Title: Liquid Biopsy for Early DiagNosis of Squamous cell Carcinoma of the HeAd and NeCk rEgion (ENHANCE Study)

Short Title: ENHANCE Study

Protocol Version:	v1.1
Dated:	18.07.2022

RESEARCH REFERENCE NUMBERS

IRAS Number:	292104
EudraCT Number:	N/A
ISRCT Number/Clinical trials.gov Number:	N/A
Sponsor Number:	CCR5610
Funders Number:	829/R1554 – RM Partners

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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 Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.
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 study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

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Signature:

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Date:

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Statistician:
Signature:

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Principle Investigator:
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1. BACKGROUND AND RATIONALE

The 5-year survival for Head and Neck squamous cell carcinoma (HNSCC) across all TNM stage groups is approximately 50% [1]. Patients who are present with stage I & II disease have significantly better survival. When a patient presents to their general practitioner (GP) with symptoms suggestive of HNSCC, they may be referred for urgent specialist input through the suspected cancer referral (SCR) pathway, which include dedicated neck lump clinics. Majority of the patients diagnosed via the SCR pathway present with Stage III &IV disease, which has a direct impact on outcomes.

HNSCC is classified as an uncommon cancer and as such diagnosis and treatment is undertaken in specialist tertiary referral centres. The care of patients initially diagnosed with HNSCC in smaller secondary care hospitals is transferred to these specialist tertiary referral centres via the Inter Trust Transfer (ITT). ITT can introduce delays in treatment pathways resulting in a failure to achieve cancer treatment targets. Southwest London HNSCC diagnostic pathway review which was undertaken in February 2021 demonstrated that 40% of ITTs received were greater than 38 days after referral and that 68% of the 62 day target breaches were in patients with ITT.

HNSCC is known to shed fragments of DNA, called circulating tumor DNA (ctDNA) into the bloodstream [2]. We have developed novel ultra-sensitive (>90% sensitivity) next generation sequencing (NGS) assay for circulating HPV DNA [3] in patients with non-metastatic locally advanced head and neck cancer. Our current work involves ctDNA detection to cover the spectrum of genetic alterations in HNC using a single sequencing workflow to detect copy number aberrations (CNAs), HPV DNA (to cover 99.9% of HNC related HPV) and somatic mutations.

The use of ultra-sensitive NGS assay for detection of ctDNA using a simple blood test (liquid biopsy) holds a great promise for cancer screening and early diagnosis and can lead to better survival results and less disease burden. This has been proven in proof of principle studies in nasopharyngeal cancer [4] (Chan N Engl J Med 2017; 377:513-522). Furthermore, this test can be administered in smaller secondary care hospitals in parallel to the ITT. With a quicker turnaround (1-2 weeks), the liquid biopsy can help expedite the patient journey through the cancer pathways reducing the incidence of cancer target breaches. In order to design studies to test this hypothesis we require preliminary data quantifying sensitivity and specificity of our assay in this setting.

2. STUDY OBJECTIVES

2.1 Hypothesis

The NGS assay has a high sensitivity and specificity for detection of ctDNA in early HNSCC

2.2 Aims

To test the feasibility of the NGS assay for detection of ctDNA in patients with early HNSCC

3. STUDY SETTING

3.1 Study Design

This is a prospective observational sample collection study in patients referred to neck lump clinics via the SCR.

3.2 End Points

- Primary endpoint – Measure the sensitivity of the NGS assay for detection of ctDNA at baseline in patients with early HNSCC

- Secondary end-point - Measure the specificity of the NGS assay for detection of ctDNA at baseline in patients with early HNSCC

3.3 Inclusion/ Exclusion Criteria

Inclusion Criteria

- Patients referred for an Ultrasound guided fine needle aspiration (USFNA) in the neck lump clinic.

Exclusion Criteria

- Patient found to have a lump in the thyroid gland at the time of USFNA
- Unable to give informed consent for biological sample collection.
- Unable to safely participate in clinic sample collection

4. TRIAL PROCEDURES

4.1 Patient Identification and Consent

The proposed observational sample collection study will recruit patients referred to the neck lump clinics via the SCR pathway. Patients are classified as low and high risk in the neck lump clinic following clinical history and examination by the clinician. Patients classified as high risk are

referred for an USFNA. These patients will be approached for screening by a designated study research nurse and be provided with a patient information sheet (PIS). They will be made aware that definite eligibility will be determined following USFNA. Patients will only be eligible if they do not have a lump in their thyroid gland after undergoing USFNA. Following USFNA, eligible patients will be approached by the research nurse to address any concerns and to sign an informed consent.

Blood sample will be collected from patients who sign the informed consent. As per the study procedure, the patient will be consented on the same day i.e. the day of the neck lump clinic, that they receive the consent form. They will be given adequate time to consider consenting to the study and have the opportunity to ask questions.

The blood sample will be transferred to the Biobank at RMH via first class post for processing and storage. These samples will then be transferred in batches to ICR or CMP and analysed for the presence of and to quantify the levels of ctDNA. With advances in technologies for analysis we will also perform a complete genomic analysis.

The study will be run at head and neck lump clinics at Kingston Hospital NHS Foundation Trust and London North West University Healthcare NHS Trust.

4.2 Withdrawal criteria

Patients may withdraw from the study at any time at their own request, or they may be discontinued at the discretion of the Principal Investigator. This can occur for the following reasons:

- Patient decision
- Ineligibility
- PI decision

If the patient is withdrawn from the study, the primary reason as well as the date of withdrawal will be recorded in the in the Excel Data Tool (combined eCRF and database). Should a patient withdraw consent for their samples to be used in the study, the samples will be destroyed. Data collected at the point of withdrawal will continue to be used by the study team.

4.3 Study completion and end of trial definition

The end of study accrual will be when the requisite numbers of patients have been recruited and the last blood sample is taken as per trial protocol.

5. DATA AQUISTITION AND HANDLING

5.1 Clinical data

We will be collecting identifiable data as part of the study . This information will be held by the study sponsor on an excel spread sheet stored on a Trust computer in line with local data governance policies and GDPR. Each patient enrolled in the study will have a unique study ID generated.
The full dataset is available in an associated Excel Data Tool (combined eCRF and database). The dataset is divided into the following areas:

1. General patient details: Demographics, smoking/alcohol
2. Diagnosis – Histolgy/cytology from the USFNA

5.2 Samples and Molecular data

Biological specimens will be identified only using the study ID (link-anonymised). As such, no patient identifiable data will be processed outside of RMH.

6. STORAGE AND ANALYSIS OF SAMPLES

6.1 Specimens

A 20ml blood sample (2x 10ml blood collection tube, Streck or other appropriate) for plasma extraction and collection of buffy coat or PBMCs will be taken.The blood will be centrifuged to separate the plasma and buffy coat and then labelled and stored at -80^{o}C.

6.2 Storage, labelling and postage of biological samples

All the samples will be labelled in accordance with good clinical practice and local protocols. If the patient consents to participate in the study, the name of the study, the study ID and the date of acquisition should also be added to the label. Sample storage will be in compliance with the Human Tissue Act (HTA).

The blood sample will be collected by the research nurse that attends the neck lump clinic. They will consent the patient, collect the sample and will arrange for the sample to be transferred to the Biobank. The research nurse will be adequately trained in the correct procedures for sample collection and transfer, ensuring procedures are compliant with the HTA. The blood sample will be transferred to Biobank at RMH via a courier or royal mail special delivery in an approved packaging for processing and storage. They will be link-anonymised and transferred to ICR for

analysis, where samples will be stored in secure facilities. Samples labelled in an anonymised fashion with no identifiable features may be shared with external institutions and collaborators. Biological samples retained for the study will be encoded with a unique identifier and other patient identifiers will be removed prior to storage in order to maintain patient confidentiality.
Laboratory researchers will not have access to any details that identify the patient in almost all circumstances. Certain key individuals within the study team will be able to link the unique identifier with the patient's identification details. This will allow the collection of clinicopathological data to complement the molecular data results from the study.
Extracted nucleic acids will be stored for future analysis in secure facilities at the Institute of Cancer Research. All samples will be held in compliance with the Human Tissue Act (HTA).

The chief investigator will have overall responsibility for the samples and will delegate responsibilities to appropriately trained staff. While the samples are stored at the RMH Biobank , the biobank manager will have overall responsibility for the samples. The scientific officer based at ICR will request the samples to be transferred for analyses. The samples will be tracked at all times. Excess samples which are left over will be transferred back to the biobank and will be stored as per their SOPs, which include transfer to off-site facilites for long term storage.

6.3 Molecular analyses

Nucleic acid extraction

Nucleic acids will be extracted from plasma with buffy coat used for germline nucleic acid sequencing. DNA samples will be subjected to sequencing analysis for identification of ctDNA.

7. STATISTICS AND DATA ANALYSIS

7.1 Statistical Considerations

This is a pilot study that aims to investigate the feasibility of using HPV DNA as a biomarker of early diagnosis of HNSCC. To this effect, we will focus on identifying the sensitivity of the NGS assay in patients who been diagnosed with invasive cancer. A sensitivity of greater than 70% would be necessary for further investigation in a larger study. We hope to recruit 49 patients who undergo USFNA and are diagnosed with HNSCC and a further 121 patients who undergo USFNA and do not have invasive HNSCC. The standard ctDNA test will be compared against the tissue diagnosis of invasive cancer as per the tumour biopsy.

7.2 Sample Size

Assuming disease prevalence at baseline of 99%, if ctDNA is detectable in at least 39 out of 49 patients, this will enable us to demonstrate a sensitivity of at least 70% (i.e. rule out the maximum futility proportion), and assume a true sensitivity of 85% (the minimum efficacy proportion), with 80% power and a one-sided type I error of 0.05."
If ctDNA is undetectable in at least 112 out of 121 patients, this will enable us to demonstrate a specificity of at least 85% (i.e. rule out the maximum futility proportion), and assume a true specificity of 95% (the minimum efficacy proportion), with 90% power and a one-sided type I error of 0.01 (Single stage phase II A'Hern design, software used nQuery v8.7.2).

7.3 Statistical analysis

The statistical analyses will be performed by Dr Ben O'Leary, who is a co-investigator on the project using the R statistical software package. Given, that the study will involve simple statistical analyses, an expert statistician will not be required to perform the analyses. However, we will ask the study statistician to check the analyses for accuracy.
All quantitative data will be analysed for normality and presented accordingly as number of observations, measure of central tendency for HPV DNA copy number and variance. Qualitative data will be presented as a number of observations and percentages
A Chi square test will also be performed to check for the association between plasma ctDNA and the diagnosis of viable invasive HNSCC.
Sensitivity at baseline is defined as the proportion of participants with invasive HNSCC who have ctDNA detected.

7.4 Study duration

The study will continue until up to 49 patients with invasive HNSCC and 121 patients without invasive SCC, have been recruited. The study will be completed once all 170 patients have been recruited and samples analysed.
The study will be run at the head and neck lump clinics at Kingston Hospital NHS Foundation Trust and London North West University Healthcare NHS Trust. Based on previous year's figures, approximately 200 patients are referred each year for USFNA following assessment in the clinic. Of these, 30-40 patients get diagnosed with HNSCC and 10-15 with Thyroid cancer. Therefore, we anticipate that this study will complete recruitment in 9-12 months.

The primary analysis will be performed once all 49 patients with invasasive HNSCC are recruited and samples analysed, whereas the secondary endpoint analysis will be performed once 121 patients without invasive HNSCC have their samples analysed.

8. DATA HANDLING

Data collection tools and source document identification

8.1 Source Data

Clinical data will be collected directly onto the Excel Data Tool (combined eCRF and database). This will be stored in the electronic site file on the shared drive for clinical studies conducted by the H&N research team. In accordance with local data governance regulations for patient identifiable data. REDCAP might be used for data collection if it becomes available by the time the study starts to register patients.

Data collection arrangement for sites outside RMH: A research nurse employed by RMH will be present at the neck lump clinics where recruitment will take place. They will enter the study data directly onto the Excel Data Tool (combined eCRF and database) on a secure trust laptop that they will carry with them to clinic.

8.2 Data handling and record keeping

The Excel Data Tool (combined eCRF and database) will be held in a folder on computers of the Royal Marsden Hospital NHS Foundation Trust and held for 5 years. This folder will have a security system to protect against unauthorized access. Each update of the data will be recorded and saved as a separate version in the folder so there will be no deletion of the entered data. Each participant will have their own trust medical record number (MRN) and a study ID to allow for identification of all the data reported for each participant and for pseudonymisation for the molecular analysis.

The chief investigator (CI), Dr Shreerang Bhide, will be the custodian of the data submitted to the Royal Marsden Hospital. The CI and the Royal Marsden Hospital NHS Foundation Trust will keep records of all participating patients and all original signed informed consent forms.

Data storage and management for the molecular analysis will be at the Institute of Cancer Research, London. Data will be pseudonymised with the study ID, encrypted and held in line with local data governance protocols.

8.3 Access to data

Direct access will be granted to authorised representatives from the Sponsor and the regulatory authorities to permit study-related monitoring, audits and inspections, in line with participant consent.

8.4 Archiving

All study documents will be archived by the Royal Marsden Hospital NHS Foundation Trust following submission of the end of study report. The site file and essential documents will be archived for 12 months after completion of the study. These documents will be stored in a location determined by the Sponsor in line with their standard operating procedures. Any destruction of essential documents will require authorisation from the Royal Marsden Hospital NHS Foundation Trust.

9. TRIAL OVERSIGHT, MONITORING, INSPECTION AND AUDIT

9.1 Regulatory & Ethics Committee Approval

The protocol will be submitted for ethical review to the Health Research Authority's Integrated Research Application System' (IRAS).

The Study is sponsored by The Royal Marsden NHS Foundation Trust and will be approved by the Sponsor's Committee for Clinical Research (CCR). The Royal Marsden NHS Foundation Trust will ensure that the study has received ethics approval from a research ethics committee (REC) and has received Health Research Authority (HRA) approval.

9.2 Trial Management Group

A Trial Management Group (TMG) will be set up and membership will include the Translational Lead Investigator, Clinical Lead Investigator, Co-Investigators and Senior Trial Manager. Principal Investigators at Kingston Hospital NHS Foundation Trust and London North West University Healthcare NHS Trust will be invited to join the TMG as appropriate. The TMG has operational responsibility for the conduct of the trial. The TMG is responsible for monitoring recruitment, safety and governance of the trial as well as collaborating with subsequent translational sub-studies. The TMG will also review any safety concerns and can convene a meeting if significant concerns exist.

10. FINANCING, INDEMNITY & INSURANCE

Where the Royal Marsden NHS Foundation Trust is either sponsoring or collaborating with externally sponsored research the NHS Litigation Authority will cover standard clinical negligence by employees, staff and health professionals employed by the Royal Marsden NHS Foundation Trust. For more information visit the following website:
http://www.nhsla.com/Claims/Pages/Clinical.aspx.

There is unlimited liability and no excess. Insurance is provided under the Clinical Negligence Scheme for Trusts and there is no cover for non-negligence claims.

For all notification of claims please contact the Board Secretariat.

Where the Institute of Cancer Research is sponsoring the study there are no special compensation arrangements for this study. The NHS Litigation Authority covers standard clinical negligence of NHS employees, staff and health professionals under its Clinical Negligence Scheme for Trusts.

For multicentre studies each participating site is responsible for ensuring insurance and indemnity arrangements are in place to cover the liability of the Principal Investigator.

11. PUBLICATION POLICY

Data arising from the study are owned by the Sponsor. Findings will be submitted for publication in relevant H&N or Cancer peer reviewed journals. Funders will be acknowledged in any subsequent reports. Authorship will be granted in line with criteria defined by The International Committee of Medical Journal Editors.

12. REFERENCES

1. *https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/head-and-neck-cancers.*
2. *Bhide S, L.J., IGarcia-Murillas I, Cutts R, Hurley T, Grove L, Nutting CM, Newbold K, Turner, Harrington KJ, Predicting response to radical (chemo)radiotherapy (R-CRT) with circulating HPV DNA and tumor DNA (ctDNA) analysis in locally-advanced head and neck squamous cell carcinoma (LAHNC). J Clin Oncol 2017. 35 suppl; : p. abstr 6043).*
3. *Lee, J.Y., et al., Predicting response to radical (chemo)radiotherapy with circulating HPV DNA in locally advanced head and neck squamous carcinoma. Br J Cancer, 2017. 117(6): p. 876-883.*
4. *Lam, W.K.J., et al., Sequencing-based counting and size profiling of plasma Epstein-Barr virus DNA enhance population screening of nasopharyngeal carcinoma. Proc Natl Acad Sci U S A, 2018. 115(22): p. E5115-E5124.*