THE RECUT PLUS STUDY

Exploratory study of clonal evolution in cancer for patients undergoing transoral **R**obotic surgery for radiation **E**xposed residual/re**C**urrent tumours of the **U**pper aerodigestive **T**ract.

Protocol version:	1.1
Date:	18th March 2020
Sponsor:	The Royal Marsden NHS foundation Trust Fulham Road, London SW3 6JJ
CCR number:	5263
IRAS number:	280262
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Clinical Research Fellow:	Mr John Hardman Specialty Registrar in Otorhinolaryngology, The Institute of Cancer Research, London The Royal Marsden Hospital, London
Molecular analysis lead:	Dr Ben O'Leary NIHR Clinical Lecturer The Institute of Cancer Research, London The Royal Marsden Hospital, London
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1 VERSION HISTORY

Protocol version no.	Date	Version
1.0	2020/02/12	Prepared for submission to the Committee for Clinical Research
1.1	2020/03/18	Following CCR feedback

2 PROJECT SUMMARY

Study Title	Exploratory study of clonal evolution in cancer for patients undergoing transoral **R**obotic surgery for radiation **E**xposed residual/re**C**urrent tumours of the **U**pper aerodigestive **T**ract.
Short Title	The RECUT Plus Study
Study Design	RECUT Plus is an exploratory molecular analysis study to assess the selective impact of radiation therapy on H&N SCC.
Study Participants	Patients undergoing transoral robotic surgery for radiation exposed residual/recurrent/new primary tumours of the upper aerodigestive tract.
Planned Sample Size	Up to 20 participants.
Follow Up Duration	Up to 5 years clinical follow up for oncological outcomes.
Planned Trial Period	Prospective identification of patients will take place over 12 months.
Primary objective	Assess the molecular makeup of head and neck tumours that have not responded to radiotherapy treatment, or which recur having previously responded.
Secondary objectives	1. Compare the sub clonal architecture of HNSCC before and after radiotherapy. 2. Compare mutational signatures between the primary disease and resistant sub clones. 3. Assess resistant sub clones for loss of heterozygosity (LOH) at the HLA loci. 4. Report the oncological status of cases at 5 years.
Methods	• This is an exploratory molecular analysis study. • Retrospective and prospective participants will be identified from screening historic and future H&N MDT lists at RMH. • Following informed consent, germline DNA data will be obtained from blood, saliva or buccal swabs. This will be compared to biopsy samples from the original primary cancer and microdissected samples from the radiation exposed residual/recurrent/new primary cancer removed using transoral robotic surgery. • DNA will be extracted and used to create exome libraries. • Variant calls and copy number will be used to infer the clonal architecture at each sampled site within the resected specimen. • The presence or absence of sub clones before treatment and after treatment will be compared and their mutation and copy number profile changes analysed. • Mutational signatures in resistant sub clones will be inferred and assessed to see if they differ between each other and from the dominant processes driving evolution in primary disease • The resistant sub clones will also be assessed for loss of heterozygosity (LOH) at the HLA loci. • Hospital records and/or GP records will be used to record incidence of recurrence and survival for up to 5 years following surgery.

3 FUNDING

Funder	Financial and Non-financial support given
BRC Uncommon Cancer theme	Awarded £20,015 on 07 April 2020 (ref: B089)
Oracle Cancer Trust	Application for £100,000 in progress

4 SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

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 investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly 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.

For and on behalf of the Study Sponsor:

Print Name: _____

Signature: _____

Date: _____

Chief Investigator:

Print Name: Vinidh Paleri

Signature: _____

Date: _____

5 RATIONALE AND BACKGROUND INFORMATION

Radical radiotherapy for head and neck squamous cell cancer (HNSCC) can be curative, but when relapse does occur it is often within the radiotherapy-treated area.[1] It is unknown whether HNSCC cancer relapse after curative radiotherapy reflects primary resistance or evolution of resistance on treatment. Understanding this could open new avenues for improving the efficacy of radical radiotherapy and tailor the systemic treatments given after tumour relapse.

The Cancer Genome Atlas has begun to shed light on the genomic landscape of head and neck cancer[2] but little work has been done to investigate the impact of molecular characteristics on treatment with radiotherapy. Intra-tumoural heterogeneity has been demonstrated in a number of malignancies, with an early analysis of the head and neck TCGA data[2] suggesting higher levels of intra-tumoural heterogeneity may associate with poorer clinical outcome.[3] This invites the hypothesis that pre-existing sub clonal diversity may drive radiotherapy resistance.

Although many patients with head and neck cancer can be offered potentially curative treatment, we do not understand why some people are cured and others are not. There has been an explosion in genomics research in many cancers, but this remains an understudied area in head and neck cancer. There are currently no treatments available to patients with head and neck cancer based on genomic insights. This study begins to address the unmet need of patients who are failed by radical treatment and provides a starting point for more detailed investigations into the relationships between cancer genomics and clinical outcomes.

6 STUDY GOAL AND OBJECTIVES

6.1 Goal

To establish the clonal architecture and evolution of H&N cancers occurring in previously irradiated fields.

6.2 Primary objective

Assess the molecular makeup of head and neck tumours that have not responded to radiotherapy treatment, or which recur having previously responded.

6.3 Secondary objectives

1. Compare the sub clonal architecture of HNSCC before and after radiotherapy.
2. Compare mutational signatures between the primary disease and resistant sub clones.
3. Assess resistant sub clones for loss of heterozygosity (LOH) at the HLA loci.
4. Report the oncological status of cases at 5 years.

7 STUDY DESIGN AND SETTING

7.1 Study design

RECUT Plus is an exploratory molecular analysis study to assess the selective impact of radiation therapy on HNSCC.

7.2 Study setting

Participants will be recruited from the Royal Marsden Hospital (RMH), Chelsea, a tertiary referral H&N cancer unit in London, UK, specialising in transoral robotic surgery.

8 PARTICIPANT ELIGIBILITY CRITERIA

8.1 Inclusion criteria

- Aged over 18
- Previous H&N cancer treated with radiotherapy.
- Undergoing TORS as part of their management for residual, recurrent or new primary H&N cancer.

8.2 Exclusion criteria

- Where TORS is used in a diagnostic setting only
- Nasopharyngeal and thyroid head and neck cancers
- Where no tissue specimens are available from the recurrent/residual/secondary tumour for the retrospective cohort

9 STUDY PROCEDURES AND METHODOLOGY

9.1 Population

Patients undergoing transoral robotic surgery for radiation exposed residual/recurrent/new primary tumours of the upper aerodigestive tract.

9.2 Patient identification

For prospective participants, the RECUT Plus study team will screen the weekly H&N MDT meetings at the Royal Marsden Hospital (RMH).

For retrospective participants, the RECUT Plus study team will screen previous MDT lists for consecutive patients treated with transoral robotic surgery since the programme was established at RMH in September 2017.

9.3 Sampling timeframe

No limitation will be placed on the recruitment window. See procedures.

9.4 Consent

9.4.1.1 Prospective

Prospective participants will be informed of the RECUT Plus study by a member of their usual care team at RMH during their routine outpatient appointments for follow up of their H&N cancer. Participants will be provided with the **Participant Information Sheet** and copy of the **Informed Consent Form**. If they express interest in taking part, then consent will be obtained by a member of the RECUT Plus team, or an appropriately trained delegate, detailed in the Delegation Log.

9.4.1.2 Retrospective

Where patients have returned to their referring institution and are no longer under active regular follow up at RMH, the patient's GP will be contacted in order to obtain the patient's status. If the patient is alive and well, a research pack will be sent to the patient via post and email. This pack will contain the **Cover Letter**, the **Participant Information Sheet** and the **Informed Consent Form**. It will also contain a saliva collection tube and buccal swab. If there has been no response after 3 weeks, a second pack will be sent out to allow for postal losses. If there has been no response after a further 3 weeks, a final attempt to contact the patient will be made via telephone.

9.5 Follow up

The electronic patient record will be used to follow up patients for up to 5 years from the date of their robotic surgery. Where patients are no longer under active regular follow up at RMH, we will contact their GP to ask about disease recurrence and survival.

9.6 Pseudonymisation

9.6.1.1 Clinical data

We will be collecting identifiable data as part of the RECUT Plus 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.

9.6.1.2 Molecular data

Tissue specimens will be identified only using the RECUT Plus Study ID. As such, no patient identifiable data will be processed outside of RMH or by the ICR laboratories.

9.7 Dataset

9.7.1.1 Clinical data

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 and co-morbidities
2. **First cancer:** Involved sites, staging, procedures and adjuvant treatment
3. **TORS for recurrence:** Involved sites, staging, procedures, complications and adjuvant treatment
4. **Post-operative histology:** Staging and margins
5. **Outcome:** Survival, recurrence, follow-up

9.7.2 Co-morbidities

The co-morbidity score will be taken using the Adult Co-morbidity Evaluation-27 (ACE-27) index, which is validated for use in H&N cancer patients[4,5]. This rates the grade of decompensation affecting the various body systems, as well as presence of obesity and the status of any cancer or substance abuse. The grade of decompensation ranges between none (Grade 0) through mild, moderate and severe decompensation (Grades 1 to 3). The result is an Overall Comorbidity Score of 0 to 3, taken as the highest grade of decompensation in any one system/category, unless the patient scores grade 2 in two more different systems/categories, in which case they are designated Overall Score 3.

9.7.3 Classification of 'recurrent' disease

The following timeframes will be used to classify tumours treated after management of the original primary:

<12 months:	Residual disease
≥12 months, <5 years:	Recurrent disease
<5 years:	New primary disease, separate site
≥5 years	New primary disease

9.8 Specimens

9.8.1 Germline and circulating DNA samples

Patients will be asked to provide blood, saliva and/or buccal swabs. This will be used to perform germline DNA analysis. Prospectively recruited participants will be asked to provide samples pre and post operatively within a 2-week timeframe of their date of surgery. Retrospectively recruited participants will be able to provide a pre-operative sample but will be asked to provide a sample at the time of recruitment.

9.8.2 Tumour samples

All tumour specimens in this study will have been taken as part of standard care. No additional biopsies or specimens will be taken for the purpose of this study. Molecular analysis will be performed on the biopsy specimens from the original presentation of the cancer and on the resected cancer which had occurred in the irradiated field. Fresh frozen and formalin fixed paraffin embedded samples will be collected after the tumour has been resected for the prospective participants and formalin fixed paraffin embedded specimens alone will be analysed for the retrospective participants.

9.8.3 Storage, labelling and postage of blood, saliva, buccal swab and tissue samples

All RECUT Plus blood, saliva and buccal swab samples and tissue samples should be collected, labelled, stored and shipped as detailed in the RECUT Plus 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.

9.8.4 Tissue Blocks

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

Dr Ben O'Leary
2S8
Chester Beatty Labs
The Institute of Cancer Research
237 Fulham Road, London SW3 6JB

9.9 Withdrawal criteria

Patients may withdrawal 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 reason:

Patient decision
Lost to follow-up
Death
Pregnancy
Protocol violation
Ineligibility
Patient noncompliance
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 RECUT Plus their blood, saliva and buccal swabs samples will be destroyed and tissue samples returned to the site for archiving. Data collected at the point of withdrawal will continue to be used by the study team.

10 STATISTICS AND DATA ANALYSIS

10.1 Sample size

This is an exploratory study and as such will not be powered prospectively. Funding for consumables will limit the RECUT Plus sub-study to up to 20 patients initially

10.2 Study duration

The study will continue until up to 20 patients have been recruited.

10.3 Statistical analysis methods

10.3.1 Clinical data analysis

Descriptive analysis only. No statistical analysis of the clinical data will be performed in this exploratory study.

10.3.2 Molecular data analysis

Detailed description of the molecular analysis methodology is beyond the scope of this protocol.

11 DATA MANAGEMENT

11.1 Data collection tools and source document identification

11.1.1 Source documents

The electronic patient record will form the principal source for clinical data. Data may also be entered directly onto the Excel Data Tool (combined eCRF and database) form where this information would not be collected as part of standard care. As such the Excel Data Tool (combined eCRF and database) may be a source document also.

11.1.2 Excel Data Tool (combined eCRF and database)

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 surgery research team. In accordance with local data governance regulations for patient identifiable data.

11.2 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 allow identification of all the data reported for each participant and for pseudonymisation for the molecular analysis.

Professor Vinidh Paleri, the Chief Investigator, will be the custodian of the data submitted to the Royal Marsden Hospital.

The chief investigator 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.

11.3 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.

11.4 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, including the CRFs, 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.

12 ETHICAL CONSIDERATIONS

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

The RECUT Plus Study is sponsored by The Royal Marsden NHS Foundation Trust and has been 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.

12.1 Trial Management Group

A Trial Management Group (TMG) will be set up and membership will include Chief Investigator, Co-Investigators and Senior Trial Manager. Principal Investigators and other key study personnel 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.

13 DISSEMINATION 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.

14 KEY STUDY CONTACTS

Name	Vinidh Paleri
Role(s)	Chief investigator Primary academic supervisor to John Hardman
Responsibilities	Including, not limited to: Oversight of project design, conduct and reporting. Liaison with Research Ethics Committee (REC), and other review bodies, during the application process, and where necessary during, the conduct of the research. Ensure adherence to protocol. Analysis and write up.

Name	Kevin Harrington
Role(s)	Molecular analysis supervisor Backup academic supervisor to John Hardman
Responsibilities	Including, not limited to: Oversight of project design, conduct and reporting. Analysis and write up.

Name	John Hardman
Role(s)	Co-investigator Clinical research fellow
Responsibilities	Including, not limited to: Recruitment of patients. Coordination of data governance and control of the Excel Data Tool (combined eCRF and database). Analysis and write up.

Name	Ben O'Leary
Role(s)	Molecular analysis lead Associate academic supervisor to John Hardman
Responsibilities	Including, not limited to: Project design. Recruitment of patients. Molecular analysis. Analysis and write up.

This study will contribute towards the pursuit of an MD(Res) at the Institute for Cancer Research by John Hardman, the Clinical Research Fellow. Some of the data collected from this study may be used in the relevant thesis, prior to ultimate submission for publication or presentation.

15 TABLE OF ACRONYMS

Acronym	Meaning
ACE-27	Adult Co-morbidity Evaluation-27
ASA	American Society of Anesthesiologists
CCR	Committee for Clinical Research (Royal Marsden Hospital)
CT	Computed tomography
eCRF	Electronic case report form
H&N	Head and neck
HLA	Human Leukocyte Antigen
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
HRA	Health Research Authority
IRAS	Integrated Research Application System
KM	Kaplan Meier
LOC	Loss of heterozygosity
MDADI	M. D. Anderson Dysphagia Inventory
MDT	Multidisciplinary team
MRI	Magnetic resonance imaging
PET CT	Positron emission tomography and computed tomography
PSS-HN	Performance status scale for head and neck cancer patients
RMH	Royal Marsden Hospital
SCC	Squamous cell carcinoma
TORS	Transoral robotic surgery

16 REFERENCES

1. Mehanna H, Kong A, Ahmed S. Recurrent head and neck cancer: United Kingdom National Multidisciplinary Guidelines. The Journal of Laryngology & Otology 2016;130(S2):S181–90.

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3. Mroz EA, Tward AM, Hammon RJ, Ren Y, Rocco JW. Intra-tumor Genetic Heterogeneity and Mortality in Head and Neck Cancer: Analysis of Data from The Cancer Genome Atlas. PLOS Medicine 2015;12(2):e1001786.

4. Piccirillo JF. Importance of comorbidity in head and neck cancer. Laryngoscope 2000;110(4):593–602.

5. Paleri V, Wight RG. Applicability of the adult comorbidity evaluation - 27 and the Charlson indexes to assess comorbidity by notes extraction in a cohort of United Kingdom patients with head and neck cancer: a retrospective study. J Laryngol Otol 2002;116(3):200–5.