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

Statistical Analysis Plan

Version 2.0 – 4th January 2021

Based on Protocol version 3.0_11Aug2020

Trial registration: 2017-003501-18

Role	Name	Title	Signature	Date
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CONTENTS

1. INTRODUCTION

This document details the proposed data presentation and analysis for the main paper(s) and final study reports from the Cancer Research UK & PsiOxus Therapeutics Limited Multicentre of intravenous EnAdenotucirev in combination with chemoradiotherapy in the treatment of locally advanced rectal cancer (CEDAR). The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

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

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

1.1 Key personnel

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1.2 Changes from previous version of SAP

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

Version number Issue date	Author of this issue	Protocol Version & Issue date	Significant changes from previous version together with reasons
V1.0_20Jan2020	Alexander Ooms, Jane Holmes	Protocol_V2.0_20Nov2019	Not applicable as this is the 1st issue
V2.0_04Jan2021	Alexander Ooms, Jane Holmes	Protocol_V3.0_11Aug2020	Updated to be in line with most recent protocol

2. BACKGROUND AND OBJECTIVES

2.1 Background and rationale

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 - Capecitabine	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 - Tablet, 900mg/m2, oral administration
Other interventions:	Neoadjuvant chemoradiotherapy	
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)	

2.2 Objectives

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 enadenotucirev	• 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

Note: Objectives in grey are not described in this SAP and will not be undertaken by the trial statistician.

3. STUDY METHODS

3.1 Trial Design/framework

Provide a brief description of trial design including type of trial (e.g. parallel group, multi-arm, cross-over, factorial), and brief description of the interventions.
Describe the framework such as superiority, non-inferiority, equivalence including which comparisons will be presented on this basis.

This is a dual-endpoint dose finding phase I trial. 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.

3.2 Randomisation and Blinding

This is not a randomised trial.

3.3 Sample Size

At most, 30 patients will be recruited. Simulations have been used to justify this sample size, results are given in Appendix 1.

3.4 Statistical Interim Analysis, Data Review and Stopping guidelines

The TMG will meet as necessary to discuss toxicity data and to decide on dose escalation. The first interim analysis will occur after the first 2 patients have been followed for 13 weeks. Subsequently there will be an interim analysis each time a dose-escalation decision is required. The TMG will include Chief Investigator, Co-Investigators, clinical Trial Manager, Trial Statistician and others as required. TMG membership and decision making procedures are documented in the TMG charter (V1.0, 26th November 2019) and is stored in the TMF.

Dose-escalation decisions will be based on all currently available data and guided by the pre-specified model. Analysis will follow the methods for primary analysis and are described in Section 6.2.1

The template for the first TMG dose decision report is given in Appendix 2. Changes may be made to this template as the trial progresses, but note that the original template will not be updated as it refers only to the first dose decision meeting.

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 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 is stored in the TMF and 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.

RIOC (Radiotherapy and Imaging Oversight Committee) will act as the TSC and convenes roughly every 6 months. The role of RIOC is to provide oversight for the trial on behalf of the Sponsor and Funder(s) as described in the charter. 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.

The trial may stop early for safety. In the event that all dose levels are toxic, the trial will stop 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.

3.5 Timing of Final Analysis

Primary and secondary outcomes will be analysed after all patients have completed their end of treatment visit, usually at week 13. Analyses for all tertiary outcomes will be documented elsewhere and will not be undertaken by the trial statistician.

3.6 Blinded analysis

This is not a blinded trial.

3.7 Statistical Analysis Outline

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.

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.

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.

4. STATISTICAL PRINCIPLES

4.1 Statistical Significance and Multiple Testing

There will be no statistical significance level defined for CEDAR as it is a dose-finding trial and schedule recommendations will be based on the posterior probabilities calculated by the dose-toxicity and dose-response models using all available data at each time.

4.2 Definition of Analysis Populations

Main: all patients who received at least one dose of enadenotucirev.
Surgical: All patients who underwent surgery after treatment
Sensitivity: Patients who received the full prescribed dose of enadenotucirev, i.e. did not miss any dose, or had a DLT. If a patient is still on treatment and has received their full prescribed dose of enadenotucirev so far, they will be included in this population.

For dose-escalation decisions during the trial, the population analysed will be the main and sensitivity populations. However, for the efficacy endpoint, patients who have not reached this time point yet will not provide any information to the efficacy model. Patients who have reached the time point for this endpoint and did not have the evaluation, withdrew or died prior to evaluation will be treated as non-responders.

5. TRIAL POPULATION AND DESCRIPTIVE ANALYSES

5.1 Representativeness of Study Sample and Patient Throughput

The flow of participants through each stage of the trial, including numbers of participants assigned to a schedule, receiving intended treatment, completing the study protocol, and analysed for the primary outcome is provided following CONSORT. Protocol violations/deviations and information relating to the screening data including the number of ineligible patients entering the study, together with reasons will be reported. Information on number of participants screened, found to be ineligible (with reasons where available), refused to participate (with reasons where available) will also be included.

A CONSORT diagram will be prepared, an example CONSORT diagram is given in Appendix 3.

5.2 Withdrawal from treatment and/or follow-up

Withdrawals/loss to follow-up together with reasons will be reported by treatment schedule.

5.3 Baseline Characteristics

Baseline characteristics, including important prognostic, demographic and clinical variables will be reported overall for the main population.

Numbers (with percentages) for binary and categorical variables and means (and standard deviations), or medians (with lower and upper quartiles) for continuous variables will be presented.

5.4 Unblinding

The trial is not blinded.

5.5 Description of Compliance with Intervention

A summary of enadenotucirev received will be presented by assigned treatment schedule. Compliance to chemotherapy and radiotherapy is a secondary outcome and will be presented as described in Section 6.2.

5.6 Reliability

The NAR score will be hand checked for 5 patients.

6. ANALYSIS

6.1 Outcome Definitions

Primary outcome:

- *Safety:* Binary toxicity measure defined by the occurrence of a DLT or not. This is recorded during the 13 weeks of follow-up after starting treatment. The definition of a DLT is given Appendix 4.
- *Efficacy:* MRI tumour regression grade. This is a 5 point scale that is coded as a binary variable, with scores of 1 or 2 defining a responder and scores of 3, 4 or 5 a non-responder. It is measured at 13 weeks. Patients who did not have the evaluation, or who withdrew or died prior to evaluation will be treated as non-responders. During the trial, for dose-escalation decisions a patient who has not yet reached this time point will not be included in analysis at this decision point. See Appendix 5 for the definition of MRI tumour regression grade

  See Section 6.2.1 Primary analysis for definition of the optimal dose.

  For dose-escalation interim analyses the safety and efficacy data is taken from the patient status update CRF. For the final determination of the MTD at the end of the trial, the safety outcome will be taken from the *BW135_DLT_Assssment* and *BW166_Adverse_Events* CRFs and the efficacy outcome taken from the *BW156_Week13_Pelvic_MRI* CRF.

Secondary outcomes:

- Treatment tolerance. This is the amount of capecitabine and radiotherapy received, coded as completing at least 80% of the intended capecitabine dose and at least 20 fractions of radiotherapy. Measured at week 9. Compliance is taken from the *BW150_Capecitabine* and *BW145_Radiotherapy* CRF.
- MRI tumour regression grade. See definition for primary outcome, but here it will not be dichotomised. MRI tumour regression grade is taken from the *BW156_Week13_Pelvic_MRI* CRF.
- Pathological complete response. This variable relates to the resected tumour so will only be observed for patients undergoing surgery. It is measured on a 4 point scale, with a score of 1 denoting pathological complete response, and scores 2:4 denoting no pathological complete response. Pathological complete response is taken from the *BW156_Week13_Pelvic_MRI* CRF.
- Neoadjuvant Rectal (NAR) score. This score ranges from 0-100 with a higher score equating to a worse prognosis. It is a derived outcome which components measured on the resected tumour and at initial tumour staging. Therefore, this will only be observed for patients undergoing surgery. It is defined as

- $NAR = \frac{[5pN-3(cT-pT)+12]^2}{9.61}$, where
- cT is clinical tumour stage and is an element of the set {1, 2, 3, 4}
- pT is pathological tumour stage and takes a value in {0, 1, 2, 3, 4}
- pN is pathological nodal stage is in {0, 1, 2}
- pT and pN are taken from the *BW160_Surgery_Details* CRF and denoted ypT and ypN, cT is taken from the *BW070_Screen_Pelvic_MRI* CRF.

Tertiary outcomes:

- IHC staining of hexon protein coat, viral gDNA and viral mRNA gene expression in tumour cells – not covered in this SAP as analysis is not carried out by the trial statistician
- Whole genome RNA expression via RNA sequencing/ Nanostring gene expression analysis – not covered in this SAP as analysis is not carried out by the trial statistician
- ctDNA analysis exploring clearance of ctDNA and any/or emerging changes in persisting ctDNA – not covered in this SAP as analysis is not carried out by the trial statistician
- Extraction of DNA and 16S sequencing and meta-transcriptomics from faecal samples – not covered in this SAP as analysis is not carried out by the trial statistician
- Multiplex immunohistochemistry of immune cell markers – not covered in this SAP as analysis is not carried out by the trial statistician

6.2 Analysis Methods

6.2.1 Primary analysis – optimal dose and frequency of enadenotucirev that can be administered with chemoradiation

The optimal dose and frequency of enadenotucirev will be estimated using toxicity and efficacy endpoints using the main population. The same analysis is used during dose escalation as well as for final analysis.

Toxicity endpoint

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. 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. The weight function is defined in the equation below as w_j.

There are 4 treatment schedules which are coded as i = (1, 2, 4, 6):4. Assuming dose 6 is the reference dose, d*, we have treated *J* patients with dose x_j, *j=1, … J*, then we model the probability of a DLT occurring using the weighted likelihood given by

$$L(\theta) = \prod_{j=1}^{J} \left[g\left(\theta_{x_j}\right) * w_j \right]^{y_j} \left[1 - g\left(\theta_{x_j}\right) * w_j \right]^{1-y_j}$$

where

y_j = 1 if a DLT occurred and 0 otherwise and

$g\left(\theta_{x_j}\right)$ = logit(θ_{x_j}) = log(α) + β log(x_j/d^*)

$$w_j = \begin{cases} 1 \text{ if } y_j = 1 \\ \frac{1}{2}\left(\frac{u_j}{T}+\frac{v_j}{D_i}\right) \text{ if } y_j=0 \end{cases}$$

$u_j =$ time patient has been followed for, T = total DLT window = 13 weeks

$v_j =$ amount of dose recieved, D_i = total dose for treatment schedule i

We assume priors for α and β of

$$\log(\alpha) \sim \text{Normal}(\log(0.3/0.7), \text{var} = 1.44)$$
$$\log(\beta) \sim \text{Normal}(-0.1, \text{var} = 0.25)$$
$$corr(\alpha, \beta) = 0$$

We can then calculate the posterior probability of toxicity for each treatment schedule.

See Neuenschwander, Branson and Gsponer (2008) and Cheung YK and Chappell R (2000) for descriptions of the model.

Efficacy endpoint

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.

Let z_j denote 1 if the patient responded and 0 otherwise. Then we assume

$$z_j|x_j \sim \text{Bernoulli}(\emptyset_{x_j})$$
$$\text{logit}(\emptyset_{x_j}) \sim \gamma_1 + \gamma_2 \log(x_j/d^*) + \gamma_3[\log(x_j/d^*)]^2$$
$$\pi(\gamma_1, \gamma_2, \gamma_3) \sim MVN\left(\begin{pmatrix} -0.5 \\ 0.5 \\ 0 \end{pmatrix}, \begin{pmatrix} 7 & 0 & 0 \\ 0 & 7 & 0 \\ 0 & 0 & 4 \end{pmatrix}\right)$$

We can then calculate the posterior probability of efficacy for each treatment schedule.

Definition of MTD

At each analysis the optimal dose is defined as 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. The optimal dose is then the minimum of these 2 doses.

Dose-escalation rules

The dose suggested for the next patient is the optimal dose as defined above. However, escalation to an untried dose is subject to no dose skipping, and is only permissible if at least 2 patients have been given the dose immediately below for at least 8 weeks. There is no restriction on de-escalation.

6.2.2 Secondary and tertiary analyses

There will be no formal analysis of the secondary or tertiary objectives. See template in appendix 3 for tables and figures that will be presented.

6.3 Missing Data

Every effort will be made for complete collection and recording of data. To minimise missing data, a dedicated CRF has been designed which captures only that data required to for dose-escalation decisions.

No data imputation is planned.

6.4 Sensitivity Analysis

Sensitivity analyses will be carried out during the trial for dose-decision meetings and also for final analysis for estimating the optimal dose. In addition to repeating the analysis using the sensitivity population defined in Section 4.2, we will also repeat the analysis using different weight functions. Therefore the 2 sensitivity analyses are:

1) Sensitivity population and analysis using weights according to length of follow-up only, i.e. not taking into account how much dose has been received
2) Main population but with the most toxic scenario, i.e. we assume that all patients currently in follow-up within the DLT Window of 13 weeks have a DLT

6.5 Pre-specified Subgroup Analysis

No subgroup analyses are planned.

6.6 Supplementary/ Additional Analyses and Outcomes

There are no supplementary or additional analyses planned.

6.7 Harms

This analysis will be based on the main population and will be presented as given in Appendix 2.

6.8 Health Economics and Cost Effectiveness (where applicable)

There is no health economics and cost effectiveness analysis.

6.9 Meta-analyses (if applicable)

No meta-analysis is planned.

7. VALIDATION OF THE PRIMARY ANALYSIS

Before the trial starts, code for generating dose decisions will be validated by creating 2 large datasets, one for efficacy and one for toxicity, and comparing results from analysis using standard logistic regression with the trial code. Although results will not be identical due to the differences between frequentist and Bayesian

analyses, with fairly vague priors and a large dataset parameter estimates should be similar. Validation will be stored in the statistical eTMF.

8. SPECIFICATION OF STATISTICAL PACKAGES

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

9. REFERENCES

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.

Cheung YK, Chappell R. Sequential designs for phase I clinical trials with late-onset toxicities. Biometrics. 2000;56:1177–82.

Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Dore C, Williamson PR, Altman DG, Montgomery A, Lim P. Berlin K, Senn S, Day S, Barbachano Y, Loder E. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. **JAMA.** 2017 Dec 19;318(23):2337-2343. doi: 10.1001/jama.2017.18556.

APPENDIX: GLOSSARY OF ABBREVIATIONS

SAP	Statistical Analysis Plan
DSMC	Data and Safety Monitoring Committee
TSC	Trial Steering Committee
CI	Chief Investigator
CRF	Case Report Form

10. APPENDIX 1 SIMULATION RESULTS AND DOSE DECISION TREE

This is a separate document which is signed by the trial statistician, senior statistician and CI

11. APPENDIX 2 TEMPLATE FOR FIRST DOSE DECISION MEETING

This is a separate document which is signed by the trial statistician, senior statistician and CI

12. APPENDIX 3 TEMPLATE TABLES AND FIGURES FOR FINAL STATISTICAL REPORT

This is a separate document which is signed by the trial statistician, senior statistician and CI

13. APPENDIX 4 DEFINITION OF DLT

Definition of DLT as given in Protocol V3.0, 11th August 2019

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) 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, 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 > 6 days
- Febrile neutropenia grade 4 (fever of unknown origin without clinically or microbiologically documented infection) (ANC <1.0 x 109/L, fever >38.5°C)
- 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 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
- 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.

14. APPENDIX 5 DEFINITION OF MRI TUMOUR REGRESSION GRADE

Definition as given in Protocol V3.0, 11th August 2020

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.