic agents (glipizide, tolbutamide, rosiglitazone); NSAIDS (indomethacin, celecoxib, diclofenac, ibuprofen)
- Concomitant use of allopurinol with capecitabine should be avoided

11 DRUG MANAGEMENT

11.1 Drug supplies

Enadenotucirev will be supplied free of charge to the local hospital pharmacies. Enadenotucirev is provided as single use vials containing 1.4 x 10^{12} viral particles in 0.7mL aliquots in 2mL type 1 borosilicate glass vial with a butyl stopper and an aluminium crimp seal. Each vial of enadenotucirev is issued in a plastic tamper-evident container.

Capecitabine as a tablet for oral use should be supplied from trial site's own stock and funded locally.

All supportive medication is to be sourced and funded locally.

11.2 Drug ordering

Initial supplies of enadenotucirev are dispatched by Fisher Bioservices, on behalf of Psioxus after Psioxus have been informed by OCTO that all approvals are in place and the site is open to recruitment. Subsequent supplies should be ordered from OCTO by site pharmacy using the drug request form provided. Email the completed form to octo-cedar@oncology.ox.ac.uk.

The Principal Investigator and Pharmacy are responsible for liaising to ensure that adequate stock levels are held as necessary to supply the recruited patients.

If a vial of enadenotucirev is accidentally destroyed, i.e. by dropping the vial or through contamination, the pharmacist can contact octo-cedar@oncology.ox.ac.uk for replacement patient supplies and submit a drug request form if required.

11.3 IMP Receipt

Sites should contact the trials office via email (octo-cedar@oncology.ox.ac.uk) to confirm receipt of enadenotucirev and complete confirmation of receipt as per the supplier's instructions.

If supplies are damaged on arrival contact the Trial Office. Damaged supplies should be destroyed on site as per local policy and a drug destruction form completed. See section 11.9 – drug destruction.

11.4 Handling and storage

Enadenotucirev should remain frozen solid at all times during transit and storage. The enadenotucirev vial should be kept inside a secondary container and should be quickly transferred to the designated, controlled-access freezer for storage below -80°C (with normal ±-10°C tolerance).

The temperature will be monitored during transport and site storage and logs will need to be reviewed at site before the virus is dispensed. Temperature excursions should be reviewed and the trials office notified. The virus should be quarantined until a decision has been made that it is still fit for purpose or that it must be destroyed.

Enadenotucirev is a live replicating oncolytic adenovirus, it is considered a BioSafety Level 1 (BSL-1) infectious substance. Appropriate precautions should be followed for a BSL-1 substance, as per section 8.4 and local site policy.

11.5 Labelling

Enadenotucirev

Enadenotucirev vials supplied to sites will have been labelled for clinical trial use as per the requirements of Eudralex Volume 4: Annex 13 'Investigational Medicinal Products' of the European Union guide to Good Manufacturing Practice (GMP).

Site pharmacies are permitted to add additional labels to the IMP at site as per standard local procedures (e.g. addition of site and patient identifiers) providing the original label is not obscured.

Capecitabine

Labelling will be as per local practice.

11.6 Dosing dispensing

Enadenotucirev is a live replicating oncolytic adenovirus, it is considered a BioSafety Level 1 (BSL-1) infectious substance. Appropriate precautions should be followed for a BSL-1 substance, as per section 8.4 and local site policy.

The enadenotucirev infusion solution should be made up as per the administration protocol. Once diluted in the infusion bag/syringe it **must be administered to the patient within 6 hours of preparation**. The diluted solution is stable at room temperature (20-26^{o}C) for up to 1 hour. If not administered within 1 hour it can be stored at +2°C to 8^{o}C for no longer than 6 hours until administration.

11.7 Drug accountability

Drug accountability is the responsibility of the site pharmacist listed on the trial delegation log. Full drug accountability records must be maintained for capecitabine and enadenotucirev. Hospitals may amend the Drug Accountability Logs provided by the trial office or use their own documentation if it captures all the information requested on the Drug Accountability Logs and has been approved by the Trial Office in advance.

Copies of completed accountability logs must be supplied to the trial office on request.

11.8 Drug returns from patients

Site staff will collect and count patient returns of capecitabine. Returns should be reconciled against the patient diary and the reason for any discrepancy documented. Returns must be recorded in the patient's CRF and accountability log. The returns can then be disposed of according to local policy.

11.9 Drug destruction

Enadenotucirev:

Used vials	Disposal at site according to local hospital policy.
Expired Virus	Disposal at site according to local hospital policy. A dated certificate of disposal should be completed and retained in the Pharmacy File and a copy emailed to the trial's office.
Virus left unused	At the end of the trial, once authorised to do so, any unused drug should be disposed of at site according to local hospital policy. A dated certificate of disposal should be completed. The original should be placed in the Pharmacy File and a copy emailed to the Trials Office.

Capecitabine:

Patient returns will be disposed of as per local hospital policy.

11.10 Occupational safety

Enadenotucirev is a live replicating oncolytic adenovirus; it is considered a BioSafety Level 1 (BSL-1) infectious substance. Appropriate precautions should be followed for a BSL-1 substance, as per section 8.4 and local site policy.

12 RADIOTHERAPY (AND CHEMORADIOTHERAPY)

Patients registered for the trial will receive standard chemoradiation treatment which consists of Capecitabine 900mg/m2 orally twice a day in equal doses (Mon-Fri) on the days of radiotherapy for 25 daily treatments. The radiotherapy protocol mandates the use of intravenous contrast CT simulation with minimum 3 mm CT slices. Patients are immobilised supine with customised pelvic immobilization equipment, with a comfortably full bladder.

50Gy in 25 fractions will be delivered to the primary tumour and macroscopically involved lymph nodes as a simultaneous integrated boost and 45Gy in 25 fractions to the pelvis/mesorectal nodes and elective pelvic lymph nodes at risk, prescribed according to recommendations by the International Commission on Radiation Units and Measurements (ICRU-50/62), to be delivered Monday to Friday as an intensity modulated radiotherapy planned single-phase treatment. An adapted atlas will be provided to aid with radiotherapy localization (Elective Clinical Target Volumes in Anorectal Cancer: An RTOG Consensus Panel Contouring Atlas). Each case will be reviewed to ensure consistency of radiotherapy delineation and radiotherapy dose delivery as described in section 12.3. Patients are reviewed weekly during RT (as per standard of care) and prior to each administration of enadenotucirev.

Please refer to the accompanying radiotherapy trial document for details on volumes, margins, organ at risk constraints', plan optimisation and quality assurance to ensure that standard of care is consistent across the sites.

12.1 Management of unscheduled gaps

CEDAR patients will be treated as RCR category 1 patients. Unscheduled gaps in radiotherapy should be limited to no more than 2 consecutive planned fractions where possible. Any toxicity resulting in delay in radiotherapy for greater than one week is considered a DLT (see section 9.1).

12.2 Radiotherapy toxicity

The acute adverse events expected for chemoradiotherapy are summarised in the following table. This data is derived from TROG 01.04 which randomised patients, in a neoadjuvant setting, to either 25Gy in 5 fractions or 50.4Gy in 28 fractions with continuous fluorouracil(4). The management of radiation side effects should be as per local protocol. Where a side effect is attributable to either capecitabine or radiation, capecitabine should be held first and continue with daily fractions of treatment.

	Long course chemoradiation*			
Toxicity	**Grade**	**Rate (%)**	**Grade**	**Rate (%)**
Radiation dermatitis	3	5.6	4	0
Diarrhoea	3	13.6	4	0.6
Proctitis	3	3.7	4	0
Pain	3	1.9	4	0
Dysuria	3	1.2	4	0
Urinary Frequency	3	1.9	4	0
Haematuria	3	0	4	0
Neuropathic pain	3	0	4	0
Perineal pain	3	1.9	4	0
Nausea	3	3.1	4	0
Vomiting	3	1.9	4	0
Fatigue	3	3.7	4	0

*Data from TROG 01.04 (39)

12.3 Radiotherapy quality assurance

In the first instance, any queries regarding radiotherapy quality assurance for CEDAR should be addressed to the national Radiotherapy Trials QA group (RTTQA) contact (CEDAR.RTTQA@wales.nhs.uk or www.rttrialsqa.org.uk).
Real time review of all patients will be required for the first patient treated in each site and there will be timely retrospective review of radiotherapy for the rest of the patients. All images, outlines, plan and dose data (DICOM) should be submitted to the RTTQA contact who will co-ordinate review of the data to check protocol compliance as each patient is recruited.

13 EVALUATION OF RESPONSE

13.1 Tumour assessment

A clinical endoscopic and magnetic resonance imaging (MRI) evaluation of the rectal cancer, and in line with the protocol, must be performed before starting the trial treatment. To ensure compatibility, the radiological assessments used to assess response must be performed using identical techniques. Imaging based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumour effect of a treatment.

Baseline evaluations

These will include radiological measurements of the extent of disease by MRI scan, in accordance with best practice guidelines (41) by referencing the UICC 8th edition of the TNM staging classification (42). All areas of disease present must be mentioned (even if specific lesions are not going to be followed for response) and the measurements of all measurable lesions must be recorded on the scan reports. Any non-measurable lesions must be stated as being present. For clinical measurements, documentation by colour photography at endoscopy including an estimate of the size of the lesion is recommended.

13.2 MRI Tumour response

Following treatment, a repeat MRI should be performed, approximately six weeks after the last fraction of chemoradiation. This is considered to be standard of care in the UK. The MRI should be reported according to MRI assessed Tumour Regression Grading (mrTRG). This has previously shown excellent prediction of favourable and unfavourable histopathology (43). It has also been shown to predict disease free survival and overall survival (44). The full mrTRG 5 point scale is included in Appendix 2 and will be grouped for purposes of analysis and dose escalation. Scores of 1 or 2 will be classified as responders and scores of 3, 4 or 5 will be classified as non-responders.

A CT scan (Chest, Abdo, Pelvis) may also be completed after treatment if local site policy specifies, but is not required as part of the trial.

13.3 Pathological Tumour response

The final resected tumour should be assessed by an experienced lower gastrointestinal pathologist and staged according to the Royal College of Pathologists recommended standard datasets as per standard of care. A pathological complete response (pCR) is staged as ypT0N0.

Toxic death: Any death to which IMP toxicity is thought to have a major contribution.
Early death: Death during the first three weeks of treatment that is not a toxic death.

14 SAFETY REPORTING

The Investigator will monitor each patient for clinical and laboratory evidence of adverse events on a routine basis throughout their participation in the trial. Should an Investigator become aware of any trial drug related SAEs following this period these must also be reported as stated below. Adverse event monitoring starts from the time the patient consents to the trial until they have completed their 4-6 weeks post-surgery visit. All reportable AEs will be followed to a satisfactory conclusion. Any reportable drug-related AEs that are unresolved at the end of treatment visit are to be followed up by the Investigator until resolution or stabilisation.

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

14.1 Adverse Event Definitions

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

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

Results in death	
Is life-threatening	This refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires in-patient hospitalisation or prolongs existing inpatient hospitalisation	In general, hospitalisation signifies that the subject has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether hospitalisation occurred or was necessary, the AE should be considered serious.
Results in persistent or significant incapacity or disability	This means a substantial disruption of a person's ability to conduct normal life functions. It does not include experiences of relatively minor medical significance or accidental trauma (e.g. sprained ankle), which do not constitute a substantial disruption.
Is a congenital anomaly or birth defect	
Is any other medically other medically important event	Defined as an event that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above. Any new primary cancer must be reported as an SAE.

An Adverse Drug Reaction (ADR) is an AE which is considered to be causally related to any dose of the IMP. This means that a causal relationship between the IMP and the AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

An Unexpected Drug Reaction is an adverse drug reaction, the nature or severity of which, is not consistent with applicable product information (referring to information in SPC or IB).

A Suspected Unexpected Serious Adverse Drug Reaction (SUSAR) is a serious adverse drug reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or SPC for an approved product).

14.2 Clinical laboratory abnormalities and other abnormal assessments as AEs and SAEs

Abnormal laboratory findings (e.g., clinical chemistry, haematology and urinalysis) or other abnormal assessments (e.g., ECGs, X-rays and scans) that are judged by the investigator as clinically significant will be recorded as AEs or SAEs if they meet the definitions given above.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the patient's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

14.3 Determining adverse event causality

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

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

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

14.4 Summary of trial safety reporting requirements

Event	DLT	SAE	AE/SAE	AE/SAE
AE/SAE defined as Dose limiting toxicity (DLT) defined as per NCI CTCAE v4.03	**Email reporting form within 24 hours**		**Report in AE CRF**	**Non reportable**
Absolute neutrophil count (ANC) <0.5 x 10^9/L for ≥7days	x	x	x	
Febrile Neutropenia (ANC <1000/mm3 with a single temperature of >38.3 degrees C or a sustained temperature of >=38 degrees C for more than one hour.)	x	x	x	
Infection (documented clinically or microbiologically) with Grade 3 or 4 neutropenia (absolute neutrophil count <1.0 x 10^9/L)	x	x	x	
Thrombocytopenia grade 3 (Platelet count < 50 x 109/L) in the presence of bleeding or requiring platelet transfusion	x	x	x	
Platelets <25 x 10^9/L (Grade ≥4)	x	x	x	
Clinically significant bleeding attributed to grade 3 thrombocytopenia or requiring platelet transfusion or other ≥3 grade clotting disorder or occurring concurrently with Grade 2 or 3 aPTT prolongation, unless there is a clear explanation for the event, such as tumour-related bleeding in the presence of Lupus Anticoagulant	x	x	x	
Clotting event (i.e. deep vein thrombosis [DVT], pulmonary embolism [PE]) occurring concurrently with Grade 2 or 3 aPTT prolongation	x	x	x	
Grade ≥3 diarrhoea for more than 3 consecutive days during within 2 weeks of starting radiotherapy	x	x	x	
Grade ≥3 pneumonitis	x	x	x	
Grade ≥3 cytokine release syndrome	x	x	x	
A concurrent elevation of ALT or AST >3 × ULN and total bilirubin >2 × ULN in whom there is no evidence of biliary obstruction or other causes that can reasonably explain the concurrent elevation	x	x	x	
Grade ≥3 nausea or vomiting not controlled by optimal outpatient anti-emetic treatment	x	x	x	
Grade ≥3 diarrhoea despite optimal outpatient anti-diarrhoeal medication use	x	x	x	
Other grade ≥ 3 effects thought to be directly treatment related to the combination of enadenotucirev with radiotherapy or chemotherapy	x	x	x	
Missing 3 consecutive fractions of radiotherapy, related to enadenotucirev, as judged by the investigator	x	x	x	
Any toxicity causing a delay of radiotherapy completion by greater than one week	x	x	x	
Missing 2 consecutive doses of enadenotucirev due to toxicity	x	x	x	
Death due to drug related complications	x	x	x	
Medically important events in the context of this trial (considered dose limiting and possibly, probably or definitely related to enadenotucirev combined with Radiotherapy +/- chemotherapy	**Email reporting form within 24 hours**		**Report in AE CRF**	**Non reportable**
Any AE not listed above that is grade ≥3	x	x	x	
AE considered more severe than expected	x	x	x	

AE Grade <3 that is **unexpected** and thought to be directly treatment related to the combination of enadenotucirev with radiotherapy +/- chemotherapy	**X**	**X**	**X**	
AE resulting in withdrawal	**X**	**X**	**X**	
Any late grade ≥3 toxicities – onset 12 weeks after completion of radiotherapy	**X**	**X**	**X**	
Any late grade 2 toxicities – onset 12 weeks after completion of radiotherapy			**X**	
Acute Hypersensitivity reaction to enadenotucirev	**X**	**X**	**X**	
Expected non-dose limiting toxicities	**Email reporting form within 24 hours**		**Report in AE CRF**	**Non reportable**
See section 9.2		**If grade≥4**	**X**	
All other AEs, abnormal assessments or laboratory results	**Email reporting form within 24 hours**		**Report in AE CRF**	**Non reportable**
All other AEs, assessments, abnormal laboratory results, if clinically significant		**If grade≥3**	**X**	
AE is life-threatening		**X**	**X**	
AE requires in-patient hospitalisation or prolongs existing inpatient hospitalisation		**X**	**X**	
AE results in persistent or significant incapacity or disability		**X**	**X**	
AE is a congenital anomaly or birth defect		**X**	**X**	
AE is any other medically important event		**X**	**X**	
AE related to surgery (wound dehiscence, life threatening complication requiring ICU management, single organ dysfunction including dialysis)		**X**	**X**	
Disease progression and resultant death	**Email reporting form within 24 hours**		**Report in AE CRF**	**Non reportable**
Hospitalisation (for progression or procedures planned prior to informed consent)				**X**
Clinical symptoms of progression				**X**
Death		**Possibly related directly to enadenotucirev or the combination of enadenotucirev + radiotherapy +/- chemotherapy**	**Report death and reason on death notification CRF**	

14.5 Reference safety information (RSI) for assessment of expectedness

The reference safety information (RSI) for the trial is:

- For enadenotucirev: section 6.2 of the IB for enadenotucirev, which lists all the expected side effects associated with the use of enadenotucirev
- For capecitabine: the SPC version provided by OCTO (approved for use in this trial by the MHRA). It is not specified that any particular brand of Capecitabine must be prescribed, however irrespective of the brand prescribed, the RSI to be referenced is provided by OCTO. N.B: This may not be the latest SPC version available online.

A copy of the current approved version of the RSI document must be held in the Site File for reference. Any change or update to the RSI during the trial will be made via substantial amendment.

Expected AEs due to radiotherapy are listed in section 12.2.

14.6 Suspected Unexpected Serious Adverse Drug Reactions (SUSARs)

All SUSARS must be reported to the responsible Authority and main REC by the Trial Office within the required timelines:

- Fatal or life threatening SUSARs will be reported within 7 days of the Trial Office receiving the initial report. Any additional information will be reported within eight days of sending the first report.
- All other SUSARs will be reported within 15 days of the Trial Office receiving the initial report

In addition, other safety issues qualify for expedited reporting where they might materially alter the current risk assessment of an IMP or be sufficient to change IMP administration or the overall conduct of the trial.

14.7 Expedited reporting of SAEs

The following SAE reporting requirements apply regardless of the Investigator's assessment of the causality or expectedness of the SAE. All SAEs should be reported on the trial SAE Report Form (see SAE Report Form and completion guidelines).

If a Serious Adverse Event occurs that requires reporting, a Serious Adverse Event reporting form should be completed and emailed within 24 hours of becoming aware of the event to:

Pharmacovigilance Office, OCTO: octo-safety@oncology.ox.ac.uk
Tel no: +44 (0) 01865 617082

If the SAE has not been reported within the specified timeframe, a reason for lateness must be provided when sending the SAE Report Form.

Investigators should also adhere to their local Trust policy for incident and SAE reporting in research.

14.8 Follow-up of Serious Adverse Events

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

If new or amended information on a reported SAE becomes available, the Investigator should report this on a new SAE Form using the completion guidelines. If using the original form to notify further information, you must initial and date all new or amended information so that all changes are clearly identified.

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

14.9 Reporting Adverse Events on the CRF

All AEs, including Serious AEs must be recorded on the case report forms (CRF) for that patient (unless otherwise specified in section 14). The information provided will include date of onset, event diagnosis (if known) or

sign/symptom, severity, time course, duration and outcome and relationship of the AE to trial drug. Any concomitant medications or other any therapy used to treat the event must be listed. The Investigator will provide an "other" cause for serious AEs considered to be unrelated to the trial drug. Sites should ensure data entered into the CRF is consistent with the SAE report information where applicable.

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

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

AEs which are serious and occur from the first dose of trial treatment up to and including 30 days after administration of the last dose of trial treatment must be reported to OCTO. Any SAE that occurs at any time after completion of trial treatment or after the designated follow-up period that the sponsor-investigator and/or co-investigator consider to be related to any trial drug must be reported to OCTO.

Terms and Grading of Events
All adverse events and toxicities must be graded according to the NCI Common Terminology Criteria for adverse events (NCI-CTCAE) Version 4.03.

14.10 Events exempt from being reported as AE/ SAEs

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

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

Death on trial
Death due to disease under trial is to be recorded on the Death CRF providing the death is not unexpected or if a causal relationship suspected. The investigator must clearly state whether the death was expected or unexpected and whether a causal relationship to the trial IMP or other protocol treatment intervention is suspected.

Elective admissions and supportive care
Elective admissions to hospital for patient convenience or for planned procedures or investigations or treatment as specified in this protocol and standard supportive care are not SAEs, and do not require SAE reporting.

14.11 Informing Investigators of new safety information

The Trial Office or the Chief Investigator will ensure that all investigators are kept informed in a timely manner, as new safety profile information becomes available. Principal Investigators are responsible for briefing their trial team and onward transmission to their R&D office as appropriate.

15 PREGNANCY

Pregnancies (in a participant or partner) occurring while participating in this trial require expedited reporting. This includes any pregnancy (also applies to female partners of male trial subjects) occurring within 6 months of the last administration of trial drug even if participant is discontinued from the trial. A Pregnancy Form should be completed and sent to the trial office within the same timelines as an SAE. All reported pregnancies should be followed and the outcome reported using the same form. If the outcome of the pregnancy meets any of the criteria for seriousness, it must also be reported as an SAE. Examples of pregnancy outcomes that are SAEs include reports of:

- Congenital anomalies or developmental delay, in the foetus or the child.
- Foetal death and spontaneous abortion.
- Suspected adverse reactions in the neonate that are classified as serious

Women who become pregnant should be withdrawn from trial treatment immediately.

16 DEFINING THE END OF TRIAL

For this trial the end of the trial is defined as: "The last visit of the last patient undergoing the trial (LPLV)" i.e. when (i.e. 4-6 weeks post-surgery visit) or has progressed or died.

Recruitment will be stopped when either:

- Sufficient patients have been recruited to achieve the primary and secondary objectives of the trial, as per the trial design & statistical model detailed in sections 2 and 17 (maximum 30 patients) ***or***
- When a defined stopping rule is met.

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

17 STATISTICAL CONSIDERATIONS

17.1 CEDAR: Evaluation of treatment schedules

The trial will use a model-based approach using the toxicity and efficacy primary endpoints to recommend the treatment schedule for future patients, and to recommend the optimal dose at the trial's conclusion.

We are using both efficacy and toxicity endpoints because although toxicity is expected to increase with increasing dose, it is possible that the relationship between efficacy and dose may not be a monotonically increasing one. This means that the optimal dose may not be the maximum tolerated dose. However, there will be no formal combination of the efficacy and toxicity data, both endpoints will contribute information independently to dose choice via dose escalation rules.
Toxicity data can be used continuously throughout the trial through the use of the TiTE-CRM. We will use a 2-parameter logistic regression model to model the relationship between dose and toxicity. Data for patients who have completed the 13 week toxicity window or experienced a dose limiting toxicity will contribute full information to this model. Only partial information is known for patients who are currently on trial within the toxicity window, or those who withdraw during treatment for reasons other than a DLT i.e. patient choice. This partial information contributes to the model, weighted proportionally to the observed portion of their toxicity time window and the proportion of treatment received, and treating them as not experiencing a dose limiting toxicity.

We will use a 3-parameter logistic regression model to model the relationship between dose and efficacy. Patients are assessed for the efficacy endpoint in week 13. Patients who have not reached this time point yet will not provide any information to this model. Patients who have reached the time point for this endpoint and did not have the evaluation, or who withdrew or died prior to evaluation will be treated as non-responders.

Specification of priors and simulation results are given in the SAP.

17.2 Dose selection during the trial

The first two patients will be assigned to treatment schedule 1 and followed to week 13. Thereafter, the toxicity and response models will be fitted every time a new patient is screened and the treatment schedule recommended for the next patient will be the minimum of the doses recommended by the toxicity and efficacy models.

Define:

- a target toxicity interval as (0.2, 0.35)
- a safety criterion such that a dose is considered safe to give if the posterior probability that the DLT rate is greater than 0.35 on the dose is less than 35% (i.e. P(risk of DLT > 0.35 |Dose, Data) < 0.35)

Then the dose that is recommended by the toxicity model is the dose with the highest posterior probability of DLT rate lying in the target toxicity interval among those doses that satisfy the safety criterion.

The treatment schedule recommended by the efficacy model is the dose with the highest posterior mean probability of response.

A further restriction on escalation is that an untried dose can only be given if at least 2 patients have been given the dose below for at least 8 weeks.

The toxicity and efficacy models are for guidance only and the TMG may overrule the recommendation, but only in favour of a lower dosing schedule than the model recommends.

See Neuenschwander, Branson and Gsponer (2008). Critical aspects of the Bayesian approach to phase I cancer trials. Stats in Med, **27**:2420-2439 for details of the toxicity model.

17.3 Stopping the trial early

In the event that all dose levels are toxic, the trial will have a built in rule to stop the trial before reaching the maximum number of patients. If P(risk of DLT > 0.35|dose = 1, current data) > 0.65 for the lowest dose level and at least three patients have complete data for the toxicity endpoint (a DLT or have completed the toxicity window) we will stop the trial.

17.4 Importance of rapid data return

Patient Status Update CRF
Analysis will be performed in real-time based on all relevant available data to best inform the treatment schedule to allocate to the next patient. The patient status update CRF is a custom built CRF that will capture all of the required data and should be entered promptly upon request. E.g. the next working day.

Data entered will also be used for safety monitoring. For these reasons, it is important that data is collected and made available on OpenClinica swiftly and no more than 5 working days of the initial event and within 14 days of receipt of a data query unless otherwise specified.

18 STATISTICAL ANALYSIS PLAN

For all analyses, patients will be included according to the treatment schedule to which they are assigned. All patients who receive at least one dose of enadenotucirev, regardless of how much treatment received and follow-up completed, will contribute to analysis. It is therefore important that every effort is made to encourage patients, including those patients who do not receive/complete their allocated treatment, to attend for follow-up clinic visits to avoid bias in the analysis of the results.

Further details can be found in the Statistical Analysis Plan.

18.1 Inclusion in analysis

All patients enrolled in the trial and who received at least one dose of enadenotucirev will be accounted for and included in the analyses. The number of patients who were not evaluable, who died or withdrew before treatment began will be recorded.

Baseline characteristics will be summarised for all enrolled patients. Patients who died or withdrew before treatment started or do not complete the required safety observations will be described and evaluated separately.
Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment.

18.2 Interim Analyses

The trial will use the model based design to propose treatment schedules and will be updated prior to each TMG meeting to recommend a treatment schedule for each patient recruited.

18.3 Procedures for reporting any deviation(s) from the protocol

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

18.4 Final analysis

Primary analysis will be after all patients have completed their 13 week, end of treatment visit.

19 TRIAL COMMITTEES

19.1 Trial Management Group (TMG)

The Chief Investigator will chair a TMG responsible for overseeing the successful conduct and publication of the trial. The TMG will include Chief Investigator, Co- Investigators, Clinical Trial Manager, Trial Statistician and others as required. The TMG will meet as necessary to discuss toxicity data and to decide on dose escalation. TMG membership and decision making procedures will be documented in the TMG charter.

19.2 Safety Review Committee (SRC)

There is no independent Data and Safety Monitoring Committee (DSMC) for this trial. The Safety Review Committee (SRC) will be convened as required to review DLTs and dose escalation decisions, made by the TMG. In the event of the TMG being unable to conclude on a dose recommendation, the SRC will meet to decide. The main outcomes will be analysed as stated in the analysis plan and will not be analysed as an interim analysis. The SRC will consist of:

1. Trial Statistician
2. OCTO trial management representative
3. Either:
    a. One Medical Oncologist and one Clinical Oncologist or
    b. Two Clinical Oncologists

The SRC Charter document for this trial will define the exact membership and who should be present for decisions to be made. Further internal or external experts may be consulted by the SRC, as necessary. Any PI can request an ad hoc SRC meeting at any time in order to facilitate the immediate communication of any emerging safety issues during the course of the trial.

19.3 Independent Radiotherapy & Imaging Oversight Committee (RIOC)

RIOC will act as the TSC. The role of RIOC is to provide oversight for the trial on behalf of the Sponsor and Funder(s). The RIOC will provide overall supervision of the safe and effective conduct of the trial. The RIOC will review trial progress against agreed milestones, adherence to protocol, and patient safety, and consider new information. The RIOC has responsibility for deciding if the trial needs to be stopped early on grounds of safety or efficacy.

20 DATA MANAGEMENT

20.1 Database considerations

Data management will be performed via a web-based, bespoke trial database (OpenClinica). OpenClinica is a dedicated and validated clinical trials database designed for electronic data capture. See: http://www.openclinica.org. The trial office will provide sites with instructions and a video link for training purposes.

The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will NOT be included in any trial data electronic file.

20.2 Case reports forms (CRFs)

The Investigator and trial site staff will ensure that data collected on each subject is recorded in the CRF as accurately and completely as possible. All appropriate laboratory data, summary reports and Investigator observations will be transcribed into the CRFs from the relevant source data held in the site medical record(s). CRFs entries will not contain any source data (unless otherwise specified in the completion instructions provided by the trial office). It is important to ensure that:

- the relevant CRFs are completed.

- The key data items (Patient Status Update CRF) that are used in the statistical model to calculate next recommended dose will be completed for all patients by the deadline requested by OCTO; this data return is essential for TMG decision making
- all CRF data are verifiable in the source documentation or the discrepancies must be explained.
- CRF sections are completed in a timely fashion, as close to the visit or event being recorded as possible.
- Data queries are resolved and documented by authorised trial staff in a timely fashion. The reason for the change or correction should be given where appropriate.
- As much data as possible is entered and cleaned in preparation for each trial database lock point.

Note: 'in a timely fashion' means within no more than 5 working days of the initial event and within 14 days of receipt of a data query unless otherwise specified.

The above considerations also apply to patients who are withdrawn early. If a patient withdraws from the trial, the reason must be noted on the appropriate form and the patient must be followed-up as per protocol.

20.3 Accounting for missing, unused, or spurious data.

The statistical analysis plan will describe the procedure for accounting for missing, unused or spurious data.

21 CLINICAL TRIAL REPORT

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

22 TRIAL SITE MANAGEMENT

22.1 Trial site responsibilities

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

22.2 Trial site set up and activation

The Principal Investigator leading the investigational trial site is responsible for providing all required core documentation. Mandatory Site Training organised by the trial office must be completed before the site can be activated. The Trial Office will check to confirm that the site has all the required trial information/documentation and is ready to recruit. The site will then be notified once they are activated on the trial database and able to enter patients.

22.3 Trial documentation

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

23 REGULATORY AND ETHICAL CONSIDERATIONS

The Sponsor and Investigators will ensure that this protocol will be conducted in compliance with the UK Clinical Trials Regulations[1] and the applicable policies of the sponsoring organisation. Together, these implement the ethical principles of the Declaration of Helsinki (1996) and the regulatory requirements for clinical trials of an investigational medicinal product under the European Union Clinical Trials Directive.

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

23.1 Ethical conduct of the trial and ethics approval

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

23.2 Regulatory Authority approval

This trial will be conducted under a UK Medicines and Healthcare Products Regulatory Agency (MHRA) Clinical Trials Authorisation (CTA). Approval to conduct the trial will be obtained from the Responsible Authority prior to initiating the trial.

23.3 NHS Research Governance

Investigators are responsible for ensuring they obtain local Trust management agreement to conduct the trial in accordance with local arrangements and policies.

23.4 Protocol amendments

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

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of the investigational medicinal product(s) used in the trial.

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

All amendments will be generated and managed according to the trial office standard operating procedures to ensure compliance with applicable regulation and other requirements. Written confirmation of all applicable REC, regulatory and local approvals must be in place prior to implementation by Investigators. The only exceptions are for changes necessary to eliminate an immediate hazard to trial patients (see below).
It is the Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented if appropriate.

23.5 Urgent safety measures

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

The notification must include:

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

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

23.6 Temporary halt

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

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

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

23.7 Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations require the Sponsor to notify any "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach. A serious breach is defined as “A breach of GCP or the trial protocol which is likely to effect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial”

Investigators must notify the Trials Office within one working day if any serious breach of GCP is suspected. The Trial Office will review the event and, if appropriate will report a serious breach to the REC, Regulatory Authority and the NHS host organisation within 7 days of the Trial Office becoming aware of the breach.

23.8 Trial Reports

This protocol will comply with all current applicable Regulatory Authority, Research Ethics Committee and Sponsor reporting requirements.

The trial office will determine which reports need to be circulated to Principal Investigators and other interested parties. Trial sites are responsible for forwarding trial reports they receive to their local Trust as required.

24 EXPENSES AND BENEFITS

Reasonable travel expenses for the additional trial related visits, in excess of standard of care as per local practice, will be covered by PsiOxus Therapeutics (Up to a total of £120.00 per patient)

The local arrangements will also be explained to the patient during the informed consent discussions prior to trial entry. E.g. hospital transport or parking permits.

25 QUALITY ASSURANCE

25.1 Risk assessment

A risk assessment and a monitoring plan will be prepared before the trial opens and will be reviewed throughout the trial if necessary in the light of significant changes while the trial is on-going or in response to outcomes from monitoring activities. Monitoring plans will be amended as appropriate.

25.2 Monitoring

Regular monitoring will be performed according to the monitoring plan. Data will be evaluated for compliance with the protocol, completeness and accuracy. The investigator and institutions involved in the trial will permit trial - related monitoring and provide direct on-site access to all trial records and facilities if required. They will provide adequate time and space for the completion of monitoring activities.

Trial sites will be monitored centrally by checking incoming data for compliance with the protocol, consistency, completeness and timing. The case report data will be validated using appropriate set criteria, range and verification checks. The trial site must resolve all data queries in a timely manner. All queries relating to key outcome and safety

data and any requiring further clarification will be referred back to the trial site for resolution. For other non-critical data items, OCTO staff may resolve data queries centrally providing the correct answer is clear. Such changes will be clearly identified in the CRF and the trial site informed.

Trial sites will also be monitored remotely and/or by site visit as necessary to ensure their proper conduct of the trial. OCTO staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have. Monitoring reports will be sent to the site in a timely fashion. The Investigator is expected to action any points highlighted through monitoring and must ensure that corrective and preventative measures are put into place as necessary to achieve satisfactory compliance.

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

- Participant screening log
- Anonymised baseline and post-treatment MRI reports

25.3 Audit and Regulatory Inspection

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

26 RECORDS RETENTION & ARCHIVING

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable the conduct of a clinical trial and the quality of the research data to be evaluated and verified. All essential documents must be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed. The medical files of trial subjects must be retained in accordance with applicable national legislation and the host institution policy.

Retention and storage of laboratory records for clinical trial samples must also follow these guidelines.
Retention and storage of central laboratory records supporting PK or PD endpoints and the disposition of samples donated via the trial must also comply with applicable legislation and Sponsor requirements.

It is the University of Oxford's policy to store data for a minimum of 5 years. Investigators may not archive or destroy trial essential documents or samples without written instruction from the trial office.

Data from this trial may contribute towards a marketing authorisation and as a result, essential clinical trial documents (including CRF's), other than the patients' medical notes, will need to be held for a minimum of 15 years after completion or discontinuation of the trial.

27 PATIENT CONFIDENTIALITY

Personal data recorded on all documents will be regarded as confidential, and to preserve each patient's anonymity, only their initials and year of birth will be recorded on the CRFs.

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

28 TRIAL FUNDING

The CEDAR trial is funded in part by the Clinical Research Committee (Early Phase & Feasibility Studies) on behalf of Cancer Research UK (CRUK/17/015). The Oncology Clinical Trials Office is supported by Cancer Research UK core funding. PsiOxus Therapeutics Limited are providing a grant and enadenotucirev to support the trial. This trial is further supported via the University of Oxford core clinical and research infrastructure underpinned by strategic research programme grant funds. This trial is on the NIHR portfolio; local research network support should be available at each site taking part to support entry of participants into this trial.

29 SPONSORSHIP AND INDEMNITY

29.1 Sponsorship

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

29.2 Indemnity

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

29.3 Contracts/Agreements

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

30 PUBLICATION POLICY

The sponsor will retain ownership of all data arising from the trial. The intention is to publish this research in a specialist peer reviewed scientific journal on completion of the trial. The results may also be presented at scientific meetings and/or used for a thesis. The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the trial and retain final editorial control. Authors will acknowledge that the trial was Sponsored by and performed with the support of the Sponsor and other funding bodies as appropriate.

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APPENDIX 1: ECOG PERFORMANCE SCALE

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

APPENDIX 2: MRI ASSESSED TUMOUR REGRESSION GRADING (mrTRG)

TRG score	mrTRG description
1	No/minimal fibrosis visible (tiny linear scar) and no tumour signal
2	Dense fibrotic scar (low signal intensity) but no macroscopic tumour signal *(indicates no or microscopic tumour)*
3	Fibrosis predominates but obvious measureable areas of tumour signal visible
4	Tumour signal predominates with little/minimal fibrosis
5	Tumour signal only: no fibrosis, includes progression of tumour

For the purposes of trial analysis and dose escalation, scores of 1 or 2 will be classified as responders and scores of 3, 4 or 5 will be classified as non-responders.

APPENDIX 3: NEOADJUVANT RECTAL SCORE

$$NAR = \frac{[5\ pN - 3(cT - pT) + 12]^2}{9.61}$$

Where:

cT is an element of the set {1, 2, 3, 4},
pT is in {0, 1, 2, 3, 4},
pN is in {0, 1, 2}.
cT clinical tumour stage,
pT pathologic tumour stage,
pN pathologic nodal stage

The neoadjuvant rectal score (47) outperforms pCR at predicting DFS and OS in clinical trials using neoadjuvant therapy for rectal cancer. It similarly performs well at predicting DFS and OS in trials using pre-op chemo and chemoradiotherapy (TNT). It is an NCI-approved short-term endpoint for phase II clinical trial performance. The final score ranges from 0-100 where a higher score equates to a worse prognosis.