

PEARL: PET-BASED ADAPTIVE RADIOTHERAPY CLINICAL TRIAL

VERSION 3.0

15TH OCTOBER 2021

Sponsor:	Velindre University NHS Trust Velindre Road Whitchurch Cardiff CF14 2TL
Sponsor ref:	2018/VCC/0029
Funder:	Cancer Research Wales Velindre Radiotherapy Charitable Funds (Moondance)
Funder ref:	2495
REC ref:	18/WA/0391
IRAS number:	242633
ClinicalTrials.gov ref:	NCT03935672
Q-Pulse Document Template Number:	TPL/003/2

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the relevant trial regulations, GCP guidelines, and CTR's SOPs.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies from the trial as planned in this protocol will be explained.

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General Information This protocol describes the PEARL clinical trial and provides information about the procedures for entering participants into the trial. The protocol should not be used as a guide, or as an aide-memoire for the treatment of other participants. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the known Investigators in the trial. Problems relating to the trial should be referred, in the first instance, to CTR.

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This protocol has been developed by the PEARL Trial Management Group (TMG).

For **all queries** please contact the PEARL team through the main trial email address. Any clinical queries will be directed through the Trial Manager to either the Chief Investigators or the Co-Investigators.

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Enrolment

Sites should provide a delegation log to the CTR PEARL team during local trial set-up.

Site staff delegated to patient enrolment and/or data entry duties will be granted access to an online enrolment portal and MACRO data entry system.

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Glossary of abbreviations

ABPI	Association of the British Pharmaceutical Industry
AE	Adverse Event
AR	Adverse Reaction
AS	Automatic Segmentation
AT	Adaptive thresholding
ATLAAS	Automatic decision Tree-based Learning Algorithm for Advanced Segmentation
bGTV	Biological Gross Tumour Volume
C&V UHB	Cardiff & Vale University Hospital Board
CBCT	Dental Cone-beam Computed Tomography
CCRT	Concurrent Chemoradiotherapy
CERR	Computational Environment for Radiotherapy Research
CF	Consent Form
ctDNA	Circulating tumour DNA
CHI	Community health index
CI	Chief Investigator
CI_n	Confidence Interval
CIDA	Cancer Imaging and Data Analytics
CMR	complete metabolic response
CRF	Case Report Form
CRUK	Cancer Research United Kingdom
CSG	Clinical Studies Group
CPA	Clinical Pathology Accreditation
CT	Computerised tomography
CTA	Clinical Trials Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTIMP	Clinical Trial of Investigational Medicinal Product
CTR	Centre for Trials Research
CTV	Clinical Target Volume
CU	Cardiff University
DICOM	Digital Imaging and Communications
DNA	Deoxyribonucleic acid
DSC	Dice Similarity Coefficient
DWI	Diffusion Weighted Imaging
EORTC	European Organisation for Research and Treatment of Cancer
EPSRC	Engineering and Physical Sciences Research Council
FCM	Fuzzy C-means
FDG	¹⁸ F-fluorodeoxyglucose
FFPE	Formalin-Fixed Paraffin Embedded
GCM	Gaussian Fuzzy C-means
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
GTV	Gross Tumour Volume
HB	Health Board
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human Papillomavirus
HRA	Health Research Authority
IBSI	Image Biomarker Standardisation Initiative

IC	Informed consent
ICT	Induction Chemotherapy
IDMC	Independent Data Monitoring Committee
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
IMRT	Intensity Modulated Radiotherapy
iPET	Interim Positron emission tomography
IRAS	Integrated Research Application System
ISF	Investigator Site File
ISH	In-Situ Hybridization
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
KM	Klustering-means
LAM	Lactational amenorrhoea method
LRC	Loco-regional Control
MBE	Member of British Empire
MDADI	M. D. Anderson Dysphagia Inventory
MDT	Multidisciplinary Team
MHRA	Medicines & Healthcare products Regulatory Agency
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MTA	Material transfer agreement
MTV	Metabolic Tumour Volume
MVCT	Megavoltage Computed Tomography
NCI	National Cancer Institute
NCIN	National Cancer Intelligence Network
NCRI	National Cancer Research Institute
NECKSTD	Standard neck protocol for PET-CT
NG	Nasogastric
NHS	National Health Service
NI	Number of unique discrete intensities
NICE	National Institute for Clinical Excellence
NICE IOG	National Institute for Clinical Excellence Improving Outcomes Guidance
NISCRH	National Institute for Social Care and Health Research
NPV	Negative Predictive Value
OAR	Organs at risk
OPSCC	Oropharyngeal Squamous Cell Carcinoma
OS	Overall Survival
PACS	Picture Archiving and Communication System
PCM	Pharyngeal Constrictor Muscles
PCR	Polymerase Chain Reaction
PCU	Permissions Co-ordinating Unit
PEARL	PET based Adaptive Radiotherapy Clinical Trial
PERCIST	Positron Emission Tomography (PET) Response Criteria in Solid Tumors
PET	Positron Emission Tomography
PET-AS	Positron Emission Tomography Automated Segmentation
PET-CT	Positron Emission Tomography/Computed Tomography
PETNECKFX	FX neck protocol for PET-CT
PFS	Progression Free Survival
PFS	Progression Free Survival

PI	Principal Investigator
PIS	Participant Information Sheet
PMR	Partial metabolic response
PSS-H&N	Performance Status Scale for Head & Neck Cancer
PTV	Planning Target Volume
QA	Quality Assurance
QL (QoL)	Quality of Life
R&D	Research and Development
REC	Research Ethics Committee
RG	Region growing
RSI	Reference Safety Information
RT	Radiotherapy
RTOG	The Radiation Therapy Oncology Group
RTP	Radiotherapy treatment planning
RTTQA	Radiotherapy Trial Quality Assurance
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SOP	Standard Operating Procedure
SSI	Site Specific Information
SUV	Standardized uptake value
SUVpeak	Standardized uptake value peak
TBR	Tumour to background ratio
TBRpeak	Tumour to background ratio peak
TLG	tumour lesion glycolysis
TMF	Trial Master File
TMG	Trial Management Group
TNM	tumour, node and metastasis
TSC	Trial Steering Committee
UICC	Union for International Cancer Control
UKCRC	United Kingdom Clinical Research Collaboration
UW-QOL	University of Washington Quality of Life Questionnaire
VMAT	Volumetric Arc Therapy
WCISU	Welsh Cancer Intelligence and Surveillance Unit
WHO	World Health Organisation
WOCBP	Women of Child Bearing Potential
WST	Water swallow test
WT	Watershed transform
XNAT	Extensible Neuroimaging Archive Toolkit

1 Amendment History

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment No.	Protocol version no.	Date issued	Summary of changes made since previous version
Not Applicable	1.0	13th July 2018	Not applicable, first version of protocol
1	2.0	9 th June 2020	Diabetes Mellitus removed from exclusion criteria TMG membership updated Additional information regarding translational sample collection Amendment to radiotherapy planning Additional reference to COVID-19 guidance documentation
2	3.0		Termination of the trial section updated Patient age of eligibility to participate lowered to 16 years Eligibility to include N2

2 Synopsis

Short title	PET BASED ADAPTIVE RADIOTHERAPY IN LOCALLY ADVANCED HPV POSITIVE OROPHARYNGEAL CANCER
Acronym	PEARL
Internal ref. no.	TBC
Development phase	II
Funder and ref.	Cancer Research Wales Velindre Radiotherapy Charitable Funds (Moondance)
Trial design Overview	<p>PEARL is a prospective, interventional, non-randomised, phase II feasibility study for patients with good prognosis Human Papillomavirus (HPV)-associated oropharyngeal squamous cell cancer (OPSCC) who are suitable for treatment with concurrent chemoradiotherapy (CCRT).</p> <p>PEARL will explore the feasibility of individually adapting the radiotherapy plan for each patient after 2 weeks of radical CCRT, based on biological changes in tumour activity seen on an interim FDG-PET-CT scan, carried out early on during a course of treatment. The aim is to reduce the dose of radiotherapy received by surrounding normal tissues to ultimately reduce toxicity.</p> <p>The study will establish the progression free survival rate (PFS) in patients who receive biologically adapted radiotherapy. Furthermore, it will also explore whether changes seen on PET-CT scan during treatment correlate with outcome and with changes in potential blood-based biomarkers of response. Toxicity rates will be assessed, particularly the effect of treatment on swallowing function.</p>
Trial participants	Patients diagnosed with T1 – T3 N0 – N2 (TNM8) HPV-positive squamous cell carcinoma of the oropharynx (HPV-positive OPSCC) and appropriate for radical treatment with concomitant chemoradiotherapy
Planned sample size	Approximately 50 PATIENTS
Planned number of sites	4

<p>Main inclusion criteria</p>	<ol style="list-style-type: none"> 1. Histologically confirmed squamous cell carcinoma of the oropharynx 2. Positive p16 Immunohistochemistry on local testing 3. UICC TNM (8th edition) stage T1 – T3 N0 – N2 M0 4. Multidisciplinary team (MDT) decision to treat with primary chemoradiotherapy 5. Patients considered fit for radical treatment with primary chemoradiotherapy (including sufficient renal function (GFR>50ml/min)) 6. Aged 16 years or older 7. Not smoked in the last 2 years 8. Written informed consent provided 9. Patients with reproductive potential (male or female), who are sexually active during the duration of the trial consent to using a highly effective method of contraception for at least six months after the last dose of chemoradiotherapy. Effective forms of contraception are described in section 15.5.
<p>Main exclusion criteria</p>	<ol style="list-style-type: none"> 1. Known HPV negative squamous cell carcinoma of the head and neck 2. T1 – T3 tumours where primary treatment with concomitant chemoradiotherapy is not considered appropriate 3. T4 disease 4. N3 (TMN 8th edition) nodal disease 5. Distant metastatic disease 6. Current smokers or smokers who have stopped within the past 2 years. Vaping is permitted and should be considered as non-smoking status. 7. Any pre-existing medical condition likely to impair swallowing function and/or a history of pre-existing swallowing dysfunction prior to index oropharyngeal cancer 8. Previous radiotherapy to the head and neck

	<p>9. History of malignancy in the last 5 years, except basal cell carcinoma of the skin, or carcinoma in situ of the cervix</p> <p>10. Tumour non-avid on PET-CT or not visible on cross sectional imaging</p>
Treatment duration	Concomitant chemoradiotherapy will take place over 6 weeks in 33 daily fractions (sessions).
Follow-up duration	2 years in study. Clinical follow-up for at least 5 years.
Planned trial period	January 2020 – January 2022
Primary objective	To maintain a high progression free survival rate with biologically adapted radiotherapy in patients with good prognosis HPV positive OPSCC
Secondary objectives	<ol style="list-style-type: none"> 1. To demonstrate feasibility of recruitment 2. To test if individualized, adaptive, biologically-based radiotherapy planning is feasible and results in a significant change in the radiotherapy plan. 3. To maintain high complete response rates 3 months after treatment. 4. To assess acute and late toxicity rates and the effect of treatment on swallowing function.
Primary endpoints	2 year Progression Free Survival (PFS)
Secondary endpoints	<ul style="list-style-type: none"> • Swallowing panel measurements including qualitative and quantitative swallowing assessments (MDADI, PSS-H&N, water swallow test) and feeding tube rate dependency at 1 year. • Quality of life (EORTC QLQ C30, HN35 and UW-QOL questionnaires) • Acute and late toxicity (NCI CTCAE criteria v4.03) • Complete response rate on PET-CT scan (postPET) 3 to 4 months after treatment.
Intervention	<p>Whilst COVID-19 is a public health issue, please refer to the latest version of the COVID-19 PEARL recruitment policy to ensure optimal care in the safest possible environment.</p> <p>Screening assessments:</p>

- **Patients with oropharyngeal squamous cell carcinoma (OPSCC) are eligible for the study. Following informed consent, patients will be registered into the study and diagnostic biopsies will be sent for central HPV testing (by p16 IHC and HR-HPV ISH); results will be available within 3 working days. HPV positivity must be confirmed before any trial-specific assessments are carried out. For centres where p16 and/or HPV testing is standard practice and the patient has been confirmed as HPV positive locally, trial specific assessments can be carried out prior to receiving confirmation of central HPV testing result.**
- **Disease Assessment**
- **Pregnancy test**

Baseline (pre-treatment) assessments:

The following assessments should be carried out prior to chemoradiotherapy:

- **Perform a PET-CT scan (prePET) and use this alongside staging CT and MRI scans and clinical examination to create an initial Gross Tumour Volume (GTV) for radiotherapy planning.**
- **MDADI score**
- **PSS-H&N**
- **Water swallow test**
- **Record made of feeding tube use to be performed at baseline and then weekly during chemoradiotherapy**
- **Clinical review**
- **Quality of life questionnaires: EORTC QLQ-C30, EORTC QLQ-H&N35 and UW-QOL**
- **Toxicity assessment:**

Toxicity should be assessed according to NCI CTCAE criteria version 4.03 at baseline and weekly during chemoradiotherapy. Toxicity should also be recorded 4 weeks (+/- 2 weeks), 12 weeks (+/- 2 weeks), 6 months (+/- 4

weeks), 12 months (+/- 4 weeks) and 24 months (+/- 8 weeks) after the end of treatment.

- Perform a PET-CT scan (iPET) and disease assessment after 10 fractions of radiotherapy and adapt the radiotherapy plan based upon the remaining avid biological GTV (bGTV_iP) on the iPET.

Post-treatment assessments:

All post-treatment assessment time points should be timed from the end of chemoradiotherapy.

4 weeks (+/- 2 weeks) post-treatment assessment:

- As described above for pre-treatment assessments apart from the PET-CT scan

12 weeks (+/- 2 weeks) post-treatment assessment:

- As described above for pre-treatment assessments including a post treatment PET-CT scan (PostPET). The postPET can be done within 10 and 16 weeks.
- Disease assessment

6 months (+/- 4 weeks) post-treatment assessments:

- As described above for pre- treatment assessments apart from the PET-CT scan

12 months (+/- 4 weeks) post-treatment assessments:

- As described above for pre-treatment assessments apart from the PET-CT scan
- Swallowing Support Summary

24 months (+/- 8 weeks) post-treatment assessments:

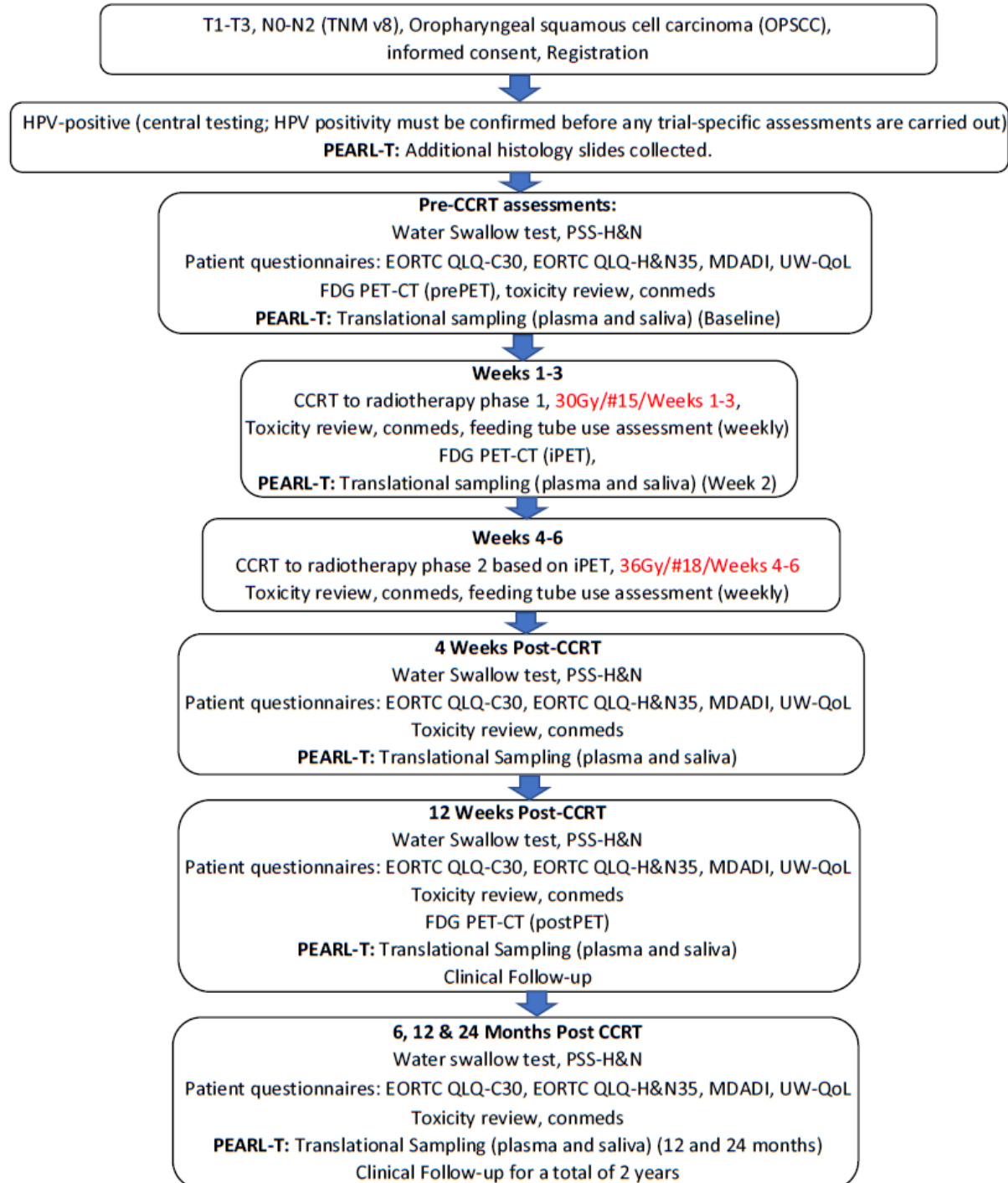
- As described above for pre-treatment assessments apart from the PET-CT scan

Participants consenting to PEARL-T will have their blood and saliva samples at baseline then at two weeks into chemoradiotherapy (after 10 fractions) and 4 weeks, 12 weeks,



	<p>12 months and 24 months after completion of chemoradiotherapy. Histology slides from the diagnostic biopsy will also be collected as part of PEARL-T.</p>
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3 Trial summary and schema



3.1 Trial lay summary

The incidence of oropharyngeal cancer (OPSCC) caused by Human Papillomavirus (HPV) infection (HPV-positive OPSCC) is increasing in the UK. It tends to affect younger patients and has a better outcome than most other head and neck cancers.

A large proportion of patients diagnosed with HPV-positive OPSCC will undergo non-surgical treatment. This usually involves 6 to 7 weeks of chemo-radiotherapy, with chemotherapy being given weekly or during the first and fourth week of the radiotherapy course (CCRT). We know that HPV-positive OPSCC responds better to radiotherapy and CCRT than HPV-negative OPSCC. Because of their favourable prognosis, many patients with HPV-positive OPSCC are cured of their disease but often have to live for several decades with the side effects of their treatment. Although every effort is made to minimise side effects, they can be permanent and have a significant impact on the patient's quality of life. Side effects from radiotherapy are usually caused because normal tissues surrounding the cancer receive radiation whilst the cancer itself is being treated. The way that radiotherapy is planned and delivered is improving all the time and a variety of ways are currently being researched to better target cancers, whilst sparing normal tissues, to try to reduce the severity of side effects, without reducing the success of treatment.

PET-CT scans are able to look at the metabolic (or biological) activity of cells and so can offer extra information compared to CT alone, or MRI. They can be better at detecting cancer cells and are already used routinely in the diagnosis and management of a number of cancers. PET-CT scans are currently recommended in the UK for response assessment after a patient has completed radiotherapy for a head and neck cancer but, as far as we know, have not yet been used routinely to adapt radiotherapy according to the individual patient's response during radiotherapy.

The PEARL study will recruit approximately 50 patients with HPV-positive OPSCC who are about to undergo primary treatment with CCRT in the United Kingdom, over a 2 year period. The main aim is to see whether it is feasible to perform a PET-CT after 2 weeks of radiotherapy and re-plan the radiotherapy based on this PET scan, to re-distribute the dose of radiotherapy being delivered, so that a smaller area of normal tissues in the mouth and throat is treated to a high dose of radiotherapy. This could potentially lead to reduced side effects from the treatment, and an improved quality of life in the long term. The re-planning of the radiotherapy will be done whilst the patient is having their third week of radiotherapy and so there won't be any delay or gap in the treatment course. Every patient recruited to the study will have three PET-CT scans, one before starting treatment, another after 2 weeks of radiotherapy and one 3 months after treatment has finished.

Patients recruited to PEARL will be asked for their permission to collect several blood and saliva samples before, during and after treatment. Participants will also be asked for their permission for tissue from their diagnostic samples to be made available for future research. We will be looking at the scans and blood tests taken during the study to see how they correlate with each other and to see if they can help us predict who will respond best to treatment. The results of the study will potentially be used as a basis

for larger studies in the future, designed to prove that PET-based radiotherapy plan adaptation can reduce side effects for patients. This part of the study is called PEARL-T.

4 Background

4.1 HPV Positive Oropharyngeal Cancer

Oropharyngeal squamous cell carcinoma (OPSCC) affects the tonsils, soft palate and base of tongue. The incidence of OPSCC in the UK has doubled in the last 10 – 15 years and in Wales the incidence has tripled in the past 15 years (1). Historically the predominant risk factors for OPSCC were smoking and alcohol. Over the past decade or so however, OPSCC associated with Human Papilloma Virus (HPV-positive OPSCC) has made up a large proportion of cases. Currently, over 70% of OPSCCs in Europe are HPV-positive (2).

HPV-positive OPSCC generally has a younger demographic and appears to be more sensitive to chemotherapy (3). Patients with good prognosis HPV-positive OPSCC have nearly double the survival of HPV negative OPSCC with 93% vs 46% patients alive at 3 years following radical treatment (4).

The current standard management of locally advanced OPSCC is non-surgical. Typically, patients undergo 6 or 7 weeks of radiotherapy receiving a dose biologically equivalent to 70 Gray (Gy) in 2Gy daily fractions to the high dose volume around the primary tumour and involved lymph nodes and 50-56Gy to the region being treated prophylactically. When appropriate, systemic treatment is included, either concomitant platinum-based chemotherapy or cetuximab (CCRT). Toxicity rates, both during and after treatment, are high. These include mucositis (ulcers in the mouth and throat), xerostomia (dry mouth), osteoradionecrosis (bone death due to damaged blood vessels caused by radiation), dysphagia (swallowing difficulty) and a dependency on feeding tubes.

In this population of patients where the average age at diagnosis is relatively young and survival rates are good, survivorship issues are a priority. In order to reduce long term sequelae of treatment, various strategies are being looked into in order to reduce the acute and chronic toxicity of radiotherapy and systemic therapy. Late toxicity rates (which persist or appear >3months after treatment) take precedent as they can be lifelong and significantly impact upon the patient's quality of life (QOL). The symptom which has the most significant impact on QOL is dysphagia (5).

4.2 Existing strategies for the de-intensification of treatment and reduction of toxicity in HPV positive oropharyngeal cancer

In addition to patient and tumour-specific variables such as the patient's age and the stage of the tumour, the magnitude of late toxicity is dependent upon the radiotherapy planning technique for example, 3D-Conformal Radiotherapy versus Intensity Modulated Radiotherapy (IMRT) (6). There is an established correlation between the rate of late dysphagia and the mean dose of radiotherapy delivered to the critical swallowing structures including the glottis/supraglottic larynx and the pharyngeal

constrictor muscles (7). IMRT is able to deliver a highly conformal dose distribution allowing better sparing of the normal tissues in the vicinity of the target volume. An analysis of pre-IMRT-era RTOG studies found that 43% of Head and Neck cancer patients treated with CCRT experienced severe (grade 3 or 4) late toxicity (8). Published IMRT toxicity rates vary but some studies have demonstrated grade 3 or 4 late toxicity rates of 25% and less (9), especially when salivary- and swallowing-sparing methods are used (10).

Several studies have demonstrated the improved response to chemo-radiation and improved outcomes in HPV-positive OPSCC patients (11). In view of this, a number of studies have been set up across the world to investigate the potential for treatment de-intensification in this patient group. The aim of these studies is to maintain high loco-regional control and survival rates, but to reduce life-long side effects for surviving patients.

One potential way of de-intensifying treatment is to lower the total dose of radiotherapy prescribed to the tumour which would subsequently reduce the dose received by the surrounding normal tissues.

Recently there have been 3 studies published in HPV-positive OPSCC patients showing comparable rates of loco-regional control after reduced dose radiotherapy in patients who have responded to initial (induction) chemotherapy.

In one single arm phase II study 44 patients with stage I-III (TNM8) HPV-positive OPSCC (12), patients who had a complete or partial response to two cycles of induction chemotherapy with Carboplatin and Paclitaxel, received 54Gy in 27 fractions of radiotherapy to the planning target volume (PTV) encompassing the primary tumour and involved nodes and 43Gy to the prophylactically treated uninvolved nodes, representing a ~20% reduction in dose compared to standard doses of radiotherapy. Weekly Paclitaxel chemotherapy was given along with the radiotherapy. The trial demonstrated a 2-year progression free survival (PFS) rate of 92% (95% CI: 77-97%) and 2-year loco-regional control (LRC) rate of 95% (95% CI: 80-99%), which compared favourably with historical studies, in addition to a reduced toxicity profile.

In another single arm phase II study (E1308) of 80 patients with TNM8 Stage I (50 – 60%), Stage II (30 – 40%) and Stage III (10% T4 – no N3) HPV-positive OPSCC (13), patients underwent 3 cycles of induction chemotherapy with Docetaxel, Cisplatin and 5-Fluorouracil. The 70% of patients who had a complete clinical response went on to receive 54Gy in 27 fractions of radiotherapy to the primary tumour and involved nodes and 51.3Gy to the uninvolved nodes, together with concurrent cetuximab. The trial demonstrated a 2-year PFS rate of 80% (95% CI: 65-89%) which was lower than expected. However, when they analysed the data, patients with T1-T3 N2 (stage I-II TNM8) disease who had a minimal (≤ 10 pack/year) smoking history did extremely well, with a 2-year PFS and 2-year overall survival (OS) rate of 96% (95% CI: 76-99%). This study illustrates the importance of selecting the right patients for de-intensification. It also demonstrated better swallowing and nutritional outcomes compared with controls.

In a third single arm phase II study, OPTIMA (14) 62 patients with HPV-positive OPSCC were stratified as having low risk disease ($\leq T3$, $\leq N2b$, ≤ 10 pack year smoking history) and high-risk disease ($T4$, $\geq N2c$, >10 pack year smoking history). Patients received 3 cycles of induction chemotherapy (ICT) with nab-paclitaxel and carboplatin and radiotherapy treatment was based upon response to ICT. Low risk patients with more than 50% response to ICT had 50Gy RT (RT50). Low risk patients with 30 – 50% response, or high-risk patients with $>50\%$ response received CCRT with 45Gy (CRT45). All other patients received standard CCRT with 70Gy. All patients who underwent de-escalated treatment had a biopsy of the primary and a neck dissection following treatment. The pathological complete response (pCR) rate for RT50 and CRT45 was 94.7% and 89.3% respectively. After a mean follow up of 1.5 years, 2year PFS was 100% for the low-risk patients and 93.5%, 97% for the high-risk patients. Acute toxicity and long-term feeding tube use was significantly reduced.

Other studies have investigated the role of reduced dose radiotherapy in the treatment of patients with HPV-positive OPSCC, without prior use of any induction therapy. In one prospective single-arm phase II study (15), 43 patients with stage I-II (TNM8) HPV-positive OPSCC and a minimal (≤ 10 pack/year) smoking history were treated with reduced dose IMRT (60Gy/30 fractions to the primary tumour and involved nodal PTV) and reduced dose (30mg/m²) concomitant weekly Cisplatin. The updated results were disclosed at an oral presentation. Biopsies of tumour sites following treatment demonstrated complete pathological response rate of 98% at the primary site and 84% in the neck nodes. The one case of a positive primary site biopsy was resected and no viable tumour was found. At a median of 36 months follow up for 42 patients regional control was 100%, local control was 100% and OS was 95%. Treatment was associated with reduced toxicity rates when compared with contemporary studies (e.g. PARADIGM) where patients received 70Gy of radiation: 39% of patients (compared to 85%) required a feeding tube for a median of 15 weeks (range 5-22 weeks).

Another larger study that has used a lower dose of radiotherapy (without induction chemotherapy) to treat good prognosis HPV-positive OPSCC is the NRG HN-002 study. In this randomised phase II study 295 patients with T1-2 N1 and T3 N0-N1 (stage I-II TNM8) HPV-positive OPSCC and a minimal (≤ 10 pack/year) smoking history were randomised to receive reduced one of two de-intensified CCRT treatment regimens, either reduced dose IMRT, 60Gy in 30 fractions over 6 weeks with concurrent weekly Cisplatin (40mg/m²) or moderately accelerated reduced dose IMRT alone, 60Gy in 30 fractions over 5 weeks. The study is currently in follow-up, with 2-year PFS and Grade 3 dysphagia as the primary endpoints.

As well as these studies of reduced dose radiotherapy, other studies have investigated other means of de-intensifying treatment and reducing side effects in patients with HPV-positive OPSCC. The largest of these studies are the US phase III RTOG 1016 study, which recruited 987 patients, and the UK phase III De-escalate HPV study, which recruited 304 patients. Patients in both studies were randomised to receive either Cisplatin or Cetuximab concurrent with standard dose radiotherapy, with the aim of seeing if Cetuximab reduces toxicity compared to Cisplatin, whilst maintaining high survival rates. Both

studies reported superior outcomes with standard of care Cisplatin-based CCRT compared to Cetuximab and radiotherapy (16, 17) and demonstrate the importance of not modifying treatment prematurely.

The NICE guidelines on management of patients with cancers of the upper aerodigestive tract, which were updated in 2016 (18), have a section on De-intensification of treatment in HPV-positive OPSCC which states: “Do not offer de-intensification of curative treatment to people with HPV-positive cancer of the oropharynx, unless it is part of a clinical trial”. Carefully planned and well-monitored studies like PEARL are therefore a fundamental part of the effort to improve future treatments for HPV-positive OPSCC.

4.3 The role of PET Imaging in radiotherapy planning

Rather than reducing the total dose of radiotherapy being delivered to patients, another strategy for reducing the dose received by normal tissues is to adapt the radiotherapy plan based on the biological response of the tumour seen on functional imaging (PET-CT), during CCRT. The aim of this strategy is to decrease the volume of tissue receiving the highest dose of radiotherapy, guided by the disease response in an individual to initial chemoradiotherapy.

¹⁸F-fluorodeoxyglucose (FDG) PET is the most widely available method of functional imaging in the UK and the most commonly used in head and neck cancer. FDG is a radio-labelled analogue of glucose which is preferentially taken up by cells with a high level of metabolic activity. FDG PET combined with CT can be helpful in the diagnosis of head and neck cancers; 90 - 100% of them can be detected using FDG-PET (19). Most relapses after CCRT occur in areas of tumour that were initially FDG-avid (18). In addition, PET avid volumes have been shown to represent tumours seen in histopathological samples following surgery more accurately than CT and/or MRI (21).

Gross tumour volumes (GTVs) of head and neck tumours based on PET scans are significantly smaller than GTVs based upon CT and MRI. This is true for pre-treatment imaging and on imaging performed during radiotherapy. In one study, the average FDG PET defined volume of 8 patients was shown to reduce during radiotherapy to approximately 70% of its original volume after 14Gy and to 55% of its original volume after 25Gy (22). Re-imaging with CT and MRI during treatment led to a less prominent reduction in GTV. Importantly, the FDG PET based GTV translated into subsequent reductions in both prophylactic and therapeutic CTVs and PTVs.

Residual FDG activity during CCRT appears to be predictive for unfavourable local control and survival (23). FDG-PET CT scans therefore offer an imaging modality which could enable us to target the part of the PTV which remains avid, and therefore most likely to relapse, after a proportion of radiotherapy treatment has been given.

One study looked at 18 patients with head and neck squamous cell cancer and repeated the FDG PET scan after a mean dose of 46Gy (24). They demonstrated that the irradiated volume defined by FDG PET

was reduced by 15 – 40% when adaptive planning based on interim PET was used. It also demonstrated that by using FDG-PET based conformal radiotherapy, significant dose sparing to the ipsilateral parotid could be achieved (mean dose 38.6% vs 30.7% with a p value = 0.004). So adaptive radiotherapy based on interim PET-CT also has the potential to reduce dose to critical structures and lower toxicity.

Key to the definition of residual PET-avid disease, is the use of a validated system to define PET avidity in the context of background radiation-induced changes to normal tissue. The Hopkins Criteria (25), provides a semi-quantitative scoring system with a 5-point output, and uses 'background' activity in normal liver and blood-pool as internal reference values, with 'blood pool' measured in an internal jugular vein. The Hopkins Criteria has been used to evaluate the prognostic value of FDG PET performed in the 3rd week of radical radiotherapy of the head and neck (24). In this study, the interim PET (iPET), in addition to the post treatment PET (postPET), was predictive of tumour control in their cohort of 69 head and neck squamous cell cancer patients, using a score of 1, 2 or 3 as negative for residual tumour. Importantly, the negative predictive value (NPV) of the iPET for locoregional recurrence-free survival was 100% and based on this, the authors suggested iPET may well be considered as a basis for defining volumes suitable for dose de-escalation.

Traditionally, the aim of radiotherapy was to deliver a homogenous radiation dose to a defined tumour volume. The response of tumour cells to radiation within the same tumour is heterogenous, and a different approach, known as 'dose-painting', has been developed in a number of settings in order to match up the magnitude of dose with the appropriate parts of the tumour.

Re-planning based on changes in the disease seen on imaging during treatment offers the opportunity to dose-paint or re-distribute the dose to residual active tumour, whilst reducing the dose to other areas of the initial tumour which have already responded to treatment.

PEARL will complement other UK and European 'dose-painting' studies using FDG PET-CT scans. A UK phase I study (FIGARO) [which recruited patients from Guys and St Thomas' Hospital and Velindre] used pre-treatment PET scans to identify the biological GTV left after 1 cycle of induction chemotherapy for a 10% dose escalation, in patients with poor prognosis (predominantly HPV-negative) OPSCC.

An ongoing European phase II study (ARTFORCE) is recruiting patients diagnosed with lung or head and neck cancers. The head and neck project is a randomised phase II study with a factorial design. It is comparing weekly concomitant cisplatin with weekly cetuximab, and conventional radiotherapy (70Gy/35F) with a dose redistribution arm that redistributes dose based upon a pre-treatment PET CT with adaptive re-planning after 2 weeks using a repeat CT.

PEARL will be the first study of its kind to re-distribute radiotherapy in patients with HPV-positive OPSCC, based on FDG PET CT scans carried out during a course of radiotherapy.

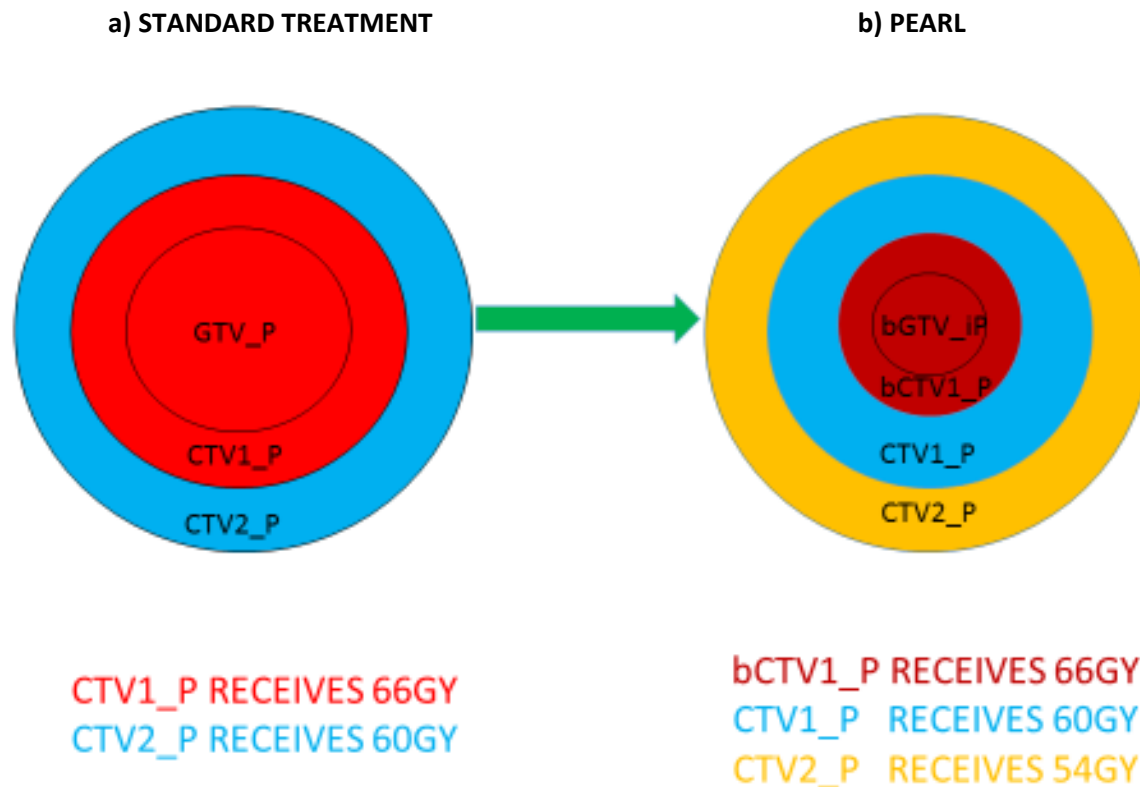
PEARL will be exploring the feasibility of individually adapting the radiotherapy plan after 2 weeks of radical CCRT for good prognosis HPV-positive OPSCC patients, based on an interim FDG PET CT scan. We



will reduce the volume of tissue receiving the highest dose of radiotherapy to encompass only the residual FDG-avid (metabolically active) tumour on the iPET (fig.1). The adapted plan will be implemented from the start of the fourth week of radiotherapy and therefore delivered over the second half of the treatment course. Reducing the volume which receives the highest dose of radiation for the second half of treatment should reduce the total dose to the surrounding normal tissues, including the swallowing structures and the major and minor salivary glands of the oral cavity, and could potentially lower late toxicity for patients.

It is crucial for accurate radiotherapy planning that imaging is performed at the most appropriate time. The planning FDG PET-CT (prePET) must be performed no more than three weeks prior to the start of radiotherapy in order to minimise inaccurate representation of the volumes. The timing of the iPET scan has been based both on the predictive nature of the scan at this time point as demonstrated by other groups, and also to minimise the impact of radiation-induced inflammation on PET image interpretation. After 2 weeks, the high dose volume will have received 18Gy. A variety of different research groups have found background inflammation to be significant only after three weeks of radiotherapy and others have found the 2 week time point favourable due to low levels of radiation-induced inflammation at that point (25). One study in particular showed no statistically significant effect in the background FDG uptake with radiation up to 66Gy (26).

Fig. 1 Comparison of final primary dose levels and volumes in standard treatment (a) and PEARL (b)



The total prescription dose remains the same in PEARL (66Gy high dose) but the volume of tissue receiving 66Gy is reduced if there is a biological response is seen on PET-CT scan after 2 weeks of treatment. The original CTV1_P receives at least 60Gy (intermediate dose), the original CTV2_P receives at least 54Gy (prophylactic dose).

4.4 Predicting response with PET CT

One barrier to using PET images to define treatment volumes has been due to the heterogeneity of clinician's interpretations of the PET signal. The Hopkins Criteria (25) is a qualitative interpretation system that visually assesses therapy response and survival outcome in head and neck squamous cell cancer patients. It has been validated in biopsy-proven HNSCC patients to define its reader reliability, accuracy and predictive ability for PFS and OS outcomes particularly in HPV-positive OPSCC. It has been demonstrated that there is significant inter-observer homogeneity when used to assess post-RT PET images, with inter-reader agreement in the region of 90% and a NPV of 92 - 96.5%, and is predictive of PFS and OS. Additionally, it was able to play a role in the post-treatment follow up of patients, detecting residual disease in 19.5% of patients who were otherwise not clinically under suspicion.

In the UK it is standard practice to offer HNSCC patients a PET-CT scan 3 months after radiotherapy to assess the tumours response to treatment and determine any residual disease. A large meta-analysis in 2011 (29) involving 2335 patients across 51 trials demonstrated that PET CT scans at this point after treatment have a NPV of 95.1% for residual disease at the primary site. Based on this evidence, it is viewed as reassuring if this PET CT is negative for any avid disease. All patients entered into PEARL will have a PET CT scan in the first instance, rather than CT alone, 3 months after the completion of CCRT. Should this demonstrate any concerning features for residual disease, the patient will be investigated with the standard investigations used in this scenario including clinical evaluation with nasoendoscopy and a biopsy.

4.5 Automatic decision Tree-based Learning Algorithm for Advanced Segmentation, ATLAAS

Being able to reliably identify and outline or segment the metabolically active tumour on a PET scan is of paramount importance. This can be done manually by a clinician, or it can be done automatically, using an automatic segmentation algorithm (AS).

Manual outlining is prone to intra- and inter-observer variability but, despite its limitations, is still regarded as the 'gold standard' for clinical use. It is generally recommended that the PET-avid region of interest is delineated under the guidance of a PET radiologist, in conjunction with an oncologist, and this will be done in PEARL.

A variety of PET automated segmentation (PET-AS) methods have been proposed to overcome the limitations of manual outlining. However, one of their limitations is the large number of algorithms available and a lack of a standard protocol between centres. In addition to this, head and neck cancers are highly heterogenous and may not all be best segmented with the same PET algorithm. Fixed thresholding PET-AS techniques, as well as adaptive iterative thresholding are unable to differentiate between tumour and background. Further, the accuracy of thresholding techniques have been shown to be dependent upon the maximum uptake within the tumour and PET-AS methods perform differently in differing tumour characteristics.

ATLAAS is a machine learning tool, designed and developed by our co-investigators (308) using simulated and phantom based PET images; it can select the optimal PET-AS method for use in a given clinical setting as it contains nine algorithms that perform differently in differing conditions. ATLAAS estimates the tumour characteristics in the given PET images and uses a predictive model to select the most appropriate segmentation methodology for the given PET image.

The PEARL study will be used to apply ATLAAS for the first time to PET-CT scans taken during treatment with CCRT and to further develop the ATLAAS methodology by integrating hybrid PET-CT image segmentation in the machine learning algorithm as outlined in section 18.1

4.6 Translational Studies

In addition to the clinical study, we will be carrying out translational laboratory sub-studies (PEARL-T). This will involve the collection and analysis of blood and saliva (so called 'liquid based biopsies') from participants consenting to PEARL-T. Additional histology slides will also be taken from the original biopsy from consenting participants. Samples will be analysed for molecular markers and circulating tumour DNA (ctDNA).

Liquid based biopsies are potentially a non-invasive and convenient method of diagnosing and monitoring disease. Tumour-specific mutations, as well as genomic characteristics such as methylation patterns, can help determine if DNA isolated from the blood and/or other bodily fluids is derived from tumour cells. Sequential liquid biopsies have the potential to provide a timely reflection of disease burden, through longitudinal monitoring of cancer biology. Optimizing prognostication could mean more personalised management plans for patients. Because the half-life of ctDNA is approximately 2 hours, it may reflect the real time burden of disease and also offer the opportunity to detect sub-clinical disease rather than relying upon symptoms, clinical evaluation and radiological signs which are relatively late in evolving.

Levels of DNA shed into the circulation after cell death correlate with stage of disease and can offer insight into prognosis (31). High risk HPV, especially the HPV 16 subtype, is an independent risk factor for OPSCC (32). HPV DNA is detected in most HNSCC patients before treatment and falls during and after successful management (33).

Multiple uses of ctDNA currently being explored include screening, monitoring, evaluating response to treatment and detection of minimal residual disease and recurrence.

Saliva-based diagnostics offer the potential for a non-invasive and easily carried out technique for diagnosing several diseases. Due to their location, DNA from necrotic OPSCCs can enter saliva and this therefore offers an additional source of biomarkers for this particular tumour group.

Wang et al (34) looked at 93 head and neck squamous cell cancer patients and used real time polymerase chain reaction (PCR) to detect if any DNA derived from the HPV-16 E6 and E7 genes was present in their saliva and plasma. Of the 93 patients, 34 had OPSCC of which 29 were HPV positive on histopathology. HPV was detected in 47% of the saliva samples of the HPV-positive OPSCC patients and in 91% of the plasma samples. Although overall, more plasma samples contained DNA than saliva, combining plasma and salivary DNA analysis increased the sensitivity of DNA as a screening tool prior to treatment versus using plasma alone to 96%. The group also demonstrated that persistence of DNA in liquid biopsies taken after treatment was associated with poorer outcomes, including lower PFS and OS. In 3 out of 4 patients who had eventual macroscopic recurrence, tumour DNA was detected in blood and/or saliva 4 – 8 months after treatment and this pre-dated clinical evidence of recurrence by up to 19 months. In the 5 patients who did not recur, no tumour DNA was detected in either blood or saliva following completion of treatment.

There is currently no consensus regarding the best liquid biomarkers to use in HPV-positive OPSCC so we are taking the opportunity to analyse ctDNA in the plasma and saliva of patients enrolled to PEARL to collect further data regarding the role of ctDNA in this disease setting and, in addition, to look at the correlation of ctDNA levels in blood and saliva with functional data from PET-CT images.

Tissue made available from the diagnostic biopsy will be used for genome analysis which will determine the presence of a panel of common genes known to be associated with oropharyngeal squamous cell carcinoma and their correlation with patient outcome. Participants will be asked to consent to excess tissue, blood and saliva remaining following these studies to be stored for future research.

4.7 Summary of the rationale for the PEARL study

HPV-positive OPSCC generally has a younger demographic and is more sensitive to CCRT. Patients with good prognosis HPV-positive OPSCC have nearly double the survival of high-risk HPV-negative OPSCC following radical treatment.

In this population of patients where the average age at diagnosis is relatively young and survival rates are good, survivorship issues are a priority. In order to reduce long term sequelae of treatment, PEARL will be exploring the feasibility of individually adapting the radiotherapy plan after 2 weeks of radical CCRT, based on an interim FDG PET CT (iPET). By redefining the high dose primary volume midway through treatment, we aim to reduce the volume receiving the highest dose for the second half of treatment and reduce dose to the surrounding normal tissues. This could potentially lower late toxicity rates if dose to the swallowing structures is minimised.

In addition to our main study, we will be carrying out translational sub-studies (PEARL-T) as described in section 4.6 and 11. Optimizing prognostication could mean more personalised management plans for this disease in future and avoidance of unnecessary toxicity, whilst preserving good outcomes.

5 Trial objectives/endpoints and outcome measures

5.1 Primary objective

To maintain a high PFS rate with biologically adapted radiotherapy in patients with HPV-positive OPSCC.

The hypothesis: Patients receiving biologically-based adaptive radiotherapy will have comparable 2 year PFS to patients undergoing standard treatment.

5.2 Secondary objectives

- To demonstrate feasibility of recruitment
- To test if individualized, adaptive, biologically-based radiotherapy planning is feasible and results in a significant change in the radiotherapy plan.
- To assess complete response rates on PET-CT scan at 3 months after treatment
- To assess acute and late toxicity rates and the effect of treatment on swallowing function.

5.3 Primary outcomes measure

- Progression free survival at 2 years.

5.4 Secondary outcomes measures

- Recruitment rates will be monitored
- Percentage reduction in dose to organs at risk (OARs) will be recorded and plans where there was 10% or greater reduction in dose OARs categorised as significantly changed.
- Swallowing panel measurements including qualitative and quantitative swallowing assessments (MDADI, PSS-H&N, water swallow test) and feeding tube rate dependency at 1 year.
- Quality of life (QOL) (EORTC QLQ C30, HN35 and UW-QOL questionnaires)
- Acute and late toxicity (NCI CTCAE criteria v4.03)
- No residual tumour on post-treatment PET-CT scan (postPET, -3 months after treatment, Hopkins criteria score 1-3)

6 Trial design and setting

PEARL is a multicentre, prospective, single arm feasibility study. Patients will be recruited from cancer centres within the United Kingdom.

Patients eligible for the study must have biopsy proven OPSCC clinically staged T1-T3 N0-N2 M0 (TNM8). The MDT must have decided that CCRT is an appropriate treatment for the patient. Approximately 50 patients will be recruited to the study over 2 years.

After informed consent, HPV-positivity will be confirmed by central testing of the diagnostic biopsy specimen. Patients who have had p16-positivity (a surrogate marker for HPV) confirmed locally can undergo baseline assessment of QOL and swallowing function prior to the central laboratory results.

Additional samples of the diagnostic biopsy specimen will be collected from participants who have consented to PEARL-T. Participants consenting to PEARL-T will also have their baseline blood and saliva collected and stored. Samples will be used for future translational analysis and research. Participants will be required to consent to the use of their samples for future research.

Patients will undergo baseline planning FDG PET CT scan (prePET) which will be used to define the biological GTV (bGTVpreP). The bGTV_preP will be defined by a nuclear medical physician and consultant clinical oncologist using information from the prePET. They may also incorporate the bGTV_preP_ATLAAS produced by the ATLAAS software. The bGTV_preP, alongside the diagnostic CT, MRI and clinical information, will be used to define the anatomical volume which represents the macroscopic tumour (GTV_P). Margins will be added to form the CTV1_P and CTV2_P.

All patients will undergo swallowing and saliva-sparing RT, delivered using Volumetric Arc Therapy (VMAT) (RapidArc), which the UK DARS clinical trial team demonstrated reduced RT dose to the pharyngeal constrictors more effectively than IMRT (35).

Patients will start their 6 weeks of CCRT within two to three weeks following the planning scans. Cisplatin chemotherapy will be administered as per site specific protocols. 33 daily fractions of radiotherapy will be delivered over 6 weeks.

A second FDG-PET-CT scan (iPET) and repeat blood plasma and saliva collections will be carried out after 2 weeks of CCRT (on RT days 9 – 12) and the iPET assessed for residual FDG-avid disease. The biological GTV will be re-outlined based on the residual avid region of the tumour on the second PET-CT to create bGTV_iP. Consistently with the outlining process used to outline the bGTV_preP, this will be done by an expert nuclear medicine radiologist and clinical oncologist using the iPET in addition to the bGTV_iP_ATLAAS produced by the ATLAAS software if available. The new bGTV_iP, with the addition of a margin to form bCTV_P, will receive the maximum dose of 66Gy/33F but the non-avid region which was originally encompassed within CTV1_P will receive a total dose of 60Gy/33F.

At the end of treatment, blood plasma and saliva samples will be taken at 4 weeks post treatment and again with the 3-month post-treatment PET-CT (postPET). In those patients who have equivocal findings

on PET, repeat imaging (CT or MRI scan) may be carried out 8-12 weeks later (as is standard practice) to check for resolution.

Swallowing and QoL assessments will be repeated 4 weeks (+/- 2 weeks) after treatment and will be repeated at 6, 12 and 24 months post-treatment. The plasma and saliva sample will be repeated at 12 and 24 months.

Clinical follow up within the trial will be for 2 years. Clinical follow-up will be for at least 5 years in accordance with National guidelines (NICE IOG guidance 2004).

6.1 Risk assessment

A Trial Risk Assessment has been completed to identify the potential hazards associated with the trial and to assess the likelihood of those hazards occurring and resulting in harm. This risk assessment document includes:

- The known and potential risks and benefits to human subjects
- How high the risk is compared to normal standard practice
- How the risk will be minimised/managed

This trial has been categorised as a medium risk where the level of risk is somewhat higher than the risk of standard medical care. A copy of the trial risk assessment may be requested from the Trial Manager. The trial risk assessment is used to determine the intensity and focus of monitoring activity (see section 24.1).

7 Site and Investigator selection

PEARL aims to recruit 50 patients over 2 years from cancer centres within the United Kingdom. These sites will be required to complete a registration form to confirm that they have adequate resources and experience to conduct the trial. The TM will liaise with sites to ensure the site is opened in accordance with the CTR procedures for site activation.

Patients will be identified and recruited at the participating hospital sites. Each participating site will:

- Have an identified PI
- Be provided with protocol specific training before being activated for recruitment
- Be provided with a local document package in line with HRA guidance (<http://www.hra.nhs.uk/resources/hra-approval-nhs-organisation-guidance/>). For sites in England this package will be provided simultaneously to both the study delivery team and the

research management team. For sites in Scotland and Wales the package will be provided as required by the devolved administrations.

- Be provided with copies of the REC and HRA approvals for the trial. The approval process includes granting favourable opinion of the host care organisation/PI.

The following documents must be in place and copies sent to the PEARL Trial email account (see contact details on page 4):

- The approval letter from the site's R&D Department
- A signed Trial Agreement including relevant MTA clauses
- Current Curriculum Vitae and GCP training certificate of the Principal Investigator (PI)
- Completed Site Activity Delegation Log and Roles and Responsibilities document
- Full contact details for all host care organisation personnel involved, indicating preferred contact
- A copy of the most recent approved version of the Participant Information Sheet(s) and Consent Form(s) on host care organisation headed paper
- A copy of the most recent approved GP letter on host care organisation headed paper
- Returned Source Data Agreement signed by the PI
- Returned copy of the Self-Evident Correction Log signed by the PI.
- RT process document for RTQA
- Confirmation that the site license is authorised for research and covers all procedures listed on the PEARL research study ARSAC approval

Upon receipt of all the above documents, the Trial Manager will send written confirmation to the Principal Investigator/lead Research Nurse detailing that the centre is now ready to recruit participants into the trial. This letter/email must be filed in each site's Site File. Along with the written confirmation, the site should receive their trial pack holding all the documents required to recruit into the Trial.

Occasionally during the trial, amendments may be made to the trial documentation listed above. CTR will issue the site with the latest version of the documents as soon as they become available. It is the responsibility of the sites to ensure that they obtain local R&D approval for the new documents.

Site initiation will be by attendance at PEARL initiation meetings or by teleconference.

8 Participant selection

Participants are eligible for the trial if they meet all of the following inclusion criteria and none of the exclusion criteria apply. All queries about participant eligibility should be directed to the Trial Manager before randomisation/registration.

8.1 Inclusion criteria

1. Histologically confirmed squamous cell carcinoma of the oropharynx
2. Positive p16 Immunohistochemistry on local testing
3. UICC TNM (8th edition) stage T1 – T3 N0 – N2 M0
4. Multidisciplinary team (MDT) decision to treat with primary CCRT
5. Patients considered fit for radical treatment with primary CCRT (including sufficient renal function (GFR>50ml/min)
6. Aged 16 years or older
7. Not smoked in the last 2 years
8. Written informed consent provided
9. Patients with reproductive potential (male or female), who are sexually active during the duration of the trial consent to using a highly effective method of contraception for at least six months after the last dose of CCRT. Effective forms of contraception are described in section 15.5.

8.2 Exclusion criteria

1. Known HPV negative squamous cell carcinoma of the head and neck
2. T1 – T3 tumours where primary treatment with CCRT is not considered appropriate
3. T4 disease
4. N3 (TMN 8th edition) nodal disease
5. Distant metastatic disease

6. Current smokers or smokers who have stopped within the past 2 years. Vaping is permitted and should be considered as non-smoking status.
7. Any pre-existing medical condition likely to impair swallowing function and/ or a history of pre-existing swallowing dysfunction prior to index oropharyngeal cancer
8. Previous radiotherapy to the head and neck
9. History of malignancy in the last 5 years, except basal cell carcinoma of the skin, or carcinoma in situ of the cervix
10. Tumour non-avid on PET-CT or not visible on cross sectional imaging

8.3 Central review of HPV status

Following informed consent and registration with the PEARL Trial Office, sections from the diagnostic biopsy (6 x 5µm unstained sections mounted on superfrost plus slides or equivalent; 3 slides for staining, 3 slides for repeat tests if necessary), along with a completed “PEARL HPV sample form”, should be sent by the participating site to the Central HPV Laboratory Service via courier or tracked Royal Mail post. Additional sections should also be taken at this point for translational research if the participant consented to PEARL-T (please see section 11.5 for more details).

Central laboratory address:

*PEARL Clinical Trial
Dr Max Robinson
Department of Cellular Pathology
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP
Tel: 0191 2824445
Fax: 0191 2825892*

Diagnostic biopsies should be tested for HPV by both p16 IHC and either ISH or validated PCR technique. These could be performed locally where testing is available. In the UK, ISH can be performed by the central service at Newcastle-upon-Tyne Hospitals Foundation Trust if unavailable locally (section 6.4). For UK centres, any ambiguity regarding HPV status at local sites may be resolved by recourse to central testing in Newcastle. Consensus interpretation and final report will be composed in iLAB Pathology System. A pdf of the iLab report will be emailed to the Co-ordinating Centre and the participating centre. Results will be available within three working days from receipt of the unstained slides.

For those sites conducting all HPV testing locally, a retrospective quality assurance review of 20% of patients will be conducted. This will include the first 2 cases from each site. Sites will be informed as to which cases will be centrally reviewed and will be asked to send the local pathology report and slides (or secure scans for which separate guidance will be provided) for the specimen (from the primary excision and nodal dissection), along with a completed “PEARL sample form” to Dr Max Robinson at the Central HPV Laboratory Service. The central laboratory address is given in section 10.0. Once reviewed, the slides will be returned to participating sites.

The HPV tests will be carried out at Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust.

The HPV tests will be tracked through and reported on the iLAB pathology system, Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust. The documentation and slides will be archived in Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust.

Patients whose biopsies are HPV negative on central review will be withdrawn from the study and treated as per local practice. Patients who are HPV-positive on central testing will proceed to have their baseline swallowing and QOL assessments followed by treatment.

9 Recruitment, Screening and registration

9.1 Participant identification

Potential participants will be identified at regional multidisciplinary meetings. Patients are eligible for the trial if they have biopsy proven oropharyngeal squamous cell carcinoma (OPSCC), all inclusion criteria are met (section 8.1) and none of the exclusion criteria apply (section 8.2). For centres where p16 Immunohistochemistry (IHC) and/or HPV DNA testing (by In Situ Hybridization [ISH]) is standard practice, the results may be used to help screen patients, prior to informed consent for the trial. The patient’s written informed consent must be obtained before registration and for any trial related procedures are undertaken.

Once informed consent has been obtained:

- The patient must be registered into the trial using the web-based registration portal. The CTR can be contacted as an alternative.
- Diagnostic biopsies should be sent to the central pathology laboratory for central testing of HPV status. HPV positivity must be confirmed by the central laboratory before any trial-specific assessments are carried out. For centres where p16 and/or HPV is positive locally, trial specific

assessments can be carried out prior to receiving confirmation of the central laboratory review. Sites should aim to send slides to the central laboratory within 7 days of consent.

- Pack year smoking history should be documented for all recruited patients. All participants should self-report any level of past smoking.
- Whilst COVID-19 is a public health issue, the latest version of the COVID-19 PEARL recruitment policy should be followed as part of the screening and study procedures.

The local PI must confirm the potential eligibility of a patient in the patient's medical notes prior to registration. Any queries about whether a patient is potentially eligible to enter the trial should be discussed with the CTR **before registration**. Any issues will be raised with the Chief Investigators or one of their delegates in their absence. It may be possible for participants to also be recruited into other clinical trials, but this should be discussed with the CTR before this is considered. Baseline demographic data for all patients considered for the trial should be included in the PEARL screening log together with reasons for ineligibility/lack of consent for those who did not enter the trial. This log must be returned to CTR upon request.

9.2 Screening logs

A screening log of all ineligible and eligible patients who are not consented/not approached will be kept at each site so that any biases from differential recruitment will be detected. When at site, logs may contain identifiable information but this **must** be redacted prior to being sent to the CTR. The screening log should be sent to the PEARL@cardiff.ac.uk every month (see section 24 for further detail on data monitoring/quality assurance).

9.3 Recruitment rates

A total of 50 participants will be recruited.

9.4 Informed consent

The participant's written informed consent must be obtained using the PEARL trial Consent Form, which follows the Participant Information Sheet. The participant should be given at least 24 hours after the initial invitation to participate before being asked to sign the Consent Form. Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial. Consent may be taken by a member of the trial team who is GCP trained, suitably qualified and experienced, and who has been delegated by the PI to undertake this activity. Consent may be taken only after a full explanation given of the treatment options, including the conventional and generally accepted methods of treatment has been given. All patients must be informed of the aims of the study, the possible adverse effects, the procedures and possible hazards to which they may be exposed. They



will be informed of the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorised individuals other than their treating physician.

One copy of the ICF will be given to the participant, the original copy will be kept in the ISF, and a further copy will be kept with the participant's hospital notes.

The consent process includes some optional consent points which are in addition to the standard informed consent. The participant may choose not to consent to any, or all, of these optional parts without being excluded from the trial. These optional consents are for the collection and storage for future use of blood, tissue and saliva samples (PEARL-T).

Trial and clinical data may be shared outside of the trial team for research purposes by national and international organisations. The data will be pseudonymised so that the participants cannot be identified outside of Cardiff University and the Sponsor through reasonable measures. Participants consenting to the trial will be consenting to future use of their pseudonymised data. Please note, only when written informed consent has been obtained from the participant and they have been enrolled into the trial can they be considered a trial participant.

Patient's consent will be sought to notify their GP of their involvement in the trial. Patients should be given as long as they require after being given the trial Participant Information Sheet to consider and discuss participation in the trial with friends and family. A contact number for someone at the site should be given to the patient should they wish to discuss any aspect of the trial. Following this, the investigator should determine that the patient is fully informed of the trial and their participation is in accordance with the principles of Good Clinical Practice. Participants should always be asked to sign a consent form. One copy should be given to the participant but the original copy should be kept in the investigator site file and a further copy should be kept with participant's hospital notes.

New safety information may necessitate changes to the PIS and ICF. In this event, it may be necessary to ask some, or all, participants to decide whether to re-consent or withdraw from the trial. Decisions on whether, and which, participants need to re-consent will be made by sponsor / TMG and the decision will be documented in the TMF. Timelines for the re-consent process to be completed will be set. The CTR will communicate local requirements to participating sites and initiate a process to track progress.

The right of the participant to refuse to participate in the trial without giving reasons must be respected. After the participant has entered the trial, the investigator must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. However, the reason for doing so should be recorded and the participant will remain within the trial for the purpose of follow up and data analysis according to the treatment option to which he/she has been allocated. Similarly, the participant must remain free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing his/her further treatment.

Reasonable endeavours will be made to destroy data and or samples collected prior to the point of withdrawal should this be requested by the patient. However, this may not be possible in certain cases.

9.5 Enrolment

Participant enrolment will be performed centrally by the CTR. Patients will be registered to the single arm of the study with no randomisation involved.

Enrolment

Patient enrolment for this trial will be through the use of a web-based system. Details of how to access the system will be supplied to investigators as part of the trial set up.

Alternatively, please contact the trial team; PEARL@cardiff.ac.uk

The participant's care will not be affected at any time by declining to participate or withdrawing from the trial.

If a participant initially consents but subsequently withdraws from the trial, clear distinction must be made as to what aspect of the trial the participant is withdrawing from. These aspects could be:

1. Withdrawal of Trial Treatment/ Intervention
2. Withdrawal from questionnaires
3. Withdrawal from PEARL-T (plasma/saliva/tissue sample collection). Participants will have the option to destroy or keep samples collected prior to withdrawal date
4. Withdrawal from follow-up assessments
5. Withdrawal of consent to all of the above (participant fully withdrawn)
6. Withdrawal from all of the above, plus destroy/delete data collected prior to withdrawal where possible.

In some cases it may not be able to delete/destroy data or samples if they have already been used in analysis or anonymised.

Furthermore, it is important to collect safety data ongoing at the time of withdrawal, especially if the participant withdraws because of a safety event. There is specific guidance on this contained in the Participant Information Sheet but briefly:

If a participant wishes to stop taking part in the trial completely, they may need to be seen one last time for an assessment. Any SAEs will need to be followed up until resolution.

A participant may withdraw or be withdrawn from trial intervention for the following reasons:

- Withdrawal of consent for treatment by the participant
- Any alteration in the participants condition which justifies the discontinuation of the intervention in the Investigator's opinion
- Non-compliance
- Intolerance to treatment
- Safety reasons as judged by an investigator
- Lost to follow-up
- Other

In all instances participants who consent and subsequently withdraw should complete a PEARL Participant withdrawal form (see Withdrawal Form in trial pack) or the PEARL withdrawal form should be completed on the participant's behalf by the researcher/clinician based on information provided by the participant. This withdrawal form should be kept at site and not returned to the CTR – please complete the Withdrawal form on MACRO (or complete the Withdrawal CRF and email it to the CTR if MACRO is unavailable). Any queries relating to potential withdrawal of a participant should be forwarded to the trial manager at the CTR.

10.2 Lost to follow up

If a participant is lost to follow up the CTR will request that the site contacts the participant's GP to obtain information on the participant's status unless they have completely withdrawn from the trial. The minimum information we will aim to collect is the participant's cause and date of death.

11 Trial Intervention

Whilst COVID-19 is a public health issue, the latest version of the COVID-19 PEARL recruitment policy should be followed as part of the screening and study procedures.

11.1 PET-CT Scans

Once recruited to the PEARL trial, patients will have 3 PET-CT scans regardless of whether a diagnostic PET-CT was performed as part of their work-up. For the first 2 trial PET-CTs, the patient will be scanned in the radiotherapy treatment position, in a customized thermoplastic shell. This shell will be used to keep the patient in the correct position throughout radiotherapy treatment.

- The 1st scan (prePET) is a baseline diagnostic scan. The patient is in a thermoplastic shell and the PET CT will be used to define a bGTV_{preP}. The bGTV_{preP} will then be used as an adjunct to help us delineate the GTV_P.
- The 2nd scan (iPET) takes place following 2 weeks (10 fractions) of chemo-radiotherapy. The patient is in a thermoplastic shell and the PET CT will be used to delineate the remaining avid disease (bGTV_{iP}).
- The 3rd scan (postPET) takes place 10 to 16 weeks following the last dose of radiotherapy. It will be used to ascertain whether any avid disease remains and may inform the need for further treatment.

More detail on the PET acquisition protocol is available as a Standard Operating Procedure in Appendix 7 and 11.

11.2 Outlining the biological primary GTVs (bGTV_{preP} and bGTV_{iP})

The biological primary GTVs (bGTV_{preP} and bGTV_{iP}) will be created by a nuclear medicine radiologist and a clinical oncologist. They may also be informed by the automatic delineation by ATLAAS (bGTV_{preP}_ATLAAS and bGTV_{iP}_ATLAAS respectively) if available.

Both bGTV_{preP} and bGTV_{iP} will consist of the high FDG uptake volume based on visual assessment whilst using suitable windowing levels. Any differences in contouring will be settled either by the two doctors reaching a consensus or by a third doctor if differences between the first two cannot be resolved.

Further information regarding volume definition can be found in the PEARL Radiotherapy Guidance Document.

11.3 Blood samples for circulating tumour DNA analysis

Details for the processing, labelling and storage of blood, saliva and tissue samples are provided in the PEARL Laboratory Manual. The key points are summarised below.

1 x 10 ml sample to be collected from consenting patients in CellSave preservation tubes at baseline, 2 weeks from start of treatment, four weeks after treatment and then at 3, 12 and 24 months in follow up. Samples should be gently inverted at least 10 times to ensure full mixing of the blood and preservative.

The current laboratory policy is that blood samples in CellSave preservative tubes received for ctDNA testing must be received within 96 hours from the time of sampling. Samples received in the Laboratory after this point will be destroyed. This policy is in place to maximise the ctDNA yield from the sample and to maintain the integrity of any ctDNA in the blood sample. Therefore, samples should be dispatched with a copy of the sample form to the AWGL as soon as possible to reach the lab within 24 hours in Royal Mail Blue Guaranteed Delivery boxes provided. The AWGL should be notified that the sample has been sent by email at: lab.genetics@wales.nhs.uk and PEARL@cardiff.ac.uk.

Address to send tissue, blood and saliva samples:

PEARL Clinical Trial
All Wales Medical Genetics Laboratory (AWGL)
Institute of Medical Genetics
University Hospital of Wales
Heath Park
Cardiff
CF14 4XW

The AWGL will extract the ctDNA using in-house SOPs and stored for future analysis.

Note: Due to the time critical nature of these samples, please DO NOT collect and send blood samples to the laboratory on Fridays. The blood sample should not be frozen or refrigerated.

11.4 Salivary samples for circulating tumour DNA analysis

DNA will be isolated from whole mouth saliva (unstimulated) from patients.

The patients will be asked to sit in a comfortable upright position and rinse their mouth with water to remove food debris. They will then tilt their heads down and pool saliva in the mouth for 2 to 5 minutes.

Saliva samples should be collected in sterile falcon tubes and transported to the laboratory as described above for the blood samples. Saliva samples should be collected from the patient at baseline, 2 weeks after the start of treatment, four weeks after the end of treatment and then at 3, 12 and 24 months in follow up.

11.5 Tissue samples for circulating tumour DNA analysis

Histology slides (4 to 6 x 10µm cuts, unstained) of the diagnostic tissue biopsy should be made at the same time as the HPV testing histology slides (see section 8.3). Histology slides should be sent to the

AWGL at the address above with a copy of the anonymised pathology report and the sample form. The DNA from the sample provided will be extracted for future analysis.

12 Radiotherapy

Patients will undergo primary radical radiotherapy 66Gy in 33# over 6 weeks with concurrent chemotherapy.

Whilst COVID-19 is a public health issue, the latest version of the COVID-19 PEARL recruitment policy should be followed as part of the screening and study procedures.

12.1 Introduction to radiotherapy

Detailed description of the target volume definition, verification and radiotherapy quality assurance are contained in a separate PEARL Radiotherapy Guidelines document, which should be used for RT planning. PEARL uses a geometric approach to define target volumes and all patients should be planned using Intensity Modulated Radiotherapy (IMRT), specifically rotational arc therapy techniques. Below is an outline of the key radiotherapy principles for the trial.

12.2 Scheduling

Patients should ideally start radiotherapy within 4 weeks and no later than 6 weeks (42 days) from the date of diagnosis. Patients who are unable to start their RT within the time constraints should be discussed with the Chief Investigators (CI), Clinical Research Fellow or member of the PEARL RTTQA team via the Centre for Trials Research (CTR) in order to decide whether they can be enrolled or continue in the trial.

Patients are managed as category 1 patients. RT should be completed within 6 weeks (42 days) as per the Royal College of Radiologists (RCR) guidelines (36). Planned interruptions (machine servicing; bank holidays) and unplanned interruptions should be managed as per the RCR guidelines.

The primary tumour will be treated in 2 phases each lasting 3 weeks. All patients will be re-scanned after #10 with a repeat PET-CT (iPET). Re-planning will take place during #10 – #15 so that there is no break in the continuity of treatment.

12.3 Participant positioning and planning PET-CT scan acquisition

Patients should be immobilised in a thermoplastic shell with the neck in a neutral position and have a planning PET-CT scan in the shell, reconstructed at appropriate slice thickness (2 to 3 mm). Use of intravenous contrast is recommended to facilitate accurate delineation. The scan should include both shoulders and should extend from the vertex to the liver as a minimum.

12.4 Definition of treatment volumes

Primary Tumour Categorisation

The primary tumour should be categorised as lateralised or non-lateralised, based on the site of the primary, T stage and the extent of involvement of midline structures, assessed clinically and radiologically, as follows:

Lateralised tumour

- Tonsillar tumour confined to the tonsillar fossa or extending onto or into the adjacent base of tongue and/or soft palate by **less than 1cm**

Non-lateralised tumour

- Tonsillar tumour that involves the adjacent base of tongue and/or soft palate by **more than 1cm**
OR
- A tumour that arises from a midline structure (base of tongue, soft palate or posterior pharyngeal wall).

Treatment of the neck

The trial protocol requires that all patients with non-lateralised tumours should undergo bilateral neck radiotherapy.

Neck nodes levels for prophylactic radiotherapy should be outlined according to consensus guidelines (34).

12.5 Target volume definition

Diagnostic imaging, clinical findings including pan-endoscopy reports, and pathology information should be used to delineate target volumes. Outlining will be carried out using a geometric approach as per the current international consensus guidelines (37) and further guidance on how to create the volumes is provided in the RT guidance document. Co-registration of the diagnostic CT and/or MRI scans with the first planning PET CT scan is recommended.

Treatment will be prescribed in 2 phases. Phase 1 includes #1 – 15 (week 1 to 3 of treatment) and will be prescribed prior to the start of treatment based on the plan created on the prePET. Phase 2 includes #16 – 33 and will be prescribed after the radiotherapy plan has been adapted based on iPET.

During phase 2, treatment of the nodes will not be adapted based on biological tumour activity seen on iPET. However, the nodal volumes will be re-outlined on the CT component of iPET without reference to avidity.

PRIMARY TUMOUR TARGET VOLUME DELINEATION

FIRST PHASE

Primary Gross Tumour Volume (GTV_P)

This volume includes the primary tumour. It will be delineated taking into consideration all the information available from the diagnostic CT (and MRI if available) as well as the prePET scan (bGTV_preP) and findings from clinical examination including the panendoscopy report.

Primary Clinical Target Volume 1 (CTV1_P)

This volume includes the primary tumour (GTV_P) with a margin of 5mm, edited for anatomical barriers. The CTV margin allows for potential microscopic spread around the primary tumour.

Primary Clinical Target Volume 2 (CTV2_P)

This volume includes the primary tumour (GTV_P) with a margin of 1cm, edited for anatomical barriers.

SECOND PHASE

Phase 2 will commence at #16.

Primary biological Gross Tumour Volume (bGTV_iP)

The region of the GTV_P that remains avid on PET-CT after 10# of radiotherapy will be re-named bGTV_iP.

Primary biological Clinical Target Volume (bCTV_iP)

This volume includes the bGTV_iP with a margin of 5mm.

Primary Clinical Target Volume 1 (CTV1_iP)

This volume includes the primary tumour (GTV_P) transferred from the prePET and re-grown on iPET with a margin of 5mm, edited for anatomical barriers. The CTV margin allows for potential microscopic spread around the primary tumour.

Primary Clinical Target Volume 2 (CTV2_iP)

This volume includes the primary tumour (GTV_P) transferred from the prePET and re-grown on iPET with a margin of 10mm, edited for anatomical barriers. The CTV margin allows for potential microscopic spread around the primary tumour.

NODAL TARGET VOLUME DELINEATION

In the scenario where there is concern about contralateral pathological nodes on the prePET or iPET, these should be outlined as GTV_N_CONTRALAT or GTV_iN_CONTRALAT respectively and grown with margins as for the ipsilateral GTV_N. Although the presence of contralateral pathological nodes deems the patient ineligible for PEARL, the patient's data will be included in the intention-to-treat analysis of results.

FIRST PHASE

Nodal Gross Tumour Volume (GTV_N)

This volume includes the pathologically involved nodes. It will be delineated taking into consideration all the information available from the diagnostic CT (and MRI if available) as well as the prePET scan and findings from clinical examination.

Nodal Clinical Target Volume (CTV1_N)

This volume includes the GTV_N with a 5mm margin in all directions edited for anatomical barriers. The CTV margin allows for potential microscopic spread around the involved nodes.

Nodal Clinical Target Volume (CTV2_N)

This volume includes the GTV_N with a 10mm margin in all directions edited for anatomical barriers.

Prophylactic Nodal Clinical Target Volume (CTV3_N)

This volume includes the rest of the involved nodal level(s) and all at risk non-pathological nodal levels appropriate for prophylactic irradiation as defined by the updated consensus guidelines and atlas (36).

SECOND PHASE

The nodal GTVs will be re-contoured for phase 2 based upon the anatomical appearances on the CT component of iPET. They will be regrown with the margins as defined below.

Nodal Gross Tumour Volume (GTV_iN)

This volume includes the pathologically involved nodes. It will be delineated taking into consideration all the information available from the diagnostic CT (and MRI if available) as well as the prePET scan and findings from clinical examination.

Nodal Clinical Target Volume (CTV1_iN)

This volume includes the GTV_iN with a 5mm margin in all directions edited for anatomical barriers. The CTV margin allows for potential microscopic spread around the involved nodes.

Nodal Clinical Target Volume (CTV2_iN)



This volume includes the GTV_iN with a 10mm margin in all directions edited for anatomical barriers.

Prophylactic Nodal Clinical Target Volume (CTV3_iN)

This volume includes the rest of the involved nodal level(s) and all at risk non-pathological nodal levels appropriate for prophylactic irradiation as defined by the updated consensus guidelines and atlas (36). It can be based upon the CTV3_N from the prePET and edited on iPET for any anatomical changes.

CONVERSION OF Clinical Target Volume (CTV) TO Planning Target Volume (PTV)

A margin will be added to each CTV in all directions, to produce the corresponding Planning Target Volumes (PTV), in line with IRCU50, 62 and 83 and should not be edited. The PTV margin allows for day-to-day variations in patient anatomy and positioning. The magnitude of this margin (typically 2 – 5mm) should reflect the geometric accuracy of the immobilisation system used by the local centre. Radiotherapy dose is prescribed to the PTV and minimum standards for PTV coverage are given in section 12.6.3.

12.6 Dose Prescription and Fractionation

12.6.1 Primary tumour radiotherapy dose and fractionation

PHASE 1	DOSE (Gy)	FRACTIONATION
PTV1_P	27.3	15
PTV2_P	24.5	15

PHASE 2	DOSE (Gy)	FRACTIONATION
bPTV1_P	38.7	18 (Total 66Gy/33F)
PTV1_P	32.7	18 (Total 60Gy/33F)
PTV2_P	29.5	18 (Total 54Gy/33F)

12.6.2 Nodal radiotherapy dose and fractionation

PHASE 1	DOSE (Gy)	FRACTIONATION
PTV1_N	27.3	15
PTV2_N	27.3	15
PTV3_N	24.5	15

PHASE 2	DOSE (Gy)	FRACTIONATION
PTV1_N	38.7	18
PTV2_N	32.7	18
PTV3_N	29.5	18

12.6.3 Dose volume constraints for volumes of interest

VOLUME (%)	DOSE (%)					
	bPTV1_P	PTV1_P	PTV2_P	PTV1_N	PTV2_N	PTV3_N
99	>90	>90	>90	>90	>90	>90
98	Unspecified but must be reported	Unspecified but must be reported	Unspecified but must be reported	Unspecified but must be reported	Unspecified but must be reported	Unspecified but must be reported
95	>95	>95	>95	>95	>95	>95
50	= 100	= 100	= 100	= 100	= 100 +/- 1Gy	= 100 +/- 1Gy
5	<105	<105	<105	<105	As low as possible	As low as possible
2	<107	<107	<107	<107	As low as possible	As low as possible

12.7 Organs at Risk

The following organs at risk (OARs) should be delineated: spinal cord, brainstem, parotid glands (contralateral and ipsilateral), submandibular glands. A 3-5mm margin will be added to the OARs to produce Planning Target Volumes (PRVs) for radiotherapy planning.

The following swallowing related structures (SWOARS) will also be outlined for every patient according to published guidelines (37) and the PEARL atlas for contouring swallowing related structures. These structures include the pharyngeal constrictor muscles (superior PCM, middle PCM and inferior PCM), supraglottic/glottic larynx, cricopharyngeus, oesophageal inlet, cervical oesophagus and oral cavity. The superior and middle pharyngeal constrictor muscles will often be in the treated volume but the other SWOARS can all be used for treatment plan optimisation.

The dose constraints for OARs and PRV are given below. No constraint is given for the ipsilateral parotid gland as it often overlaps or abuts the PTV; this should be kept as low as possible but not at the expense of PTV coverage.

Structure	Volume Constraint	Optimal Dose Constraint
Spinal cord	Max	<48Gy*
	1cm ³	<46Gy*
Spinal cord PRV	1cm ³	<48Gy*
Brain stem	Max	<55Gy*
	1cm ³	<54Gy*
Brain stem PRV	1cm ³	<55Gy*
Contralateral Parotid (Lateralised Tumour)	Mean	<14Gy
Contralateral Parotid (Non-lateralised Tumour)	Mean	<24Gy
Ipsilateral Parotid	Mean	ALARP
Contralateral Submandibular Gland	Mean	<35Gy
Supraglottic Larynx	Mean	<55Gy
Glottic Larynx	Mean	<45Gy
Superior Pharyngeal Constrictor Muscles	Mean	<50Gy
Middle Pharyngeal Constrictor Muscles	Mean	<50Gy
Inferior Pharyngeal Constrictor Muscles	Mean	<20Gy
Cricopharyngeus/oesophageal inlet	Mean	<20Gy
Cervical oesophagus	Mean	<20Gy
Oral Cavity (low priority for optimisation)	Mean	<30Gy

*Mandatory dose constraint

Further information regarding OAR delineation is available in the PEARL radiotherapy guidance document.

12.8 Treatment plan design

The trial protocol mandates that all patients within the PEARL study will undergo swallowing and saliva-sparing RT, delivered using Volumetric Arc Therapy (VMAT) (RapidArc), which the UK DARS clinical trial team demonstrated reduced RT dose to the pharyngeal constrictors more effectively than IMRT (34). Each participating trial centre must detail their method of treatment planning and delivery in their radiotherapy process document (as described in the RT Guidance Document). The number of beams and beam arrangement should ensure uniform coverage of the PTV and satisfy dose constraints to OARs.

12.9 Treatment verification

The PEARL protocol mandates daily imaging with online cone beam CT for the majority of fractions (33 +/- 20%) for on treatment verification.

12.10 Radiotherapy quality assurance

The quality assurance programme for the PEARL trial will be co-ordinated by the National Radiotherapy Trials Quality Assurance (RTTQA) group. This will consist of the following:

1. Each site must complete a pre-accrual outlining benchmark case. This will be reviewed by the PEARL QA team and any amendments will be fed back to the clinician. If the centre has already completed the quality assurance process for other VMAT or PET-based planning trials there is no need to repeat this.
2. Real time review of the first patient recruited by each centre will be carried out by the PEARL QA team before treatment starts, both for outlining and planning and any amendments required will be fed back to the site.
3. Completion of a facility questionnaire describing equipment, software and techniques to be used in the trial.
4. Verification of electronic transfer of data to ensure data can be anonymised and transferred to the QA centre.
5. Dosimetry audit visit would consist of output measurement and dosimetric measurements of a representative treatment plan. The need for a visit by RTTQA team would depend on each centre's prior participation in a credentialed trial.

The details of the RTTQA programme and the necessary forms and details on how to upload plan data can be found via the PEARL link at www.rtrialsqa.org.uk. Further information is also available in the PEARL RT guidance document.

12.11 PET PROCESS QA

RAW data of all PET scans (i.e. data before image reconstruction) must be saved and made available with the reconstructed PET scans as part of standard trial data collection.

13 Chemotherapy

All patients must have sufficient renal function (GFR>50ml/min) to receive concurrent chemotherapy with the radiotherapy. If Cisplatin is not appropriate, carboplatin may be substituted.

Chemotherapy will be administered according to local practice in each centre. Precise scheduling and dose reductions are at the discretion of the treating oncologist.

Whilst COVID-19 is a public health issue, the latest version of the COVID-19 PEARL recruitment policy should be followed as part of the screening and study procedures.

13.1 Scheduling

Cisplatin is delivered as per local practice in each centre, according to the following recommended schedule: Cisplatin 100 mg/m² administered intravenously on day 1 for 2 cycles, that is day 1 (day 1 of cycle 1) and day 22 (day 1 of cycle 2) of the RT schedule. Cisplatin given within 24hrs of the required day for cycle 1 and within 48hrs for cycle 2 is acceptable.

On the day of chemotherapy, Cisplatin should, whenever possible, be administered prior to RT.

In the event of Cisplatin substitution by Carboplatin, Carboplatin is delivered as per local practice in each centre, using AUC5 to calculate dose.

14 Trial Assessments

Whilst COVID-19 is a public health issue, the latest version of the COVID-19 PEARL recruitment policy should be followed as part of the screening and study procedures. This may include some trial visits or assessments being done remotely.

14.1 Baseline (pre-chemoradiotherapy) assessments

Patients should undergo the following assessments prior to chemoradiotherapy*:

- PET-CT scan (prePET)
- Toxicity assessment

- Quality of life questionnaires - EORTC QLQ-C30 and EORTC QLQ-H&N35 (Appendix 4) and UW-QOL (Appendix 5)
- A panel of swallowing assessments (Appendix 2)
 - MDADI score (MDADI questionnaire is located in Appendix 3)
 - PSS-H&N
 - 100mL Water swallow test
- Recording of feeding tube use
- Clinical Review

*For centres where p16 and/or HPV testing is standard practice and the patient has been confirmed as HPV positive locally, trial specific assessments can be carried out prior to receiving confirmation of central HPV testing result.

Questionnaire-based assessments should be conducted prior to the water swallow test so that responses to questionnaires are not influenced by this procedure. The timing of assessments will be documented in the CRF. All assessments can be carried out on the same day or staged, according to local resources. Speech and Language Therapists in individual centres may delegate functional assessments to appropriately trained research nurses or other members of the team. A member of the site Speech and Language Therapist team should oversee those performing study assessments to ensure temporal consistency in the results.

Baseline translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub study. Additional histology slides taken from the diagnostic biopsy should be sent to the AWGL (see section 4.6 and 11 for more details).

14.2 Assessments during chemoradiotherapy

Toxicity

The worst grade toxicity as per CTCAE criteria v4.03 should be recorded weekly during chemoradiotherapy. CTCAE V4.03 can be found at the following link:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

Toxicity should also be recorded 4 weeks (+/- 2 weeks), 6 months (+/- 4 weeks), 12 months (+/- 4 weeks) and 24 months (+/- 8 weeks) after the end of chemo-radiotherapy.

Recording of feeding tube to be performed weekly during chemoradiotherapy.

In addition, at two weeks into CCRT, translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study.

PET-CT scan and disease assessment to be performed after 10 fractions of radiotherapy (at 2 two weeks into CCRT) and the radiotherapy plan adapted based upon the remaining avid biological GTV (bGTV_iP) on the iPET.

14.3 Post-chemoradiotherapy assessments (4 weeks and 6, 12 and 24 months post-radiotherapy)

All post-radiotherapy assessment time points should be timed from the end of chemoradiotherapy.

After 4 weeks post-treatment, visits can be conducted telephonically, and questionnaires emailed or posted where this remains in the best interest of the participant and the visit does not include statistically relevant assessments where hospital attendance is needed for them to be carried out.

4 weeks (+/- 2 weeks) post-chemoradiotherapy assessments:

As described above for pre-chemoradiotherapy assessments apart from the FDG-PET-CT scan. CTCAE toxicity should be recorded. Translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study.

12 weeks (+/- 2 weeks) post chemoradiotherapy assessments:

As described above for pre-chemoradiotherapy assessments including the post treatment PET-CT scan (postPET). The PostPET can be completed within 10 and 16 weeks. Disease assessment should also be performed. Translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study.

6 months (+/- 4 weeks) post-chemoradiotherapy assessments:

As described above for pre-chemoradiotherapy assessments apart from the FDG-PET-CT scan. CTCAE toxicity should be recorded.

12 months (+/- 4 weeks) post-chemoradiotherapy assessments:

As described above for pre-chemoradiotherapy assessments apart from the FDG-PET-CT scan. CTCAE toxicity should be recorded. Clinical review and Swallowing support summary is also required. Translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study.

24 months (+/- 8 weeks) post-chemoradiotherapy assessments:

As described above for pre-chemoradiotherapy assessments apart from the FDG-PET-CT scan. CTCAE toxicity should be recorded. Clinical review is also required. Translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study.

As before, questionnaire-based assessments should be conducted prior to water swallowing testing so that responses to questionnaires are not influenced by these investigations.

14. 4 Follow-up

Clinical follow up after treatment (for disease recurrence/death) should be carried out as per routine practice for at least 5 years in accordance with National guidelines (NICE IOG guidance 2004). Specifically, patients should undergo regular full examination of the head and neck according to the following schedule: year 1 – every 4 – 6 weeks; year 2 – every 8 – 10 weeks; year 3 – every 3 - 5 months; years 4 and 5 – approximately every 6 months. The patient will be followed up by the trial team for the first 2 years after treatment. Patient status will be collected at the end of the trial in order to obtain up to date PFS data. Translational blood and saliva samples should be collected from participants who consented to take part in the PEARL-T sub-study as per Section 14.5 Trial assessment table.

Procedures	Screening	During CCRT			Post CCRT				
		Baseline	Weekly	Fraction 10	4 Weeks	12 weeks	6 months	12 months	24 months
Informed consent	x								
Demographics	x								
Medical History	x								
Eligibility Assessment	x								
Pregnancy test*	x								
Central HPV testing		x							
FDG PET-CT		x		x		X***			
MDADI Score		x			x	x	X	x	x
PSS- H&N		x			x	x	X	x	x

Procedures	Screening	During CCRT			Post CCRT				
		Baseline	Weekly	Fraction 10	4 Weeks	12 weeks	6 months	12 months	24 months
Water Swallow test		X			X	X	X	X	X
Feeding tube use		X	X		X	X	X	X	X
Swallowing support summary								X	
QoL (EORTC QLQ-C30, EORTC H&N35 and UW-QOL)		X			X	X	X	X	X
CTCAE Toxicity (v 4.03)		X	X		X	X	X	X	X
Clinical review		X	X		X	X	X	X	X
Disease assessment	X			X		X			

Procedures	Screening	During CCRT			Post CCRT				
		Baseline	Weekly	Fraction 10	4 Weeks	12 weeks	6 months	12 months	24 months
Translational Blood Sampling		X		X	X	X		X	X
Translational Saliva sampling		X		X	X	X		X	X
Histology slides from the diagnostic biopsy		X**							

*As applicable

** Histology slides should be cut for PEARL-T at the same time as cutting the slides for the HPV centralised test provided the participant has consented to tissue collection.

***PostPET scan can be completed within 10 to 16 weeks post treatment.

15 Safety Reporting

The Principal Investigator is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section.

All SAEs must be reported immediately (and within 24 hours of knowledge of the event) by the PI at the participating site to the CTR PV and safety specialist or Trial team unless the SAE is specified as not requiring immediate reporting.

15.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which are not necessarily caused by or related to that product or intervention.
Serious Adverse Event (SAE)	Any adverse event that - <ul style="list-style-type: none"> • Results in death (Grade 5) • Is life-threatening* • Required hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Other medically important condition***
Serious Adverse Reactions (SARs)	Any SAE occurring in a clinical trial participant for which there is a reasonable possibility that it is related to the trial treatments.

<p>Suspected Unexpected Serious Adverse Reactions (SUSARs)</p>	<p>A SAR, the nature and severity of which is not consistent with the Reference Safety Information (RSI) for the trial treatments – Radiotherapy or chemotherapy</p>
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***Note:** The term 'life-threatening' in the definition of serious refers to an event in which the trial participant was at risk of death at the time of the event or it is suspected that used or continued used of the product would result in the subjects death; it does not refer to an event which hypothetically might have caused death if it were more severe.

**** Note:** Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened, or elective procedures, does not constitute an SAE.

***** Note:** other events that may not result in death, are not life-threatening, or do not require hospitalisation, may be considered as an SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

15.2 Trial Specific SAE Reporting requirements

In addition to the SAE reporting requirements above, for the purposes of this trial the following events will also be considered SAEs and must be captured on the SAE form and reported to the CTR with 24 hours of knowledge of the event:

- Any event which fulfils the definitions of an SAE except for those listed below as not requiring immediate reporting.
- All deaths within 30 days of chemotherapy or radiotherapy.
- Positive COVID-19 test result within 30 days of CCRT.

For the purposes of this trial the following events will not require reporting as SAEs:

- Hospitalisation as a result of expected toxicities of radiotherapy (grade 1 – 3) and/or chemotherapy (grade 1 – 3).

These should be completed in the participant's notes and on the relevant toxicities CRF page and forwarded to the CTR in the normal timeframes for CRFs.

For expected toxicities of radiotherapy please refer to section 15.4 (list of expected events for radiotherapy) and for expected toxicities of chemotherapy please refer to this table:

Intervention	SPC to be used as reference for list of expected toxicities	Relevant section of SPC to be used as reference for list of expected toxicities
Cisplatin	Cisplatin 1mg/ml Concentrate for Solution Infusion Accord Healthcare Limited (Updated 22-Feb-2020)	Section 4.8
Carboplatin	Carboplatin 10mg/ml concentrate for solution for infusion Accord Healthcare Limited (Updated 31-Aug-2018)	Section 4.8

Note: For the purposes of this protocol anticipated treatment related SAEs of grade ≤ 3 which do not result in hospitalisation are not subject to expedited reporting but are recorded in the CRFs.

15.3 Causality

Causal relationship will be assessed for the intervention:

Intervention and Procedures: Concurrent Chemoradiotherapy where the radiotherapy is adjusted partway through treatment. Chemotherapy is delivered as standard of care.

The Principal Investigator (or another delegated medically qualified doctor from the trial team) will assess each SAE to determine the causal relationship and the Chief Investigator (or another appropriately qualified member of the Trial Management Group) can also provide this assessment where necessary:

Relationship	Description	Reasonable possibility that the SAE may have been caused by the intervention?
Unrelated	There is no evidence of any causal relationship with the intervention	No
Unlikely	There is little evidence to suggest there is a causal relationship with the intervention (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).	No
Possible	There is some evidence to suggest a causal relationship with the intervention (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).	Yes
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	Yes
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	Yes

The causality assessment given by the Principal Investigator (or delegate) cannot be downgraded by the Chief Investigator (or delegate), and in the case of disagreement both opinions will be provided.

15.4 Expectedness

The Chief Investigator (or another delegated appropriately qualified individual) will assess each SAE to perform the assessment of expectedness.

The expectedness assessment should be made with reference to the current Reference Safety Information (RSI) for radiotherapy for each SAR. Expectedness decisions must be based purely on the content of the RSI; other factors such as the participant population and participant history should not be taken into account. Expectedness is not related to what is an anticipated event within a particular disease.

SAEs which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected events. For example, an event more specific or more severe than that described in the protocol is considered unexpected.

The following lists the expected events in relation to radiotherapy and this should be used as the RSI when assessing the expectedness of SAEs causally related to radiotherapy:

It is expected that patients receiving primary radiotherapy for oropharyngeal cancers may require admission for symptom control of the following:

Mucositis	grade >3
Dysphagia	grade >3
Pain	grade >3
Anaemia	grade >3
Nausea and vomiting	grade >3
Weight loss	grade >3
Poor oral intake	grade >3
Tinnitus (chemotherapy related)	grade >3
Infection	grade >3

They may require intravenous hydration and/or nasogastric feeding tube (NG) feeding and pain control.

15.5 Reporting procedures

15.5.1 Participating Site Responsibilities

The PI (or delegated appropriately qualified doctor from the trial team) should sign and date the SAE CRF to acknowledge that he/she has performed the seriousness and causality assessments. Investigators should also report SAEs to their own health boards or trust in accordance with local practice.

A completed SAE form for all events requiring immediate reporting should be submitted via fax or email to the CTR within 24 hours of knowledge of the event. A separate form must be used to report each event, irrespective of whether or not the events had the same date of onset.

The participant will be identified only by trial number, partial date of birth (mm/yyyy) and initials. The participant's name should not be used on any correspondence.

It is also required that sites respond to and clarify any queries raised on any reported SAEs and report any additional information as and when it becomes available through to the resolution of the event. Additionally, the CTR may request additional information relating to any SAEs and the site should provide as much information as is available to them in order to resolve these queries.

Serious Adverse Event (SAE) email address:

CTR-Safety@cardiff.ac.uk

Please also copy in the PEARL trial team:

pearl@cardiff.ac.uk

SAE Fax number:

0203 0432 376

Serious adverse events should be reported from time of signature of informed consent, throughout the treatment period up to, and including 30 days after the participant receives the intervention.

Adverse events (AE) should be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

The toxicity grades should be recorded on the toxicity part of the CRF.

An SAE form is not considered as complete unless the following details are provided:

- Full participant trial number
- An Adverse Event
- A completed assessment of the seriousness, and causality as performed by the PI (or another appropriately medically qualified doctor registered on the delegation log).

If any of these details are missing, the site will be contacted and the information must be provided by the site to the CTR within 24 hours.

All other AEs should be reported on the CRF following the CRF procedure described in Section 17.

15.5.2 The CTR responsibilities

Following the initial report, all SAEs should be followed up to resolution wherever possible, and further information may be requested by the CTR. Follow up information must be provided on a new SAE form.

The CTR should continue reporting SAEs until 30 days after the participant receives the last part of the intervention.

Once an SAE is received at the CTR, it will be evaluated by staff at the CTR and sent to the Chief Investigator (or their delegate) for an assessment of expectedness.

For all non-CTIMP studies, only reports of related and unexpected Serious Adverse Events (SAEs) should be submitted to the REC. These should be sent within 15 days of the chief investigator becoming aware of the event. There is no requirement for annual safety reports in addition to the information provided through the annual progress report.

15.6 Contraception and pregnancy

15.6.1 Contraception

Women of Child Bearing Potential (WOCBP) entering into this trial must agree to use a highly effective method of contraception preferably with low user dependency for at least six months after the intervention. A highly effective method of contraception is considered as having a failure rate of less than 1% per. Some acceptable contraception methods are listed below;

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
 - oral
 - intravaginal
 - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
 - oral
 - injectable
 - implantable*
- Intrauterine device (IUD)*
- Intrauterine hormone-releasing system (IUS)*
- Bilateral tubal occlusion*
- Vasectomised partner*
- Sexual abstinence defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments.

N.B. periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception.

**These contraception methods are considered to be low user dependency.*

Male participants with a WOCBP partner should use condom during treatment and at least until six months after the treatment. For a non-pregnant WOCBP partner, contraception recommendations should also be considered.

15.6.2 Pregnancy reporting whilst participating in the trial

Pregnancy, or the pregnancy of a partner occurring whilst participating in the trial, is not considered an SAE, however, a congenital anomaly or birth defect is. Other cases (e.g. termination of pregnancy without information on congenital malformation, and reports of pregnancy exposure without outcome data) should not normally be reported as such. When pregnancy occurs in a trial, either in a female participant or the female partner of a male participant, this should be followed up until at least the end of pregnancy, whether that is a live birth, abortion etc. Without follow-up of the pregnancy, it would not be possible for the CTR to know if a congenital anomaly or birth defect occurred, and therefore if there was an SAE that must be included in the safety evaluation of the intervention. Information on a pregnancy in a trial participant will be captured on the CTR Pregnancy Report Form supplied to sites by the CTR and a participant will be followed up as per standard of care.

Sites should report pregnancy occurring within SAE reporting periods stipulated in the trial protocol (for example, some trial protocols may state that SAEs should be reported during the trial treatment period and up to 30 days after the last date of treatment, this timeline would also apply to the reporting of pregnancies). Congenital anomalies or birth defects are considered an SAE and so these events must also be reported to the CTR on a trial-specific SAE form. Congenital anomalies or birth defects related to the intervention and unexpected with respect to the Reference Safety Information (RSI) must be submitted by the CTR within expedited time frames to the relevant REC.

15.6 Urgent Safety Measures (USMs)

An urgent safety measure is an action that the Sponsor, Chief Investigator or Principal Investigator may carry out in order to protect the subjects of a trial against any immediate hazard to their health or safety. Any urgent safety measure relating to this trial must be notified to the Research Ethics Committee immediately by telephone, and in any event within 3 days in writing, that such a measure has been taken. USMs reported to the CTR will be handled according to CTR processes.



16 Statistical considerations

16.1 Sample size

To be certain that we are not having a negative impact on PFS by adapting the RT plan we will ensure that PFS is at least as high as expected after treatment with chemo-radiotherapy in patients with similarly staged HPV-positive OPSCC. The E1308 trial (13) estimated the 2 year PFS to be between 76% and 99% with 95% confidence in the subgroup of patients who will be recruited to PEARL (T1 – T3, N1 (TNM8), low smoking history). If we assume that our point estimate may be as high as 90% then 44 patients will allow us to calculate confidence intervals that exclude 76% (77-97%). Comparable Phase II studies (12,13) have used similar assumptions. We will recruit 50 patients to allow for ~10% loss to follow up.

16.2 Procedures for reporting deviation(s) from the original SAP

These will be submitted as substantial amendments where applicable and recorded in subsequent versions of the protocol and SAP.

16.3 Termination of the trial

To alleviate concern that we are having a detrimental impact on treatment efficacy, we will have an early stopping rule based on complete response at the primary site at 3 months following treatment (defined as having no disease at the 3 month PET and no disease on any subsequent biopsy). A recent study (15) found that 43/44 (98%, 95% CIs: 88%-100%) of patients had complete response. This correlated with excellent outcomes at 2 years with local control, regional control, cancer specific survival, distant metastasis free survival and overall survival rates at 100%, 100%, 100%, 100% and 95% respectively. Any accumulated data will be reviewed regularly at IDMC meetings. When presenting data to the IDMC we will generate 95% confidence intervals around our estimate of 3 month complete response rate and ensure that they include 88%. We will conduct an interim analysis after 15 patients complete 3 months of follow-up and are assessed for complete response and the IDMC will have the mandate to stop the trial for harm if we see less than 10 complete responders.

16.4 Main analysis

All patients who had an interim PET-CT (iPET) will be included in the main analysis.



16.4.1 Primary endpoint

Progression free survival will be calculated using Kaplan Meier estimation methods with 95% confidence intervals.

16.4.2 Secondary endpoints

1 *To demonstrate feasibility of recruitment*

Recruitment over 2 years will be presented.

2 To test if individualized, adaptive, biologically-based radiotherapy planning is feasible and results in a significant change in the radiotherapy plan.

Percentage reduction in mean dose to OAR (superior pharyngeal constrictor muscles, contralateral parotids, contralateral submandibular gland, salivary glands) as a result of PET-CT during treatment. Percentage change to PTV will also be presented.

3 *To maintain high complete response rates 3 months after treatment*

The proportion of patients who have no residual tumour at 3 months as per Hopkins criteria will be presented.

4 *To assess acute and late toxicity rates and the effect of treatment on swallowing function*

Cumulative acute CTCAE toxicity score percentages during and up to 3 months after treatment will be presented. Toxicity scores will be presented at 6, 12, 18 and 24 months. Water swallow test, MDADI and QoL scores will be plotted over time.

17 Data Management

Source Data is defined as “All information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents.” There is only one set of source data at any time for any data element, as defined in site source data agreement.

Source data include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. Sites will retain all original source of data from these investigations for future reference. On all trial-specific

documents, other than the signed consent form, the participant will be referred to by the trial participant ID, not by name.

17.1 Completion of Electronic CRFs

It is intended that data recording for this trial will be through use of a web-based system. This is a secure encrypted system accessed by an institutional password and complies with General Data Protection Regulation 2016.

Details of how to access the system will be supplied to investigators as part of site set up. A user password will be supplied to investigators upon completion of all processes required prior to opening.

A paper CRF will be used to collect the patient reported outcomes via the Quality of Life questionnaires (EORTC QLQ-C30, EORTC QLQ-H&N35 and UW-QOL).

Participating sites will be provided with training and instructions on how to complete and return the CRFs. The CTR will send reminders for any overdue data. It is the site's responsibility to submit complete and accurate data in timely manner.

18 Associated Research

18.1 ATLAAS Sub Study

Most PET-based clinical studies currently use manual delineation of PET-avid volumes, relying on freehand outlining by clinicians. This is time consuming and prone to both intra-and inter- observer variability. Automatic segmentation (PET-AS) by computers is an objective and reproducible alternative to delineating PET-avid volumes, but a large number of automatic algorithms are available and their utility varies in different clinical settings. The ATLAAS supervised learning algorithm has already been validated on phantom and synthetic 'ground truth' data and applied to radiotherapy planning PET-CT scans of head and neck squamous cell carcinoma patients. In the POSITIVE study (30) ATLAAS automatically produced FDG-avid volumes in 20 patients that were similar to the manually outlined PET volumes delineated by an experienced PET-radiologist and clinical oncologist. The main limitation of PET-AS is that they are designed to segment PET-avid volumes with no input from the anatomical component of the hybrid PET-CT scans. With this sub study we now aim to extend the functionality of ATLAAS by developing a method for the joint simultaneous segmentation of both functional (FDG-PET) and anatomical (CT) images.

18.1.1 ATLAAS Training

To train ATLAAS to simultaneously segment both the anatomical and the functional component of a PET-CT scan we will use the PET-CT scans of fillable phantoms already included in the original ATLAAS



training database (39). These scans already provide reference volumes segmented on the CT scan. We will also use a cohort of retrospective head and neck scans. This cohort will include:

- Data acquired during the POSITIVE study (20 PET-CT scans with labelled volumes including the GTV outlined by a team of expert radiologists and oncologists on the combined information provided by PET, CT and MRI scans).
- Planning CT scans acquired through clinical practice in Velindre since 2010 (200/year, all CT scans provide labelled volumes including the GTV outlined by a team of expert oncologists).
- Diagnostic PET-CT scans of patients treated with radical RT in Velindre with unknown primary tumours (2 -5 /year, with labelled volumes including the GTV outlined by a team of radiologists and oncologists based on the combined information provided by PET and CT scans).

In this phase we will explore the use of several methods including object/background seed localization method (40, 41), and machine learning/artificial intelligence.

18.1.2 ATLAAS Validation

Following the training phase, we will validate the ATLAAS simultaneous PET-CT segmentation. We will apply the newly developed ATLAAS simultaneous PET-CT auto-segmentation method to the PET-CT scans acquired during the PEARL trial. The results of the segmentation (bGTV_preP_ATLAAS and bGTV_iPET_ATLAAS) will be compared to clinician-outlined volumes. For each patient recruited to PEARL we will use the Primary Gross Tumour Volume volumes (bGTV_preP) outlined in Phase 1 and the Biological Primary Gross Tumour Volume volumes (bGTV_iP) outlined in PHASE 2.

18.1.3 Data analysis

We will use an assessment method based on the Turing Test (42) to evaluate the auto-contouring capabilities of ATLAAS.

We will ask a panel of external expert nuclear medicine radiologists and clinical oncologists to assess contours drawn by clinicians and ATLAAS on the same scan.

We will ask the panel three questions:

1. Is the contour human-drawn or ATLAAS-drawn?
2. Is the contour clinically acceptable?
3. If clinically acceptable, which contour do they prefer?



18.1.4 Main outcomes

To extend the capability of ATLAAS to simultaneously segment both the anatomical and the functional component of a PET-CT scan.

To validate the new ATLAAS joint PET-CT segmentation method on both baseline and interim CCRT PET-CT scans.

- Determine the rate of correct identification of contouring source
- Record the acceptance rate of the ATLAAS and clinician-drawn contours
- Determine which source of contouring was preferred overall

18.2 PET Response and Radiomics Sub Study

In addition to potential prognostic information from baseline FDG-PET-CT, a small number of studies have investigated the role of FDG-PET-CT during radical radiotherapy for head and neck cancer in predicting response to treatment and prognosis. They have published some conflicting results. As described in a recent systematic review, various published reports demonstrate differing degrees of predictive and prognostic capabilities of FDG-PET-CT in the setting of head and neck cancer and it is yet to be determined at what time point(s), and with which parameters, PET scans during radiotherapy should be performed (43).

In 2002, Brun et al (44) performed an FDG-PET-CT at baseline (PET1) and 5 – 10 days into radical head and neck cancer treatment (PET2) with radiotherapy (n=45) or chemotherapy (n=2) in a heterogenous head and neck cancer patient group. At a median follow up of 3.3 years, LCR was 80%, 5 year OS 54%. Within this small population of patients, a low metabolic rate at PET2 in the primary tumour was strongly associated with complete remission, LRC and survival. Survival was 72% and 35% in patients who had a low metabolic rate (MR) at PET2 vs a high one respectively. The hazard ratio for death was 4.5 vs 2.8. In equal quartiles of subgroups based on MR there was a statistically significant increase in the hazard ratios between each quartile and the reference.

These results were supported by more recent work performed by another group on a cohort of 37 patients with advanced head and neck cancer. Hentschel et al (23) found that the decrease in SUV max from baseline FDG-PET-CT to the FDG_PET_CT performed after week 1 or 2 into radical radiotherapy, to be a potentially prognostic biomarker. 2 year OS and 2 year LRC were improved for patients who demonstrated a decrease in SUV max of the primary tumour of over 50% at the second FDG-PET-CT (88% vs 38% and 88% vs 40% respectively).

In addition to these studies a variety of groups have looked to validate a visual therapy response interpretation criteria, The Hopkins Criteria, for use on FDG-PET-CTs performed on head and neck patients during a course of radiotherapy. This objective and standardised visual grading system was intended to reduce inter-observer variability in assessing PET scans. Non-CMR on iPET is classed as

avidity equal to or greater than the focal uptake of the liver and non-CMR on post-PET is classed as avidity equal to or greater than the mediastinum. Min et al (26) looked at 69 patients with head and neck cancer treated with radical radiotherapy. An FDG-PET-CT was performed at baseline, during week 3 of radiotherapy, and post-completion of treatment after a median interval of 13 weeks. They assessed the residual FDG uptake using the 5 point visual grading system and concluded that The Hopkins Criteria was a useful predictor of response to treatment and patient outcome based both on the post-treatment FDG-PET-CT and the interim FDG-PET-CT. Whilst it did not demonstrate the level of prognostic power as the post-treatment FDG-PET-CT, the interim PET had a very high NPV. In the patients who had a CMR on the interim FDG-PET-CT, the Hopkins Criteria had an NPV of 91% for the primary site alone, and 100% if both primary and nodal disease demonstrated a CMR. The group postulated that it potentially offered a mechanism on which to base adaptive de-escalated radiotherapy. They also established optimal thresholds for the definition of the non-CMR group as focal grade 3 uptake more than, or equal to that of the liver. This is a higher threshold than that of the post-treatment FDG-PET-CT to account for elements of increased FDG-uptake attributable to radiotherapy treatment.

Despite the above findings Ceulemans et al (45) concluded that they could not replace the post-treatment FDG-PET-CT with an earlier, intra-treatment scan. When they compared FDG-PET-CT conducted at the end of week 4 of radiotherapy (47Gy) to that of a scan at 4 months post-completion of treatment, the sensitivity, NPV and accuracy of detecting complete response was reduced (28.6% vs 42.5%, 31.0% vs 60.0% and 80.0% vs 88% respectively). Although the PPV and specificity was slightly higher in the week 4 scan (81.8% vs 75.0% and 80.0% vs 77%), this was not enough to warrant a change of imaging scheduling, One possible explanation of this difference in findings in the higher amount of radiation the Ceulemans patients were exposed to. It is now widely considered that after 2 – 3 weeks of radiotherapy, background radiation-induced inflammation can become an issue for the interpretation of FDG-based imaging.

Castaldi et al (46), published their prospective study conducted on 26 head and neck cancer patients of varying subsites. Patients underwent a baseline FDG-PET-CT and then another at 2 weeks into radiotherapy and finally one at 8 – 12 weeks post-completion of treatment. The post treatment FDG-PET-CT was predictive of clinical outcome, correlating with both relapse free survival (RFS) and disease specific survival (DSS). The group was unable to confirm a role of the mid-treatment FDG-PET-CT which did not have any features that significantly correlated with patient outcome.

Another important area of debate surrounds the choice of parameter to use when using interim FDG-PET-CT for predictive and prognostic means. In addition to whether or not there is CMR on the iPET, parameters most widely studied include SUVmax, Metabolic Tumour Volume (MTV) and Total Lesion Glycolysis (TLG). Most work on this has been performed on the pre_PET.

The primary tumour SUVmax on pre-PET has been demonstrated to be predictive of survival regardless of the size and stage of the tumour (47) although studies differ on their reported cut-off values, which

range from SUVmax 4 – 10. The work by Hentschel et al mention above used a relative reduction of 50% in SUVmax between pre_PET and iPET.

The MTV has been shown to be more accurate in the prediction of outcome compared to SUVmax (46). One suggested explanation for this is that SUVmax may have less bearing on outcome because highly avid disease may be expected to have a better response to radiotherapy treatment. The MTV can be broadly determined in two ways. The first by a fixed background SUV cut off eg MTV2.5, or 2SD of normal liver activity; or by using the SUVmax of individual tumour site regions up to a prefixed percentage of the SUVmax eg MTV40%. Dibble et al (49) showed the MTV (and TLG) on pre-PET to be significantly associated with survival and outcome. An MTV equal or greater than 7.7ml was predictive of time to OS. Kao et al (508) looked at the prePET of 64 patients with mixed pharyngeal tumours and found the MTV2.5 to be predictive of primary recurrence and DFS. More relevant to our study, Lim et al (51) looked at 176 patients with oropharyngeal cancer. They used an SUV42% to delineate MTV and compared SUVmax, MTV and TLG. They found the MTV and TLG to be strongly correlated with the development of distant metastases or death. An MTV of over 19.7cm³ was strongly predictive of a high risk of death. Abgral et al (52) looked at 80 patients with mixed HNSCC and demonstrated a prePET MTV5.0 to be the best predictor of recurrence and death following treatment and an MTV5.0 of over 4.9ml to be predictive of poor event free survival (EFS) and OS.

So far studies have been predominantly made up of rather heterogenous patient groups, using a variety of parameters and methods of defining them. This may go some way to explain the disparate conclusions and cut offs that the research has produced. We intend to build upon the small amount of published data available by analysing the iPET data for our well-defined patient cohort of a specific demographic, and investigate correlations already seen by other research groups as well as more novel work comparing iPET parameters to those of prePET and clinical outcomes.

This sub-study is a prospective, observational study designed to assess the potential benefit of radiomics in patients with head & neck cancer, specifically HPV-positive OPSCC, treated with adaptive radiotherapy regimes in the PEARL study. This study will assess the predictive value of radiomics as imaging biomarkers to predict response to radiotherapy and radiation toxicity. The imaging data will not inform patient management but can be validated against current clinical, imaging and biological methods. This is the first head and neck cancer radiomics study that we are aware of in which patients are treated with an adaptive radiotherapy protocol.

Radiomics are advanced imaging techniques that allow non-invasive, high-throughput, 3-dimensional extraction of large numbers of descriptive features from a tumour (53). The use of radiomics in cancer research is currently of great interest and the number of publications using these techniques are rising exponentially. Radiomics include image features that describe the spatial frequency and distribution of voxels (3D pixels) within a region of interest. Variations in image features of the primary tumour are thought to reflect underlying tumour biology, such as areas of hypoxia, necrosis, angiogenesis and proliferation (54, 55). These image features could enhance the diagnosis, staging, treatment planning

and monitoring of cancer patients. The Cardiff University Cancer Imaging and Data Analytics (CIDA) group participates to the Image Biomarker Standardisation Initiative (IBSI)¹ to develop standardised radiomics algorithms and reporting guidelines that can make radiomics analyses reproducible and comparable (56).

Our previous work includes the development and validation of a prognostic model in 403 patients with oesophageal cancer that showed the additional value of radiomic techniques compared to current staging methods (57). The patient cohort, including both adenocarcinomas and squamous cell carcinomas were all staged with PET-CT. Radiomics were obtained from the primary oesophageal tumours segmented using ATLAAS (39). As expected, age at diagnosis, the TNM stage and treatment with curative intent were all independent predictors of overall survival. In addition, 3 imaging features were also independently associated with survival; tumour lesion glycolysis (TLG), Histogram Energy and Histogram Kurtosis. TLG quantifies the volume and avidity of the tumour. Energy describes the intensity variation, whilst Kurtosis describes the 'peak' of the histogram, indicating the range of voxel intensities. A schematic of a typical radiomics pipeline including ATLAAS automated image segmentation and IBSI feature extraction is shown in Figure 2.

This study developed and validated a prognostic model from baseline PET-CT data. Arguably, of more clinical benefit, is a predictive model which could be used to help clinicians decide upon the best treatment regime and to guide subsequent management. Changes in image features can be assessed between intervals. 'Delta' Radiomics quantifies the change in features between imaging examinations and has been shown to have clinical predictive value for patient outcomes (58). Early responders to treatment may be identified, which in turn could facilitate the implementation of an adaptive therapy regime. Alternatively, a radiomics model could predict patients who will develop radiation toxicity (59). Changes in Radiomic signals may also match corresponding changes in underlying tumour biology. A reduction in circulating cell-free DNA may correspond to changes in imaging phenotype.

18.2.1 Methods

a) Data Collection

The following imaging examinations will be collected for all patients recruited into PEARL.

- i. pre_PET
- ii. iPET
- iii. PET_post
- iv. Staging CT
- v. Staging MRI (including diffusion weighted imaging (DWI) sequences
- vi. Radiotherapy planning simulation CT, RTDOSE, RTSTRUCT and RTPLAN

¹ Image biomarker standardisation initiative <https://arxiv.org/abs/1612.07003v6>

vii. Daily online cone-beam CT (CBCT)

b) Data Repository

Once the imaging examinations have been transferred and collected from the Velindre PACS system, anonymised images will be exported to the secure CIDA server for analysis using the established Radiomics pipeline (Fig. 2). The scans will be collected in the CIDA Extensible Neuroimaging Archive Toolkit (XNAT) platform, the most widely-used informatics platform for imaging research (www.xnat.org). This is in-line with the approach used in other well established imaging centres such as the CRUK-EPSRC Cancer Imaging Centre at the Institute of Cancer Research.

We plan to test a pre-defined and published radiomics signature developed from a predictive model by Vallières et al (60). The signature was developed and validated in 300 patients with head & neck cancer treated with chemo-radiation or radiotherapy alone from 4 different centres in Canada. The radiomic signature that will be tested in this sub-study is found in the published article.

c) Definition of response on PET-CT

As per The Hopkins Criteria Scores of 1-3 are considered negative for tumour, but we will also analyse for complete metabolic response (score 1), including scores of 1 and 2 at interim PET.

Responders are classified as having either a CMR or a non-CMR

- CMR for iPET: Uptake below that of the liver (score 1 or 2)
- CMR for post-PET: Uptake below that of the mediastinum (score 1)

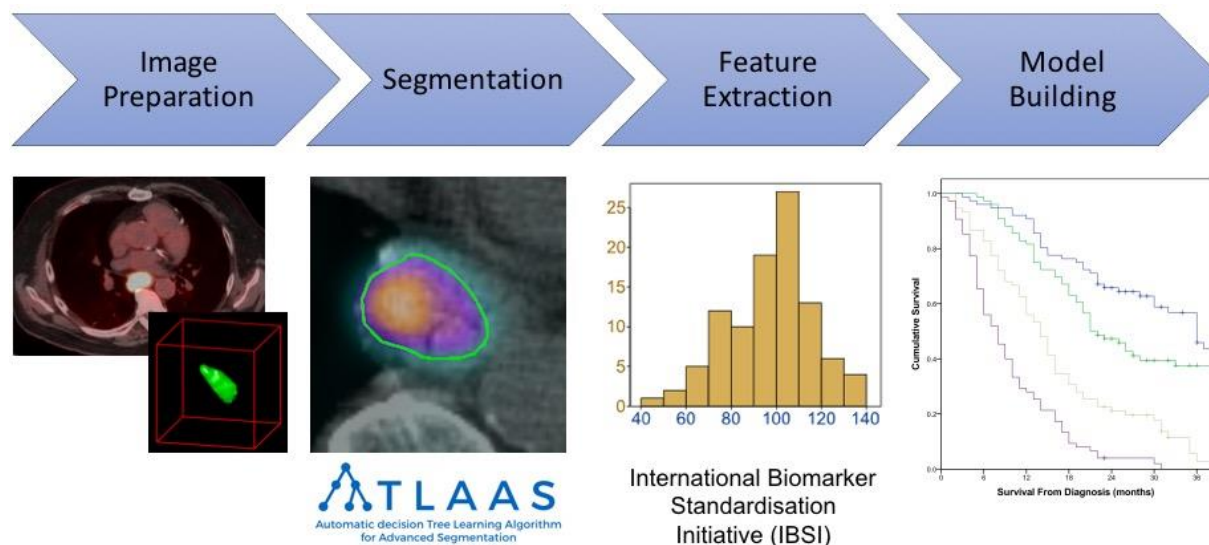


Figure 2. A schematic demonstrating the main steps in the radiomics pipeline.

The PET-CT scan acquisition protocol and supporting information for the PET-CT scanning report can be found in Appendix 7 and 11.

18.2.2 Main outcomes

Primary objectives

To determine the NPV, PPV, specificity and sensitivity of the response on iPET to predict CMR on FDG-PET-CT at 3 months post-treatment.

To evaluate the ability of a pre-defined radiomic signature to predict the percentage reduction in SUVmax defined by ATLAAS after 2 weeks of radiotherapy.

Secondary objectives

To evaluate the ability of a pre-defined radiomic signature to predict the percentage reduction in other PET parameters, including the volume of the bGTV_iP_ATLAAS defined by ATLAAS, after 2 weeks of radiotherapy.

To assess whether this radiomic biomarker signature, or a discreet quantitative image biomarker, correlates with response rates seen on PET-CT scan 3 months after chemo-radiotherapy and loco-regional control rate at 2 years.

To explore possible correlation between delta radiomics on PET and daily online CBCT done during radiotherapy.

To evaluate whether radiomics can predict which patients will develop radiation toxicity at 12 months e.g. do xerostomia rates correlate with salivary gland changes seen on imaging during/after radiotherapy?

To evaluate whether changes in image features correspond to changes in ctDNA.

Hypotheses

- a) A radiomics prediction model based on iPET can improve prediction of clinically important outcomes, such as who will respond early to radiotherapy
- b) A radiomics prediction model can predict patients who will develop radiation toxicity at 12 months
- c) PET-based response to radiotherapy (as defined by The Hopkins Criteria) will correspond to a reduction in ctDNA

18.3 Tissue, Plasma and Salivary DNA Sub Study: PEARL -T

18.3.1 Background

The clinical detection of residual or recurrent primary oropharyngeal carcinoma is often difficult because of the complex anatomy of the oropharynx and surrounding structures.

As previously mentioned, in the post-treatment setting of non-surgically managed HPV-positive HNSCC, FDG-PET-CT has been demonstrated to have a high NPV of over 90% for the detection of residual disease. The PPV however, is less robust, and this uncertainty can lead to a proportion of patients undergoing unnecessary surgical procedures intended to offer a form of salvage. The PET-NECK study (619) reported a severe surgical complication rate of 20 – 26% following neck dissection. Consequently, there has been increasing interest in developing additional novel biomarkers which can diagnose disease and identify recurrence prior to radiological and clinical signs and symptoms becoming apparent.

HPV-positive OPSCC is often driven by the integration of high risk HPV-16. This produces synthesis and shedding of E6 and E7, viral oncoproteins which promote tumour growth via inactivation of the p53 and retinoblastoma tumour suppression pathways.

Circulating tumour DNA (ctDNA) in blood has been demonstrated to correlate with the presence or absence of disease.

In patients treated for OPSCC, or who are at high risk of developing OPSCC, blood and salivary sampling is a particularly attractive possibility for large population screening. It has been demonstrated that combining HPV DNA results from contemporaneous samples of saliva and plasma may lead to a higher sensitivity of disease detection in the pre- and post-treatment setting. One group found that whilst if analysed separately, plasma and saliva had reduced sensitivity compared to post-treatment FDG PET at 3 months, when combined, the sensitivity of plasma plus saliva tests was higher (34). This was based on a retrospective study of 93 patients, 21 of whom had with HPV-positive head and neck cancer.

More recently, a prospective study looked at the plasma and tumour block DNA of 55 patients with locally advanced head and neck cancer using an in-house ultra-sensitive HPV DNA next generation sequencing (NGS) assay (62). 27 patients had HPV-positive disease confirmed from tumour blocks. Patients had baseline plasma samples and underwent primary radical CCRT. Patients were then followed up with serial plasma samples at 6 and 12 weeks after completion of treatment. An FDG-PET-CT was also performed at 12 weeks. The group were able to demonstrate a correlation of the NGS assay with the clinical response following treatment and validated the assay with an independently recruited prospective observational trial. The tracking of HPV DNA in samples throughout treatment and follow up was predictive of residual disease and response to treatment and could theoretically reduce the number of unnecessary 'salvage' surgical procedures.

In order to contribute to our understanding of how disease processes may be monitored in a less invasive and less morbid manner, we will be collecting blood and saliva samples prior to, during, and after the radical treatment of OPSCC in PEARL, to see if there is correlation with disease status and FDG-PET-CT response.

18.3.2 Methods

All patients enrolled in PEARL will be eligible for the translational sub studies of PEARL (PEARL-T). They will be given the option to consent to inclusion in PEARL but decline participation in PEARL-T.

The consent form will itemise specific biosources to be collected and patients will be asked to consent to each type of biosources they are prepared to consent to the collection of. Consent to PEARL-T is included in the main PEARL consent form that forms part of the PEARL Participant Information Sheet (PIS).

Salivary rinses and plasma samples of patients will be collected at baseline, 2 weeks after the start of treatment, four weeks after treatment and then at 3, 12 and 24 months after treatment (follow up). Histology slides of the diagnostic tissue biopsy will also be collected (4 to 6 x 10µm unstained slides).

Please refer to the laboratory manual for further details on samples collection, processing and storage. A summary has been included in section 4.6 and 11 of the protocol.

18.3.3 Data Analysis

Exploratory analyses will be undertaken to determine the presence of a panel of common genes known to be associated with oropharyngeal squamous cell carcinoma both in the diagnostic tissue and at various time points in the sequential blood and saliva samples to see if they correlate with clinical and radiological outcomes.

All samples will be allocated laboratory numbers assigned by the laboratory. Data will be appropriately pseudonymised.

The results of genetic analysis will be held in the laboratory but not returned to participants.

18.3.4 Main outcomes

Primary objectives

- To determine the sensitivity, specificity, negative predictive value, positive predictive value of single and combined pre-treatment plasma and saliva HPV-16 ctDNA for detecting tumour HPV-status.



- To assess the association of post-treatment HPV DNA status with clinical outcomes including recurrence-free survival and 2 year progression free survival.

Secondary objectives

- To determine any correlation of tissue sample mutations with clinical outcomes.
- To explore any correlation of liquid biopsy biomarker levels with contemporaneous FDG PET-CT findings.
- To assess any correlation of liquid biopsy biomarker levels with radiomic signatures of predictive and prognostic significance.

19 Protocol/GCP non-compliance

The Principal Investigator should report any non-compliance to the trial protocol or the conditions and principles of Good Clinical Practice to the CTR in writing as soon as they become aware of it.

20 End of Trial definition

The treatment phase will be followed by a follow-up period that will continue for 2 years after the last participant completes protocol treatment.

The end of the trial is defined as the date of final data capture to meet the trial endpoints.

Sponsor must notify the main REC of the end of a clinical trial within 90 days of its completion or within 15 days if the trial is terminated early.

21 Archiving

The TMF and TSF containing essential documents will be archived at an approved external storage facility for a minimum of 15 years from trial closure date. The CTR will archive the TMF and TSFs on behalf of the Sponsor. The Principal Investigator is responsible for archival of the ISF at site on approval from Sponsor at an approved external storage facility for a minimum of 15 years. Essential documents pertaining to the trial shall not be destroyed without permission from the Sponsor.

22 Regulatory Considerations

22.1 Ethical and governance approval

This protocol has approval from a Research Ethics Committee (REC) that is legally “recognised” by the United Kingdom Ethics Committee Authority for review and approval.

This trial protocol will be submitted through DSCHR PCU which assesses governance and legal compliance for the NHS in Wales. Additional governance review and approval will be obtained through HRA as this is required to open sites in England. The HRA approval process replaces the need for local checks of legal compliance and related matters by participating sites in England—this means sites do not give site specific governance approval.

Approval will be obtained from the host care organisation who will consider local governance requirements and site feasibility. The Research Governance approval of the host care organisation must be obtained before recruitment of participants within that host care organisation.

22.2 Data Protection

The CTR will act to preserve participant confidentiality and will not disclose or reproduce any information by which participants could be identified, except where specific consent is obtained. Data will be stored in a secure manner and will be registered in accordance with the General Data Protection Regulation 2016. There will be a joint data controller responsibility shared between Velindre NHS Trust and the CTR. The translational sample custodian for this trial is the CTR.

22.3 Indemnity

- Non-negligent harm: This trial is an academic, investigator-led and designed trial sponsored by Velindre NHS Trust and coordinated by the CTR. The Chief Investigator, local Investigators and CTR do not hold insurance against claims for compensation for injury caused by participation in a clinical trial and therefore cannot offer any non-negligent harm indemnity. The Association of the British Pharmaceutical Industry (ABPI) guidelines will not apply.
- Negligent harm: In accordance with Technical Note 12 Indemnity for Clinical Research for research Sponsored by a Welsh body, Welsh Risk Pool Services provides indemnity cover against successful negligence claims arising from the management and conduct of the trial. Where NHS employees are responsible for the design of a trial, indemnity cover will also be provided for negligent harm arising from the trial design. Velindre NHS Trust does not accept liability for any breach in the other NHS Organisations duty of care, or any negligence on the part of employees of these NHS Organisations.

All participants will be recruited at NHS sites and therefore the NHS indemnity scheme/NHS professional indemnity will apply with respect to claims arising from harm to participants at site management organisations.

22.4 Trial sponsorship

The trial is being sponsored by Velindre NHS University Trust. The Trust shall be responsible for ensuring that the trial is performed in accordance with the following:

- Conditions and principles of Good Clinical Practice.

- Declaration of Helsinki (1996)
- UK Policy Framework for Health and Social Care Research
- General Data Protection Regulation 2016.
- Other regulatory requirements as appropriate.

The Sponsor has/will be delegating certain responsibilities to Cardiff University (CTR), the Chief Investigators, Principal Investigators, host sites and other stakeholder organisations as appropriate in accordance with the relevant agreement that is informed by regulation and trial type.

22.5 Funding

This study is funded by Cancer Research Wales and Velindre Radiotherapy Charitable Funds (Moondance Foundation).

23 Trial management

23.1 TMG (Trial Management Group)

The Trial Management Group (TMG) will be responsible for the day-to-day running of the trial and will meet once every three months. The TMG members will include the Chief Investigators, Co-investigators, CTR representatives, specialist advisors and consumer representatives.

TMG members will be required to sign up to the remit and conditions as set out in the TMG Charter.

23.2 TSC (Trial Steering Committee)

The Trial Steering Committee (TSC) will be a committee of independent members providing overall supervision of the trial. The role of the TSC is to act on behalf of the sponsor, to provide overall supervision for the trial, to ensure that it is conducted in accordance with GCP, and to provide advice through its independent chairperson. The TSC will review the recommendations of IDMC and will decide on continuing or stopping the trial, or modifying the protocol as required. It will meet at least annually when it will consider each report of the IDMC, as well as results of other trials and new information which has arisen and recommend appropriate action.

The TSC terms of reference, roles and responsibilities will be defined in a charter. TSC members will be required to sign up to the remit and conditions as set out in the TSC Charter.

23.3 IDMC (Independent Data Monitoring Committee)

The data will be reviewed (approximately six monthly) by an Independent Data Monitoring Committee (IDMC), consisting of at least two Clinicians (not entering patients into the trial) and an independent Statistician. The IDMC will be asked to recommend whether the accumulated data from the trial,

together with results from other relevant trials, justifies continuing recruitment of further patients. A decision to discontinue recruitment, in all patients or in selected subgroups, will be made only if the result is likely to convince a broad range of Clinicians including PIs in the trial and the general clinical community. If a decision is made to continue, the IDMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The IDMC will make confidential recommendations to the Trial Steering Committee (TSC).

IDMC members will be required to sign up to the remit and conditions as set out in the DMC Charter.

24 Quality Control and Assurance

24.1 Monitoring

The clinical trial risk assessment has been used to determine the intensity and focus of central and on-site monitoring activity in the PEARL trial. Moderate monitoring levels will be employed and will be fully documented in the trial monitoring plan.

Investigators should agree to allow trial related monitoring, including audits and regulatory inspections, by providing direct access to source data/documents as required. Participant consent for this will be obtained.

Findings generated from on-site and central monitoring will be shared with the Sponsor, CI, PI & local R&D.

24.2 Audits & inspections

The trial does not involve an Investigational Medicinal Product (IMP) or a device and therefore does not require Clinical Trial Authorisation (CTA) from the MHRA. The trial will be submitted through the Research Governance process of the host care organisation for review and approval. The research governance approval of the host care organisation must be obtained before the start of the trial within that host care organisation.

The trial may be participant to audit by Velindre NHS Trust under their remit as Sponsor.

25 Publication policy

All publications and presentations relating to the trial will be authorised by the Trial Management Group.

Data from all sites will be analysed together and published as soon as possible. Individual participating PIs may not publish data concerning their participants that are directly relevant to questions posed by the trial until the TMG has published its report. The TMG will form the basis of the writing committee and advice on the nature of publications, subject to the Sponsor's requirements.



The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the TMG, and this may also include high accruing clinicians and/or other people who contribute to the trial. All participating centres and clinicians will be acknowledged in this main publication together with appropriate staff from the CTR.

All publications should include a list of participating PIs, and if there are named authors, these should include the CI, Co-Investigators, Trial Manager, and Statistician(s) involved in the trial, as agreed by the CI and Director of CTR. If there are no named authors then a writing committee will be identified.

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27 Appendices

APPENDIX 1 - HPV testing for PEARL

1.0 Purpose and scope, applications

This is a standard operating procedure (SOP) for the clinical trial PEARL (Chief Investigator: Mererid Evans, Velindre NHS Trust, Cardiff, Wales). The purpose is to describe the tissue pathway for assessing the HPV status of oropharyngeal squamous cell carcinomas recruited to the trial. The SOP applies to all biopsies submitted to the 'Central HPV Laboratory Service' (Newcastle upon Tyne Hospitals NHS Foundation Trust) as part of the trial protocol.

2.0 COSHH / Health & Safety

Not applicable.

3.0 Personnel

Appropriately trained medical secretaries, biomedical scientists, pathologists.

4.0 Equipment / reagents

Not applicable.

5.0 References

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6.0 CPA

Standards A8.1 F2.1

7.0 Departmental policy

Not applicable.

8.0 Forms

Supplementary request form. HILF098

PEARL HPV sample form

9.0 Related Documents

Immunohistochemistry SOPs. HIIC123 HIIC124 HIMP049

10.0 Procedure

Diagnostic pathway and determination of HPV status

Following consent to take part in PEARL and registration with the PEARL Trial Office, sections from the diagnostic biopsy (6 x 5µm unstained sections mounted on superfrost plus slides or equivalent; 3 slides for staining, 3 slides for repeat tests if necessary), along with the trial specific pathology request form, will be sent by the participating site to the Central HPV Laboratory Service. Additional sections should also be taken at this point for translational research if the participant consented to PEARL-T (please see 11.5 for more details).

PEARL Clinical Trial
Dr Max Robinson
Department of Cellular Pathology
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP
Tel: 0191 2824445
Fax: 0191 2825892

Tissue pathway and timeline Day 1

Sections and trial specific pathology request form received by the medical secretaries at the Department of Cellular Pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne Hospitals Foundation Trust.

Medical secretary logs the case on the iLAB Pathology System (Trial specific study code=MRHPV. Initials=Forename. Trial number=Surname).

Case allocated to Dr Max Robinson (ROBCM).

The sections are despatched to the Immunohistochemistry Laboratory with a completed supplementary request form.

Booking form and pathology request form despatched to Dr Max Robinson.

Immunohistochemistry laboratory

Sections received with supplementary request form.

Slides stained for:

- Haematoxylin and eosin (H&E).
- p16 immunohistochemistry: p16 test with HPV control TMA on slide.
- High risk HPV in situ hybridisation (HR-HPV ISH): HR HPV ISH test with HPV control TMA on slide.
- Staining protocol completed the same day or using an overnight program.

Day2

Sections despatched to Dr Max Robinson for interpretation.

Sections passed to second pathologist (Dr A Chambers, Dr A Okpokam or Professor P Sloan) for interpretation.

Consensus interpretation and final report composed in iLAB Pathology System.

iLAB report FAXed to Participating Centre and Co-ordinating Centre.

The original pathology request form, stained slides and spare slides will be archived in the Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust.

The material will be archived and retained according to local standard operating procedures.

Methods

p16 immunohistochemistry

p16 immunohistochemistry will be carried out using a proprietary kit (CINtec histology, MTM laboratories AG, Germany) on a Ventana Benchmark Autostainer (Ventana Medical Systems Inc, USA). Test cases will be compared with a high risk HPV positive oropharyngeal SCC that shows high p16 expression (positive control) and a HPV negative oropharyngeal SCC (negative control). The p16 test will be interpreted independently by two pathologists. The p16 test will be scored as positive if there is strong and diffuse nuclear and cytoplasmic staining present in greater than 70% of the tumour specimen. All other staining patterns will be scored as negative. In cases

where the interpretation differs between the two pathologists, the slides will be re-examined and a consensus reached.

High risk HPV in situ hybridisation

High risk HPV in situ hybridisation will be carried out using a proprietary kit (Inform HPV III, Ventana Medical Systems Inc, USA) on a Ventana Benchmark Autostainer (Ventana Medical Systems Inc, USA). The Inform HPV III Family 16 Probe (B) detects a range of high risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66. Test cases will be compared with a high risk HPV positive oropharyngeal SCC (positive control) and a HPV negative oropharyngeal SCC (negative control). The HPV test will be interpreted independently by two pathologists. The HPV test will be scored as positive if there is evidence of a blue reaction product that co-localises with the nuclei of malignant cells. In cases where the interpretation differs between the two pathologists, the slides will be re-examined and a consensus reached.

Quality assurance and test interpretation

The diagnostic pathway described forms part of the routine diagnostic service in the Department of Cellular Pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne Hospitals Foundation Trust. The tests are CE-marked for diagnostic purposes, are subject to laboratory quality assurance and form part of a Clinical Pathology Accreditation (CPA (UK) Ltd) approved diagnostic service. The tests were introduced in our laboratories in 2009 (Thavaraj et al., 2011). Four pathologists (Dr M Robinson, Dr A Chambers, Dr A Okpokam and Professor P Sloan) deliver a head and neck pathology specialist service at Newcastle-upon-Tyne Hospitals Foundation Trust and all have accumulated experience of interpreting the tests. There is high inter-observer correlation between the pathologists (Intraclass correlation 0.964; 95% CI 0.949-0.975; $p < 0.001$). The involvement of three pathologists at one site means that delivering the diagnostic protocol is feasible. The quality objective is to provide the HPV status within 3 working days from receipt of the unstained slides. We will audit the accrual of trial material against this quality object and seek feedback from the participating centres.

Safety measures

Any concerns regarding laboratory testing carried out in Newcastle should be directed to Dr Max Robinson and will be investigated by Emma Doran (Quality Manager, Department of Cellular Pathology).

APPENDIX 2 – FUNCTIONAL OUTCOME PANEL GUIDELINES AND STANDARD OPERATING PROCEDURES

MD Anderson Dysphagia Inventory

Patients will be given the written MDADI questionnaire to complete independently, at their research visit. The assessor will be available to help should the patient require assistance, but will not direct any answers. The standard instructions for completion of the MDADI are as follows ‘This questionnaire asks for your views about your swallowing ability. This information will help us understand how you feel about swallowing. The following statements have been made by people who have problems with their swallowing. Some of the statements may apply to you. Please read each statement and circle the response which best reflects your experience in the past week’. Scoring of the MDADI will be conducted by the CTR. The trial database will calculate all MDADI scores (19-item composite/total, 1-item global, and subscales – physical, emotional, functional). SALTs should verify completion of MDADI questionnaires at the time of assessment. Patients should be probed for any missing items. MDADIs with missing items must be dropped from analysis. Patients who are nil by mouth should still complete all questionnaires. The complete MDADI questionnaire is reprinted in Appendix 3.

Water swallow test

The water swallow test (WST) can be conducted by speech and language therapists or a research nurse trained by a speech and language therapist, with reliability checks on ten volunteers. The person conducting the WST at each centre will remain consistent throughout the course of the study, as far as possible.

The test requires a stopwatch, syringe or measuring cup, plastic cup and tap water. The assessor measures 100 mls of tap water into a plastic cup using a syringe or measuring cup. The water temperature should be neither warm nor chilled. Instruct the patient to drink the water as quickly as possible, without making themselves uncomfortable. Ask them not to talk during the assessment. The assessor simultaneously places their fingers on the thyroid cartilage to measure the number of laryngeal elevations. Timing starts from the moment the water touches the patient’s bottom lip and stops on the last swallow, as the larynx returns to a final resting position (this is usually accompanied by other signals such as exhalation, phonation or opening of the mouth). Include any final clearance swallow. Make a note of any throat clearing or coughing during or after the assessment as these are clinical signs of aspiration or laryngeal penetration. Stopping for breath in between swallows is counted into the overall timing.

Record:

1. The number of swallows taken
2. The time taken to complete task (rounded up or down to the nearest seconds)
3. The presence/absence of throat clear or cough



The WST should not be attempted on individuals who have been recommended by their managing clinician to remain nil by mouth. However, the test may form part of a clinical swallowing examination to determine the patient's readiness for return to oral feeding, if deemed appropriate by the managing clinician. If there are overt signs of significant aspiration (explosive coughing, prolonged coughing) or the patient is becoming distressed, stop the assessment and measure the remaining amount in the cup and record. Measures of swallow capacity (mls/time) and swallow volume (mls/number of swallows) are derived from the data. Non-completion of the test will be recorded. Nil by mouth patients will automatically score a zero on both measures. Signs of aspiration/penetration (throat clear/ coughing / wet voice) are recorded as YES/NO.

Performance Status Scale for Head & Neck Cancer (PSS-HN)

The PSS-HN can be rated by health professionals including speech & language therapists and research nurses. The person collecting this data will remain consistent, as far as is feasible, throughout the course of the study. Scores are determined following an unstructured interview. Interviewer instructions for each subscale are outlined below:

Normalcy of Diet subscale

Begin by asking the patient what kinds of foods (s)he has been eating. Ask what foods are difficult to eat. Based on the patient's response, choose an item at the low end of the scale. Move up the scale giving examples of foods in each category and asking the patient if (s)he is eating those food items. Even if the patient says that (s)he eats everything, enquire about specific items beginning with 50, soft chewable foods and moving upwards. Stop at an item at, and above which the patient cannot eat. The patient then receives the score below that. If the patient indicates that (s)he is eating a full diet, also enquire whether (s)he needs to drink more liquids than usual with meals; eating a full diet with intake of extra fluids is scored 90. If the patient can take foods orally, but is also using a feeding tube, score based on solid food intake and check the box provided. Also use this guideline when rating patients who can eat some foods but cannot take oral liquid. Possible scores for the Normalcy of Diet subscale are:

- 100 Full diet with no restrictions
- 90 Full diet with liquid assistance
- 80 All meats
- 70 Carrots, celery (crunchy)
- 60 Dry bread & crackers
- 50 Soft, chewable foods (pasta, canned soft fruits, fish)
- 40 Soft foods requiring no chewing e.g. mashed potato, apple sauce
- 30 Puree
- 20 Warm liquids
- 10 Cold liquids
- 0 Non oral



Public Eating subscale

Score the Public Eating scale by asking the patient where (s)he eats (in a restaurant, at home, at friends/relatives' homes, etc.) and with whom (s)he eats (always alone, with family/friends, etc). Ask patient if (s)he chooses different foods (softer, less messy, etc.) when eating with others. When was the last time the patient ate in a restaurant, cafe, pub, picnic, family reunion? Choose the score beside the description that best fits the patient. A patient on a restricted diet, (e.g., tube feeding, pureed foods) who does not eat in public but will join others in a public eating setting should be rated 75. The Eating in Public Scale is not applicable for hospital inpatients as they generally have little opportunity to eat with others or leave their ward. Participants in PEARL are highly unlikely to be an in-patient at the specified data collection time points. However, sub- sections can be represented individually i.e. the absence of one sub-section does not affect the validity of the other sections.

Possible scores for the Eating in Public subscale are:

- 100 No restriction of place, food or companion (eats out at any opportunity)
- 75 No restriction of place, but restricts diet when in public (eats anywhere, but may limit intake to less "messy" foods (e.g., liquids)
- 50 Eats only in presence of selected persons in selected places
- 25 Eats only at home in presence of selected persons
- 0 Always eats alone

Understandability of Speech

This scale is scored based on the interviewer's ability to understand the patient during conversation (in this case, based on conversation about patient's diet and social activities). Choose the score beside the description that best fits the patient. See if you can understand the patient if you are looking away while (s)he is talking. Possible scores for the Understandability of Speech subscale are:

- 100 Always understandable
- 75 Understandable most of the time; occasional repetition necessary
- 50 Usually understandable; face-to-face
- 25 Never understandable; may use written
- 0 No communication

Current Feeding Tube Status

Feeding tube use will be assessed at each functional assessment interval.

Does patient have a feeding tube? (Circle one)

- 0 No tube
- 1 Yes – total dependency on tube, nil by mouth
- 2 Yes – tube dependency with minimal oral intake
- 3 = Yes tube feed supplements oral intake
- 4 Not currently being used

Percentage of overall nutritional requirement taken by feeding tube _____

Swallowing Support Summary

At the 12 month assessment interval, the SALT will document the following variables in reference to feeding tube placement and swallowing therapy.

Did the patient require a feeding tube during or after treatment? (Circle one)

- 0) No
- 1) Yes

Date insertion
Date removal

Type of feeding tube? (Circle one)

- 0) None
- 1) Nasogastric only
- 2) Nasogastric converted to gastrostomy
- 3) Gastrostomy only
- 4) Jejunostomy

Did the patient receive preventive swallowing exercise therapy?

- 0) No
- 1) Yes

If yes, did the patient report adherence to preventive swallowing exercise therapy?

- 0) No
- 1) Yes (specify, average # sets/day _____, duration adherence in weeks: _____)

Number of SALT sessions where the main purpose is swallowing rehabilitation (outside of protocol diagnostics): _____

European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire and University of Washington Quality of Life Questionnaire

Patients will be given the written EORTC QLQ-C30/H&N35 questionnaire (Appendix 4) and UW-QOL (Appendix 5) to complete independently, at their research visit. The assessor will be available to help should the patient require assistance, but will not direct any answers. The standard instructions on the questionnaire are as follows 'Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential'. Patients are asked to reflect on their symptoms, functioning and quality of life over the previous week.



APPENDIX 3 – MDADI QUESTIONNAIRE

The M.D. Anderson Dysphagia Inventory This questionnaire asks for your views about your swallowing ability. This information will help us understand how you feel about swallowing. The following statements have been made by people who have problems with their swallowing. Some of the statements may apply to you. Please read each statement and circle the response which best reflects your experience in the past week.

1. My swallowing ability limits my day-to-day activities.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

2. I am embarrassed by my eating habits.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

3. People have difficulty cooking for me.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

4. Swallowing is more difficult at the end of the day.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

5. I do not feel self-conscious when I eat.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

6. I am upset by my swallowing problem.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

7. Swallowing takes great effort.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

8. I do not go out because of my swallowing problem.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

9. My swallowing difficulty has caused me to lose income.

Strongly Agree Agree No Opinion Disagree Strongly Disagree



10. It takes me longer to eat because of my swallowing problem.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

11. People ask me, "Why can't you eat that?"

Strongly Agree Agree No Opinion Disagree Strongly Disagree

12. Other people are irritated by my eating problem.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

13. I cough when I try to drink liquids.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

14. My swallowing problems limit my social and personal life.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

15. I feel free to go out to eat with my friends, neighbours, and relatives.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

16. I limit my food intake because of my swallowing difficulty.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

17. I cannot maintain my weight because of my swallowing problem.

Strongly Agree Agree No Opinion Disagree S Strongly Disagree

18. I have low self-esteem because of my swallowing problem.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

19. I feel that I am swallowing a huge amount of food.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

20. I feel excluded because of my eating habits.

Strongly Agree Agree No Opinion Disagree Strongly Disagree

APPENDIX 4 – EORTC QUESTIONNAIRE H&N35 AND EORTC QLQ-C30

ENGLISH



EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

--	--	--	--	--	--	--	--	--	--

Your birthdate (Day, Month, Year):

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Today's date (Day, Month, Year):

31																			
----	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a <u>long</u> walk?	1	2	3	4
3. Do you have any trouble taking a <u>short</u> walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page



ENGLISH

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your <u>social</u> activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor

Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor

Excellent



EORTC QLQ - H&N35

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week:	Not at all	A little	Quite a bit	Very much
31. Have you had pain in your mouth?	1	2	3	4
32. Have you had pain in your jaw?	1	2	3	4
33. Have you had soreness in your mouth?	1	2	3	4
34. Have you had a painful throat?	1	2	3	4
35. Have you had problems swallowing liquids?	1	2	3	4
36. Have you had problems swallowing pureed food?	1	2	3	4
37. Have you had problems swallowing solid food?	1	2	3	4
38. Have you choked when swallowing?	1	2	3	4
39. Have you had problems with your teeth?	1	2	3	4
40. Have you had problems opening your mouth wide?	1	2	3	4
41. Have you had a dry mouth?	1	2	3	4
42. Have you had sticky saliva?	1	2	3	4
43. Have you had problems with your sense of smell?	1	2	3	4
44. Have you had problems with your sense of taste?	1	2	3	4
45. Have you coughed?	1	2	3	4
46. Have you been hoarse?	1	2	3	4
47. Have you felt ill?	1	2	3	4
48. Has your appearance bothered you?	1	2	3	4

Please go on to the next page

During the past week:

	Not at all	A little	Quite a bit	Very much
49. Have you had trouble eating?	1	2	3	4
50. Have you had trouble eating in front of your family?	1	2	3	4
51. Have you had trouble eating in front of other people?	1	2	3	4
52. Have you had trouble enjoying your meals?	1	2	3	4
53. Have you had trouble talking to other people?	1	2	3	4
54. Have you had trouble talking on the telephone?	1	2	3	4
55. Have you had trouble having social contact with your family?	1	2	3	4
56. Have you had trouble having social contact with friends?	1	2	3	4
57. Have you had trouble going out in public?	1	2	3	4
58. Have you had trouble having physical contact with family or friends?	1	2	3	4
59. Have you felt less interest in sex?	1	2	3	4
60. Have you felt less sexual enjoyment?	1	2	3	4

During the past week:

	No	Yes
61. Have you used pain-killers?	1	2
62. Have you taken any nutritional supplements (excluding vitamins)?	1	2
63. Have you used a feeding tube?	1	2
64. Have you lost weight?	1	2
65. Have you gained weight?	1	2

APPENDIX 5 – UNIVERSITY OF WASHINGTON QUALITY OF LIFE QUESTIONNAIRE (UW - QOL)

This questionnaire asks about your health and quality of life over the past seven days. Please answer all of the questions by checking one box for each question.

1. **Pain.** (Check one box:)

I have no pain.

There is mild pain not needing medication.

I have moderate pain - requires regular medication (codeine or nonnarcotic). I have severe pain controlled only by narcotics.

I have severe pain, not controlled by medication.

2. **Appearance.** (Check one box:)

There is no change in my appearance.

The change in my appearance is minor.

My appearance bothers me but I remain active.

I feel significantly disfigured and limit my activities due to my appearance. I cannot be with people due to my appearance.

3. **Activity.** (Check one box:)

I am as active as I have ever been.

There are times when I can't keep up my old pace, but not often.

I am often tired and have slowed down my activities although I still get out. I don't go out because I don't have the strength.

I am usually in bed or chair and don't leave home.

4. **Recreation.** (Check one box:)

There are no limitations to recreation at home or away from home.

There are a few things I can't do but I still get out and enjoy life.

There are many times when I wish I could get out more, but I'm not up to it.

There are severe limitations to what I can do, mostly I stay at home and watch TV. I can't do anything enjoyable.

5. **Swallowing.** (Check one box:)

I can swallow as well as ever.

I cannot swallow certain solid foods.

I can only swallow liquid food.

I cannot swallow because it "goes down the wrong way" and chokes me.

6. **Chewing.** (Check one box:)

I can chew as well as ever.
I can eat soft solids but cannot chew some foods.
I cannot even chew soft solids.

7. **Speech.** (Check one box:)

My speech is the same as always.
I have difficulty saying some words but I can be understood over the phone. Only my family and friends can understand me.
I cannot be understood.

8. **Shoulder.** (Check one box:)

I have no problem with my shoulder.
My shoulder is stiff but it has not affected my activity or strength.
Pain or weakness in my shoulder has caused me to change my work. I cannot work due to problems with my shoulder.

9. **Taste.** (Check one box:)

I can taste food normally.
I can taste most foods normally.
I can taste some foods.
I cannot taste any foods.

10. **Saliva.** (Check one box:)

My saliva is of normal consistency.
I have less saliva than normal, but it is enough. I have too little saliva.
I have no saliva.

11. **Mood.** (Check one box:)

My mood is excellent and unaffected by my cancer.
My mood is generally good and only occasionally affected by my cancer. I am neither in a good mood nor depressed about my cancer.
I am somewhat depressed about my cancer.
I am extremely depressed about my cancer.

12. **Anxiety.** (Check one box:)

- I am not anxious about my cancer.
- I am a little anxious about my cancer.
- I am anxious about my cancer.
- I am very anxious about my cancer.

Which issues have been the most important to you during the past 7 days? Check up to 3 boxes.

- | | | |
|------------|------------|---------|
| Pain | Swallowing | Taste |
| Appearance | Chewing | Saliva |
| Activity | Speech | Mood |
| Recreation | Shoulder | Anxiety |

GENERAL QUESTIONS

Compared to the month before you developed cancer, how would you rate your health-related quality of life? (check one box:)

- Much better
- Somewhat better
- About the same
- Somewhat worse
- Much worse

In general, would you say your **health-related quality of life** during the past 7 days has been: (check one box:)

- Outstanding
- Very good
- Good
- Fair
- Poor
- Very poor

Overall quality of life includes not only physical and mental health, but also many other factors, such as family, friends, spirituality, or personal leisure activities that are important to your enjoyment of life. Considering everything in your life that contributes to your personal well-being, rate your **overall quality of life** during the past 7 days. (check one box:)



Outstanding
Very good
Good
Fair
Poor
Very poor

Please describe any other issues (medical or nonmedical) that are important to your quality of life and have not been adequately addressed by our questions (you may attach additional sheets if needed).

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APPENDIX 6 – WHO PERFORMANCE SCORE CRITERIA

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



APPENDIX 7 - PET-CT SCAN ACQUISITION PROTOCOL

Patients will have three scans during the trial performed:

- The 1st scan (pre_PET) is a baseline diagnostic scan. The patient is in a thermoplastic mask and PET CT will be used as an adjunct to help us delineate the tumour.
- The 2nd scan (iPET) takes place following 2 weeks (10 fractions) of chemo-radiotherapy.
- The 3rd scan (post_PET) takes place 10 to 16 weeks following the last dose of radiotherapy.

Scans 1 & 2

As per routine FDG Oncological scan (**PT 3150**) but with the following differences. Check if the most recent Creatinine/GFR results have been received . Change patient into a hospital gown and insert Pink Venflon for radiopharmaceutical and IV contrast administration. 60 or 90 minute uptake period (± 5 minutes) using a silence protocol, with blankets around neck and shoulders to reduce brown fat uptake. Subsequent scans should be performed within ± 5 minutes of the baseline exam uptake time. 4MBq/kg, with a maximum dose of 600MBq and a minimum dose of 300MBq. Flat bed, Q-fix board & mask must be used for both scans for reproducibility. Radiotherapy planners will accompany patient to set-up equipment and patient. Start positioning patient at 45 minutes of uptake.

The baseline diagnostic scan range is between Vertex and mid thighs. The interim scan 2 weeks following radiotherapy range is between vertex to liver. Both scans performed in radiotherapy planning position. Following the PET/CT scan a high resolution contrast CT for RTP will be acquired from Vertex to Carina. 100mls of Omnipaque 350 at 3ml/s with 50second delay – no breathing or swallowing during CT acquisition. Check for contrast reaction before patient leaves the department. On RADIS, in procedure details, enter PEARL (as opposed to LUNG, COLORECTAL etc)

Scan 3

As per routine FDG Oncological scan (**PT 3150**) with 60 or 90 minute uptake (and ± 5 minutes of the 2 previous scans) and use blankets to keep patient warm especially neck and shoulders. Not necessary to use RTP table.

Scan Protocol 6.1 - Scan from Vertex to mid-thighs.

NO high resolution contrast CT

On RADIS, in procedure details, enter PEARL (as opposed to LUNG, COLORECTAL etc).



Data Transfer

All files except RAW should be archived as standard and send to MIMS. Once archived use **EDIT PATIENT** to change the local hospital number to the Velindre hospital number and transfer all data except the RAW PET via **NETWORK** to PROSOMA in Velindre. Once this transfer is successful **ANONYMISE** the examination and send all data to the HERMES server via **NETWORK** and select the Hermes server.

APPENDIX 8 - DEFINITION OF bGTV ON VELOCITY

1. Select CT as primary data set (NECKSTD)
2. Select PET as secondary data set (PETNECKFX)
3. Select pre-set SUV/BW (body weight) levels for 0 – 10
4. Check registration
5. Deselect CT (primary)
6. Convert greyscale to inverse grey
7. Name file
8. Select secondary in 'threshold'
9. Adapt region of interest box around the target so it is tight fitting in all three dimensions
10. Change the threshold through visual assessment to better cover the target. This does not need to be consistent between scans of the same patient.
11. Assess coverage
12. Save

APPENDIX 9 – THE ATLAAS ALGORITHM

An **Automatic Decision Tree-based Learning Algorithm for Advanced Image Segmentation (ATLAAS)** is a segmentation methodology designed to select the most accurate positron emission tomography automated segmentation (PET-AS) method for a provided Positron Emission Tomography (PET) image [1]. The chosen PET-AS method is selected from a list of nine advanced PET-AS algorithms built into the system. In the literature, it has been shown that PET-AS methods perform differently based upon different tumour characteristics [2], [3]. When ATLAAS is provided an image, it computes the tumour characteristics that influence segmentation accuracy. The following tumour characteristics were identified as classifiers for the performance of PET-AS accuracy:

- Metabolic Tumour Volume (MTV) in mL.
- Tumour to background ratio peak (TBR_{peak}): ratio between the target objects SUV_{peak} , calculated as the mean value in a 1 cm^3 sphere centred on the maximum standardised uptake value (SUV) in the target object, and the background SUV, calculated as the mean intensity in a 0.5cm extension of the target object contour [4]. For multiple maximum SUV in the target object, the maximum SUV_{peak} is chosen.
- The number of unique discrete intensities (NI): a region texture feature related to the intensity distribution in the target object. The NI in the target object is obtained from a grey level co-occurrence matrix. NI is the number of different discrete intensity values in the target object, after resampling the object image to 64 discrete level as defined by Haralick et al [5].

For each PET-AS method the predicted accuracy for the MTV delineation is calculated for the computed tumour characteristics. Accuracy is defined by the Dice Similarity Coefficient (DSC) [6], where a value of 1 means a perfect delineation and 0 means a disjointed volume has been delineated. The DSC is calculated as twice the intersection of two volumes divided by the sum of the two volumes, as shown in (1) where X and Y are the number of elements in the samples.

$$DSC = \frac{2|X \cap Y|}{|X| + |Y|} \quad (1)$$

The prediction of the best performing PET-AS method is completed using decision trees (DT) that define a predictive model. The DT's are developed during the training process of ATLAAS and are generated from a training dataset consisting 200 PET scans, all with known ground truths (GT). Figure 1 shows the workflow for the training and clinical application of ATLAAS. The parameters of the dataset vary within a pre-defined range calculated from MTV delineations observed within the clinical environment. The mean MTV in the training dataset is 16.05 mL [0.02 – 51.71 mL], mean NI is 55.35

[1 – 65] and mean tumour to background ratio (TBR) is 2.56 [0.49 – 2.78]. The distribution of the MTV, TBR and NI is shown in Figures 2 - 4.

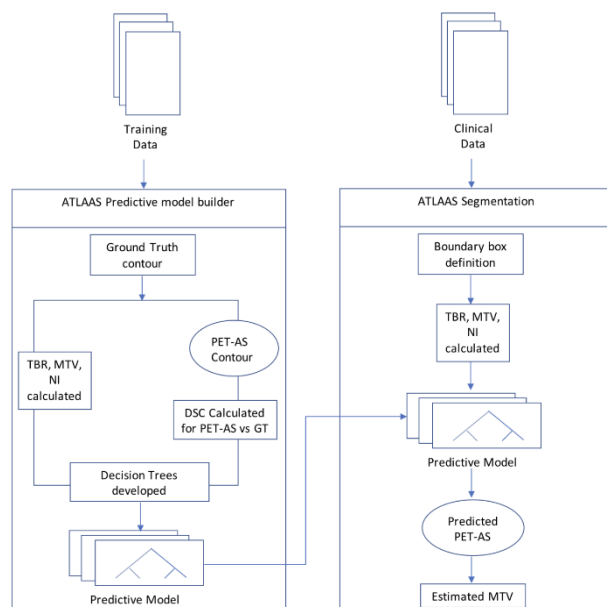


Figure 1: The workflow for the training and application of the ATLAAS segmentation methodology on clinical imaging.

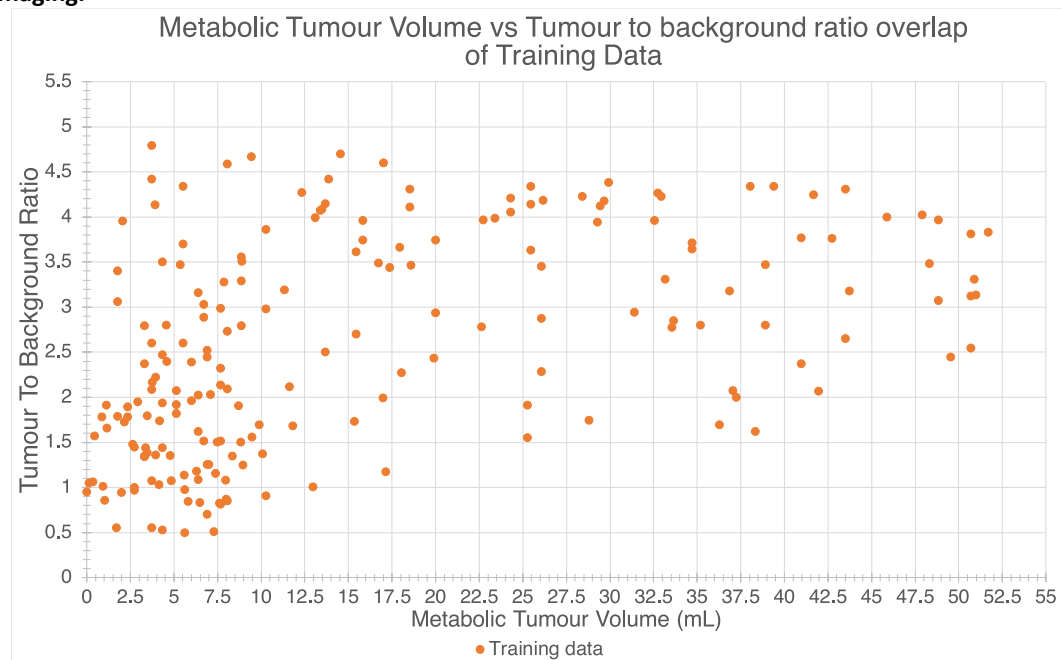


Figure 2: The distribution of the metabolic tumour volume of the training data mapped to the tumour to background ratio of the training data.

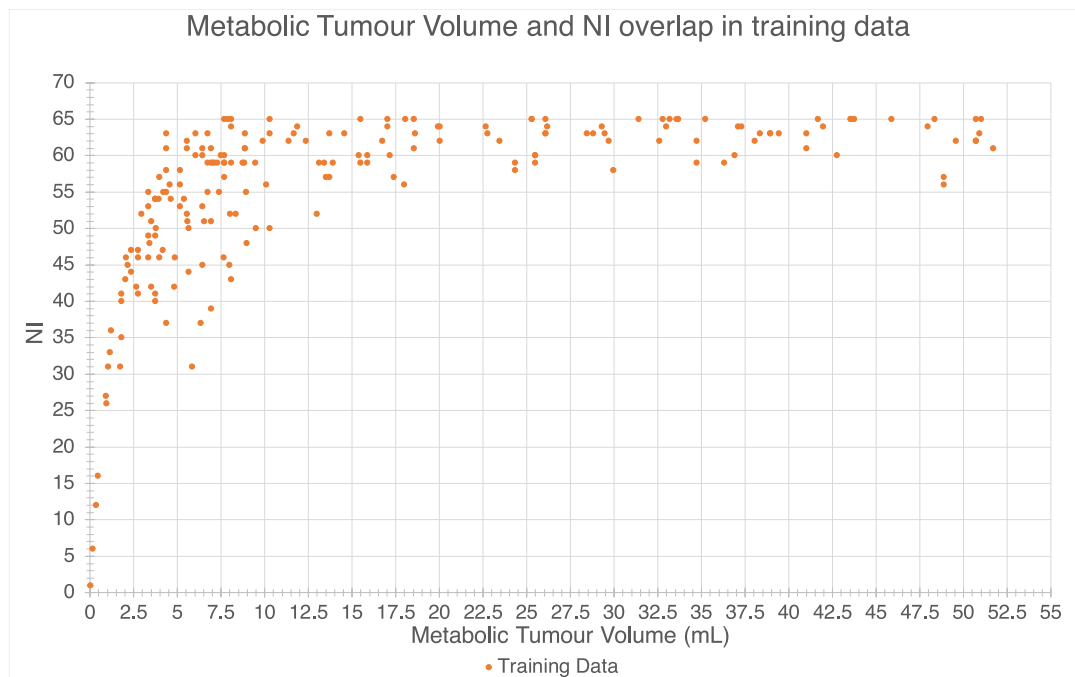


Figure 3: The distribution of the metabolic tumour volume of the training data mapped to the number of discrete intensities of the training data.

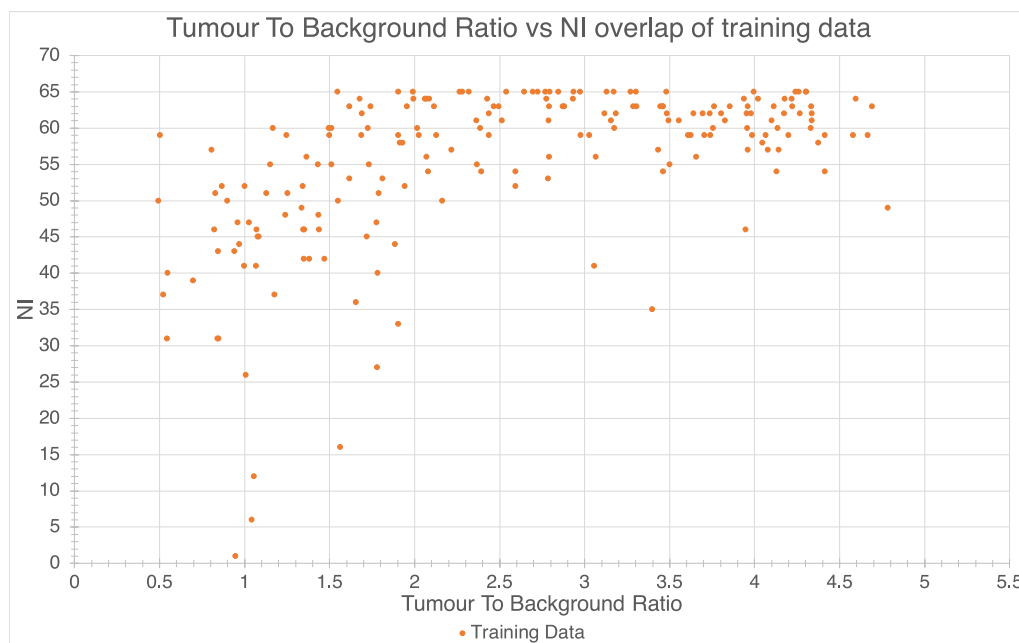


Figure 4: The distribution of the tumour to background ratio of the training data mapped to the number of discrete intensities of the training data.

Segmentation methods adopting clustering techniques such as Fuzzy C-means (FCM), Gaussian Fuzzy C-means (GCM) and K-means (KM) using 2,3 and 4 clusters (FCM2, GCM3 - 4, KM2 - KM4), as well as region growing (RG) and watershed transform (WT) methods, are promising segmentation methods in the delineation of the MTV. These segmentation methods are reviewed in detail in the report by Hatt et al [7] and are summarised in Table 1. These segmentation methods have been included in the ATLAAS methodology.

Table 1: Name and description of PET-AS methods used in this study, with references of published work using similar segmentation approaches

Algorithm	Description	Key References
AT	3D Adaptive iterative thresholding, using background subtraction	Jentzen et al [8], Drever et al [9]
RG	3D Region-growing with automatic seed finder and stopping criterion	Day et al [10]
KM	3D K-mean iterative clustering with custom stopping criterion	Zaidi and El Naqa [11]
FCM	3D Fuzzy C-mean iterative clustering with custom stopping criterion	Belhassen and Zaidi [12]
GCM	3D Gaussian Mixture Models based clustering with custom stopping criterion	Hatt et al [13]

WT Watershed Transform-based
algorithm, using sobel filter

Geets et al [14], Tylski et al [15]

PET-AS methods have been shown to perform differently in different conditions [7]. A range of PET-AS methods have been included in the ATLAAS segmentation methodology to represent a range of different segmentation approaches. The segmentation approaches include binary classification approaches, adaptive thresholding (AT), region growing (RG), and watershed thresholding (WT). Further, clustering-based methods, Klustering-means (KM), Fuzzy-clustering means (FCM), and General-clustering means (GCM) with 2, 3 and 4 clusters (KM2-4, GCM2-4 and FCM2-4) respectively are also included in the predictive model.

AT applies an initial fixed threshold; the generated contour is updated iteratively by applying successive thresholds that have calculated from iterative estimations of the background mean uptake as follows:

$$T = RT * (SUV_{max} - B_{mean}) + B_{mean} \quad (2)$$

T is the final absolute threshold to apply to the image, RT the relative threshold, SUV_{max} the maximum SUV value inside the lesion and B_{mean} the mean background intensity. The implementation of the AT algorithm is initialised with a mean background value calculated on voxels with an intensity lower than 50% of the maximum intensity and a value of 40% of the maximum intensity for the RT. Equation (2) is applied iteratively until the difference between delineations changes by one voxel or less.

WT find the crests within an image by simulating a water level rising from the local minima in the image. The water level rises iteratively until two clusters remain separating the background and tumour. Region growing selects the SUV_{max} as a seed and grows a region from that voxel dependent upon the intensity of the voxel adjacent to it. The criterion for inclusion as tumour is based upon the mean tumour uptake and the standard deviation from the μ . The inclusion criterion is continuously updated on each iteration. The region stops growing once there is no change between one iteration and the next. KM assigns each voxel to a cluster with mean intensity value closest to its own value. In Fuzzy C-means clustering each voxel is assigned to a cluster with a membership value. The membership value quantifies how close the included voxel intensity is to the clusters mean value. General clustering means assumes each cluster has a Gaussian intensity distribution. The mean and standard deviation of the distribution is calculated at each iteration. The membership of the voxel to the cluster is determined by the probability of the Gaussian intensity distribution generating the voxel intensity value. Further, clustering methods test first order statistics to produce homogenous volumes within the cluster.

For each of the PET-AS methods included in the system the performance of segmentation is mapped to the tumour characteristics of the tumour. This results in the development of multiple DT's within the predictive model. For application of the predictive model to clinical imaging, estimated values for the tumour characteristics from which the DT's are built are computed. This requires the estimation



of the MTV. The PET-AS method used for the estimation of the MTV is AT. The estimated MTV (mL), TBR and NI are then computed from the estimated MTV volume. These estimated characteristics are used as the input parameters in the predictive model. The output of the predictive model for the input parameter values is the most appropriate segmentation methodology for the given characteristic values. The segmentation methodology outputted from the predictive model is used to delineated the MTV.

APPENDIX 10 – OUTLINING WITH ATLAAS

To outline PET imaging using ATLAAS, patient images are required to be exported from Velocity and imported into CERR.

1. Export patient scan from Velocity.
 - a. Find the patient to export in Velocity using search tools
 - b. Right click, export patient and save to folder with patient name
2. Open Matlab
3. Load CERR
4. Import patient DICOM files (Figure 1).
 - a. Find the patient folder you exported from Velocity as above and click open

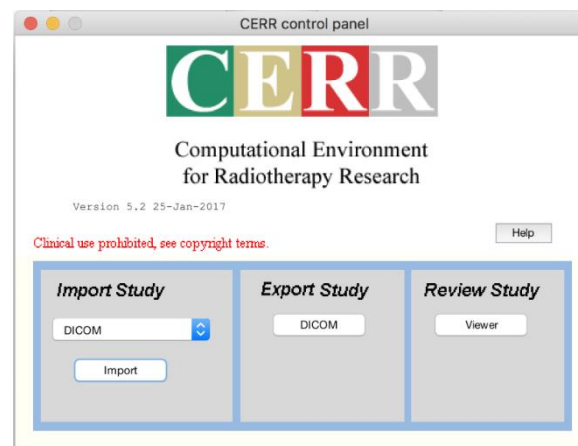


Figure 1: The splash screen for CERR, click import to start loading patient DICOM files.

b. Once CERR has finished scanning, click import All (Figure 2)

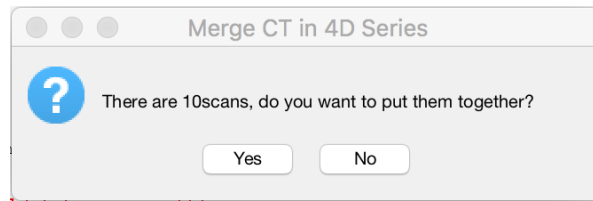


Figure 2: You are able to merge scans into a 4D series, click no.

c. Do NOT merge scans into 4D series (Figure 3)

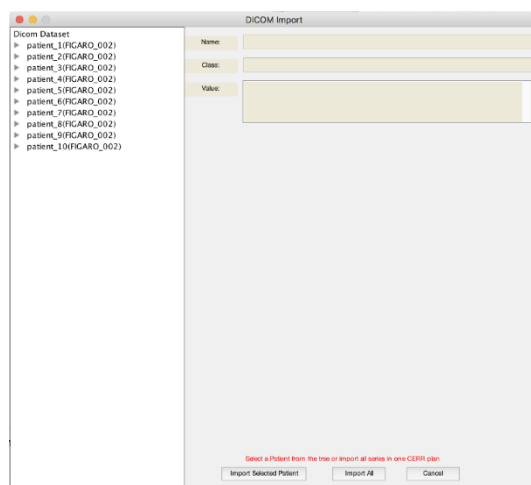


Figure 3: Imaging within the folder exported from Velocity, click import all.

d. Save CERR file with patient ID to suitable folder

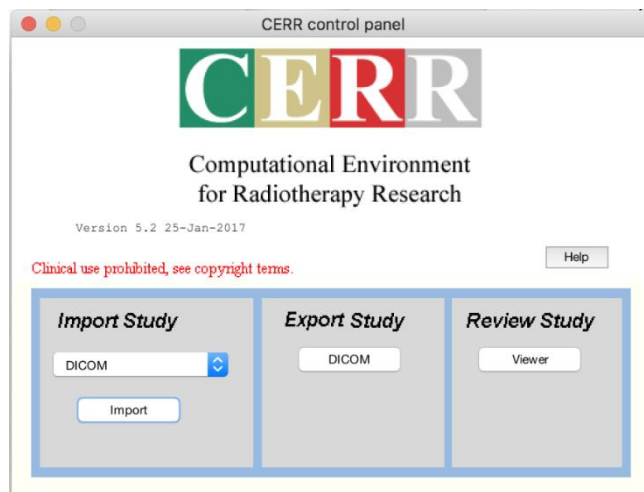


Figure 4: Load CERR, click viewer.

5. Open CERR, click viewer
6. Import patient CERR file you saved earlier.
 - a. Click File, Open
 - b. Find the CERR file you saved with the patient ID as above
7. Open the PET scan to outline the primary tumour on, by clicking scan and then clicking the appropriate scan.
8. Click PET-STAT -> Start Segmentation (Figure 5).

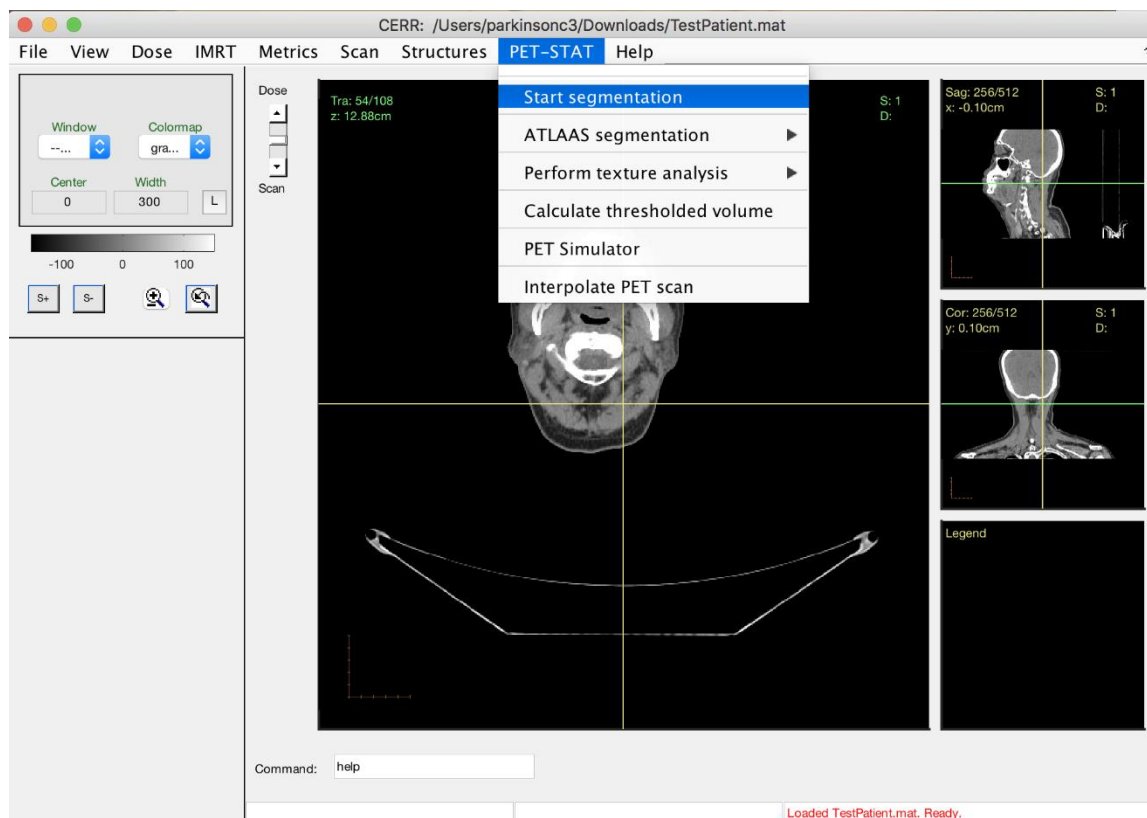


Figure 5: Open the Segmentation screen by clicking start segmentation in the PET-STAT menu.

9. Change the heat map of the PET scans by left clicking once the appropriate colour scheme on the right-hand side.

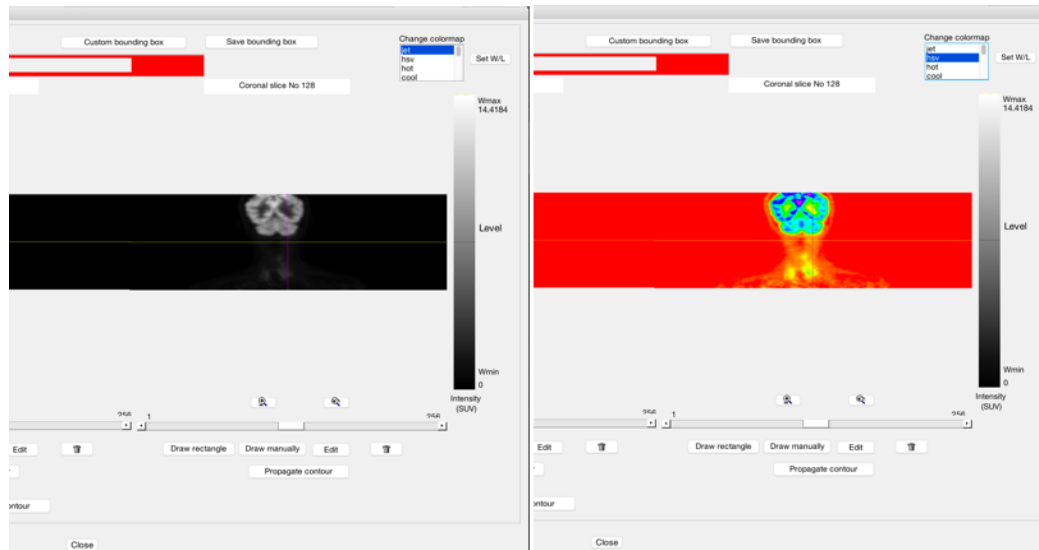


Figure 6: Change the heat map to visualise the primary tumour.

10. Automated delineation of the primary tumour volume requires definition of a boundary box

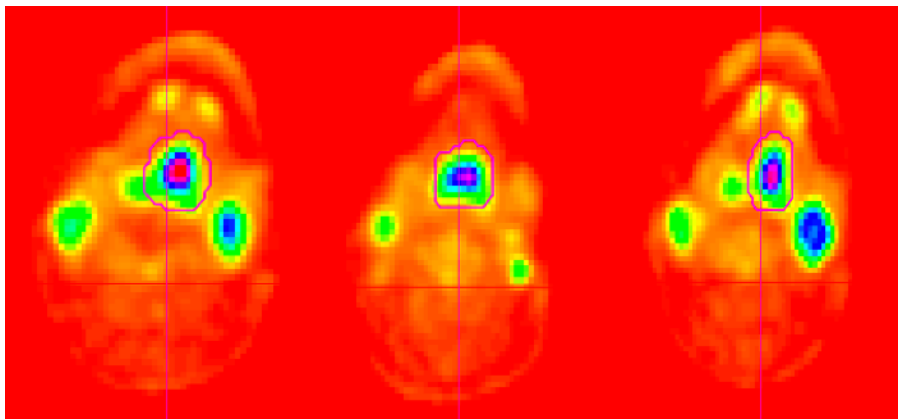


Figure 7: Boundary box definition to improve accuracy of automated segmentation.

(Figure 7) to provide optimal delineation results.

- a. Move through the transverse slices to find the extremity slices of the primary tumour volume.

- i. Once extremity slice is chosen, left click Select Seed point and then left click on the extremity of the primary tumour, it doesn't need to be exact. Further, it is necessary to press the enter key after placing each seed point on the PET image.
- ii. Repeat for the lower, upper and middle slices a maximum of 6 seed points per tumour is required, 2 seed points minimum. (Figure 8).

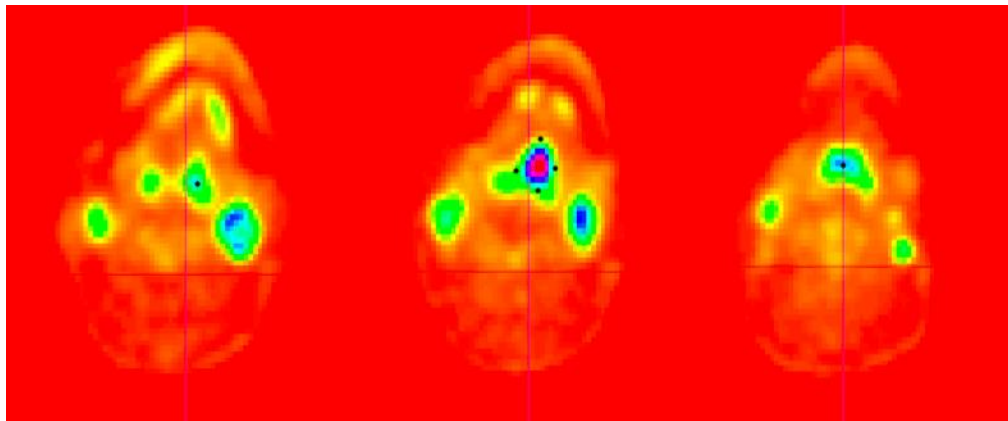


Figure 8: Seed point selection for definition of boundary box.

- iii. Select Auto generate mask (Figure 9) to generate the boundary box (Figure 7).

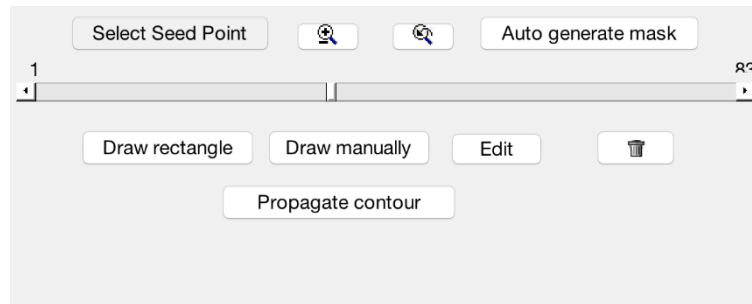


Figure 9: Auto generate mask button generates a boundary box based upon the seed point defined earlier.

11. Save the boundary box with the nomenclature patientID_BB (Figure 10).

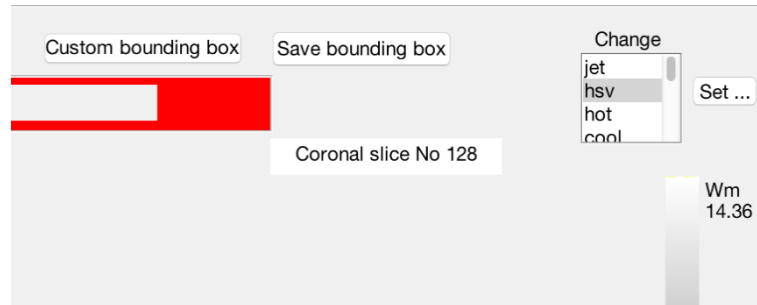


Figure 10: Save bounding box button allows you to save the boundary box, making the delineation repeatable.

12. To delineate the primary GTV once the boundary box has been delineated, click the chosen PET-AS method (Figure 11). In this case we are interested in **ATLAASv1**. Click the Generate contour button.

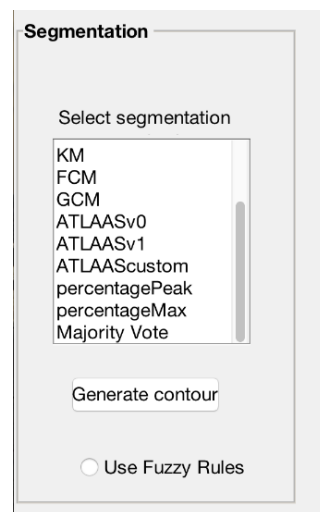


Figure 11: Save bounding box button allows you to save the boundary box, making the delineation repeatable.

13. To save the contoured primary GTV, the contour needs to be selected and the name to save the contour is required, the suggested nomenclature is GTV_ATLAAS for ATLAAS.

Results

Contour No 1 : ATLAASv1.

Enter structure name

Figure 12: Saving the delineated GTV contour requires a specified nomenclature

APPENDIX 11 – PEARL PET-CT Scan Reporting

PEARL study patients will have 3 scans. Please also see Appendix 7 (scan acquisition protocols).
Patients with HPV+ve (p16 IHC) Oropharyngeal SCC, T1-3 N \leq 1 M0 (N1 = single ipsilateral node \leq 3cm)

pre_PET	Baseline (RTP with mask and table, IV contrast)
iPET	Interim during chemoradiotherapy, and may be used to adjust field. (scan after 2weeks / 10fractions, RTP with mask and table, reduced cover vertex to liver, IV contrast)
post_PET	Follow-up Response assessment, 10 to 16 weeks after End of Treatment

Baseline

Please review and report as standard oncology staging study

Please include tumour SUVmax

Nodal or distant lesions that increase stage

Incidental findings

Interim

Please include residual SUVmax,

and overall score according to Hopkins criteria(REF), *details below*.

Please report any new sites of disease or incidental findings.

Follow-up

Please review and report as normal for ENT end-of-treatment response assessment.

Please include residual SUVmax,

and overall score according to Hopkins criteria.

Please report any new sites of disease or incidental findings.

Table 1 from Marcus et al. JNM 2014; 55:1411

TABLE 1
Five-Point Qualitative Posttherapy Assessment Scoring
System (Hopkins Criteria) for Head and Neck PET/CT

Score	¹⁸ F-FDG uptake pattern	Response category
1	¹⁸ F-FDG uptake at the primary site and nodes less than IJV.	Complete metabolic response
2	Focal ¹⁸ F-FDG uptake at the primary site and nodes greater than IJV but less than liver.	Likely complete metabolic response
3	Diffuse ¹⁸ F-FDG uptake at the primary site or nodes is greater than IJV or liver.	Likely postradiation inflammation
4	Focal ¹⁸ F-FDG uptake at the primary site or nodes greater than liver.	Likely residual tumor
5	Focal and intense ¹⁸ F-FDG uptake at the primary site or nodes.	Residual tumor

Scores 1, 2, and 3, which represent complete metabolic response, likely complete metabolic response, and likely post-radiation inflammation, respectively, were considered negative for tumor. Scores 4 and 5, which represent likely residual tumor and residual tumor, respectively, were considered positive for tumor. New lesion would be considered as progressive disease.