INOVATE

Investigation of novel plasma Human Papilloma Virus DNA assay for treatment response estimation in head and neck cancer.

PROTOCOL

Version: 2.0
Date: 27 September 2021

Chief Investigator:	Dr Shreerang Bhide
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The INOVATE study is part of the National Institute for Health Research Clinical Research Network Trial Portfolio

ICR The Institute of Cancer Research

MRC Medical Research Council

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Study Coordination

ICR-CTSU (a UKCRC registered NCRI cancer clinical trials unit) is responsible for the central coordination of the study on behalf of the Sponsor.

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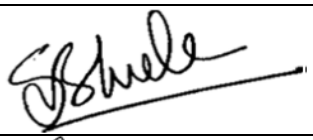
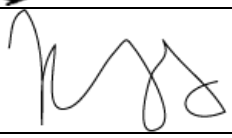
This protocol describes the INOVATE study and provides information about procedures for entering patients and for the collection of tissue, blood samples and data. No treatments are prescribed by this study protocol. Any questions relating to this protocol should be addressed in the first instance to the INOVATE Project Manager within ICR-CTSU.

Protocol Authorised by:

Name & Role	Signature	Date
Dr Shreerang Bhide (Chief Investigator)		27 September 2021
Dr Maggie Cheang (ICR-CTSU Scientific/Methodology Lead)		27 September 2021

Every care was taken in the preparation of this protocol, but corrections or amendments may be necessary. Protocol amendments will be circulated to participating sites as they occur, but sites entering patients for the first time are advised to contact ICR-CTSU to confirm they have the most recent version.

HISTORY OF CHANGES

PROTOCOL VERSION AND DATE	SUMMARY OF CHANGES
Version 1.0 23 October 2019	Original approved version
Version 2.0 27 September 2021	• Updated to allow flexibility around patient withdrawals due to missing baseline samples. • Change to the schedule for samples collected up to 4 weeks after RT/CRT treatment bringing this in line with site 'standard of care' clinic visits. • Clarification provided around withdrawals for patients who have not completed RT treatment. • Update to contact details and further minor clarifications throughout. • Update to include exploratory objectives around T-cell clonality and diversity, and associated details on RNA extraction and analysis.

CONTENTS

1. INOVATE STUDY SUMMARY

Protocol Title	**Investigation of novel plasma Human Papilloma Virus DNA assay for treatment response estimation in head and neck cancer.**
Study Population	Patients over 18 years with newly diagnosed locally advanced squamous cell cancers of the oropharynx requiring definitive treatment with radical radiotherapy or chemo-radiotherapy (RT/CRT).
Target Disease	Confirmed T1-T2/N1-3 or T3-T4 N0-3 (AJCC TNM classification v7.0) HPV positive (HPV+) and HPV negative (HPV-) oropharyngeal cancer.
Study Objectives	**Primary objective:** validation of HPV DNA, measured using HPV-detect as a predictor of the absence of residual disease following primary RT/CRT for HPV+ positive oropharyngeal cancer. **Secondary objective:** to develop clinical pathway maps and study the cost-effectiveness of the test for future implementation into clinical practice.
Study Design	Muticentre prospective biological sample collection to validate HPV DNA as a marker of residual disease in HPV positive oropharngeal cancer.
Recruitment Target	**191 patients:** comprising 143 HPV+ oropharyngea patients and up to 48 HPV- oropharyngeal patients recruited as negative controls to demonstrate that HPV+ ctDNA is not seen sporadically in this patient group.
Primary Endpoint	The specificity of HPV-detect measuring plasma HPV DNA levels at 3 months following completion of primary RT/CRT to identify absence of disease compared with ^{18}F-FDG PET-CT at the same timepoint (gold standard).
Secondary Endpoints	• The sensitivity of HPV-detect measuring plasma HPV DNA levels at 3 months following completion of primary RT/CRT to identify presence of disease compared with ^{18}F FDG PET-CT at the same time point (gold standard). • The proportion of patients who have no residual disease on biopsy/neck dissection amongst those with residual disease according to ^{18}F FDG PET-CT but HPV-detect negative at 3 months after primary RT/CRT. • Association between plasma HPV DNA levels at different timepoints and clinical and radiological response up to a period of 12 months post-RT/CRT. • Sensitivity and specificity of HPV-detect in measuring HPV DNA levels compared with HPV DNA levels from diagnostic tissue at baseline. • Kinetics of change in plasma HPV DNA levels during and immediately following RT/CRT.
Tissue Collection	**All patients:** archival diagnostic tumour blocks (alternatively 5 paraffin slides or tumour DNA, if extracted for another study, may be provided for patients if the blocks are not available). **HPV+ patients only:** additional tissue blocks will be collected after any surgical procedure and/or biopsies which are taken post chemo-radiotherapy.
Blood Sample Collection	**HPV+ patients**: blood samples will be collected pre-RT/CRT, weekly during RT/CRT, at each standard clinic visit during the 4 weeks thereafter*, and at 6 weeks, 3, 6, 9 and 12 months post-RT/CRT. Additional blood samples will also be obtained for HPV+ patients before and after any surgical procedures or biopsies performed post-RT/CRT. *blood samples should be collected during this time in line with the 'standard of care' clinic visits at each site. **HPV- patients:** blood samples will be collected pre-RT/CRT, at 6 weeks, and at 3,6,9,12 months post-RT/CRT only. All blood samples for all patients will be collected in Streck™ tubes.

Follow up	Clinical data will be collected from routine clinical follow-up. Information on the status of disease recurrence will be collected at 3, 6, 9 and 12 months post-RT/CRT.

2. BACKGROUND AND RATIONALE

In the last decade, Human Papilloma Virus (HPV) related head and neck cancer has been proven to be a distinct disease entity in terms of epidemiology, tumour biology and response to treatment. The UK incidence of locally advanced human papilloma virus related oropharyngeal cancer (HPV+ oropharyngeal cancer) is 2000/year (Office of National Statistics in England, NHS National Services Scotland, Northern Ireland Cancer Registry, and Welsh Cancer Intelligence & Surveillance Unit). At least 50% of patients present with stage III/IV disease (AJCC 7) – so-called locally-advanced head and neck cancer (LAHNC)[1]. Organ-preserving radiotherapy (RT), or concomitant- cisplatin-based chemoradiotherapy (CRT), followed by surgical salvage if required, is a standard-of-care for LAHNC of the oropharynx[2] and, compared to conventional surgery, achieves equivalent or improved locoregional control and disease-free survival rates with favourable functional outcomes[3, 4]. Following RT/CRT, decisions regarding salvage surgery for residual primary and cervical lymph node disease are based on clinical examination and imaging. ^{18}F-FDG PET-CT, when available, is the imaging modality of choice[5], with a negative predictive value in excess of 90%[5, 6]. However, the positive predictive value is sub-optimal and 20-30%[7] of patients frequently undergo unnecessary (no viable tumour on post-operative pathology) neck dissection (ND)[8] and/or repeated biopsies from the purportedly residual primary tumour. ND causes significant morbidity (fibrosis, facial/hypoglossal nerve damage, reduced shoulder movement, swallowing problems)[9-12] and repeated biopsies to rule out residual cancer at the primary site can lead to delayed mucosal healing and significant patient anxiety. Furthermore, HPV+ patients are younger and due to the excellent treatment outcomes with the current treatments are expected to carry the burden of treatment related toxicity for life. Over the last few decades, the incidence of HPV+ oropharyngeal cancer has significantly increased and is projected to increase by up to 230% in the near future[13, 14]. Therefore, optimised predictors of residual disease are required as a means of guiding management decisions.

HPV DNA is released into the bloodstream and can potentially be used as a detection marker of HPV+ oropharyngeal cancer. In a recently completed single-centre prospective pilot study a novel next generation sequencing assay (HPV-detect) was developed for the detection of circulating HPV DNA (cHPV-DNA)[15].

A pilot study performed at the Royal Marsden Hospital/Institute of Cancer Research prospectively collected serial plasma samples in test (n=41) and independent validation cohorts (n=33) of a homogeneous group of patients with non-oral cavity LAHNC treated with primary chemo-radiotherapy. The validation cohort consisted of a separate set of patients with LAHNC, treated identically to patients in the test cohort. Pre-treatment tumour HPV status was confirmed using E7 PCR (gold standard)[16]. Circulating HPV DNA levels at 12 weeks post-CRT/CRT were correlated to residual disease assessed by ^{18}F-FDG PET-CT and surgery[16].

In the test cohort (of which 27 were HPV+), HPV-detect demonstrated 100% sensitivity and 93% specificity in detecting HPV DNA in baseline (pre-treatment) plasma samples compared with tumour biopsy HPV status. In the corresponding plasma samples in the validation cohort (of which 20 were HPV+), HPV-detect demonstrated 90% sensitivity and 100% specificity compared with tumour biopsy HPV status. 38 HPV+ patients (test & validation cohort) had pre-treatment and 12 weeks post-treatment plasma samples-set available. Thirty patients had a negative HPV-detect and negative ^{18}F-FDG PET-CT at the end of treatment and required no further treatment. In 7 patients a positive ^{18}F-FDG PET-CT post treatment indicated residual disease in the cervical lymph nodes (n=4) or at site of primary disease (n=3). The 4 patients with ^{18}F-FDG PET-

CT uptake in lymph nodes underwent a ND, which did not show pathological evidence of viable cancer. Three patients with ^{18}F-FDG PET-CT uptake at primary site underwent repeated biopsies that were all negative for residual cancer. All seven patients had undetectable HPV DNA in the plasma samples at the end of treatment. Therefore, HPV DNA can potentially predict for the "true" absence of residual disease in patients with positive or equivocal ^{18}F-FDG PET-CT. The inclusion of plasma HPV DNA levels in addition to ^{18}F-FDG PET-CT to the algorithm for diagnosis of presence of residual disease following potentially curative treatment will help avoid unnecessary ND and tumour biopsies.

Wang et al. had a detection rate of 86% (18/21) of HPV DNA in plasma using digital PCR (ddPCR) for E7 in patients with HPV+ LAHNC (confirmed by E7 RNA) [17]. However, in that study, post-treatment monitoring of HPV DNA was not reported. Studies by Dahlstrom et al. (RT-PCR), Cao et al. (qPCR) and Ahn et al. (q-PCR) examined HPV DNA in baseline plasma in HPV+ LAHNC using PCR for E6 and/or E7 protein following initial confirmation of HPV integration in the primary tumour [18-20]. Using these methods, HPV DNA at baseline was detected in 60-65% of patients. Mazurek et al. based the diagnosis of HPV+ LAHNC solely on the presence of plasma HPV DNA quantified using E6/E7 PCR. Our NGS-based approach was developed and validated in a prospective patient cohort receiving identical treatment, delivered a high sensitivity and specificity for HPV DNA detection in plasma following confirmation of HPV status in primary tumour using the current gold-standard.

The specificity and accuracy of the assay, requires validation in a multi-centre study, with potential to change practice or transition to a Phase III randomised trial. The primary objective of the proposed study is validation of HPV DNA, measured using HPV-detect, as a predictor of absence of residual disease following primary chemo-radiotherapy for HPV+ oropharyngeal cancer. HPV negative oropharyngeal cancer patients will be recruited as negative controls to demonstrate that HPV-DNA is not seen sporadically (false positives) in this patient group at baseline.

HPV+ tumours generally have a better response to treatment, which has led to clinical trials with the intention of de-escalating their treatment [21]. However, for reasons not completely elucidated, some HPV+ patients have a worse prognosis, similar to HPV- cases [22]. There is a clinical need to identify those patients that are at risk of treatment failure. Patients mount an anti-cancer immune response via T-cell lymphocytes. T-cell responses against HPV are found in HPV+ patients and the density of tumour-infiltrating lymphocytes has been correlated with improved clinical response to standard therapy. It is now thought that the recruitment and activation of HPV-specific T cells offers a functional link to better prognosis of HPV-driven HNSCC. Components of the tumour microenvironment (TME) also modulate the anti-tumoral immune response. Analyzing the T-cell receptor (TCR) profile, identifying tumour antigen-specific cytotoxic T cells and TME presents an important and promising opportunity and a "probe" to explore the evolution of the immune response against cancer. Genomic analysis can now be performed on T-cells isolated from tumour and peripheral blood lymphocytes (PBMCs) present in buffy coat. We will aim to study these in samples obtained in the study and these will inform exploratory end-points.

2.1 Description of Population

Male and female over 18 years with newly diagnosed locally advanced squamous cell cancer of the oropharynx requiring definitive treatment with RT/CRT. Patients should be registered in the study after consent but before any RT/CRT commences.

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of the proposed study is the validation of plasma HPV DNA, measured using HPV-detect, as a predictor of absence of residual disease following primary chemo-radiotherapy for HPV+ oropharyngeal cancer.

3.2 Secondary Objectives

- To validate plasma HPV DNA, measured using HPV-detect, as a predictor of presence of residual disease following completion of primary chemo-radiation for HPV+ oropharyngeal cancer.
- To investigate the proportion of patients who have no residual disease on biopsy/neck dissection amongst those with residual disease according to ^{18}F-FDG PET-CT but HPV-detect negative (undetectable levels of plasma HPV DNA) at 3 months after primary RT/CRT.
- To investigate the utility of plasma HPV DNA levels for disease surveillance following RT/CRT up to a period of 12 months post-treatment.
- To validate whether the detection of plasma HPV DNA at baseline measured by HPV-detect correlates with tumour biopsy HPV status.
- To investigate the response of plasma HPV DNA levels to RT/CRT/CRT.

3.3 Exploratory Objectives

- To develop clinical pathway maps with a view to implementing the assay in clinical practice.
- To study the cost-effectiveness of the test for future implementation into clinical practice.
- To study changes in the T-cell clonality and diversity in plasma (buffy coat) at baseline and longitudinally using T-cell receptor genomic sequencing.
- To study the T-cell clonality and diversity and tumour microenvironment in tumour tissue using genomic sequencing.

4. STUDY DESIGN

This is a multicentre, prospective biological research study involving the collection of tissue (blood and tumour) and routine clinical data. Any reference to “treatment” in this protocol refers to RT/CRT treatment delivered as per standard of care, and does not constitute an intervention defined by the study protocol. The only intervention of this study protocol is the collection of tissue and blood samples.

Samples will be collected from newly diagnosed patients with T1-T2/N1-3 or T3-T4/N0-3 carcinoma of the oropharynx, with known tumour HPV status and due to receive radical RT/CRT. Patients who are suitable for RT/CRT will be registered in the study after diagnosis (defined as confirmed histological diagnosis, and staging) and before administration of RT/CRT. Initially the study will be open to both HPV+ and HPV- patients (negative controls), but following the recruitment of 48 HPV- patients sites will be notified that no further HPV – patients should be approached about the study. The frequency and timing of donated blood and tissue samples will depend on whether the patient is HPV+ or HPV- and is summarized in the study schema and in Table 1.

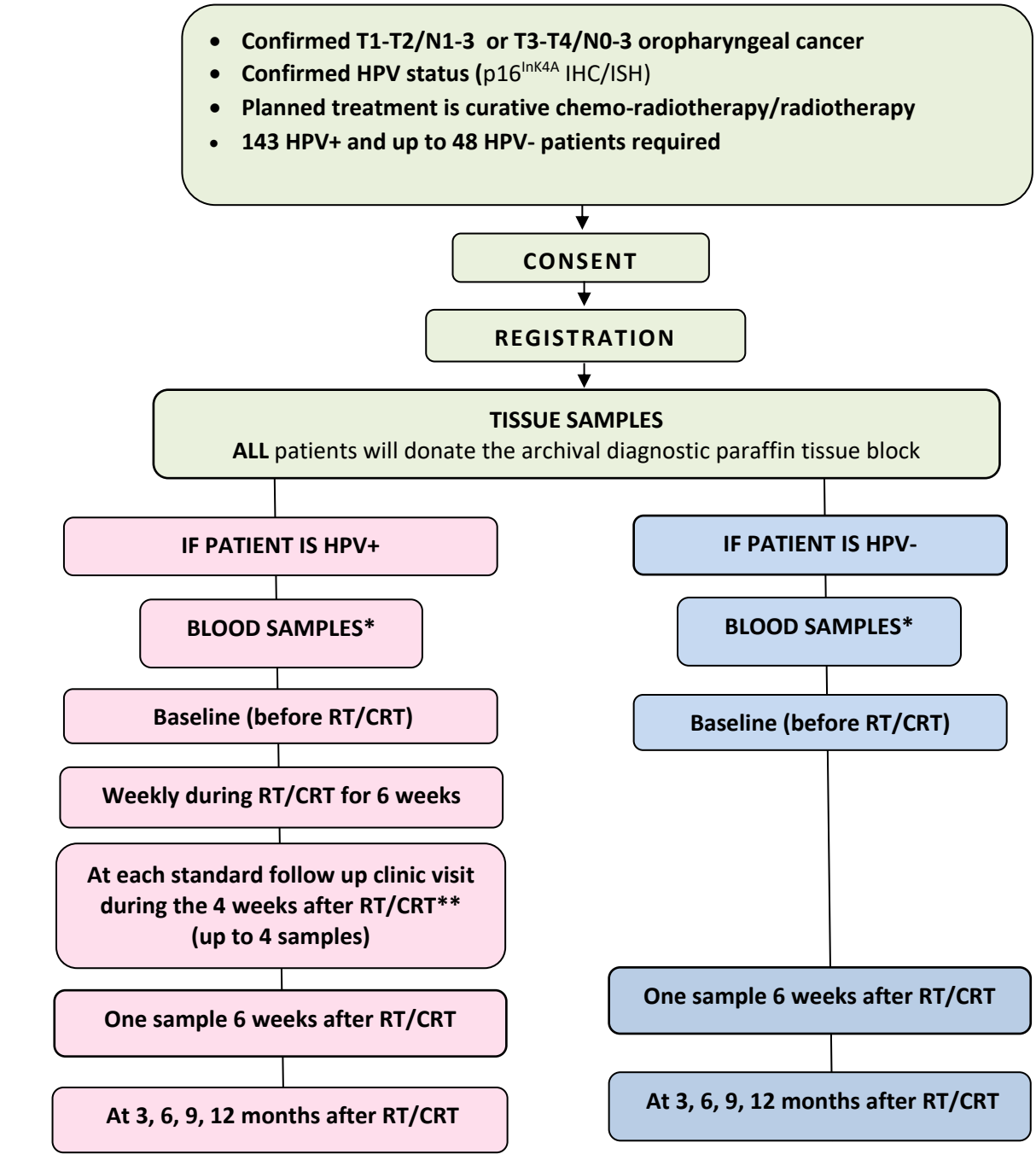
4.2 Clinical Follow up.

Sites will be required to provide clinical data from routine follow-up. Assessment of local control at 3 months following the completion of RT/CRT will be assessed using ^{18}F-FDG PET-CT.
Data will be collected from ^{18}F-FDG PET-CT scans performed as part of routine practice. Sites are requested to send a copy of the report for the 3 month ^{18}F-FDG PET-CT scan to the ICR-CTSU office. This report should be redacted to protect the patient's personal data.
Please note that ICR-CTSU may request redacted reports of other ^{18}F-FDG PET-CT scans carried out during the lifetime of the study.
For further details regarding the submission of these reports please see the INOVATE study guidelines.

Data on the status of disease recurrence will be requested from routine clinical assessment at 3, 6, 9 and 12 months post-RT/CRT. It is anticipated that further radiological review will be carried out as per standard practice if clinical assessment raises the suspicion of recurrent disease. It is anticipated that patients with confirmed residual disease, disease recurrence or progression will be recommended to have a biopsy and/or neck dissection (See Section 9).

It is important to note that the clinical management of the patients is based on routine ^{18}F-FDG PET-CT PET-CT scans only. As treating clinicians will NOT be notified of the results of the HPV-detect assay the patients' treatment plans will not be altered based on these results.

4.1 Study Schema



Additional samples will be taken in HPV+ patients before and following any surgical procedures and/or biopsies performed post RT/CRT ie

A blood sample **before** and **after** surgery
A paraffin tissue block collected after any surgical procedure and/or biopsies.

**All blood samples should be collected in Streck™ tubes.*

***Blood samples should be collected during this time in line with the 'standard of care' clinic visits at each site.*

5. STUDY ENDPOINTS

5.1 Primary Endpoint

The specificity of HPV-detect measuring plasma HPV DNA levels at 3 months following completion of primary RT/CRT to identify absence of disease compared with ^{18}F-FDG PET-CT at the same timepoint (gold standard).

5.2 Secondary Endpoints

- The sensitivity of HPV-detect measuring plasma HPV DNA levels at 3 months following completion of primary RT/CRT to identify presence of disease compared with ^{18}F FDG PET-CT at the same time point (gold standard).
- The proportion of patients who have no residual disease on biopsy/neck dissection amongst those with residual disease according to ^{18}F FDG PET-CT but HPV-detect negative at 3 months after primary RT/CRT.
- Association between plasma HPV DNA levels at different timepoints and clinical and radiological response up to a period of 12 months post-RT/CRT.
- Sensitivity and specificity of HPV-detect in measuring HPV DNA levels compared with HPV DNA levels from diagnostic tissue at baseline.
- Kinetics of change in plasma HPV DNA levels during and immediately following RT/CRT.

5.3 Exploratory Endpoints

- Description of clinical pathway maps with a view to implementing the assay in clinical practice.
- Cost-effectiveness of the test for future implementation into clinical practice.

6. PATIENT SELECTION & ELIGIBILITY

6.1 Number of Participants

The aim is to recruit 191 participants; comprising 143 HPV+ and up to 48 HPV- oropharyngeal cancer patients (negative controls). Negative controls are recruited to demonstrate that HPV+ ctDNA is not seen sporadically in this patient group.

6.2 Source of Participants

Participants will be recruited from approximately 20 participating sites in the UK. Potential participants will be identified and discussed at Multi-Disciplinary Team (MDT) meetings in oncology clinics.
Patients will be considered eligible for registration into INOVATE if they fulfil all the inclusion and none of the exclusion criteria listed below.

6.3 Inclusion Criteria

- Aged 18 years or above
- Newly diagnosed patients with T1-T2/N1-3 or T3-T4/ N0-3 squamous cell carcinoma of the oropharynx.
- Availability of tissue (or extracted DNA) from one archival diagnostic tumour tissue block
- Confirmed HPV status (p16^{InK4A} IHC/ISH)
- Patients must be candidates for and willing to undergo curative RT/CRT*
- Written informed consent.

*Only patients receiving primary RT/CRT as part of their standard care are eligible for participation in this study. Patients receiving post-operative RT/CRT are not eligible. It is anticipated that the RT/CRT will be delivered with

radical (curative) intent (radiotherapy dose fractionation and concomitant treatment will be at the discretion of the local centre).

6.4 Exclusion Criteria

- Previous or concurrent illness, which in the investigator's opinion would interfere with collection of the complete sample collection.
- Any invasive malignancy within previous 5 years (other than non melanomatous skin carcinoma or cervical carcinoma in situ).
- Clinical evidence of metastatic disease.

6.5 Participation in Clinical Trials

Patients participating in interventional trials can be co-enrolled in INOVATE provided tissue or tumour DNA is available from one archival diagnostic tissue block (Section 8.1).

7. REGISTRATION/STUDY ENTRY

7.1 Consent

Once a diagnosis of malignancy has been confirmed, and after the patients have been scheduled to receive RT/CRT patients should be offered to consent to the study. Patients should be given the up to date ethics approved INOVATE patient information sheet for their consideration. Patients should only be asked to consent to the study after they have had sufficient time to consider the study, and the opportunity to ask any further questions. All consent forms must be countersigned by the Principal Investigator or a designated individual. A signature log of delegated responsibilities, listing the designated individuals and the circumstances under which they may countersign consent forms, must be maintained at the participating site. This log, together with original copies of all signed patient consent forms, should be retained in the Site Investigator File.

Confirmation of the patient's consent and the informed consent process must be documented in the patient's medical notes. A copy of the signed consent form should be provided to the patient and the original retained in the investigator site file, which must be available for verification by ICR-CTSU study staff.
Participants must be registered centrally with ICR-CTSU before any tissue and blood samples are sent to the INOVATE central laboratory.

7.2 Procedure for Patient Registration

Patients should be registered by emailing ICR-CTSU to request a call back on:
randomisation-icrctsu@icr.ac.uk
09.00-17.00 (UK time) Monday to Friday
A registration and eligibility checklist must be completed prior to registration.

The following information will be required at registration:

- Name of hospital, consultant and person registering the patient
- Confirmation that the patient is eligible for the study by completion of the eligibility checklist ie confirmation that
  - patient has T1-T2/N1-3 or T3-T4/N0-3 squamous cell carcinoma of the oropharynx
  - HPV status has been confirmed by $p16^{InK4A}$ IHC/ISH

- planned treatment is curative radiotherapy/chemo-radiotherapy
- patient has given written informed consent for study participation

- If the patient is participating in another study, name of the study and confirmation whether the site can provide the archival tumour tissue and any blocks collected after any surgical procedure and biopsies (if relevant).
- Patient's initials, hospital number, date of birth, and NHS/CHI number. The caller will be given the patient's unique Study ID.

ICR-CTSU will send confirmation of patients' entry into the study to the Principal Investigator and the data management contact at the recruiting site.

8. BIOLOGICAL SAMPLE COLLECTION

The number and timing of samples collected in INOVATE will depend on whether the patient is HPV+ or HPV- and is summarised in Table 1.

8.1 Tissue Collection

All patients: archival diagnostic tumour paraffin blocks will be collected once the site has completed standard pathological assessment and pathology guided management plans have been formulated. If the tumour blocks are not available sites may provide 5 paraffin slides or tumour DNA if extracted for another study.

HPV+ patients only: additional tissue blocks will be collected after any surgical procedure and/or biopsies which are taken post chemo-radiotherapy.

Table 1: Sample collection timepoints

	HPV positive		HPV negative	
Sample collection timepoints	Blood sample	Tissue Sample	Blood Sample	Tissue Sample
Baseline (study entry) i.e. after registration, before treatment	✓	✓	✓	✓
Weeks 1 - 6* (every week during treatment)	✓			
At each standard clinic visit during the **4 weeks** after treatment	✓			
6 Weeks after the end of treatment	✓		✓	
3 Months after the end of treatment	✓		✓	
6 Months after the end of treatment	✓		✓	
9 Months after the end of treatment	✓		✓	
12 Months after the end of treatment	✓		✓	
Before any necessary biopsies or surgery	✓			
After any necessary biopsies or surgery	✓	✓		

**If radiotherapy treatment extends into week 7 sites are requested to collect an additional sample at the end of treatment.*

8.2 Blood Sample Collection

All blood samples will comprise one 20 mL blood sample collected in 2 x 10 mL Streck™ tubes.
As the blood samples are collected in Streck™ tubes they should NOT be stored in the refrigerator at any time. For further details on the use of Streck™ tubes please refer to the INOVATE Sample Collection Guidelines.

The majority of blood samples will be taken at the same time as routine blood collection. However all patients will be required to donate a number of samples after the 6 week time point which may be in addition to standard practice (depending on local practice at the recruiting centre).

8.2.1 Additional blood Samples for HPV+ patients pre and post surgery.

HPV+ patients only: an additional 20 mL blood sample (2 x 10 mL Streck™ tubes) for HPV DNA analysis will be collected before and after any surgical procedures or biopsies performed post-RT/CRT. Samples may be collected at any time between confirmation of residual disease/recurrence and surgery and before hospital discharge after surgery.

8.2.2 Sample scheduling and missing samples

- Weekly blood samples collected during RT/CRT for HPV+ patients should be collected on the same day each week where possible.
- Where HPV+ patients have their radiotherapy treatment extended into week 7 it is requested that an additional blood sample is collected at the end of RT/CRT treatment.
- For HPV- patients only - if the baseline sample is not collected prior to RT/CRT treatment, the patient should be withdrawn from the INOVATE study.
- For HPV+ patients only - if the baseline sample is not collected prior to RT/CRT treatment and both the RT week 1 and RT week 2 samples are missing, the patient should be withdrawn from the INOVATE study.
- Patients who miss sample collection at any of the time-points (other than missing all three of: baseline, RT week 1 and RT week 2) will continue in the study and will be included in final analysis.
- As the 12 week post-RT/CRT blood sample informs the primary endpoint it may be collected anytime between 11 – 18 weeks post-RT/CRT.
- If a patient is found to have residual disease, recurrence or progression the blood samples should still be collected according to the time lines specified in the protocol.
- If the patient does not complete RT for any reason, they should be withdrawn and sample collection will cease.

8.3 Storage, labelling and postage of blood and tissue samples

All INOVATE blood samples and tissue samples should be collected, labelled, stored and shipped as detailed in the INOVATE Sample Collection Guidelines.

All samples must be labelled with the patient's study identifier (patient Registration Number), date of birth and date of sample to enable cross-referencing.

All samples should be posted to the central laboratory at the Institute of Cancer Research in the packaging provided.

Tissue Blocks

Archival blocks will be collected in batches. ICR-CTSU will notify the sites when block collection is required. All samples should be sent by post to the following address:

Room 35
CBO, Chester Betty Laboratories
Targeted Therapy Team
Cancer Biology
The Institute of Cancer Research
237 Fulham Road
London. SW3 6JB

Research Blood Samples

All research blood samples should be sent by post **within 48-72 hours** to the following address:

Room 35
CBO, Chester Betty Laboratories
Targeted Therapy Team
Cancer Biology
The Institute of Cancer Research
237 Fulham Road
London. SW3 6JB

Samples labelled in an anonymised manner may be shared with external institutions, including those outside the UK and EU, for future research.

8.4 Clinical Data Collection

Electronic (e) Case Report Forms (CRFs) will be used for the collection of study data. Clinical data will be stored and analysed at ICR-CTSU who will provide guidance to sites to aid the completion of the eCRFs.

The following data will be collected on electronic case report forms:

At baseline: age, sex, smoking history, detailed tumour staging, HPV (p16Ink4A) status.

Post RT/CRT treatment: radiotherapy start date and schedule.

At 12 week post RT/CRT: results of 12 week ^{18}F FDG PET-CT scan (and any repeated scans), carried out as per local practice for assessment of local control. If an HPV+ patient is found to have residual disease at the 12 week assessment and is scheduled to receive surgery/biopsy this should be reported to the ICR-CTSU trials office in an expedited fashion on the appropriate eCRF as soon as the surgery/requirement for biopsy is is confirmed**.**

During follow up: Information on the status of disease recurrence will be collected at 3, 6, 9 and 12 months post-RT/CRT. It is anticipated that further radiological review will be carried out as per standard practice if clinical assessment raises the suspicion of recurrent disease.

9. PROCEDURE FOLLOWING CONFIRMATION OF RESIDUAL DISEASE (AT 12 WEEKS), DISEASE PROGRESSION OR RECURRENCE

If the confirmation of residual disease following the 12 week ^{18}F FDG PET-CT scan or later confirmation of disease progression/recurrence will require the patient to undergo surgery/biopsies this should be reported to the ICR-CTSU trials office in an expedited manner on the appropriate eCRF.

It is anticipated that patients with residual disease, disease progression or recurrence who meet the following criteria following radiological assessment will undergo neck surgery:

a) positive PET-CT and/or
b) equivocal PET-CT with lymph node > 1cm.

If the 12 week ^{18}F FDG PET-CT scan is repeated following equivocal results, details of the repeat scan should be notified to ICR-CTSU on the appropriate eCRF.

It is anticipated that patients who have residual disease at primary site will undergo a biopsy of this site. However if the 12 week ^{18}F FDG PET-CT scan is repeated (according to local practice) following equivocal results, details of the repeat scan should be notified to ICR-CTSU on the appropriate eCRF.

Prior to opening, centres will be required to confirm that they fulfil minimal surgical quality assurances stipulated by the INOVATE surgical co-investigators and that they will adhere to the surgical recommendations in Section 9.1.

Data will be requested from routine follow-up visits relating to surgical outcome and disease status.

9.1 Surgical recommendations for INOVATE patients requiring neck surgery (as per national guidelines).

- The surgeon performing the procedure will work within a recognised MDT within a cancer network in the UK.

It is recognised that the extent of neck dissection varies across geographical regions, with an increasing trend for less extensive neck dissections. In addition, the number of nodes in a radiated neck will be low, and will not serve as a quality indicator as it does in the previously untreated neck. Thus the criteria for this study will take two forms:

A. Neck dissection levels

1. Based on surgeon preference and the size and site(s) of abnormality as seen on the PET-CT/CT scan, current practice varies from a nidusectomy alone to multilevel dissection. Hence, from a pragmatic perspective, the following procedures are recommended:

    a. In instances where a solitary focus is the abnormality on PET-CT, the procedure should include at a minimum the abnormal nidus (lymph node).
    b. In the presence of multiple foci, but at the same neck level, a minimum of two adjacent levels in addition to the abnormal node being targeted is recommended (see Table 2).

Table 2: Study specific neck dissection recommendations

Abnormal node level	Levels to be removed by neck dissection
IIa	IIb and III
III	II and IV
IV	III and Vb
I	II and III

c. In the event of multiple abnormal nodes at several levels, a 5 level dissection is recommended.

2. The abnormal nodes should be clearly identified and orientated in the specimen sent to the lab.

B. Correlation of the dissected specimen with the radiological findings

The neck treated by radiation can be affected by fibrosis making for a challenging procedure. To ensure that the abnormal nodes have been removed by the surgical procedure, the INOVATE study team will randomly choose 20% of neck dissections and correlate the sizes of the nodes identified in the histology report with the radiological findings. Allowing for a 30% shrinkage with specimen fixation, the maximum dimension of the nodes identified as being abnormal in the specimen should correlate with the maximum dimension on radiologic imaging.

Any further clinical treatment is at the discretion of the treating clinician and should follow standard of care.

10. DISCONTINUATION FROM SAMPLE COLLECTION AND FOLLOW UP

Patients may discontinue from sample donation at any time at their own request, or they may be discontinued at the discretion of the Principal Investigator. If all three of the baseline (pre-RT/CRT), RT week 1 and RT week 2 samples are not collected, the patient will be withdrawn from the study and an additional patient recruited.

If a patient wishes to withdraw from the study and has donated both baseline and 12 week blood samples it should be clarified whether they no longer wish to donate any further samples but agree that information about them may still to be sent to the ICR-CTSU, or whether they have withdrawn consent for further information about them to be collected within the study. A 'Change of Study Status' form should be submitted to ICR-CTSU stating the nature of withdrawal.

Should a patient withdraw consent for their samples to be used in INOVATE their blood samples will be destroyed and tissue samples returned to the site for archiving following receipt of written confirmation from the site to ICR-CTSU.

11. BIOLOGICAL SAMPLE PROCESSING AND ANALYSES

All samples will be transported to the central ICR laboratory for storage within 48-72 hours. Samples will be pseudo-anonymised and allocated a unique study reference number. All samples will be processed and analysed in accordance with Good Clinical Practice Laboratory (GCLP) standards using pre-defined standard operating procedures (SOPs). The laboratory staff will be blinded to the clinical data including the HPV status.

11.1 Tissue samples

Tumour will be identified from the formalin fixed paraffin-embedded (FFPE) tissue samples and DNA will be isolated using micro dissection. DNA and RNA will be extracted from the plasma using a standard, commercially available Qiagen Circulating Nucleic Acid kit.

11.2 Plasma samples

The collected blood (Streck™ tubes) will be centrifuged (1600 rpm X 20 min) on receipt at the central laboratory. 1 mL of plasma will be aliquoted into separate cryovials labelled as per the sample bottle. Buffy coat will be separately aspirated and stored. The plasma will be frozen at -80°C and stored at the ICR. Prior to extraction, plasma samples will be further centrifuged at 14000 rpm for 10 minutes. DNA and RNA will be extracted from 5mL of plasma and buffy coat using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according manufacturer's instructions. DNA and RNA will be eluted in 50µL of AVE buffer at stored at -20°C.

11.3 Tumour Characteristics

11.3.1 DNA quantification and sequencing (tissue and plasma)

Plasma and tissue DNA will be quantified using a Bio-Rad QX100 ddPCR machine- using ribonuclease P (RNase P) as a reference gene. This will be sequenced for HPV DNA using HPV-detect. Samples will be classified into HPV DNA positive and negative based on the thresholds generated in the pilot study (*Lee*, *Br J Cancer, 2017*).

11.3.2 RNA analysis

RNA will be extracted from biological samples (tumour tissue and plasma samples (buffy coat) and will be subjected to high-throughput techniques such as RNA-sequencing or other molecular techniques to identify changes relevant to cancer biology and resistance to treatment. The analysis will focus initially on the identification of genomic changes in the tumour, which can be identified in the contemporaneous plasma samples (buffy coat) at the outset of treatment. The changes will then be followed in a longitudinal manner throughout treatment in plasma and other biological samples. This will allow analysis for exploratory end-points.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design and Sample Size Justification

The primary aim of the proposed study will be to establish the potential of HPV-detect to predict absence of residual disease (high specificity). To demonstrate specificity compared with ^{18}F-FDG PET-CT of at least 85%, assuming that the true specificity is 95%, then with 80% power, given two-sided type I error of 0.05 and assumed prevalence of residual disease of 25% on ^{18}F FDG PET-CT, the study will require recruitment of 100 HPV+ oropharyngeal cancer patients undergoing RT/CRT. Assuming a rate of patient dropout and sample loss of 30%, 143 HPV+ oropharyngeal cancer patients will need to be registered into the study. Forty-eight (N=143/3≈48) HPV- oropharyngeal cancer patients will also be recruited as negative controls to demonstrate that plasma HPV DNA is not seen sporadically in this patient group. This number will allow estimation of an upper one-sided 95% confidence bound of 6%, given proportion observed of 0% in HPV- oropharyngeal cancer patients. The total sample size is therefore 191 patients.

12.2 Endpoint Definitions

12.2.1 Primary endpoint

The specificity of HPV-detect (using plasma HPV DNA levels) in correctly identifying those with no residual disease, at 3 months following completion of primary RT/CRT is defined as the proportion patients who are HPV-detect negative among those with no residual disease according to 18F-FDG PET-CT at the same time point.

12.2.2 Secondary endpoints

- The sensitivity of HPV-detect (using plasma HPV DNA levels) in correctly identifying those with residual disease at 3 months following completion of primary RT/CRT, is defined as the proportion of patients who are HPV-detect positive among those with residual disease according to ^{18}F-FDG PET-CT at the same time-point.
- The proportion of patients who have no residual disease on biopsy/neck dissection amongst those with residual disease according to 18F FDG PET-CT but HPV-detect negative at 3 months after primary RT/CRT.
- Repeated measures of plasma HPV DNA levels up to a period of 12 months post RT/CRT (time points defined in the protocol) will be associated with the clinical and radiological response.
- Sensitivity and specificity of HPV-detect in measuring plasma HPV DNA levels compared with HPV DNA levels from diagnostic tissue at baseline are defined as the proportions of true positives and the proportions of true negatives, respectively.
- The pattern of plasma HPV DNA responses to RT/CRT will be presented by calculating the percentage change in HPV DNA level at each assessment time points from baseline.

12.2.3 Exploratory endpoints

- Description of clinical pathway maps with a view to implementing the assay in clinical practice.
- Cost-effectiveness of the test for future implementation into clinical practice.
- Changes in molecular features between the paired tumour tissue and plama samples (buffy coat)

12.3 Statistical Analysis Plan

Analysis of the primary endpoint will include all patients in the HPV+ oropharyngeal cancer cohort who are evaluable i.e. have both HPV-detect (plasma DNA) and ^{18}F-FDG PET-CT results at 3 months following completion of primary RT/CRT. Specificity will be calculated and presented with associated exact 95% confidence interval. Primary analysis will be performed when the last patient registered has reached the 3-month time point. Sensitivity will be analysed similarly.

Based on the high negative predictive value of HPV detect (>90%), a small proportion of patients with residual disease as per ^{18}F-FDG PET-CT will potentially have a negative HPV-detect. We will report these as a proportion with associated exact 95% confidence intervals.

We will not alter the clinical management of these patients, but aim to collect samples for all patients up to one year, to track HPV DNA in these patients and correlate this to disease outcome.

Secondary endpoints of sensitivity, and the proportion of patients with no residual disease on biopsy/neck dissection amongst those with residual disease according to 18F FDG PET-CT but HPV-detect negative at 3 months following completion of primary chemo-radiation will be analysed using the same patient population and as per the primary endpoint. The proportions will be presented with associated exact 95% confidence interval

Association between plasma HPV DNA levels at different timepoints and clinical and radiological response up to a period of 12 months post-RT/CRT will be investigated using logistic regression models.

Analysis of sensitivity and specificity of HPV-detect in measuring HPV DNA levels at baseline will be calculated and presented with associated exact 95% confidence interval from all patients with paired data from diagnostic tissue and HPV-detect at baseline and will include both HPV+ and HPV- (negative control) patients.

Analysis of the molecular features/characteristics of the tumour tissues and to determine changes/conservation of these features in paired plasma samples.

Kinetics of change in plasma HPV DNA levels during and immediately following RT/CRT will be displayed graphically, separately for HPV+ and HPV- patients.

Full details of analyses of all primary and secondary endpoints will be described in the Statistical Analysis Plan in accordance with ICR-CTSU Standard Operating Procedures.

NIHR-DEC will undertake health-economic, stakeholder, end-user and downstream pathway analysis. Details of this will be fully developed in a specific Analysis Plan.

12.4 Interim Analyses and Stopping Rules

Specificity will be assessed once 40 HPV+ patients are evaluable for the primary endpoint, i.e. have both 'HPV-detect' and ^{18}F-FDG PET-CT results at 3 months following completion of primary RT/CRT. If at this point specificity is <60% it is unlikely that the true specificity at the end of the study will be >85% and therefore consideration will be given to stopping the study due to futility.
The sample size was therefore calculated as follows: Using an A'Hern single stage design with p0 = 0.6 and p1 = 0.85, alpha = 0.05 and power=90%, 27 ^{18}F-FDG PET-CT negative patients are needed. If 21 or more of

the 27 are also HPV-detect negative, it will be assumed that the true specificity is greater than 60% and the study will continue. Assuming a prevalence of 25% of residual disease of on 18F-FDG PET-CT, 40 HPV+ patients are needed at interim analysis.

13. STUDY MANAGEMENT

13.1 Project Management Group (PMG)

A Project Management Group (PMG) will be set up and will include the Chief Investigator, ICR-CTSU Scientific Lead, Co-investigators and identified collaborators, the Study Statistician and Project Manager. Where possible, membership will include a lay/consumer representative. The PMG will meet at regular intervals, and at least annually. Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the PMG have operational responsibility for the conduct of the study. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

13.2 Independent Data Monitoring and Steering Committee (IDMSC)

A joint Independent Data Monitoring and Steering Committee (IDMSC) will be set up to, monitor the data produced by the study, put these data into overall context and supervise the progress of the study towards its interim and overall objectives. The IDMSC will comprise an independent Chairman and at least two further independent members with clinical or statistical expertise (at least one member must be a statistician).

The IDMSC will meet in confidence at regular intervals, and at least annually. A summary of findings and any recommendations will be produced following each meeting. This summary will be submitted to the PMG and if required, the main REC.

The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

14. RESEARCH GOVERNANCE

14.1 Sponsor Responsibilities

The Sponsor of this study is the Institute of Cancer Research (ICR).

14.2 Participating Site Responsibilities

Responsibilities of participating sites are defined in an agreement between the individual participating site and the Sponsor.

15. STUDY ADMINISTRATION & LOGISTICS

15.1 Site activation

Before activating the study, participating sites are required to sign an agreement accepting responsibility for all study activity which takes place within their site.

Sites may commence recruitment once the site agreement has been signed by all required signatories, the required study documentation is in place (as specified by ICR-CTSU), a site initiation (teleconference) has taken place and ICR-CTSU has confirmed in writing that the site is officially open to recruitment.

15.2 Data Acquisition

Electronic (e) Case Report Forms (CRF) will be used for the collection of study data. ICR-CTSU will provide guidance to sites to aid the completion of the eCRFs. The Project Management Group reserves the right to amend or add to the eCRF template as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by sites in accordance with the guidelines provided by ICR-CTSU.

The clinical data should be reported on the INOVATE eCRFs to the ICR-CTSU in a timely manner. Specific guidance on how data will be collected will be detailed in study guidance notes. On receipt at ICR-CTSU, eCRFs will be recorded as received and any missing data will be reported to the originating site.

15.3 Central Data Monitoring

Once data has been entered on the eCRF by the site personnel, ICR-CTSU will review it for compliance with the protocol, and for inconsistent or missing data. Should any missing data or data anomalies be found, queries will be raised for resolution by the site.

15.4 Completion of the Study and Definition of Study End Date

The study end date is deemed to be the date of last data capture.

15.5 Archiving

Essential study documents should be retained according to local policy and for a sufficient period for possible inspection by the regulatory authorities (at least 5 years after the date of last data capture). Documents should be securely stored and access restricted to authorised personnel.

16. PATIENT PROTECTION AND ETHICAL CONSIDERATIONS

16.1 Study Approvals

INOVATE has been approved by the Sponsor's Committee for Clinical Research (CCR).

ICR-CTSU, on behalf of the Sponsor, will ensure that the study has received ethics approval from a research ethics committee and Health Research Authority (HRA) approval. Before entering patients, the Principal Investigator at each site is responsible for submitting this protocol and Site Specific Information to gain either confirmation of capacity and capability (for participating sites in England) or local Research and Development approval (for participating sites in Scotland, Wales or Northern Ireland).

16.2 Study Conduct

This study will be conducted according to the approved protocol and its amendments, supplementary guidance supplied by the Sponsor and in accordance with the UK Policy Framework for Health & Social Care and the principles of GCP.

16.3 Patient Confidentiality

Patients will be asked to consent to their initials being collected at study entry in addition to their date of birth, hospital number, postcode and NHS number or equivalent to allow linkage with routinely collected NHS data.
Biological samples will be labelled with date of birth and study ID to ensure accuracy in handling the samples.

Each investigator should keep a separate log of all participants' Study IDs, names, addresses and hospital numbers. The investigator must retain study documents (e.g. participants' written consent forms) in strict confidence. The investigator must ensure the participants' confidentiality is maintained at all times.

Representatives of ICR-CTSU may require access to participants' hospital notes for quality assurance purposes. ICR-CTSU will maintain the confidentiality of participants at all times and will not reproduce or disclose any information by which participants could be identified.

16.4 Data Protection

All parties must comply with all applicable data protection laws.

16.5 Liability

Indemnity to meet the potential legal liability of investigators participating in this study is provided by the usual NHS indemnity arrangements.

17. FINANCIAL MATTERS

This study is investigator designed and led and has been approved by the Medical Research Council Developmental Pathway Funding Scheme.

ICR has received funding from the Medical Research Council for the central coordination of the study. In the UK, the study meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England. The study is part of the National Institute for Health Research Clinical Research Network (NCRN) portfolio by virtue of its approval by the MRC. NCRN resources should therefore be made available for the study to cover UK specific research costs.

18. TISSUE SHARING POLICY

The custodian of the samples will be the Project Management Group. Proposals for future research projects involving the material will be considered by the PMG.

19. PUBLICATION POLICY

The main study results will be published in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, consisting of members of the PMG. Participating clinicians may be selected to join the writing group on the basis of intellectual and time input. All participating clinicians will be acknowledged in the publication.

Any presentations and publications relating to the study must be authorised by the PMG. Authorship of any secondary publications e.g. those relating to sub-studies, will reflect intellectual and time input into these studies.

No investigator may present or attempt to publish data relating to the INOVATE study without prior permission from the PMG.

20. ASSOCIATED STUDIES – STAKEHOLDER ANALYSIS AND HEALTH ECONOMICS STUDY

The National Institute for Health Research London in vitro Diagnostics Cooperative (NIHR London IVD) at Imperial will undertake stakeholder analyses to understand risks, adoption barriers and assess potential impact of HPV-detect in the NHS. Actual end-users' and potential key stakeholders will be interviewed to identify and understand perceived barriers to adoption, needs for improvement adaptation of HPV-detect and potential impact for the NHS.

Workflow and clinical pathway mapping analysis will be undertaken in order to understand the impact of implementing HPV-detect on hospital resources. Health economic modeling for cost effectiveness analysis will be carried out by generating costs for clinical pathways with, and without HPV-detect. The cost benefits will be captured in a decision analytic model with consequences for the patients (changes in short and long term QOL) and cost savings to the NHS. The impact of test sensitivity and specificity on cost will be modelled using sensitivity analyses.

The pathway mapping and health economic analyses will be carried out in parellell with the clinical study in order to model the future impact of HPV-detect implementation. These analyses will be carried out over a period of **9 months**. The stakeholder analyses will require 6 months and will be conducted during the study. The health economic analyses will require 3 months and will be performed once the final results of the study become available prior to study publication.

Further details of the stakeholder analysis and health ecomonics sub-study will be added to the protocol by substantial amendment. Sites will be notified by ICR-CTSU when the sub-study will commmence.

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