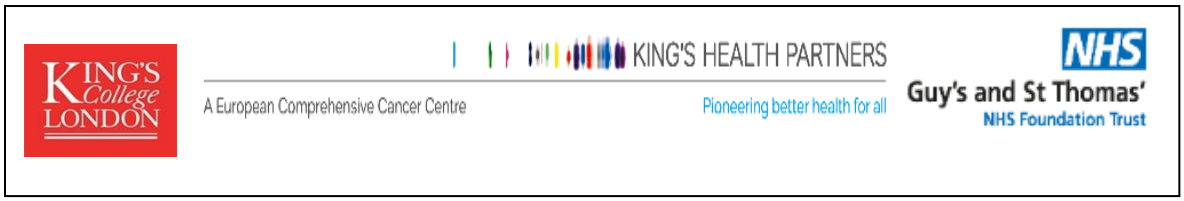

1 Protocol details

1.1 Title

SOTO study: Prospective study to correlate the treatment Sensitivity of patient-derived Organoids with Treatment Outcomes in head and neck cancer patients

Short title - SOTO: treatment Sensitivity of Organoids to predict Treatment Outcome

1.2 Names (titles), roles and contact details of:

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1.3 Protocol details

Sponsor's/Protocol number: 305689
Protocol Version number 1.0
Date: 14/03/2022

2 Signature Page

The Chief Investigator and the R&D (sponsor office) have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol

The investigator agrees to conduct the trial in compliance with the approved protocol, EU GCP, the UK Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework (2005' 2nd Edition; as amended), the Sponsor's SOPs, and other regulatory requirements as amended.

Chief investigator

[Insert name of CI]

Signature Date

Sponsor Representative

R&D to Add
GSTFT

Signature Date

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4 List of Abbreviations and Definitions

(Delete as appropriate)

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
MA	Marketing Authorisation
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
Participant	An individual who takes part in a clinical trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

5 Summary/Synopsis

Trial Title	Prospective study to correlate the treatment sensitivity of patient-derived organoids with clinical outcomes in head and neck squamous cell carcinoma (HNSCC) patients	
Internal ref. no. (or short title)	SOTO: treatment Sensitivity of Organoids to predict Treatment Outcome in head and neck cancer patients	
Protocol Version number and Date	Version 1.1 14th March 2022	
Study Phase if not mentioned in title	NA. It is an observation study	
IRAS Number	305689	
REC Reference	22/NW/0023	
Study Duration	1 year of recruitment followed by standard of care follow-up for 5 years	
Sponsor name	King's College London Guy's and St. Thomas Foundation NHS trust (co-sponsor)	
Chief Investigator	Dr. Anthony Kong	
Funder	Guy's and St Thomas' (GSTT) charity via a generous philanthropic donation from Charles Wilson and Rowena Olegario	
Medical condition or disease under investigation	Head and neck squamous cell carcinoma (HNSCC)	
	Objectives	Endpoints
Primary	To assess the success rate of generating organoids from tissues in patients with HNSCC	Rates of successful organoid cultures
Secondary	1) To assess the sensitivity of radiotherapy, cisplatin chemotherapy or cetuximab or their combination in PDOs 2) To correlate the treatment sensitivities of PDOs above with the treatment outcome	The ability of these treatment(s) to decrease tumour growth and kill the organoids ex-vivo (number and size of alive organoids after treatments) To correlate the IC50 doses and dose response

	undergoing **primary surgery** and **adjuvant radiotherapy** +/- concurrent platinum chemotherapy (**Cohort 1**)	curves above with the **recurrence rates and disease-free survival** of patients
	3) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients undergoing **primary radiotherapy** +/- platinum chemotherapy (**Cohort 2**)	The ability of the organoids to predict complete metabolic response and treatment outcome of patients
	4) To correlate the sensitivities of platinum chemotherapy and/or cetuximab of PDOs or anti-PD1 with the treatment outcome of **recurrent or metastatic** HNSCC patients (**Cohort 3**)	The ability of the organoids to predict response to the same systemic treatments that the patients are receiving systemic treatments
Number of Subjects/Patients	20 patients for year 1 pilot study	
Study Type	Observational	
Main Inclusion Criteria	- Patients with head and neck squamous cell carcinoma undergoing curative treatment (primary surgery or radiotherapy) or presenting with recurrent or metastatic cancers - Age > 18 years old	
Statistical Methodology and Analysis	This is a prospective study with a one-year pilot study aiming to recruit 20 patients, which is the realistic target for year 1. The results from year 1 will be used to estimate the sample size for future prospective observation study	
Human Tissue Samples (if applicable)	We will generate organoids from fresh tumour tissues and normal tissue when applicable and also stored unused biological samples in head and neck biobank for future translational research	
Data collected/storage (if applicable)	Patients' demographics, smoking and alcohol history, tumour characteristics, treatment details and responses, recurrences and survivals will be obtained from clinical	

	record and recorded on paper Case Report Forms (CRFs).

6 Introduction

Background

Head and neck cancers are a group of heterogeneous diseases and the most common histology being squamous cell carcinoma (1). Head and neck squamous cell carcinomas (HNSCC) tend to be associated with smoking and alcohol intake although there has been increasing incidence of oropharyngeal cancer due to HPV infection (2). Localized HNSCCs are usually treated with combined treatment modalities including surgery, radiotherapy and chemotherapy. HPV positive oropharygeal patients respond better to chemoradiation compared to HPV negative, tobacco-induced oropharyngeal cancer patients (2). These patients also have better 3-year rates of overall survival (82.4%, vs. 57.1%) compared with patients with HPV-negative tumours (3). Apart from HPV status, there is no accurate way of predicting which patients will be cured from radical treatment although patients with tumours which are resistant to radiotherapy +/- platinum cisplatin chemotherapy tend to present with locoregional recurrence and have a poorer outcome. There is no easy way to test radiosensitivity and chemosensitivity of these tumours. There is a need, therefore, to develop an accurate test to evaluate the sensitivity ex-vivo so that we can predict which patients are likely to be resistant to radiotherapy or platinum chemotherapy and therefore more likely to relapse. If so, treatment interventions such as radiation dose escalation, use of radiosensitising agents and/or other treatment strategies could be implemented.

Rationale of the study

Up to 50% of HNSCC patients may recur and the prognosis of these patients is less than one year. The first-line treatment for these patients is a platinum-based chemotherapy with cetuximab (4), which is toxic and only benefit a small number of patients. Although immunotherapy has shown great promise in recurrent or metastatic HNSCC as first-line or second-line treatments, only around 15-20% patients respond to these therapies with durable responses (5-6). There is therefore a need to ensure more effective radical curative treatment to prevent recurrences and to develop more effective targeted therapies for recurrent/metastatic HNSCC patients based on their genomic profiles.

Previously most of the preclinical models were largely based on immortalized cancer cell lines and xenografts studies from transformed cell lines. These techniques have been important in elucidating the mechanisms of late-stage tumour development as well as studying drug resistance. However, large-scale drug screens of transformed cell line panels have resulted in a high failure rate of preclinical compounds in clinical trials, which demonstrates the limitations of using these existing preclinical models based on transformed cell models. To this end, living biobanks consisting of tumour organoids, which facilitates the integration of genomic data with drug screening of patients' tumour samples to identify effective therapeutic regimens, have been established in a few tumour types including colorectal cancers, gastric cancers, liver cancers, breast cancers and more recently head and neck squamous cell carcinomas (7-10). The use of organoids in conjunction with genomic analysis from patient-derived tumour samples has the potential to stratify and identify effective

cancer therapies for individual patients by (a) comparing the response of individual tumours to specific drugs in order to provide personalized recommendations to manage patient care; (b) assessing how tumours respond and develop resistance to in order to further understand the mechanisms; (c) determining the next drug treatment option when standard clinical options are not available or not considered effective; (d) creating a database of drug sensitivity to tumour genetics to recommend potential therapeutic strategies.

In this study, we aim to assess whether PDOs can be used to predict treatment sensitivity in HNSCC patients.

7 Trial objectives and purpose

Overall aim
This study aims to generate patient-derived organoids (PDOs) from patients' tumour samples and to collect preliminary data on the ability of PDOs to predict patients' treatment response and whether their radiosensitivity and chemosensitivity can be correlated with their survival outcome.

Patient's cohorts
We will have three cohorts of HNSCC patients undergoing standard of care treatments:
1) Surgically resectable disease, followed by adjuvant radiotherapy +/- chemotherapy
2) Primary radiotherapy +/- concurrent chemotherapy
3) Recurrent or metastatic HNSCC undergoing platinum based chemotherapy +/- cetuximab

Primary objective
To assess the percentage of successful generated organoids from tissues in head and neck cancer patients

Secondary and exploratory objectives

Secondary objectives:
1) To assess the sensitivity of radiotherapy, platinum (cisplatin and/or carboplatin) chemotherapy or cetuximab or their combination in PDOs
2) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients undergoing *primary surgery and adjuvant radiotherapy* +/- concurrent platinum chemotherapy (*cohort 1*)
3) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients undergoing *primary radiotherapy* +/- platinum chemotherapy (*cohort 2*)
4) To correlate the sensitivities of platinum chemotherapy and/or cetuximab or immunotherapy of PDOs with the treatment outcome of *recurrent or metastatic* HNSCC patients (*cohort 3*)

Exploratory objectives:
1) To correlate the treatment sensitivities of PDOs with the detection rates of plasma ctDNA (all cohorts)

2) To assess and correlate the histopathological, genomic and transcriptomic features of patients' samples with PDOs
3) To assess the sensitivities of PDOs to various targeted therapies based on their genomic profiles
4) Collection of archival tissues, blood samples (whole or processed for ctDNA and PBMC), saliva (for ctDNA), urine or stool samples for other translational research

8 Study design & Flowchart

8.1 Study Design

This is a prospective observation study to generate patient-derived organoids from patients' samples to assess treatment response and to correlate with patients' treatment outcomes. We would first do a pilot study during year 1 to establish the pathways required and to assess the success rate in generating patient-derived organoids. During this time, we will also assess the willingness of patients to participate in the study, as well as that of clinicians to help recruitment. In addition, the time required to generate HNSCC organoids and to assess treatment response in order to correlate with patients' outcome will be evaluated during year 1.

Primary endpoint and outcome

Primary endpoint
The percentage of successful organoid samples generated from the tumour samples obtained

Primary outcome
The ability to generate organoid cultures successfully from tumour samples

Secondary endpoint and outcomes

Secondary endpoints
1) To determine the IC50 doses and dose response curves of radiotherapy, cisplatin or carboplatin chemotherapy and their combination of PDOs
2) To correlate the IC50 doses and dose response curves above with the recurrence rates and disease-free survival of patients
3) To correlate IC50 doses and dose response curves above with the complete metabolic response (PET-CT) and residual disease or salvage neck dissection
4) To correlate IC50 doses/dose response curves above with the objective response rates of patients undergoing same systemic treatment

Secondary outcomes
1) The ability of these treatment(s) to decrease tumour growth and kill the organoids ex-vivo (number and size of alive organoids after treatments)
2) The ability of the PDOs to predict recurrences and survival outcome of these patients based on the radiosensitivity and chemosensitivity of the PDOs
3) The ability of the organoids to predict complete metabolic response and treatment outcome of patients
4) The ability of the organoids to predict response to the same systemic treatments that the patients are receiving systemic treatments

Exploratory endpoints and outcomes

Exploratory endpoints

1) To correlate the IC50 doses/dose response curves with plasma ctDNA before and after treatment
2) Immunohistochemistry (IHC) staining, exome sequencing and RNA sequencing of PDOs and patients' samples
3) The IC50 doses of various targeted therapies based on actionable genetic mutations
4) To conduct further translational research using the patients' samples

Exploratory outcomes

1) The ability of the PDOs to predict minimal residual disease and persistent disease
2) The ability of the PDOs to recapitulate the histopathological and genomic features of human samples
3) The ability of the PDOs to predict responses to various targeted therapies based on genomic profiles
4) The ability of the PDOs to facilitate high quality translational research that will help to predict treatment response and bring benefit to patients

Table of endpoints/measures and outcomes

Objectives	Endpoints/Measures	Outcomes
PRIMARY 1) Compliance and acceptability of tissue sampling for the generation of organoids in head and neck cancer patients	Percentage of approached participants who consent to taking part	Rates of participants who agree to have samples taken to generate PDOs
2) Ability to generate organoids from patients' tissues	Percentage of individuals whose successful organoid samples are obtained	Rates of successful organoid cultures
SECONDARY 1) To assess the sensitivity of radiotherapy, cisplatin chemotherapy or cetuximab or their combination in PDOs	Determine IC50 doses and dose response curves of radiotherapy, cisplatin chemotherapy and their combination of PDOs	The ability of these treatment(s) to decrease tumour growth and kill the organoids ex-vivo measured by the number and size of alive organoids after treatments.
2) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients undergoing **primary surgery** and **adjuvant radiotherapy** +/- concurrent platinum chemotherapy (**Cohort 1**)	To correlate the IC50 doses and dose response curves above with the **recurrence rates and disease-free survival** of patients	The ability of the PDOs to predict recurrences and survival outcome of these patients based on the radiosensitivity and chemosensitivity of the PDOs
	Correlating IC50 doses and	The ability of the organoids to

3) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients undergoing **primary radiotherapy** +/- platinum chemotherapy (**Cohort 2**) 4) To correlate the sensitivities of platinum chemotherapy and/or cetuximab or immunotherapy of PDOs with the treatment outcome of **recurrent or metastatic** HNSCC patients (**Cohort 3**)	dose response curves above with the **complete metabolic response** (PET-CT) and residual disease or salvage neck dissection Correlating IC50 doses/dose response curves above with the objective response rates of patients undergoing same systemic treatment	predict complete metabolic response and treatment outcome of patients The ability of the organoids to predict response to the same systemic treatments that the patients are receiving systemic treatments
EXPLORATORY 1) To correlate the treatment sensitivities of PDOs with the detection rates of plasma ctDNA (all cohorts)	To correlate the IC50 doses/dose response curves with plasma ctDNA before and after treatment	The ability of the PDOs to predict minimal residual disease and persistent disease
2) To assess and correlate the histopathological, genomic and transcriptomic features of patients' samples with PDOs	IHC staining, exome sequencing and RNA sequencing of PDOs and patients' samples	The ability of the PDOs to recapitulate the histo-pathological and genomic features of human samples
3) To assess the sensitivities of PDOs to various targeted therapies based on their genomic profiles	The IC50 doses of various targeted therapies based on actionable genetic mutations	The ability of the PDOs to predict responses to various targeted therapies based on genomic profiles
4) Collection of archival tissues, blood (whole or processed for ctDNA and PBMC) +/- saliva (for ctDNA) as well as mouth swab/stool samples for other translational research	To conduct various translational research including TMB, PDL1 test, co-culturing of PDOs with PBMC to assess immunotherapy response	The ability of the PDOs to facilitate high quality translational research that will help to predict treatment response and bring benefit to patients

8.2 Treatment visits

This is an observation study and the patients will continue their clinical visits and follow-up for 5 years as per normal standard of care. We will obtain the treatment outcome from medical records of patients.

9 Subject selection

This will be a single centre study performed at King's College London and Guy's and St Thomas' NHS foundation trust.

We will have several cohorts of HNSCC patients undergoing standard of care treatments, which we plan to include:

1) 10 patients with surgically resectable locally advanced disease, followed by adjuvant radiotherapy +/- chemotherapy

2) 5 primary radiotherapy +/- concurrent chemotherapy

3) 5 recurrent or metastatic HNSCC undergoing platinum based chemotherapy +/- cetuximab or immunotherapy.

9.1 Subject inclusion criteria

1) Patients with head and neck squamous cell carcinoma (including oral cavity, oropharynx, paranasal sinuses, hypopharynx or larynx) undergoing curative treatment (primary surgery or radiotherapy) or presenting with recurrent or metastatic cancers

2) Age > 18 years old

In addition, there are specific criteria for the three cohorts:

- **Cohort 1:**

  Patients with surgically resectable locally advanced disease who will likely to undergo adjuvant radiotherapy +/- concurrent chemotherapy

- **Cohort 2:**

  Patients suitable for primary radical radiotherapy +/- concurrent chemotherapy who agree to have additional research biopsy

- **Cohort 3:**

  Patients with recurrent or metastatic disease who agree to have additional research biopsy

9.2 Subject exclusion criteria

1) Patients unable to give informed consent e.g. mental disability or vulnerable adults

10 Study procedures

10.1 Subject recruitment

Method of recruitment

The potential participants will be identified from MDT, surgical and oncology clinics and the initial approach to the potential participants will only be made by the direct care team members including those from surgical, oncology and radiology teams. HNSCC patients suspected to have primary or

recurrent HNSCC will be asked to consent to an additional research biopsy while undergoing an Examination Under Anaethesia (EUA) or biopsy or transoral robotic resection or biopsy as standard of care. This additional research biopsy will be used to generate PDOs in the lab. For those surgical resectable patients, we may ask for additional resected sample once adequate sample have been obtained for routine diagnostic purposes. For these patients, the resectable samples will be used to obtain more PDOs regardless of whether the biopsy samples were successful in generating PDOs. Moreover, for the surgical patients we will request specimens from the normal surrounding resected tissue to create normal tissue organoids to be used as the control. Once the PDOs are successfully generated in the lab, they will be tested for sensitivities to various treatments including radiotherapy, cisplatin/carboplatin and their combination for those organoids generated from HNSCC patients undergoing curative radical treatment and also platinum based chemotherapy +/- cetuximab or immunotherapy for those organoids generated from recurrent or metastatic HNSCC patients.

When the PDOs contain sufficient cells, these cells will be treated with increasing doses of radiotherapy and/or cisplatin chemotherapy and/or cetuximab in order to determine the IC50 and to obtain dose-response curves of these organoids to the treatments. In particular, we will treat the PDOs with the same treatments that the HNSCC patients receive in order to correlate the responses to radiotherapy and/or platinum chemotherapy +/- cetuximab or immunotherapy (co-cultured with immune cells). Successfully generated tumour organoids will be stored in the lab and can be used for future research.

The response of these HNSCC-PDOs will be compared to the actual tumour response to these treatments in these HNSCC patients:

1) Cohort 1: surgically resectable locally advanced disease
The tumours of these patients would have been surgically resected and so the responses of these organoids to radiotherapy and chemotherapy cannot be directly assessed. Most of these patients may then undergo adjuvant radiotherapy +/- concurrent chemotherapy. It is anticipated that the radiosensitivity and platinum chemosensitivity may correlate with the outcome of these patients. We will therefore assess whether those PDOs with lower IC50 would have longer disease-free survivals. If PDOs could be derived from both biopsy and resected samples from these patients, we will compare the differences in responses of these PDOs generated from either biopsy or resected samples. We will use the normal tissue organoids from the periphery of the surgical specimen to confirm that response to the treatments is tumour specific.

2) Cohort 2: Primary radiotherapy +/- platinum chemotherapy
We will culture HNSCC-PDOs and treat with irradiation and the same platinum chemotherapy drugs (cisplatin or carboplatin) as the corresponding patient. A few of these patients may undergo neoadjuvant chemotherapy before definitive chemoradiation. In this case, we will assess the responses and sensitivities of these organoids to the same chemotherapy drugs, usually docetaxel, cisplatin and 5FU (TPF). For patients undergoing primary radiotherapy with or without concurrent chemoradiation, PET-CT scan is done at 3 months after the completion of treatment to assess metabolic response. We will correlate the treatment sensitivity of PDOs as determined by the IC50 and dose-response curves with the complete metabolic responses by PET-CT scan and the rates of residual disease as well as salvage neck dissection.

3)Cohort 3: recurrent or metastatic HNSCC
The first-line treatment for the recurrent or metastatic HNSCC patients had been platinum-based chemotherapy +/- 5FU chemotherapy with or without cetuximab (oral cavity SCC only).

Pembrolizumab monotherapy is now approved as a first-line treatment for HNSCC patients with PDL1 positive (Combine pathology score CPS $\geq$ 1) tumours in the UK. Patients with PDL1 negative tumours or those with rapidly progressing disease will continue to receive platinum based chemotherapy as first-line treatment. We will correlate the treatment sensitivities of PDOs to these drugs with the radiological responses from the HNSCC patients. In addition, we are currently running an EORTC study (AK is the UK chief PI) where HNSCC patients previously treated with platinum-based chemotherapy will be allocated to be treated with different targeted therapies or immunotherapy based on potential biomarkers and molecular alterations of their tumours (NCT03088059, UPSTREAM study). We will record whether the assessment of the genetic profiles of these PDOs may help to enrol these patients in the UPSTREAM study eventually.

Payment

The participants will not be paid for this study since patients will not be required to have additional visits other than their standard of care visits.

Consent

This observation study will be opened at Guy's and St Thomas' NHS foundation trust only. The Principal Investigator (PI) retains overall responsibility for the conduct of research at participating site(s) with delegation of duties to appropriate clinical sub-investigators (e.g. clinical fellow), which includes the taking of informed consent of participants at their site with the processes below:

- Checking the inclusion and the exclusion criteria
- A discussion with the potential participant about the research including the nature and objectives of this study and possible risks associated with their participation
- Patient information leaflet and consent form will be given to patients, usually at least 24 hours before consent although it can be on the same day for this observation study if required
- Potential participants will be given the opportunity to ask questions
- Assessment of the mental capacity for the participants to consent will be performed by either the PI or the appropriate clinical sub-investigators

The principal investigator will ensure that any person delegated the responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki.

Translational research: Participants will be asked to give generic consent for their samples, derived organoids and/or cell lines and linked data to be transferred and used in future research. This includes the transfer and use of samples/data to KCL and its partners including the commercial sector and overseas organisations (including genetic research and whole genome sequencing). This will include the depositing of genetic and whole genome sequencing data in international repositories and participant-level data where it would not be possible to have data subsequently deleted.

Based on this consent, archival tissue, blood, saliva, oral swab, urine and stool samples will be stored at Guy's and St Thomas' head and neck biobank beyond the ethics approval date of the project and stored under the custodial of Rhonda Hanley-Smith, biobank manager (HTA licence 12121; Licence Holder Cheryl Gillet). All material will be handled in accordance with the Human Tissue Act 2004 and other relevant legislation relating to the use of cell lines. Specific agreements will be put in place as required by the Sponsor including material transfer agreements where necessary.

Blood and other biological sample collection time points: We will ask patients to provide blood samples before (baseline) and at 3 months after the completion of their treatments. We may also request for up to additional 4 times during their treatment (e.g. at 3rd and 6th weeks of your radiotherapy) and follow-up (at 12 months after completion or at the event of recurrence), most likely at the same time that they are having blood tests as part of the standard procedures or occasionally as an additional procedure on each occasion, we will take up to 40ml of blood. We may also ask them to donate other samples such as saliva, oral swab, urine or faeces for future research at the same time points above.

Withdrawal of consent: The right of a participant to refuse participation without giving reasons will be respected. The participant will remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the trial. Patients will be made aware of the terms and conditions around withdrawal. If a patient withdraws from the study, the research team can retain any tissue samples, DNA/RNA samples and organoids/cell lines that have been created up until the time of the patient's withdrawal if the patient agrees. Any unused samples taken for research purpose can be destroyed if patients request to do so. Any demographic and medical information already provided or results from tests already performed on their samples will continue to be used in the study, however no further data or sample collection will be performed.

10.2 Screening Procedures

Potential eligible patients will be identified from surgical and/or oncology clinics and/or multi-disciplinary meetings. These patients will be checked to see whether they will meet inclusion criteria. No extra screening investigations are needed for this study since this is an observation study. Patients will undergo routine standard of care investigations and treatments apart from consenting to this study and for their samples to be used for translational research during their routine standard of care procedures.

10.3 Schedule of assessments for each visit

Patients will undergo assessments as per normal standard of care and the schedule will not be changed due to this study.

10.4 Follow up Procedures

The follow-up procedures will be as per normal standard of care for 5 years and the schedule will not be changed due to this study. We will obtain relevant clinical information from the medical records.

10.5 End of Study Definition

The patient recruitment for the initial pilot study will be one year from the commencement of the study at Guy's Hospital. We will assess whether the endpoints and outcomes are met at the end of the pilot study but the patients will be followed up for their long-term treatment outcome and survival as per normal standard of care. We will continue to obtain relevant information on patients' treatment and survival outcome from patients' medical records. The end of the study definition for the pilot study (year 1) is the end of 12 months' period after the commencement of the study regardless of the number of patients recruited. We will submit the protocol for a major amendment towards the end of year 1 so that SOTO study will continue as a prospective observation study for up to additional 4 years of recruitment if further funding is obtained. If no further funding is obtained

by the end of year 1, we will end the patient recruitment and the study. We will analyse all data and produce the final study report at the end of second year.

The criteria for progression beyond year 1 pilot study are set as below:
1) More than 50% of the approached participants will consent to taking part in SOTO study
2) At least 30% of the tissue samples will be successfully used to generate organoids
3) Further funding is obtained to continue beyond year 1

11 Laboratories

The fresh tissue samples will be provided by the head and neck biobank at Guy's Hospital to be processed for organoids within 72 hours at Dr. Kong's laboratory at Kings College London and the blood samples will be processed according to standard laboratory SOP at Dr. Kong's laboratory at Kings College London. Plasma and saliva samples will also be processed to assess ctDNA in the future. The additional biological samples including oral swab, urine or stool samples if obtained will be stored at Guy's Hospital head and neck biobank under the custodial of Rhonda Hanley-Smith, biobank manager (HTA licence is 12121; the Licence Holder is Cheryl Gillet). Further details on the sample collection, processing, storage and analysis will be provided in the laboratory manual.

11.1 Data Recording/Reporting

All staff that would directly deal with the human tissues or blood will undertake HTA training.

11.2 Sample Receipt/Chain of Custody/Accountability

Handling of the samples upon arrival at the laboratory needs to be documented. Upon receipt of the samples, the laboratory should ensure that the physical integrity of these samples have not been compromised in transit. If it has, it is important that the study teams, as well as the sponsor, are informed of this. Upon receipt of samples laboratory staff should ensure that all samples are accounted as per the labeling. All samples received should be logged in an accountability log.

11.3 Sample Transfer to sites outside the Organisation

Individual informed consent will be obtained from the patients to specify whether they would agree for their samples to be transferred to collaborating third parties including overseas and commercial laboratories on behalf of KCL. This may include but not be limited to validation of research results.

The patient samples and derived organoids (with linked data) may be shared with collaborating laboratories, nationally or internationally, for the purposes of facilitating the research aims. Specific agreements will be put in place as required by the Sponsor including material transfer agreements where necessary. Any commercialisation of the results of this study will be specified in contractual arrangements between parties where necessary and participants will be informed that they would not benefit financially.

12 Assessment of Safety

This is an observation study and the patients participated in this study will undergo routine treatments and the safety of the treatments will be assessed as per standard of care.

12.1 Ethics Reporting

No serious adverse events are expected to occur following the tissue sampling involved in this study, as they will be done during standard care procedures. Participants will be followed-up by their treating clinicians as per normal practice.

12.2 Ethics & Regulatory Approvals

Study protocol and other documentation will be submitted to the North West - Greater Manchester South Research Ethics Committee for ethical approval.

13 Compliance and withdrawal

13.1 Subject compliance

We will monitor the percentage of approached participants who consent to taking part in the study during the first year of recruitment.

13.2 Withdrawal / dropout of subjects

We will monitor the percentage of participants who withdraw consent from the study after taking part. Since the primary outcome is the rate of successful generated organoids and will not be affected by the dropouts of subjects, we will not need to recruit more participants to back fill those who may have withdrawn or dropped out once the fresh tissues are obtained from patients following consent.

13.3 Protocol Compliance

The patients will undergo routine treatments as per standard of care. We will monitor whether we will miss the blood samples from patients during specific time points.

14 Data

14.1 Data management

As this is an observation study, we will collect data from patients that are already available in the hospital record on patients' demographics, smoking and alcohol history, tumour characteristics including HPV status, proposed treatment plans. The patients' treatment details, treatment responses, recurrences and survivals will also be obtained from clinical record. They will be recorded on paper Case Report Forms (CRFs). Participant files and paper CRFs will be identified using a Participant Identification Number. Personal information will not be stored on CRFs. Screening and enrolment logs will be maintained by the research team. For enrolled participants, identifiable patient data will be stored in an Excel file for the purposes of gathering follow-up data. This file will password protected and stored on the KCL secured network, whereby access is only granted by the PI to named individuals. Paper CRFs, consent forms and the Trial Master File will be stored in locked filing cabinets within the Oral Clinical Research Unit (KCL, Guy's Hospital, London, UK). Data generated from laboratory processes related to the generation of PDOs and subsequent testing will

be kept in the research laboratory. These data will be identified with the PIN and no personal data will be used in the lab records.

Data linkage
If the participating patients have come from or will move to different health care providers, we will attempt to get either available archival tissues and/or data from the relevant health care providers after sending them the relevant patients' consent forms. We may also go to the central national databases with a patient's NHS number to get clinical data if necessary.

15 Statistical considerations

The first year of recruitment will serve as a pilot study for this observation study and no formal power calculation is performed for year 1. The number of patients chosen for each cohort is the expected number of patients that we anticipate to recruit within 12 months based on the number of HNSCC patients that we treat at Guy's. We expect more than 50% of the approached participants will consent to taking part in SOTO study and at least 30% of the tissue samples will be successfully used to generate organoids. A successful pilot study in year 1 will be defined as one that meets the pre-defined expectation. The data from year 1 pilot study will be used to calculate the sample size required to extend the observation study beyond year 1, which will be submitted as a major amendment.

16 Ethical considerations

16.1 Research Ethics Committee (REC) review & reports

Before the start of the trial, approval will be sought from an NHS REC for the trial protocol, informed consent forms, patient information sheet, and GP information letters.

Any amendments to the protocol or study documents will be reviewed by the Sponsor, sent to the REC for review and approvals. No changes will be implemented until approval has been received from the REC and approved by the Trust R&D department, if required.

All correspondence with the REC will be retained in the Trial Master File.

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The Chief Investigator's will approve the annual report which will be submitted by the CI or delegate.

The Chief Investigator will notify the REC of the end of the trial within 90 days of the end of the study. If the trial is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.

Within one year after the end of the trial, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

16.2 Peer review

This study has been previously peer reviewed when an application for was submitted to the GSTT charity for the funding of this study.

16.3 Regulatory Compliance

The trial will not commence until a Favourable REC opinion is obtained along with HRA approval and confirmation of capacity and capability from the GSTT R&D department.

All amendments to the protocol and study documents will be reviewed by the Sponsor and submitted for REC approval. Subsequently, documents and approvals will be sent to the Trust R&D department for confirmation of implementation.

17 Financing and Insurance

The study is co-sponsored by King's College London and Guys and St Thomas' NHS Foundation Trust. The sponsors will, at all times, maintain adequate insurance in relation to the study. King's College London through its' own professional indemnity (Clinical Trials) & no fault compensation and the Trust having a duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of negligence by its employees, brought by or on behalf of a study participant.

18 Reporting and dissemination

The results of this trial will be submitted for publication in peer-reviewed journals. The manuscripts will be prepared by the chief investigator and co-investigators. The authorship will be determined by mutual agreement. A copy of the publication will be provided to the participants if interested and requested.

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