Official Title	SOTO-BC: Prospective study to correlate the treatment Sensitivity of patient-derived Organoids with Treatment Outcomes in breast cancer patients with brain and/or extra-cranial metastases
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1. Protocol details

1.1 Protocol title

SOTO-BC: Prospective study to correlate the treatment Sensitivity of patient-derived Organoids with Treatment Outcomes in breast cancer patients with brain and/or extra-cranial metastases

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1.3 Protocol details

Version number 1.1
Final
Date: 23 May 2025

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 (2018), 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

Dr. Anthony Kong

Signature Date

3 Contents Page

4 List of Abbreviations and Definitions

APR	Annual Progress Report
BM	Brain metastasis
cfDNA	Circulating free DNA (deoxynucleic acid)
CI	Chief Investigator
CSF	Cerebrospinal fluid
CRF	Case Report Form
GCP	Good Clinical Practice
GP	General Practitioner
GSTT	Guy's and St-Thomas' Trust
HTA	Human Tissue Act
IC50	Half maximal Inhibitory Concentration
ICF	Informed Consent Form
KCL	King's College London
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PBMC	Peripheral blood mononuclear cell
PDO(s)	Patient derived organoid(s)
PI	Principal Investigator
PPI	Patient and public involvement
REC	Research Ethics Committee
SOP	Standard Operating Procedure
SRS	Stereotactic radiosurgery
TNBC	Triple negative breast cancer
WBRT	Whole brain radiotherapy

5 Summary/Synopsis

Trial Title	Prospective study to correlate the treatment sensitivity of patient-derived organoids with clinical outcomes in breast cancer patients with brain and/or extra-cranial metastases	
Internal ref. no. (or short title)	SOTO-BC: Sensitivity of Organoids to predict Treatment Outcome in Breast Cancer	
Protocol Version number and Date	version 1.1, 23 May 2025	
Study Phase if not mentioned in title	N/A. It is an observation study	
IRAS Number	315973	
REC Reference	24/NE/0022	
Study Duration	2.5 years (1.5 year of recruitment and 12 months' follow-up)	
Sponsor name	King's College London and Guy's & St Thomas' NHS Foundation Trust	
Chief Investigator	Dr. Anthony Kong, King's College London	
Co- investigator	Prof. Keyoumars Ashkan, King's College Hospital	
Funder	Guy's Cancer Charity	
Medical condition or disease under investigation	Breast cancer with brain and/or extra-cranial metastases	
	Objectives	Endpoints
Primary	The percentage of successful generated organoids from tissues in breast cancer patients with brain or extra-cranial metastases	Rates of successful organoid cultures
Secondary	1) To assess the sensitivity of radiotherapy in PDOs 2) To assess the sensitivity of the same systemic treatments that the patients previously had and will have in PDOs	1) To determine the IC50 doses and dose response curves of radiotherapy 2) To determine the IC50 doses and dose response curves of the previous systemic treatments that patients had and will have in PDOs

	3) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients 4) To correlate the treatment sensitivities of PDOs with the objective response rates of patients undergoing same systemic treatment	3) To correlate the IC50 doses and dose response curves above with the recurrence rates and progression-free survival of patients 4) To correlate IC50 doses/dose response curves above with the objective response rates of patients undergoing same systemic treatment
Number of Subjects/Patients	20 patients	
Study Type	Observational	
Main Inclusion Criteria	- Breast cancer patients with brain metastases who are suitable for surgical resection - Breast cancer patients with extra-cranial metastases undergoing surgical resection or biopsy - Age > 18 years old	
Statistical Methodology and Analysis	This is a pilot observation study, and the results will be used to estimate the sample size for future prospective observation study	
Human Tissue Samples (if applicable)	Tumour and normal tissues, blood, saliva, oral swabs, urine and stool	
Data collected/storage (if applicable)	Data on demographics, medical history, diagnosis, treatment plans and clinical outcomes	

6 Introduction

6.1 Background

Breast cancer is the most common cancer in the UK and nearly all breast-cancer deaths are related to metastasis rather than primary cancer. HER2 positive breast cancer and triple negative breast cancer (TNBC) can present with early recurrence and distant metastasis, whereas the luminal type of breast cancer can have a long dormancy before presenting with metastasis many years later. In addition, they can present with distinct metastasis behaviour (1). HER2-positive breast cancer can metastasize

to many organs including brain, lung, liver, bones and lymph nodes. In TNBC, brain metastasis could be the first site of distant spread in 26% patients or after dissemination to the lungs. And in luminal subtype, brain metastasis is not common and distant spread to the bones and lungs is more frequent (2-3). In a study of 222 breast cancer patients with brain metastases, the proportions of patients with HER2-positive, triple negative and luminal breast cancer were 53%, 28% and 19%, respectively (2), indicating that triple-negative and HER2-positive breast cancer have a special predisposition to brain metastases. The median survival for the entire group was 7.5 months and in HER2-positive, triple-negative and luminal subtypes, they were 9, 3.7 and 15 months, respectively. The prognosis of patients with untreated brain metastasis is extremely poor and whole-brain radiotherapy (WBRT) may increase median survival of these patients from 3-4 months to 6 months.

In patients with oligometastases in the brain and good performance status, surgical excision or stereotactic radiosurgery (SRS) with or without whole brain radiotherapy (WBRT) is the standard of care and the median survival may be up to 1–2 years for some of these patients (2-3). SRS delivers high dose radiation within a tumour while avoiding radiation to the surrounding healthy tissue, which includes a variety of different methods and systems such as Gamma knife, stereotactic Linear Accelerator (Linac) and the Cyberknife. The 1-year tumour control rates could range between 69% and 81% although lower local control rate of 60% is achieved in tumors larger than 2 cm (4). The objective response rate after SRS has been shown to be 38% in one study (5). However, the assessment of objective response after SRS is difficult and can be variable depending on the given SRS doses, tumour sizes, time points of radiological assessment and tumour types etc. Therefore, local tumour control rates (defined as absence of regrowth of a treated lesion) instead of objective responses are considered to be better measurement of the treatment outcome for SRS.

Surgical excision may be preferred for histological confirmation, single or large metastases, symptomatic mass, whereas SRS may be done for patients with smaller lesions without mass effect and if the tumours are located in surgically inaccessible regions such as brainstem (6). In an EORTC phase III trial, patients with one to three brain metastases of solid tumours were treated with either surgery or SRS and were randomly assigned to adjuvant WBRT (30 Gy in 10 fractions) or observation (OBS) (7). Although the addition of whole brain radiotherapy after surgical excision or SRS reduced intracranial relapses, it did not improve overall survival and may negatively impact on neurocognitive outcomes and quality of life (6-7). Thus, WBRT has been largely omitted after SRS or surgical resection at many centres. However, there is significantly higher local and distant brain relapse rate for patients who do not have WBRT (7). Without WBRT, the relapse rates for patients who had surgery & SRS without WBRT were 59% & 31% at initial sites, and 42% & 48% at new sites respectively, causing death due to intracranial progression in 44% of patients (7).

In a randomised phase 3 trial, patients who had a complete resection of one to three brain metastases were randomly assigned with to either SRS of the resection cavity (within 30 days of surgery) or observation. The 12-month freedom from local recurrence was 72% in the SRS group compared with 43% in the observation group and no adverse events or treatment-related deaths in either group (8). In another randomised phase 3 trial, patients with one resected brain metastasis were randomly

assigned to either postoperative SRS or WBRT with the co-primary endpoints to be cognitive-deterioration-free survival and overall survival (9). It was shown that cognitive deterioration-free survival was slightly longer in patients assigned to SRS compared to those assigned to WBRT, 3.7 months versus 3 months respectively and the cognitive deterioration at 6 months was more frequent in patients who received WBRT compared to those received SRS (85% versus 52% respectively). There was no difference in the median overall survival, 12.2 months for SRS versus 11.6 months for WBRT and the surgical bed median relapse-free survival (RFS) was similar for both groups, 7.5 months in SRS group versus 7.7 months for WBRT but SRS had inferior 6-month local control (p=0·00068) and shorter time to intracranial tumour progression (HR 2·45, 95% CI 1·62–3·72; p<0·0001) compared with WBRT (9). Although SRS to surgical cavity has been proposed by these two trials as alternative to WBRT to surgical cavity, there is still a concern of the worse intracranial control rate of SRS compared to WBRT (10).

6.2 Rationale of the study

Previously large-scale drug screens of transformed cell line panels have failed to translate most preclinical compounds in clinical trials in view of the limitations of these preclinical models. Patient-derived organoids (PDOs) have been shown to recapitulate patients' tumour characteristics more closely and could help to identify effective therapeutic regimens (11-15). We have been generating PDOs from primary breast cancer tissues based on the published protocol (16). We would now like to generate organoids from brain and/or extra-cranial metastasis samples from breast cancer patients who may subsequently undergo SRS to surgical cavity for brain metastases and/or systemic treatments. We will do a pilot observation study to assess the potential of these PDOs in predicting treatment outcome in patients.

7 Trial objectives and purpose

Overall aim and hypothesis

This study aims to address the hypothesis that it would be possible to generate patient-derived organoids (PDOs) from resected brain or resected/biopsied extra-cranial metastases and to collect preliminary data on the ability of PDOs to predict patients' treatment response and their radiosensitivity and chemosensitivity can be correlated with their survival outcome.

Patient cohorts

1) Breast cancer patients with brain metastases undergoing surgical resection with or without further radiotherapy (SRS or whole brain radiotherapy)

2) Breast cancer patients with extra-cranial metastases undergoing surgical resection or biopsy

Primary objective

To assess the percentage of successful generated organoids from resected brain or resected/biopsied extra-cranial metastases of breast cancer patients

Secondary objectives

1) To assess the sensitivity of radiotherapy in PDOs

2) To assess the sensitivity of the same systemic treatments that the patients previously had and will have in PDOs

3) To correlate the treatment sensitivities of PDOs above with the treatment outcome of patients

4) To correlate IC50 doses/dose response curves above with the objective response rates of patients undergoing same systemic treatment

Exploratory objectives

1) To assess and correlate the histopathological (including immune markers), genomic and transcriptomic features of patients' samples with PDOs

2) To assess peripheral immune responses in patients before and after radiation +/- systemic treatments and to correlate the results with those in PDOs

3) To assess the sensitivities of PDOs to various targeted therapies based on their genomic profiles and mutations

4) To assess the novel treatment combination of radiotherapy with immunotherapy and other agents in patient-derived organoids co-cultured with immune cells

5) Collection of archival tissues, blood samples, saliva, oral swab, cerebrospinal fluid (CSF), urine or stool samples for other translational research

8 Study design & Flowchart

8.1 Study Design

This is a prospective observation study to establish the pathways required to successfully generate PDOs from resected brain or resected/biopsied extra-cranial metastases and to test their treatment sensitivity in correlation with patients' treatment outcomes. In addition, the time required to generate organoids and to assess treatment response in order to correlate with patients' outcome will be evaluated.

Endpoints

This main endpoint is the success rate in generating PDOs from brain or extra-cranial metastases of breast cancer patients.

Primary endpoint

The percentage of individuals whose successful organoid samples from metastases are obtained

Secondary endpoints

1) To determine the IC50 doses and dose response curves of radiotherapy

2) To determine the IC50 doses and dose response curves of the previous and same systemic treatments that patients had or will have in PDOs

3) To correlate the IC50 doses and dose response curves above with the recurrence rates and progression-free survival of patients

4) To correlate IC50 doses/dose response curves above with the objective response rates of patients undergoing same systemic treatment

Exploratory endpoints

1) Tumour immune markers (including CD4 and CD8 T cells, FoxP3, PDL1), exome sequencing and RNA sequencing of PDOs and patients' samples

2) Peripheral immune responses (including CD8-T cell with exhaustion and cytolytic activity marker, CD4/FoxP3 Tregs) in patients before and after radiation +/- systemic treatments and correlation of the results with those in PDOs

3) The IC50 doses of various targeted therapies based on actionable genetic mutations + cell viability data of PDOs

4) The promising treatment combination of radiotherapy with immunotherapy and other agents in PDOs co-cultured with immune cells

5) To conduct further translational research using the patients' samples including assessing plasma and CSF ctDNA before and after treatments in patients

8.2 Treatment visits

This is an observation study and the patients will continue their clinical visits and follow-up as per normal standard of care.

9 Subject selection

This study will be open at King's Health Partners, including King's College London, Guy's and St. Thomas' NHS Foundation trust and King's College Hospital NHS Foundation trust. Potential eligible patients will be identified and enrolled at King's College Hospital or at Guy's Hospital. After their surgery at King's College Hospital or Guy's and St. Thomas' NHS Foundation trust, participants will receive their radiotherapy or radiosurgery at Guy's Hospital or systemic treatments at their hospitals, as per standard protocol.

The study may be opened to other centres in the future if there is an extension of the study and/or if another centre would be able to follow the protocol to generate organoids as specified. If this is the case, an appropriate study protocol amendment will be submitted and ethical approval will be obtained before another site is opened.

We will include breast cancer patients with resectable brain or extra-cranial metastases and undergoing postoperative radiotherapy (SRS and/or WBRT) or palliative radiotherapy to extracranial metastases if necessary +/- other systemic treatments after resection. Patients who will undergo a

biopsy of the extra-cranial metastases may be recruited and asked to consent for an additional research biopsy at the same time as standard of care biopsy.

9.1 Subject inclusion criteria

- Breast cancer patients with brain metastases who are suitable for surgical resection or Breast cancer patients with extra-cranial metastases undergoing surgical resection or biopsy

- Age > 18 years old

9.2 Subject exclusion criteria

- Patients unable to give informed consent e.g., mental disability or vulnerable adults

10 Study procedures

10.1 Subject recruitment

Method of recruitment

Potential eligible patients will be identified from multi-disciplinary meetings and breast clinics at King's College Hospital and Guy's Hospital. These patients will be checked to see whether they will meet inclusion criteria.

Breast cancer patients with resectable brain or extra-cranial metastases or who will undergo a biopsy of the extra-cranial metastases will be asked to consent for this study and for their resected/biopsied samples to be used to generate PDOs. We aim to recruit 20 patients in the pilot phase of the study.

When the PDOs contain sufficient cells, these cells will be treated with increasing doses of radiotherapy and/or relevant systemic treatments in order to determine the IC50 and to obtain dose-response curves of these PDOs to the treatments. In particular, we will treat the PDOs with the same treatments that the patients had or will receive in order to correlate the responses to radiotherapy and/or systemic treatments including immunotherapy (co-cultured with immune cells).

The response of these PDOs will be compared to the treatment outcome and survivals in these patients.

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 pilot study will initially be opened at King's College Hospital NHS Foundation Trust and Guy's and St Thomas' NHS foundation trust although we will open the study to other centres for participation if they would be able to follow the protocol to generate organoids from metastases as specified. 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 fellows or other

consultants or specialist registrars), 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 participants 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 PI 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 data for use 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).

Based on this consent, archival tissues may be requested, and translational research blood, saliva, oral swab, CSF, urine and stool samples will be stored at Guy's and St Thomas' biobank beyond the ethics approval date of the project and stored under the HTA licence (HTA licence is 12121; the Designated Individual is Cheryl Gillett). 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 may ask patients to provide blood samples before surgery and/or radiotherapy (baseline), during treatment (week 1), at 4 to 6 weeks, at 3 months and at 12 months after the completion of their treatments as well as at disease recurrence/progression if possible. We may also ask them to donate other samples such as saliva, oral swab, CSF, urine or faeces for future research at the same time points as above. If possible, the collection samples will normally be done during patients' routine clinical appointments. Translational research may be conducted for up to 5 years after the recruitment period.

Participants may be asked to provide other tumour samples and surrounding tissue if having further surgery and/or biopsies for tumours (primary or metastasis) located in other areas besides the brain and if having further resection of new/other brain metastasis. These samples will be used to correlate findings from the PDOs generated from resected metastasis of the brain or resected/biopsied extra-cranial metastasis with the original sample(s) taken when first consented. If the participants have other available archival tissue samples in the pathological laboratories or in the biobank, we may

request for these samples to be released (sections or blocks) for the SOTO-BC study via the Guy's and St Thomas' biobank. Some participants may have primary breast tumour tissues and/or metastasis samples stored, and this will further link outcomes between brain metastases and the primary breast tumour as well as any other available extracranial metastasis.

10.2 Screening Procedures

Potential eligible patients will be identified from 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. 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. Tumour (and occasionally normal tissue if available) will be obtained at the time of surgical resection of brain metastases or during biopsy/resection of extra-cranial metastases. We may ask patients to provide blood samples before surgery or biopsy.

10.4 Follow up Procedures

The follow-up procedures will be as per normal standard of care and the schedule will not be changed due to this study. Participants will undergo radiotherapy at the cancer centre at Guy's Hospital (Guy's and St-Thomas' Trust) and thus follow-up sample collection will occur at this site.

Blood and other biological sample collection time points: We may ask patients to provide blood samples before radiotherapy (week 0), during treatment (week 1), at 4 to 6 weeks after starting treatment, at 3 months which is usually the end of treatment, and at 12 months after the completion of their treatment, as well as at disease recurrence/progression, if possible. We may also ask patients to donate other samples such as saliva, oral swab, CSF, urine or faeces for future research at the same time points above. If possible, the collection samples will normally be done during patients' routine clinical appointments and only when patients have consented to donating these additional samples. CSF will only be obtained during standard of care procedures if the patient has consented to this and only if it is possible to obtain (5 mins if obtained during surgery and 30 mins if obtained through lumbar puncture).

Sample collection will be conducted throughout the recruitment period and the follow-up period. After this stage, we will continue to monitor treatment outcomes through the patients' medical records or through their GP for up to 5 years from their start in the study.

10.5 Radiology Assessments

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

10.6 End of Study Definition

The patient recruitment for the pilot study will last 18 months and the follow-up period for additional sample collection will last another 12 months. We will assess whether the endpoints and outcomes are met but the patients will be followed up for their long-term treatment outcome and survival for up to 5 years. The end of the study definition for the pilot study is the end of the 30 months' period after the commencement of the study regardless of the number of patients recruited. We will submit the protocol for a substantial amendment approximately 1 year after the recruitment of the first participant so that the SOTO-BC study will continue as a prospective observation study for up to additional 3 years including further recruitment or as a new main study if funding is obtained. If no funding is obtained by the end of 18 months, we will end the patient recruitment and continue to collect samples from recruited patients for 12 months.

In addition to obtaining funding, the criteria for progression beyond pilot study are set as below:
1) More than 50% of the approached participants will consent to taking part in SOTO-BC study
2) At least 30% of the tissue samples will be successfully used to generate organoids
3) Further funding is obtained to continue beyond the 18-month recruitment period

11 Laboratories and sample storage

The fresh tissue samples will be processed for organoids within 24 to 48 hours of surgery/biopsy. Once the experiments on the organoids have been completed, they will be frozen for future analysis and stored in Dr. Kong's laboratory at KCL. Blood samples will be processed according to standard laboratory SOP in Dr. Kong's Laboratory (KCL, London, UK) for PMBCs and plasma collection. Plasma and/or CSF samples will also be processed to assess ctDNA in the future. The additional biological samples including saliva, oral swab, urine or stool samples if obtained will be stored at Guy's Hospital biobank. Once collected, these samples will be stored directly with the biobank. All unused samples will be stored for 5 years from the end of the SOTO study under the biobank's HTA license (reference 12121, Designated Individual Cheryl Gillett). At the end of this storage period, the unused biological samples stored at Guy's biobank will be handled and/or destroyed according to the biobank's Standard Operating Procedure (SOP) if the agreement to store the samples has expired and/or not extended.
Further details on the sample collection, processing, storage and analysis will be found in the laboratory manual.

11.1 Data Recording/Reporting

Data generated from laboratory testing of organoids will be recorded and stored on computers at King's College London. Laboratory data will be linked to the participant through a participant trial number. No identifying information will be used on the samples or when used in the laboratory. The data obtained from the laboratory tests will not be used for clinical purposes and will not be reported to the clinical team. Separate diagnostic testing will be done by the clinical teams as per normal processes.

11.2 Sample Receipt/Chain of Custody/Accountability

A member of the research team will collect the samples and process them in Dr. Kong's Laboratory at KCL. Tumour tissues will be processed for organoid cultures and blood samples processed for PBMC and plasma storage. CSF will also be processed for storage at Dr. Kong's laboratory. Processed blood samples (PBMC isolates and plasma), and processed CSF, along with all other samples (saliva, oral swab, urine and stool) will be given to the biobank at Guy's Hospital for storage. The receipt, processing and transfer of samples will be documented through Sample Processing Forms. Upon receipt of the tumour samples, the laboratory will 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. Tumour samples will be processed for organoid cultures and will be kept in Dr. Kong's laboratory for the duration of the study and frozen for long-term storage. Upon receipt of samples laboratory staff will ensure that all samples are accounted for as per the labeling. All samples received will be logged in a study sample log.

Samples will be transferred on foot at the Guy's campus between Guy's Hospital and New Hunt's House. Samples will be transferred using the KHP staff bus when collecting samples from KCH.

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. 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.

12.1 Ethics Reporting

Annual progress reports will be submitted to the Research Ethics Committee as per normal regulatory processes.

12.2 Trial Steering Committee

No formal trial steering committee will be set up for this feasibility study.

12.3 Ethics & Regulatory Approvals

Study protocol and other documentation will be submitted to the North East - Tyne & Wear South Research Ethics Committee (REF 24/NE/0022) 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

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 patient requests 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. If a participant consents to the study but subsequently loses the capacity to consent during the study, we will withdraw the participant from sample collection. However, we will retain and make further use of any identifiable data and collected tissues until the point of withdrawal. We will stop taking any further blood or other samples from the participant although we will continue collecting information about the participant's health from the hospital record and/or GP for up to 5 years.

We will monitor the percentage of participants who withdraw consent from the study after taking part. If no longer wishing to take part, participants will consent to have their collected samples and data included in the research after they have withdrawn and to consent to follow their clinical outcomes through their medical notes. The patient recruitment for the pilot study will last 18 months with additional follow-up for another 12 months. If there is withdrawal of consent from any patients before the organoids are successfully generated from the samples, we will recruit more participants to back fill those who may have withdrawn or dropped out, provided that this is within the recruitment period stated above.

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 only be collecting data from patients that are already available in the hospital record on patients' demographics, menopausal state, tumour characteristics (including ER/PR and HER2 status), previous treatments and proposed treatment plans. The patients' treatment details, treatment responses, recurrences and survivals will also be obtained from clinical record. The data 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. Data will be entered into an electronic CRF database (Excel) designed by the research team and stored on secured servers at King's College London. Access to the database will be granted by the Chief Investigator.

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 GSTT secured network, whereby access is only granted by the site PI to named individuals.

Consent forms and an Investigator Site File will be kept at the recruiting sites. Paper CRFs, copies of consent forms and the Trial Master File will be stored in locked filing cabinets within the Dr. Kong's Laboratory (KCL, Guy's Campus) and 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.

Full dataset and linked code with patient identifiers will be kept for 5 years at local recruiting sites for governance purposes on Trust networks. This dataset will be accessed by the research team for long term follow-up of participants for up to 5 years of their recruitment to the study. Subsequently identifiable data will be deleted (name, NHS/hospital number and date of birth). A pseudonymised dataset will be kept for 5 years after study completion at King's College London which will link participant trial numbers to their age at consent, ethnicity, gender, clinical information and treatment outcome.

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. We expect more than 50% of the approached participants will consent to taking part in SOTO-BC study and at least 30% of the tissue samples will be successfully

used to generate organoids. A successful pilot study will be defined as one that meets the pre-defined expectation. The data from study will be used to calculate the sample size required to extend the observation study, 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 was previously presented to the Guy's and St. Thomas breast cancer research working party and have also sent for external peer review from experts as well as PPI members.

16.3 Regulatory Compliance

The trial will not commence until a Favourable REC opinion is obtained and R&D has provided greenlight for recruitment.

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 for confirmation of implementation.

17 Financing and Insurance

The study is linked to the SOTO study (IRAS 305689) which is a prospective pilot study looking at growing organoids from head and neck tumours. The research team working on SOTO-BC is funded through a Guy's Cancer Charity grant.

The study is co-sponsored by King's College London (KCL) and Guys and St Thomas' NHS Foundation Trust (GSTT). The sponsors will, at all times, maintain adequate insurance in relation to the study. KCL through its' own professional indemnity (Clinical Trials) and no-fault compensation and the GSTT 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.

King's College London employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University's employment.
King's College London cannot offer indemnity for non-negligent harm.

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.

19 References

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