TITLE OF THE PROTOCOL: Prospective Evaluation of AI R&D tool for patient stratification - Mechanism of action Evaluation in Triple negative breast cancer (PEAR-MET)

IRAS Reference: 316023

Sponsor:	**Ourotech Limited (trading as Pear Bio)** Representative of Sponsor: Duleek Runatanga Hill House, 1 Little New Street, London, United Kingdom, EC4A 3TR Tel: 07716558079 Email: duleek@pearbio.com
Chief Investigator:	**King's College London** Sheeba Irshad, MD PhD Guy's Cancer Centre, Innovation Hub, SE19RT Tel: 020 7188 4260 Email: sheeba.irshad@kcl.ac.uk
Sponsor Lab Lead Investigator	**Ourotech Limited (trading as Pear Bio)** Eleonora Peerani, PhD Pear Bio Imperial College London White City IHub 84 Wood Lane London, W120BZ Tel: (+39) 3319571756 E-mail: eleonora@pearbio.com
Principal Investigator(s):	

STUDY CONTACTS

Funder(s):

Ourotech Limited (trading as Pear Bio), Hill House, 1 Little New Street, London, United Kingdom, EC4A 3TR

Statistical Advice:

Pear Bio, Imperial College White City Campus, 84 Wood Lane, London, W12 0BZ.
Contact: Aston Crawley
E: aston@pearbio.com

Sponsor Laboratory:

Pear Bio, Imperial College White City Campus, 84 Wood Lane, London, W12 0BZ.
Contact: Dr Eleonora Peerani
E: eleonora@pearbio.com
Tel: (+39) 3319571756

Trial Coordination:

Pear Bio, Imperial College White City Campus, 84 Wood Lane, London, W12 0BZ.
Contact: Elli Tham
E: elli.tham@pearbio.com
Tel: 07976094236

Participating Sites:

A list of participating sites can be obtained from the PEAR-MET Trial Coordinator

PROTOCOL SIGNATURE PAGE

Signature of the Chief Investigator:

The clinical study as detailed within this research protocol (Version 2.0, Dated 03 February 2023), and any subsequent amendments, will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Name: Dr Sheeba Irshad

Signature and Date: _____ __ __ / __ __ __ / __ __ __ __

Signature of the Sponsor Lab Lead Investigator:

The clinical study as detailed within this research protocol (Version 2.0, Dated 03 February 2023), and any subsequent amendments, will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Name: Dr Eleonora Peerani

Signature and Date: _____ __ __ / __ __ __ / __ __ __ __

Signature of Statistical Advisor:
The clinical study as detailed within this research protocol (Version 2.0, Dated 03 February 2023), or any subsequent amendments, and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH E6-GCP, ICH E9 - Statistical principles for Clinical Trials, ICH E10 - Choice of Control Groups and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Name: Aston Crawley

Signature and Date: _____ __ __ / __ __ __ / __ __ __ __

Signature of the Principal Investigator:
The clinical study as detailed within this research protocol (Version 2.0, Dated 03 February 2023), and any subsequent amendments, will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Principal Investigator Name:

Principal Investigator Site**:** _____

Signature and Date: _____ __ __ / __ __ __ / __ __ __ __

TABLE OF CONTENTS

List of Tables and Figures in the Text

Tables

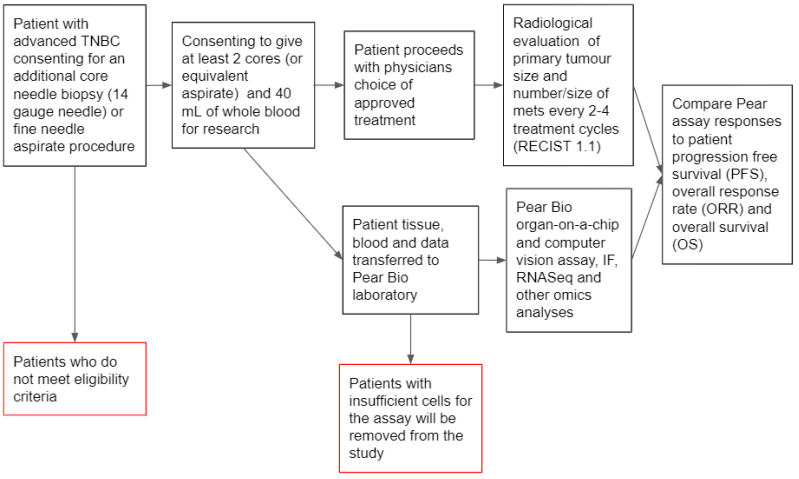
Figures

GLOSSARY OF TERMS AND ABBREVIATIONS

AE	Adverse Event
ANC	Absolute Neutrophil Count
APR	Annual Progress Report
AST	Aspartate aminotransferase
CI	Chief Investigator
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EU	European Union
FBC	Full Blood Count
GCP	Good Clinical Practice
HTA	Human Tissue Authority
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IF	Immunofluorescence
ISF	Investigator Site File
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria For Adverse Events
NRES	National Research Ethics Service
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria In Solid Tumours
RNASeq	RNA sequencing
SAE	Serious Adverse Event

SAR	Serious Adverse Reaction
SD	Stable Disease
SDV	Source Data Verification
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
TNBC	Triple Negative Breast Cancer
US	Ultrasound
WBC	White blood cell count

STUDY SYNOPSIS

Title	Prospective Evaluation of AI R&D tool for patient stratification - Mechanism of action Evaluation in Triple negative breast cancer (PEAR-MET)
Main Objectives	The primary objective of this study is to establish a functional dose of each commonly used FDA approved therapeutic/combination for advanced triple negative breast cancer (TNBC) in Pear Bio's *ex vivo* platform, and to confirm these therapies demonstrate their intended mechanism of action (direct cell killing, cell killing by immune cell activation, etc.). Secondary objectives will focus on determining the correlation between Pear Bio's *ex vivo* tumour culture response to patient outcomes against the same treatment. Overall response rate (ORR) and progression free survival (PFS) data are collected prospectively for each patient to meet the secondary objectives.
Phase	N/A
Design	Eligible patients with advanced TNBC will undergo a mandatory, study-specific core needle biopsy or fine needle aspiration of the breast tumour or metastasis before commencing their next line of therapy. The research sample will be sent to Pear Bio's lab to run Pear Bio's test whilst the patient receives therapy as per their physician's choice. The Pear Bio test measures the *ex vivo* tumour sample treatment response to each therapy option, including the therapy that will be administered to the patient. This study will not use Pear Bio's tool to inform the choice of treatment, with the treating oncologist being blinded to the test results. Treatment response data will be collected at multiple timepoints to conduct analyses on the study's secondary and tertiary objectives. 

Sample Size	30
Inclusion Criteria	1. Able to give written informed consent prior to admission to this study. 2. Female or male aged ≥18 years. 3. Histologically confirmed primary breast cancer which is triple-negative by the most recent ASCO/College of American Pathologists (CAP) guidelines. 4. Stage 4 or locally advanced breast cancer planned for first line systemic therapy, or has received prior lines of systemic therapy and is due to undergo another line of systemic therapy. 5. Willing and able to undergo a mandatory additional core needle biopsy (minimum 2 cores) or equivalent fine needle aspiration from the primary breast mass or a metastasis prior to starting the subsequent line of systemic therapy. 6. Willing and able to undergo a mandatory procedure to collect 40 mL of blood
Exclusion Criteria	1. Tumours not confirmed as triple negative breast cancer. 2. Early stage TNBC. 3. Patients with TNBC that do not intend to receive systemic therapy. 4. Patients who have already commenced systemic therapy with no plans of changing the systemic therapy after the collection of the core needle biopsy or fine needle aspirate sample. 5. Patients who are due to receive experimental therapies that are not included in the study protocol. 6. Haemoglobin levels below 80g/L prior to research sample collection. 7. Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that, in the investigator's opinion, gives reasonable suspicion of a disease or condition that may affect the interpretation of the results, render the patient at high risk from treatment complications or interferes with obtaining informed consent.

1: INTRODUCTION

1.1 Trial outline

Triple-negative breast cancer (TNBC) has the worst prognosis of all the breast cancer subtypes. Metastatic TNBC has a median overall survival of 13.3 months with treatment.[1] Patients are given chemotherapy, targeted therapy or immunotherapy to halt cancer progression as long as possible, and potentially cause tumour shrinking (partial response) or elimination (complete response). This progression free survival (PFS) lasts, on average (median), 11.9 weeks for first-line therapy.[1] Tumours continue to grow after that point and require second-line, third-line, and any further lines of treatment as necessary until patient death. Median PFS for 2nd line therapy is 9 weeks and median PFS for 3rd line therapy is 4 weeks.[1]

New therapeutics for advanced TNBC are being approved or are in clinical trials.. These include approved therapies like pembrolizumab[2] and sacituzumab govitecan[3], which have the potential to extend progression free survival (PFS) and overall survival (OS). Novel therapies with diverse mechanisms of action are being tested in clinical trials, ranging from targeted therapies (e.g., PARP inhibitors), to next-generation immunotherapies, to hormone therapies (e.g., androgen receptor inhibitors). Unfortunately, head-to-head comparisons of new drugs and use of the standard of care in control arms are not the norm in clinical drug development. As more therapies become available for advanced TNBC, it becomes critical to compare treatment options and provide each patient with a therapeutic that benefits them at that point in their care. However, to date, no predictive biomarkers exist to compare these diverse therapies and inform treatment decision-making to maximise PFS and/or OS.

Pear Bio have developed a personalised medicine assay that combines organ-on-a-chip technology and computer vision. Small patient tumour samples are taken via core needle biopsy or fine needle aspiration and are sent to Pear Bio's laboratory. Live cells are isolated from the tumour samples and cultured in multiple organ-on-a-chips. Each chip receives a potential treatment regimen being considered for that patient, and the regimen efficacy is compared by tracking a range of *ex vivo* tumour response metrics, including cell viability, cell migration and shrinking/growth of the tumour samples in the organ-on-a-chip.

This platform has been validated on patient tumour samples acquired from biobanks, and it is also being studied in another clinical study for early-stage TNBC (PEAR-TNBC). This study, PEAR-MET, will recruit advanced TNBC patients who are due to receive their next line of therapy. In this observational study, patient tumour samples will be tested in Pear Bio's laboratory while oncologists are blinded to assay results and provide treatment as per standard of care. This will enable comparisons between Pear Bio's predicted level of response to a given therapy against the actual response of the patient, without compromising the patient's safety. This study will allow Pear Bio to assess the platform's capability as a treatment response prediction tool for advanced TNBC. Future trials will recruit larger cohorts of patients to measure the potential benefits to patient response (PFS, ORR, OS, etc.) that come from using Pear Bio's technology to guide treatment choices.

1.2 Background and rationale

Breast cancer (BC) has now surpassed lung cancer as the most commonly diagnosed cancer worldwide, with an estimated 2.3 million new cases (11.7% of all cancers) in 2020.[7] This accounts for 24.2% of cancers in women.[4] Age-standardised incidence rates globally were 46.3 per 100 000 persons per year in 2018, rising to over 80 per 100 000 for Europe and North America.[4] The incidence continues to increase, with predictions of over 3 million new cases per year by 2040.[5] In 2018, over 626 000 people died from breast cancer worldwide, accounting for 6.6% of all cancer deaths, climbing to 15% in females, where it is the leading cause of cancer death.[4] Predictions estimate that breast cancer mortality will increase by ~50% by 2040.(5)

Clinically and molecularly, breast cancer is not a single entity.[3, 6] Currently, prognosis and treatment decisions are defined by whether the oestrogen and progesterone hormone receptors are expressed and whether the human epidermal growth factor receptor 2 (HER2) is overexpressed or amplified.[3, 6] Triple-negative breast cancer (TNBC) is a subtype defined by the lack of hormone receptor (HR) expression and absence of HER2 overexpression or amplification. It accounts for 10-20% of invasive breast cancers and is associated with African-Americans, younger age at diagnosis and BRCA1 mutations.[7] TNBC has a more aggressive phenotype, shorter time to recurrence and a worse overall survival, regardless of stage, compared to patients with other breast cancer subtypes.[8-10]

1.2.1 Current management of advanced triple-negative breast cancer

Systemic therapy is standard for patients with advanced TNBC. The aim of treatment is symptom relief and prolongation of life. Prophylactic palliation may be justified in some patients with minimal or no symptoms. Age, performance status, sites of disease and previous therapy(ies) affect the first-line modality used. Local therapy with radiation or limited palliative surgery may be considered where local symptoms predominate.

In unresectable locally advanced or metastatic TNBC patients, whose tumours express PD-L1 at a level of 1% or more and have not had previous chemotherapy for metastatic disease, Atezolizumab with nab-Paclitaxel (Abraxane) is considered first line treatment. For BRCA1/2 mutation carriers Carboplatin AUC6 or Cisplatin 75mg/m2 or Gemcitabine and Carboplatin may be considered as first line therapy for metastatic breast cancer. Other commonly used chemotherapy regimens for advanced disease include: single agent Epirubicin, combination EC (Epirubicin and Cyclophosphamide), CMF, Taxanes (Docetaxel or Paclitaxel), Capecitabine, Eribulin, Vinorelbine (i.v or oral), single agent Carboplatin, metronomic chemotherapy with Cyclophosphamide and Methotrexate. The exact choice of regimen will depend on a number of factors, including: 1) prior chemotherapy and previous response; 2) cumulative anthracycline exposure and 3) patient choice and fitness. More recently, patients with unresectable or metastatic triple-negative breast cancer who have received two or more prior systemic therapies, including at least one of them for advanced disease may be eligible for the newly approved treatment sacituzumab govitecan.[3]

1.2.2 Treatment response in advanced triple-negative breast cancer

Advanced (stage 4) TNBC has a median overall survival of 13.3 months with treatment.[1]

Progression free survival (PFS) lasts for a median of 11.9 weeks for first-line therapy.[1] Tumours continue to grow after that point and require second-line, third-line and any further lines of treatment as necessary until patient death. Median PFS for 2nd line therapy is 9 weeks and median PFS for 3rd line therapy is 4 weeks.[1]

Response to chemotherapy is generally reassessed after 2–4 courses and treatment should be continued beyond this point only if there is clear-cut evidence of symptom relief and/or tumour regression. Before introducing a change in systemic therapy, documentation of the nature and severity of the symptoms for subsequent subjective assessment of patient response is required. A general principle is to avoid any treatment with side effects worse than those caused by the patient's cancer. Often, marker lesions for objective assessment of patient response are nominated at the baseline scans. Bi-dimensional measurements are documented.

1.2.3 Predictive biomarkers of patient response

While more therapies have become available for the treatment of advanced TNBC, and many more are in clinical trials, the number of predictive biomarkers are limited. As TNBC patients are negative for hormone receptors and HER2, they are not able to receive the associated therapies. Checkpoint inhibitor immunotherapies, such as Keytruda, can be provided for patients with high PD-L1 expression measured through immunohistochemistry (IHC). Information like the number of tumour infiltrating lymphocytes (TILs) can be useful, but are limited in predicting potential immunotherapy response and require further validation on predictive power.

There is a lack of effective biomarker panels or other broad-range predictive tests to compare multiple therapies with different mechanisms of action and prescribe a therapeutic or combination with high potential efficacy for a specific patient.

1.3 Benefit/risk assessment

This is an observational study with patients receiving standard of care systemic therapy. The Pear Bio test will run in parallel with the patient's treatment, rather than beforehand, and the treating oncologist will be blinded to the outcome. As such, there are no benefits to the patients taking part in this trial. However, the data will be used to design future trials aimed at increasing patient response rates by using the test before systemic therapy starts in order to decide on the optimal therapeutic strategy to adopt for an individual patient.

The main risk to the patient comes from the additional core needle biopsy or fine needle aspirate procedure that is required, as fresh tissue (rather than FFPE tissue) is needed for the assay. This procedure will be done separately from the standard diagnostic core needle biopsy as only 10-20% of breast cancer cases will be TNBC, leading to 80-90% of newly diagnosed patients having unnecessary samples taken if done concurrently. The potential risks that a patient may experience from a core needle biopsy include pain (5.4%), failure to sample tumour cells (2.2-3.6%), bleeding requiring treatment (0.72%), infection requiring treatment (0.15%), haematoma requiring treatment (0.09%).[11] To mitigate this risk, patients who are not suitable for a core needle biopsy procedure will have a fine needle aspirate taken instead.

There is a risk that the patient sample will not establish a culture in the laboratory, resulting in Pear Bio being unable to run the test. During initial development, the culture success rate was 83.33%. However, these assays used biobank samples which had not been collected and stored under optimal conditions for this assay. It is expected that the culture success rate will improve to over 90% by prospectively collecting the tumour samples under specified conditions.

2: STUDY AIMS AND OBJECTIVES

2.1 Primary objectives and endpoints

Primary objective	Endpoints
The primary objective is to establish a functional dose of each commonly used FDA approved therapeutic/combination for advanced TNBC in Pear Bio's *ex vivo* platform, and to confirm these therapies demonstrate their intended mechanism of action (direct cell killing, cell killing by immune cell activation, etc.)	As the primary objective is laboratory-based, a patient outcome endpoint is not necessary. Instead, success will be determined by: 1. Observing differentiated mechanism-relevant *ex vivo* treatment response across the therapies tested on each patient's tumour sample (intra-patient comparison) 2. Observing differentiated mechanism-relevant *ex vivo* treatment response levels between the cohort of samples collected from patients on a per therapeutic basis (inter-patient comparison)

2.2 Secondary objectives and endpoints

Secondary objectives	Endpoints
Assess the accuracy of Pear Bio's assay at stratifying patient overall response rate (ORR)	ORR will be measured after 2-4 cycles (6-12 week mark) of the treatment regimen following the collection of the research sample. Overall response rate is defined as the percentage of patients achieving a partial or complete response (i.e., stable disease excluded) to the line of treatment given after research sample collection as defined by RECIST 1.1 guidelines. The prediction accuracy of Pear Bio's test is measured by: 1. Sensitivity, defined as the percentage of true responders identified by Pear's test based on the total number of patients who achieved a response in the study (PR or CR after 2-4 cycles) 2. Specificity, defined as the percentage of true non-responders identified by Pear's test based on the total number of patients who did not achieve a response in the study (SD or PD after 2-4 cycles) 3. Positive predictive value (PPV), defined as the percentage of patients correctly predicted to achieve a response by Pear's test based on all predictions of positive response (PR or CR after 2-4 cycles) 4. Negative predictive value (NPV), defined as the percentage of patients correctly predicted to not respond to therapy by Pear's test based on all predictions of non-response (SD or PD after 2-4 cycles)

Assess the accuracy of Pear Bio's assay at correlating to patient progression free survival (PFS)	Tumour response will be measured after 2-4 cycles (6-12 week mark) of the line of treatment commencing after collection of the research sample, and at all subsequent timepoints until disease progression, as per standard of care. Disease progression is evaluated by the patient's radiologist and is defined by RECIST 1.1 guidelines. Kaplan–Meier curves will be generated on the patient population, and where feasible, based on their line of treatment and for each therapeutic option (if n is sufficient). These curves will be compared to reported data to determine how representative the patient population is of past trials and clinical practice. Computer vision biomarkers will be categorised into low/high groups to determine their correlation with PFS. This analysis will not demonstrate the patient benefit of using Pear Bio's tool, but it will generate hypotheses for interventional trials designed to demonstrate patient benefit.

2.3 Exploratory objectives and endpoints

Tertiary objectives	**Endpoints**
Determine the rate of successfully established cultures from the core needle biopsy and fine needle aspirate samples.	The success of a cell isolation protocol is defined by obtaining a minimum of 100,000 viable cells per patient. Patients whose cell count after extraction is below 100,000 will be excluded from the trial. The successful culture rate is the percentage of cultures in which ≥70% of viable cells cultured on day 0 are still alive on day 3 in the control chip (no treatment) compared to the total number of research samples taken and successfully arriving at Pear Bio's lab.
Assess Pear Bio's assay ability to categorise patients for below average or above average overall survival (OS)	Patient data is collected up to death and their time from metastatic disease diagnosis to death is recorded to determine the overall survival (OS) time. This analysis will use median OS within the study cohort to differentiate patients as generally responsive or resistant to treatment. The analysis will then explore indicators/biomarkers in Pear Bio's test that can identify patients as generally responsive or resistant to treatment, agnostic of the therapeutic choice.
Assess the correlation of omics biomarkers to patient PFS, ORR and/or OS	Omics readouts taken of patient tumour samples at Pear Bio's laboratory will be used to determine whether any biomarkers can correlate to therapeutic response. Omics methods include:

	1. Immunofluorescence (IF) 2. RNASeq 3. Tumour mutational burden (TMB) and microsatellite instability (MSI) Prediction methods include: 1. Correlating baseline expression of biomarkers in the tumour sample to real-world patient outcomes 2. Comparing the change in biomarker expression after *ex vivo* treatment testing to real-world patient outcomes

3: INVESTIGATIONAL PLAN

3.1 Overall design

This is a UK-based observational study that aims to discover novel predictive biomarkers with the potential to guide treatment decision making and prolong PFS and OS in patients with advanced TNBC. Patients will undergo a mandatory, study-specific core needle biopsy or fine needle aspiration of the breast tumour or metastasis before commencing their next line of therapy. The research sample will be run on Pear Bio's test whilst the patient receives therapy as per their physician's choice. This study will not use Pear Bio's tool to inform the choice of treatment, with the treating oncologist being blinded to the test results. Treatment response data will be collected at multiple timepoints to conduct analyses on the study's secondary and tertiary objectives.

3.2 Trial schema

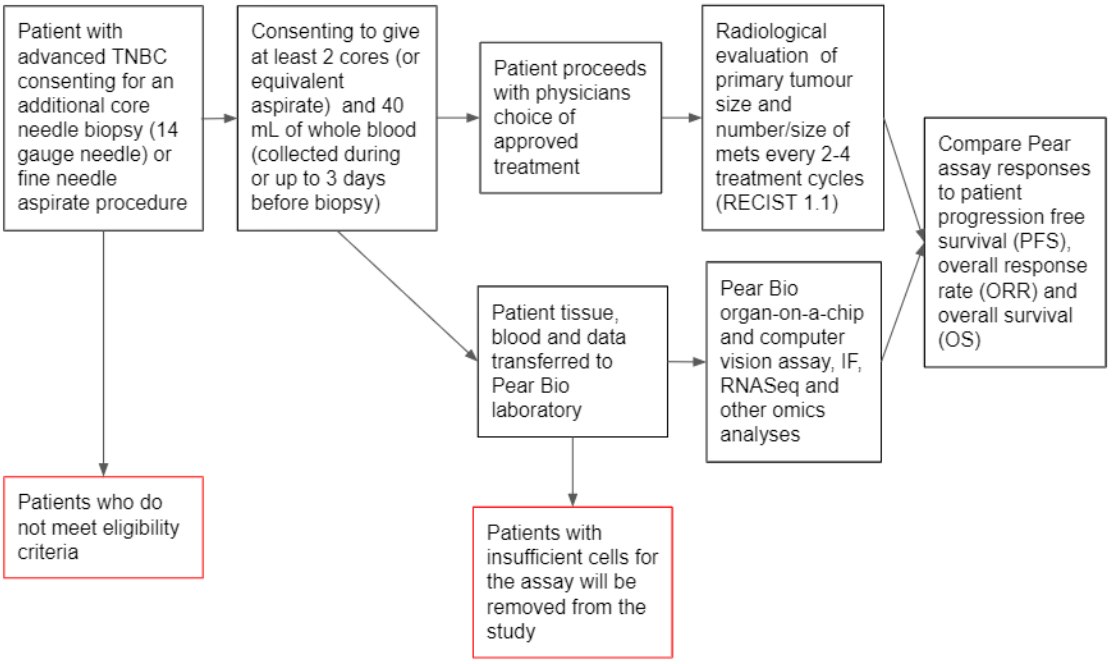


Figure 1: Trial schema

3.3 Data and tissue collected

	Biospecimens	Data
Required	1. At least 2 cores taken from an additional biopsy procedure with a 14 gauge needle, or if that is not feasible/safe, an	1. Demographic data (pseudonymised) 2. Redacted pathology reports, including all immunohistochemistry tests 3. Concomitant medications 4. Planned therapy for the next line of treatment following

	equivalent fine needle aspirate 2. 4 10mL EDTA vials of matched whole blood (collected during or up to 3 days before biopsy/aspirate)	the collection of the core needle biopsy or fine needle aspirate 5. ECOG 6. Data on previous lines of treatment and patient response (if the patient is receiving a 2nd or later line of treatment) 7. Prospective data collection on treatment regimens (therapies and doses) and patient responses (RECIST 1.1 evaluation, CT-TAP, bone scan, MRI, PET-CT, CA15-3, etc.) every 2-4 cycles until disease progression or, where feasible, until death.
Collected if available	N/A	1. Blood tests at baseline 2. Liver and kidney function tests at baseline 3. Any known mutations in the primary tumour and/or metastases

3.4 Laboratory setup

Fresh tissue resections that arrive at Pear Bio's lab will undergo processing, cell culture and various drug dosing and omics assays (depending on extracted cell numbers). Tumour samples will be processed using a cell isolation kit to retrieve a viable single-cell suspension. A minimum of 100,000 cells (10,000 viable cells per chip) will be used for staining with live and dead cell-tracking dyes. In parallel, blood vials will be processed for PBMCs and further effector cell extraction (flow cytometry, Dynabeads, etc). The remaining cells will be used for sequencing (DNA/RNA), fixed for immunofluorescence characterisation of biomarkers/receptor status or used for further omics assays (if cell numbers allow). This may include tumour mutational burden and microsatellite instability testing.

The stained cells will be cultured in a biomimetic hydrogel within Pear Bio's organ-on-a-chip to provide a physiological 3D environment for drug dosing experiments. Using a microfluidic device, samples in each chip will be exposed to approved therapies (either as monotherapy or combination therapies, as outlined below) over multiple days.

Standard set of therapies tested	
Chip 1	Control
Chip 2 (chip v7 instead of v4)	Keytruda + Abraxane
Chip 3	Gemcitabine + Carboplatin
Chip 4	Sacituzumab govitecan
Chip 5	Trastuzumab deruxtecan
Chip 6	Capecitabine
Chip 7	Olaparib
Chip 8	Epirubicin + Cyclophosphamide
Exploratory panel	
Chip 9	Cyclophosphamide + Methotrexate
Chip 10	Sacituzumab govitecan + Keytruda
Chip 11	Trastuzumab deruxtecan + Keytruda

Chip 12	Xtandi
Chip 13 (chip v7 instead of v4)	Olaparib + Imfinzi
Chip 14	Navelbine

The following therapies may also be tested if the sample has a sufficient cell count and compounds can be acquired:

- HER inhibitors
- ATR inhibitor + cisplatin
- Niraparib
- ADAR1 inhibitors

In parallel, PBMCs will be extracted from whole blood, characterised and sorted via flow cytometry and fluorescence-activated cell sorting (FACS) or magnetic beads selection. Cells of interest (e.g. $CD8^+$ T cells) will be used for culture in Pear Bio's chips jointly with cells isolated from the matched tumour sample. To test immunotherapies, tumour cells will be co-cultured with immune cells in a modified organ-on-a-chip architecture. Chips receiving immunotherapies may be tested for tumour mutational burden and/or microsatellite instability.

Confocal microscopy will be conducted daily to collect 3D image data of the cells and track their position and behaviour over time. At the end of the assay, the 3D cell cultures will be fixed for further 3D immunofluorescence analyses or used for embedding, sectioning and assessment of spatial transcriptomics. For targeted therapies, RNAseq, IF and other omics data will be integrated to confirm drug MoA and identify other potential therapeutic targets. Concurrently, 3D image data is processed through a computer vision pipeline to measure functional metrics of the *ex vivo* 3D cell cultures, including cell viability, cell culture width and cell migration, both at a bulk tumour level and at a single-cell resolution. For immunotherapies, additional metrics such as immune cell infiltration and immune cell killing will be recorded. A patient report is then generated to outline an individual patient sample's response to each therapy tested.

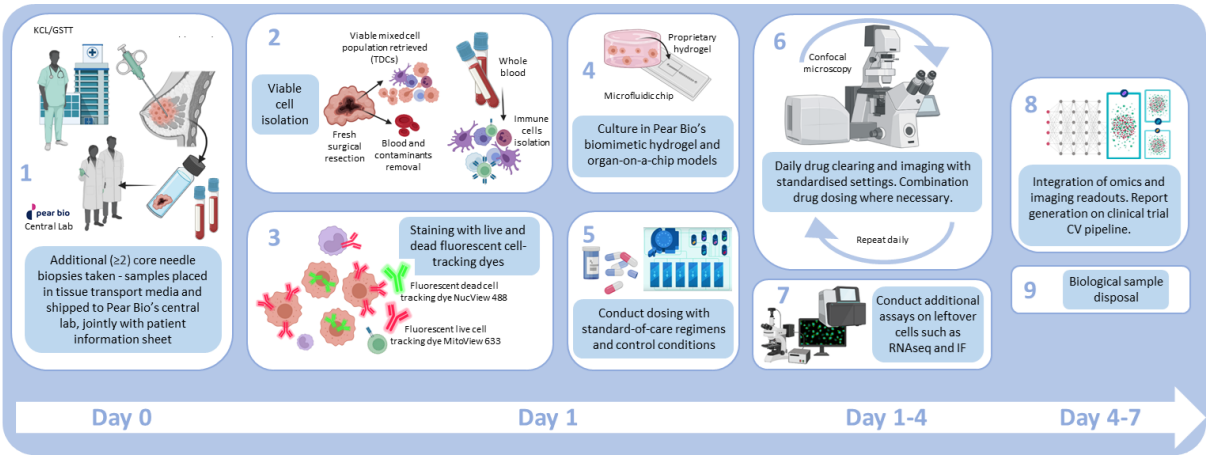


Figure 2: PEAR-MET clinical trial laboratory workflow. (1) mTNBC biopsies/aspirates are collected at trial sites and viably shipped to Pear Bio's central lab for processing. (2) A mixed cell population is isolated from tumour samples. Immune cells are isolated from whole blood. (3) Tumour-dissociated cells (TDCs) and immune cells are stained with live-cell and dead-cell fluorescent dyes. (4) The cells are cultured simultaneously in multiple Pear Bio microfluidic chips within a proprietary hydrogel and device. (5) Standard-of-care chemotherapy and targeted treatments are dosed within the device whilst (6) daily imaging allows for live tracking of cell viability and migration. (7) Wherever additional cells are available, RNAseq, TMB/MSI and IF may be run in parallel to check expression of common biomarkers and validate drug MoA. (8) Computer vision (CV) is implemented to detect changes in cell morphology, viability, and position (amongst other parameters) over time in order to make an informed comparison of differential treatment efficacies.

3.5 Patient evaluability

To be considered evaluable, patients will have to meet **all** of the following criteria:

- Meet the eligibility criteria;
- Have research biopsies/aspirates which yield a minimum of 100,000 viable cells;
- Have research biopsies/aspirates which establish a successful cell culture in the Pear Bio laboratory;
- Complete at least one cycle of subsequent systemic therapy.

3.6 Replacement of patients

Patients who do not meet the evaluability criteria set out in section 3.5 will be excluded from analysis and replaced.

3.7 Target accrual

A maximum of 30 evaluable patients will be recruited in this trial. On recruitment of the first 20 patients, the Trial Management Group (TMG) will meet to assess whether monthly recruitment targets are met, and to confirm sample quality and successful culture rates upon processing at the Pear Bio laboratory. The TMG will use the results to determine whether to increase accrual up to a maximum of 30 patients.

4: PATIENT SELECTION

4.1 Inclusion criteria

1. Able to give written informed consent prior to admission to this study.
2. Female or male aged ≥18 years.
3. Histologically confirmed primary breast cancer which is triple-negative by the most recent ASCO/College of American Pathologists (CAP) guidelines.
4. Stage 4 or locally advanced breast cancer planned for first line systemic therapy, or has received prior lines of systemic therapy and is due to undergo another line of systemic therapy.
5. Willing and able to undergo a mandatory additional core needle biopsy (minimum 2 cores) or equivalent fine needle aspiration from the primary breast mass or a metastasis prior to starting the subsequent line of systemic therapy.
6. Willing and able to undergo a mandatory procedure to collect 40 mL of blood.

4.2 Exclusion criteria

1. Tumours not confirmed as triple negative breast cancer.
2. Early stage TNBC.
3. Patients with TNBC that do not intend to receive systemic therapy.
4. Patients who have already commenced systemic therapy with no plans of changing the systemic therapy after the collection of the core needle biopsy or fine needle aspirate sample.
5. Patients who are due to receive experimental therapies that are not included in the study protocol.
6. Haemoglobin levels below 80g/L prior to research sample collection.
7. Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that, in the investigator's opinion, gives reasonable suspicion of a disease or condition that may affect the interpretation of the results, render the patient at high risk from treatment complications or interferes with obtaining informed consent.

5: STUDY PROCEDURES AND SCHEDULE OF ASSESSMENTS

5.1 Patient identification

Patients will be identified in multi-disciplinary team meetings or in outpatient clinics by their clinical care team.

5.2 Informed consent procedure

It is the responsibility of the Investigator, or clinical research staff delegated by the Investigator, to obtain written informed consent from each subject **prior** to participation in this study, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Ample time must be given for consideration by the patient before taking part. An official hospital translator will be used for any participant who is not competent or comfortable with communication in English. The translator will be asked to read through the Patient Information Sheet (PIS) and Consent Form and to translate each section for the participant. Written informed consent will only be obtained from those who the Investigator feels assured have understood the implications of participation in the study. Patients lacking mental capacity will not be included in this study. The PI must document in the patient's notes when the PIS was given to the patient and when informed consent was obtained.

If new safety information becomes available, the CI, in conjunction with the Trial Management Group (TMG), will review the study, update the PIS accordingly and resubmit for relevant approvals. The CI will review the new safety information and assess whether an urgent TMG meeting should be convened or whether this information can be reviewed at the next scheduled meeting. All patients, including those already undergoing scans, should be informed of the new information, given a copy of the revised PIS, and asked to give their consent to continue in the study. Patients will not be re-consented following amendments that do not affect safety or the number of assessments/visits required.

5.3 Patient enrolment

Principal Investigator(s) (PIs) and delegated team members at each recruiting site must keep a record of all patients screened for entry into this study, including those deemed ineligible after screening. Copies of the screening logs should be filed in the Investigator Site File (ISF). For each patient, the primary reason for exclusion should be recorded. Diagnostic data obtained as part of the patient's standard care can be used to determine eligibility provided they fall within the protocol defined timelines. Written informed consent must be obtained prior to the patient undergoing any study specific procedures.

After ensuring that a patient has consented to participate in the study, a registration electronic case report form (eCRF) must be completed. Patients will then undergo screening to confirm study eligibility. Once it has been confirmed that a patient meets all eligibility criteria, the study site will submit the patient's eligibility information to the coordinating centre. The clinical site will assign patients with a unique study ID for use in all correspondences (the Sponsor will provide a sequence of codes to assign). To ensure patient confidentiality, patients will only be identified using their assigned study ID on eCRFs,

other study specific forms and all communications to the Sponsor. It is the PI's responsibility to maintain a confidential record of the identity (i.e., full name, date of birth and hospital number) for the patients enrolled in this study and their assigned study ID. At the end of the study, this record should be archived along with the ISF.

Full details of how to enrol a patient via the PEAR-MET eCRF can be found in the eCRF completion guidance document.

5.4 Schedule of assessments

While on the study, patients will have to attend at least one additional visit for the biopsy/aspirate and blood collection and screening assessments. Due to logistical reasons, it may be difficult for the recruiting site to carry out all screening assessments in one day. Patients will be fully informed about the number of visits required to confirm eligibility in the trial. Subsequent visits will be as per standard of care at the local institution. For a summary of assessments see Table 1.

	Screening/baseline		**At each scheduled response assessment**
	Up to 1 month before subsequent line of systemic therapy	**Up to 3 days after research biopsy/aspiration**	
Informed consent and eligibility checks	X		
Demographics and medical history	X		
Height, weight, ECOG	X		
Concomitant medication	X		
Results from standard of care haematology, biochemistry assessments	X		
Cancer diagnosis[1]	X		
Tumour size evaluation	X		
Details of past line(s) of treatment given to patients	X		
40mL of whole	X		

blood (collected during or up to 3 days before biopsy/aspirate)			
Image-guided breast tumour research biopsy/aspiration[2]	X		
Details of subsequent line of treatment planned for patient[3]	X		
Adverse Events by CTCAE v5.0[4]		X	
Treatment regimen details[5]			X
Treatment response (defined by RECIST 1.1) [6]			X
Length of progression free survival[7]			X
Length of overall survival[8]			X

Table 1: Schedule of assessments

Table notes:

1. Copies of link-anonymised histology reports from the patient's diagnostic biopsy will be collected.
2. Patients must be willing to undergo a new image-guided biopsy or fine needle aspiration procedure in order to obtain fresh tissue. This procedure must be carried out as close as possible to the decision of which systemic therapy regimen will be prescribed.
3. Include full initial regimen details (drugs, doses, schedule).
4. Relating to research biopsy/aspiration and blood collection only. This can be conducted by telephone – physical examination to be done only if clinically indicated.
5. Include any changes to the initially planned regimen (drugs, doses, schedule/delays) during treatment.
6. Response types are defined by RECIST 1.1 guidelines and are applied to routine scans estimated to occur every 2-4 treatment cycles.
7. Copies of anonymised radiology reports and other measurements of disease response or progression will be collected and sent to the CI's clinical research team and the Sponsor.
8. Where possible, the length of time from metastatic disease diagnosis to death will be collected and sent to the CI's clinical research team and the Sponsor.

5.5 Procedures and measurements

5.5.1 Demographics and medical history

Demographic data collected will include age, sex and race/ethnicity. Details of medical history obtained as part of standard care will be collected, including details of any relevant medical conditions occurring prior to consent.

Details will also be collected on the patient's cancer diagnosis, including site(s), date of diagnosis, tumour size, and tumour stage.

5.5.2 Height, weight and ECOG

Baseline height (cm) and weight (kg) will be collected from the medical records.

Performance status data will be collected at baseline only using the ECOG performance score according to Table 2 and will be recorded in the e-CRF:

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Table 2: ECOG performance status

5.5.3 Concomitant medication

All medications (including prescription medications and over the counter preparations) taken by the patient during the screening period will be documented as concomitant medications. The following details will be collected at baseline: drug name, reason for treatment, dose/units, route of administration, frequency.

5.5.4 Haematology and clinical biochemistry

The results of any standard of care haematology and clinical biochemistry tests will be collected at baseline (prior to the research biopsy/aspiration or after the research biopsy/aspiration but prior to the subsequent line of treatment commencing). The date and result for each test must be recorded in the appropriate eCRF.

5.5.5 Treatment details

Patients will receive systemic therapy as per standard of care at the discretion of the treating physician. The following details will be collected at each cycle: drug name, start date and end date, dose/units, dose reductions/interruptions, reasons for any treatment changes/interruptions/dose reductions.

5.5.6 Tumour size evaluation

Standard of care clinical diagnosis of a breast tumour or metastasis amenable to 2 cores (preferable) or a fine needle aspirate (acceptable if core biopsy is not feasible or unsafe) being taken must be available prior to a patient being confirmed as eligible for this study. This can be based on any imaging assessment (US, mammogram, MRI, CT etc) carried out as per the patient's standard care prior to the start of the subsequent line of systemic therapy. Measurements will be made by clinical imaging, with the modality used dependent on local institutional guidelines. The modalities used will also depend on the location of lesions (e.g., the use of MRI for brain metastases or bone scans for bone metastases). The results of any imaging tumour assessments done while the patient is on study will be collected in this study. The imaging modality used will be recorded.

5.5.7 Adverse events

Adverse events will be restricted to those resulting from the study-mandated breast tumour biopsy/aspirate and blood collection, and will be collected up to 3 days after the collection procedure. The following details will be collected: AE term, date of onset, date of resolution, CTCAE grade (maximum intensity), seriousness, investigator causality rating against research procedures (yes or no), action taken with regard to the research procedures and outcome.

5.5.8 Breast tumour research biopsy/aspirate and blood collection

One additional image-guided core needle biopsy (minimum 14-gauge) from the primary breast tumour or a metastasis will be required, from which at least two cores are taken. If the collection of cores is not feasible or poses a safety risk, an equivalent fine needle aspirate will be taken. This sample collection must be carried out as close as possible to the decision of which systemic therapy regimen will be prescribed. Samples must be placed in tissue transport medium to be supplied by Pear Bio.

40mL of whole blood will also be collected in four 10mL EDTA tubes up to 3 days before the tumour sample collection; preferably before conducting the core needle biopsy or fine needle aspiration procedure if on the same visit.

Together, the samples are stored at 4°C before being transported by courier to Pear Bio. The time elapsed between collection and delivery to Pear Bio must be less than 24 hours.

5.5.9 Radiation dose and exposure considerations

This study is an observational study, and the majority of radiation exposure will occur as part of standard of care. However, in line with HRA guidance, many of these are classified as "research exposures" as they are significant for determining study outcomes. The majority of these scans are CT scans of the Chest, Abdomen and Pelvis, although clinicians may also use FDG-PET-CT scans as well, if clinically indicated.

In addition, patients may receive radiotherapy as part of their routine care. Although this study does not involve the use of radiotherapy, we record the use of radiotherapy in patients in the study, and lesions are deemed to be non-evaluable for endpoints such as ORR and PFS once they have been irradiated.

Patients need to undergo a biopsy as part of the enrolment process for the study. Clinical teams will decide how best to arrange this based on clinical review in the local multi-disciplinary team meeting (MDT). The biopsy process requires image guidance, and that imaging may involve the use of ionising radiation, although it might also be based on ultrasound.

Expected trial research exposures therefore may consist of:

CT scans: Delivered as part of routine care, which are then used to measure study outcomes
18-FDG PET-CT scans: Delivered as part of routine care, which may contribute to measuring study outcomes
Radiotherapy: Delivered as part of routine care

Imaging for Biopsy: This is a study-specific procedure outside of standard of care, and may involve the use of ionising radiation (CT scan or fluoroscopy) or ultrasound. This will be a one-time event only for each study participant.

5.6 Exploratory research

All patients will be consented for the collection and use of their tumour tissue and blood samples. All samples will be link-anonymised and only identified by the study ID and unique sample number allocated by the clinical site (Sponsor to provide sequence of codes to assign). These results may be reported separately from the clinical study report.

5.6.1 Chain of custody of biological samples

In all cases, patients will be consented for the collection and use of their biological samples and a full chain of custody will be maintained for all samples throughout their lifecycle. The Investigator at each site is responsible for maintaining a record of full traceability of biological samples collected from patients while these are in storage at the site; either until shipment or

disposal. Any sample receiver (e.g., sub-contracted service provider) will keep full traceability of samples from receipt of arrival to further shipment or disposal (as appropriate).

In the event that a patient withdraws their consent from the study, all samples and data collected up to that date will be used in the study, but no further data will be collected. As the Sponsor, Ourotech Limited (trading as Pear Bio) will maintain oversight of the entire lifecycle through internal procedures and monitoring of the study site(s). The Sponsor will be the custodian of the samples. Samples will be transferred from the participating site to Ourotech Limited (trading as Pear Bio). At the end of the study, unused samples (or portions of samples) will be retained for future research while all used samples (or portions of samples) will be disposed of in accordance with the Human Tissue Act 2004.

5.7 Patient withdrawal

Patients may voluntarily withdraw from the study at any time. Patients will also be withdrawn from the study if they are not able to undergo a biopsy/aspiration of the breast tumour or metastasis for any reason, the biopsy/aspirate sample yields less than 100,000 viable cells or fails to establish a culture in the laboratory, or the patient completes fewer than two cycles of systemic therapy.

6: PHARMACOVIGILANCE

6.1 Definition of an Adverse Event (AE)

An AE is any untoward medical occurrence (including deterioration of a pre-existing medical condition) in a subject who is administered any research procedure, which does not necessarily have a causal relationship with this procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with a research procedure, whether or not considered related to the procedure.

6.2 Recording of AEs

AEs will be collected from informed consent until 3 days after study sample collection. They will be followed up according to local practice until the event has stabilised or resolved. Any unresolved AEs at the patient's last visit should be followed up for as long as medically indicated, but without further recording in the eCRF. The following details will be collected in the eCRF for each AE: AE term, date of onset, date of resolution, NCI-CTCAE grade maximum intensity, seriousness, investigator causality rating against research procedures, action taken with regards to research procedures and outcome.

6.3 Severity of AEs

Severity is a measure of intensity whereas seriousness is defined by the criteria in section 6.6. Severity will be assessed using the grading scales found in the National Cancer Institute CTCAE version v5.0 (27Nov2017) for all AEs with an assigned NCI-CTCAE term. For those events without assigned NCI-CTCAE grades, the recommendation on page 1 of the NCI-CTCAE that converts mild, moderate and severe into NCI-CTCAE grades should be used. A copy of the NCI-CTCAE version 5.0 can be downloaded from the Cancer Therapy Evaluation Program website (http://ctep.cancer.gov).

6.4 Causality of AEs

The Investigator will assess causal relationships between research procedures and each AE.

6.5 Abnormal laboratory test results

Not applicable. Haematological and biochemical parameters will not be assessed throughout the study.

6.6 Definition of Serious Adverse Event (SAE)

An SAE is an AE occurring during any part of the study that meets one or more of the following criteria:

- Is fatal – results in death
    - NOTE: death is an outcome, not an event
- Is life-threatening
    - NOTE: The term ‘life threatening’ in the definition of ‘serious’ refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more serious,
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
    - NOTE: “Hospitalisation” means any unexpected admission to a hospital. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission). Elective admissions for surgery are also excluded.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other important medical events
    - NOTE: Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a subject, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

6.7 Reporting of SAEs

Rapid reporting of all SAEs occurring from consent until 3 days after study sample collection must be performed as detailed in the “SAE reporting instructions” within 24 hours of the PI or designee becoming aware of the event. If the investigator becomes aware of safety information that appears to be related to a research procedure involving a subject who participated in the study, even after an individual subject has completed the study, this should also be reported to the Sponsor. All SAEs should be reported to Sponsor using the SAE form and will be reviewed by the CI or designated representative to confirm relatedness and expectedness. Following documented assessment by a delegated investigator, the completed SAE form will be forwarded to the Sponsor by the clinical site within the pre-specified timelines.

All SAEs must be reported to the Sponsor using the PEAR-MET SAE form via email and within 24 hours of the site becoming aware of the event.

Please note all events should also be recorded in the relevant sections of the case report forms and patient medical records.

6.7.1 Non-reportable events

Due to the nature and stage of the disease in this study, the following situations that fulfil the definition of an SAE are excluded from recording/reporting on an SAE form. However, they should be recorded on the eCRF and in the medical records.

- Elective hospitalisation for treatment of cancer or its complications.

- Prolonged hospitalisation for post anti-cancer treatment complications
- Elective hospitalisation to make treatment or procedures easier.
- Elective hospitalisation for pre-existing conditions that have not been exacerbated by trial intervention(s)

6.8 Definition of an Adverse Reaction (AR)

An AR is any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease which temporarily resulted from the administration of any research procedures associated with the study. The expression “reasonable causal relationship” means to convey, in general, that there is evidence or argument to suggest a causal relationship.

6.9 Definition of Serious Adverse Reaction (SAR)

A SAR is an AR that is classed as serious as per the criteria included in section 6.6 of the study protocol.

6.10 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

If an SAE is related to the use of a medical device product or taking part in research procedures, and is not listed in the study protocol as an expected occurrence, then it is a SUSAR.

6.11 Reporting of SUSARs

Research sites will submit SUSARs to the Sponsor. It is the Sponsor’s responsibility to report SUSARs to the REC and to disseminate SUSARs to participating sites. Follow-up of patients who have experienced a SUSAR should continue until recovery is complete or the condition has stabilised.

6.12 Annual reporting

The Annual Progress Report (APR) will be sent by the CI to the Sponsor and REC using the NRES template. The APR will be submitted on the anniversary date of the “favourable opinion” letter from the REC. A copy of the APR and an associated correspondence with REC will also be sent to participating sites.

6.13 Urgent safety measures

The CI or Sponsor may take urgent safety measures to ensure the safety and protection of the clinical trial patients from any immediate hazard to their health and safety, in accordance with Regulation 30. The measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the Sponsor (via telephone for discussion with the medical assessor at the clinical trials unit) of this event **immediately**.

The Sponsor has an obligation to inform the REC in writing within **3 days**, in the form of a substantial amendment. The Sponsor must be sent a copy of the correspondence with regards to this matter.

7: STATISTICAL CONSIDERATIONS

7.1 Sample size

Up to thirty (30) evaluable patients will be recruited to this study. This study is not formally powered due to the proof-of-concept nature of the study and the lack of comparable historical data, but the patient numbers will allow for preliminary Receiver Operating Characteristic (ROC) and Time-To-Event (TTE) analysis.

7.2 Statistical analysis

7.2.1 Primary efficacy analysis

The primary objective of this study does not require patient outcomes. Instead, comparisons will be made to quantify the variability of treatment response within and between patient tumour samples. As these are purely tied to lab results of the Pear Bio test on patient samples, they are analytical comparisons rather than clinical comparisons. No correlations are made between Pear Bio's test results and patient outcomes in the primary analysis.

Comparisons of differentiated *ex vivo* therapeutic response are done on the basis of:

1. Each therapeutic demonstrating its intended mechanism of action based on a before and/or after biomarker measurement of the tumour/blood sample (e.g., IF assay measuring the level of target protein for a given targeted drug)

2. Computer vision analysis applied to confocal microscopy images of the tumour cell cultures in the organ-on-a-chips at multiple timepoints, which yields multiple phenotypic metrics of *ex vivo* tumour response, including, but not limited to:
    a. Cell viability (live cell count, dead cell count, percent viability, etc.)
    b. Cell migration distance/speed (mean, median, 5% most aggressive cells, etc.)
    c. Change in the diameter of the 3D tumour cell culture

These analytical measurements are used to conduct the following comparisons:

1. Observing differentiated *ex vivo* treatment response across the therapies tested on each patient's tumour sample (intra-patient comparison)
    a. Ranking of drug efficacy will be done for each assay metric on a per patient basis to determine agreement/disagreement between assay metrics
    b. Calculating the range/variability of response to all tested therapies for each assay metric on a per patient basis

2. Observing differentiated *ex vivo* treatment response levels between the cohort of samples collected from patients on a per therapeutic basis (inter-patient comparison)

a. Grouping of patient samples based on change in target biomarker level on a per therapeutic basis
b. Grouping of patient samples based on *ex vivo* response or resistance at a phenotypic level (quantified by computer vision) on a per therapeutic basis
c. Comparing the general efficacy of the therapeutics tested across all patient samples using box plots for each phenotypic *ex vivo* tumour response metric of interest
d. Comparing the general efficacy of the therapeutics tested across patient samples using drug efficacy ranks on each sample and using Kendall's W and Spearman ranked correlation tests to determine whether some therapies consistently outperform others *ex vivo* (on a given metric of interest)
e. Comparing the general efficacy of the therapeutics tested across patient samples using Repeated Measures ANOVA for each phenotypic *ex vivo* tumour response metric of interest
f. Comparing the relative efficacy of any 2 therapeutics tested across patient samples using a paired T-test for each phenotypic *ex vivo* tumour response metric of interest

The primary objective will be met if there are significant variations in intra-patient and inter-patient response. Due to the heterogeneity of TNBC, one therapeutic is not expected to always outperform other options across all patients. That could indicate *ex vivo* overperformance due to assay conditions that require adjustment, either in the biology workflow or response vs resistance thresholds. This information is critical to obtain before the assay is used in interventional trials to guide treatment decisions.

7.2.2 Secondary efficacy analysis

Overall response rate (ORR)

ORR will be measured after 2-4 cycles of the treatment regimen following the collection of the research sample.

Overall response rate is evaluated by the patient's radiologist and is defined as the patient achieving a partial or complete response (i.e., stable disease excluded) to the line of treatment given after research sample collection based on RECIST 1.1 guidelines. Lesions that receive radiotherapy are excluded from consideration of response from the date of radiotherapy.

The prediction accuracy of Pear Bio's test is measured with the aid of an ROC and contingency table.

Contingency table:

	Patients with response	Patients without response
Pear predicts response	A	B

Pear predicts non-response	C	D

The contingency table is used to calculate:

1. Sensitivity, defined as the percentage of true responders identified by Pear's test based on the total number of patients who achieved a response in the study (PR or CR after 2-4 cycles). Sensitivity = A/(A+C)
2. Specificity, defined as the percentage of true non-responders identified by Pear's test based on the total number of patients who did not achieve a response in the study (SD or PD after 2-4 cycles). Specificity = D/(B+D)
3. Positive predictive value (PPV), defined as the percentage of patients correctly predicted to achieve a response by Pear's test based on all predictions of positive response (PR or CR after 2-4 cycles). PPV = A/(A+B)
4. Negative predictive value (NPV), defined as the percentage of patients correctly predicted to not respond to therapy by Pear's test based on all predictions of non-response (SD or PD after 2-4 cycles). NPV = D/(C+D)

The ROC curves will enable measurements of sensitivity, specificity, positive predictive value and negative predictive value. For at least 1 assay metric, the ROC must be able to meet ≥70% sensitivity and specificity, with a partial area under the curve (pAUC) ≥70%. The ROC will be generated using the "pROC" package on R. Input metrics are extracted from Pear Bio's computer vision pipeline applied to microscope images of patient-derived organ-on-a-chip 3D cell cultures exposed to the treatment given to that patient. The analytical threshold at which sensitivity and specificity are maximised is returned, alongside the corresponding sensitivity, specificity, PPV, and NPV.

The threshold established from the ROC for a given *ex vivo* tumour response metric is used to populate a contingency table. A Fisher Exact test can be used on a 2x2 contingency table to return a p value. A p value under 0.05 (<0.05) is desired, although this study does not require statistical significance.

<u>Progression free survival (PFS)</u>

Tumour response will be measured after 2-4 cycles of the line of treatment commencing after collection of the research biopsy/aspirate, and at all subsequent timepoints until disease progression, as per standard of care.

Disease progression is evaluated by the patient's radiologist and is defined as tumour growth or increase in lesions against the line of treatment given after research biopsy/aspirate collection based on RECIST 1.1 guidelines. Lesions that receive radiotherapy are excluded from consideration of response from the date of radiotherapy.

Kaplan–Meier curves will be generated on the patient population and, where feasible, for each line of therapy or for each therapeutic option (if n is sufficient).

Low vs high biomarker groups will be correlated to PFS for each computer vision metric generated by Pear Bio, including, but not limited to:

1. *Ex vivo* cell viability on the same therapy given to a patient
2. *Ex vivo* cell migration speed on the same therapy given to a patient
    a. Mean distance/speed
    b. Median distance/speed
    c. Speed of the 5% most aggressive cells
3. Change in *ex vivo* 3D cell culture diameter on the same therapy given to a patient

Kaplan-Meier curves are generated using the “survminer” package for Kaplan-Meier curves on R.

The potential benefit of Pear Bio’s test is projected by comparing Kaplan–Meier curves of 2 patient groups for PFS. A given assay metric of Pear Bio’s test is used with a threshold to separate patients into 2 groups based on predicted benefit or non-benefit to the given therapy. Thresholds for each assay measurement are set using a receiver operating characteristics (ROC) curve, which can be generated based on a change in baseline or therapeutic efficacy greater than a control chip in the *ex vivo* cell culture. The thresholds can also be based on an ROC generated on the overall response rate (ORR) endpoint. The hazard ratio, 95% confidence interval and p-value of the 2 groups of patients separated using an analytical threshold are calculated using a log-rank test (optionally a Cox proportional-hazards model). The results of this analysis are not meant to demonstrate the benefit of Pear Bio’s test. Rather, this data will generate hypotheses for a given assay metric and analytical threshold to be used in future trials, provided that other analyses, such as box plots and ROCs, show a clear ability of the assay metric and analytical threshold to identify patients with response vs non-response to therapy.

Subgroup comparisons will be done on Kaplan–Meier curves for PFS where only patients who received the same line of treatment are analysed (e.g., only patients with first line therapy are compared to each other). This analysis will only be done if patient numbers are sufficient for analysis by line of treatment. Likewise, this method will be applied on a per therapeutic basis.

7.2.3 Exploratory analysis

<u>Successful cell culture rate</u>

A successful culture is defined as a minimum of 100,000 viable cells being extracted from the cores or aspirates, and achieving a ≥70% cell viability on day 3 in the control organ-on-a-chip (no treatment) relative to the number of viable cells plated post-cell isolation on day 0.

The successful cell culture rate is the percentage of successful cell cultures established out of all tumour samples arriving uncompromised within 24 hours of collection to the Pear Bio laboratory. Culture success rates will be compared between core needle samples and fine needle aspirate samples.

<u>Overall survival prediction</u>

Patient data is collected up to death and their time from metastatic disease diagnosis to death is recorded to determine the overall survival (OS) time.

This analysis will use median OS within the study cohort to differentiate patients into above average and below average prognosis groups. The analysis will then explore indicators/biomarkers in Pear Bio's test that can identify patients as above average or below average prognosis, agnostic of the therapeutic choice.

Thresholds for each assay metric from Pear Bio's test will be set using a receiver operating characteristic (ROC) curve to determine whether that metric/threshold can differentiate prognosis groups with high sensitivity and specificity.

Omics analysis

Omics measurements of patient samples taken at Pear Bio's laboratory will be used to determine whether any biomarkers can correlate to therapeutic response.

Omics methods include:
1. Immunofluorescence (IF)
2. RNASeq
3. TMB/MSI

Prediction methods include:
1. Correlating baseline expression of biomarkers in the tumour sample to real-world patient outcomes
2. Comparing the change in biomarker expression before and after *ex vivo* treatment testing to real-world patient response.

7.3 Interim analysis and study termination

Interim analysis will be done on the primary objective after 20 patients. However, that number of patients will be insufficient for many of the statistical tests.

On recruitment of the first 20 patients, the TMG will meet to assess whether monthly recruitment targets are met and to confirm sample quality and successful culture rates upon receipt and processing at the Pear Bio lab. The TMG will use the results to determine whether to increase accrual up to a maximum of 30 patients.

The study may be terminated early if the primary and secondary objectives are satisfied, and remaining patients who have not seen disease progression will not affect median PFS or, to a significant degree, the analysis of results. If at least 50% of the 30 total patients have seen disease progression and all remaining enrolled patients have continued PFS that is greater than the 50th percentile of the total study population, median PFS will be set at the 50th percentile.

7.4 End of study definition

The end of the trial is defined as the last patient's last data collection (e.g. disease progression), which is estimated to take place within 4 months of the patient commencing the line of therapy subsequent to the collection of the research biopsy/aspirate and blood, or 24 months after enrollment of the last evaluable patient, whichever happens first. In cases of

early termination of the trial or a temporary halt, the Sponsor will notify the REC within 15 days of the decision, and a detailed written explanation for the termination/halt will be given.

7.5 Handling of missing data

Missing data will be recorded as not available on eCRFs. Missing data points will not be imputed in the analysis for that specific endpoint.

8: DATA HANDLING AND RECORD KEEPING

8.1 Confidentiality

All information generated in the study will be kept strictly confidential. The researchers conducting the trial will abide by the Data Protection Act 1998, and the rights the patient has under this act.

Parts of the patients' medical records and the data collected for the trial will be looked at by authorised personnel from the Sponsor during audit/inspection activities. It may also be looked at by authorised personnel from the patient's NHS Trust to check that the trial is being carried out correctly. This is clearly stated on the consent form.

All the above bodies have a duty of confidentiality to the patient as a research participant and nothing that could reveal their identity will be disclosed outside the research site. All data will be stored in a locked and dedicated room only accessible by authorised personnel.

8.2 Study documents

All trial related documents should be filed in the Investigator's Site File (ISF). It should contain essential documents as per the contents page provided to the Investigator by the Sponsor. The Sponsor will inform the PI and their staff of any updates and forward any relevant documentation. It is the participating PI's responsibility to maintain this file and keep all records up to date.

8.3 Data and sample acquisition

This trial uses electronic case report forms (eCRFs). Sites will receive training for appropriate eCRF completion. The eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with the Sponsor's instructions. Any data queries arising from initial review will be sent to the relevant centre for resolution.

All eCRFs should be completed by designated, trained examining personnel or the study coordinator as appropriate. The eCRF should be reviewed and electronically signed and dated by the investigator. In addition, at the end of the study, the investigator will receive patient data for his or her site that must be kept with the study records.

The Trial Management Group (TMG) reserves the right to amend or add to the eCRFs as appropriate. Revised or additional forms should be used by centres in accordance with the guidelines provided by the Sponsor.

The PI will be responsible for monitoring the transfer of biological specimens. The Sponsor will confirm the receipt of biological specimens. Tracking forms will accompany all sample transfers to the Sponsor's central lab. The clinical site will link with the Sponsor to ensure all biological samples are collected and transferred as per the lab manual. All data will be handled, computerised and stored in accordance with GDPR.

8.4 Record retention and archiving

At the end of the trial, all documentation, as defined by GCP, should be stored by each individual site's archiving facility, until notification for destruction from the Sponsor. The location of the archiving facility must be provided to the Sponsor.

The Sponsor will arrange a 'close out' visit where all trial documentation will be prepared for archiving by that site. Records will be retained at each individual site. All records relating to the trial should be stored together, including the ISF. It is the responsibility of the Principal Investigator to ensure a full set of records is collated and documented.

In addition, source documentation (medical notes, images, results etc.) should be retained, as per Sponsor request, for the duration of the archiving period.

All this information will be stored for a minimum of 25 years. The Sponsor should be contacted prior to destruction.

8.5 Compliance

This trial will be conducted in accordance with the principles of Good Clinical Practice (GCP) as laid out in the EU directive and The Medicines for Human Use (Clinical Trials) Regulation 2004, and its amendments.

In addition, Sponsor auditors will be allowed access to eCRFs, source documents, and other trial files to evaluate the trial. Audit reports will be kept confidential.

9: STUDY MANAGEMENT

A TMG will be convened and will consist of members of the lead clinical site (CI, Trial Coordinator, Project Lead) and the Sponsor's representatives, scientists and statistician(s). The role of the TMG will be to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to, and take appropriate action to safeguard participants and the quality of the trial itself. The TMG will meet at least four times a year. Final decisions about the continuation or termination of the trial are the responsibility of the TMG.

10: CLINICAL GOVERNANCE ISSUES

10.1 Ethical considerations

The trial will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The Research Ethics Committee (REC) will review all appropriate trial documentation in order to safeguard the rights, safety and wellbeing of patients. The trial will only be conducted at sites where appropriate approval has been obtained.

The Sponsor will inform the REC of any changes to the conduct of the trial and seek approval for these changes and any amended patient materials. The Sponsor will maintain an accurate and complete record of all written correspondence to and from the REC and will agree to share all such documents and reports with the Sponsor.

The informed consent and any other documentation provided to patients will be revised if important new information becomes available that is relevant to the subject's consent. Amended documents will be approved by the REC before distribution to patients.

10.2 Summary of monitoring plan

Refer to the PEAR-MET Monitoring Plan for further details. Monitoring will involve a review of the Investigator Site File (ISF), as well as a proportion of Source Data Verification (SDV). This will involve direct access by Sponsor representatives (or other parties, see Section 8.1) to patient notes at the participating hospital sites, which will include the review of consent forms and other relevant investigational reports. Missing data will be sought, unless confirmed as not available. During these visits, the site's activity will be monitored to verify that:

- Source data transcribed onto eCRFs is authentic, accurate, and complete
- Safety, rights, and well-being of the participants are being protected
- The study is being conducted in accordance with the currently approved protocol
- Any other study agreements, GCP, and all applicable NRES requirements are met

10.3 Audit and inspection

This study may be audited by representatives from the Sponsor. The investigator and institution will be informed of the audit outcome. Investigators are obliged to cooperate in any audit; allowing the auditor direct access to all relevant documents and allocating their time and the time of their staff to the auditor to discuss any findings or issues. Audits may occur at any time during or after completion of the study. The investigator should notify the Sponsor immediately of any other audits/inspections if there are any such plans.

10.4 Reporting of serious breaches in GCP or the trial protocol

All investigators participating in the trial will promptly notify the Sponsor of a serious breach (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of. The CI is responsible for notifying the Sponsor within 24 hours of becoming aware of a serious breach.

The Sponsor is responsible, within 7 days of becoming aware of that breach, for notifying the REC in writing of any serious breach of:

- The conditions and principles of GCP in connection with the trial; or
- The protocol relating to that trial, as amended from time to time in accordance with regulations 22 to 25.

A “serious breach” is a breach which is likely to affect to a significant degree:

- The safety or physical or mental integrity of patients in the trial; or
- The scientific value of the trial.

Participating centres should contact the Sponsor for further information.

11: STUDY FINANCES

11.1 Funding sources

This trial is Sponsor-led. Funding is provided by Ourotech Limited (trading as Pear Bio).

11.2 Patient expenses/payments

The Sponsor will compensate study participants for any additional visits related to participation in this trial (i.e., visits outside standard care). This will only cover study participants in the UK, and only for UK domestic travel.

12: SPONSORSHIP AND INDEMNITY

Dr Sheeba Irshad is the Chief Investigator, with Dr Eleonora Peerani as the lead researcher representing the Sponsor. Ourotech Limited (trading as Pear Bio) is sponsoring the study. Indemnity for participating sites is provided by the Sponsor.

13: PUBLICATION POLICY

This study is sponsored by Ourotech Limited (trading as Pear Bio). The data collected in this study will not be used to licence/register any pharmaceuticals. Authorship of the final manuscript(s), interim publications, or abstracts will be decided according to active participation in statistical design, TMG, accrual of eligible patients and statistical analysis.

Contributing centres (and participating investigators) will be acknowledged in the final manuscript. Representatives of the Sponsor will be added, as appropriate, as co-authors. No participant may present data from their centre separately from the rest of the study results, unless they receive written approval from the Sponsor. The publication policy will adhere to the contractual agreement between the Sponsor and its collaborators.

14: REFERENCES

1. Kassam, F., Enright, K., Dent, R., Dranitsaris, G., Myers, J., Flynn, C., ... & Clemons, M. (2009). Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. *Clinical breast cancer*, *9*(1), 29-33.
2. https://www.keytruda.com/triple-negative-breast-cancer/clinical-trial-results/
3. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-regular-approval-sacituzumab-govitecan-triple-negative-breast-cancer
4. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.
5. Cardoso, F., Senkus, E., Costa, A., Papadopoulos, E., Aapro, M., André, F., ... & Winer, E. P. (2018). 4th ESO–ESMO international consensus guidelines for advanced breast cancer (ABC 4). *Annals of Oncology*, *29*(8), 1634-1657.
6. Boyle, P. (2012). Triple-negative breast cancer: epidemiological considerations and recommendations. *Annals of oncology*, *23*, vi7-vi12.
7. Kennecke, H., Yerushalmi, R., Woods, R., Cheang, M. C. U., Voduc, D., Speers, C. H., ... & Gelmon, K. (2010). Metastatic behavior of breast cancer subtypes. *Journal of clinical oncology*, *28*(20), 3271-3277.
8. Metzger-Filho, O., Sun, Z., Viale, G., Price, K. N., Crivellari, D., Snyder, R. D., ... & Cardoso, F. (2013). Patterns of recurrence and outcome according to breast cancer subtypes in lymph node–negative disease: results from International Breast Cancer Study Group Trials VIII and IX. *Journal of clinical oncology*, *31*(25), 3083.
9. Li, X., Yang, J., Peng, L., Sahin, A. A., Huo, L., Ward, K. C., ... & Meisel, J. L. (2017). Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer. *Breast cancer research and treatment*, *161*(2), 279-287.
10. Bruening, W., Fontanarosa, J., Tipton, K., Treadwell, J. R., Launders, J., & Schoelles, K. (2010). Systematic review: comparative effectiveness of core-needle and open surgical biopsy to diagnose breast lesions. *Annals of internal medicine*, *152*(4), 238-246.