TITLE OF THE PROTOCOL: Prospective Evaluation of image-based Artificial intelligence Research and development tool for precision neoadjuvant Triple-Negative Breast Cancer treatment (PEAR TNBC)

IRAS Reference: 295589

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

PROTOCOL SIGNATURE PAGE

Signatures of the Chief Investigator:

The clinical study as detailed within this research protocol (Version 3.0, Dated 11 November 2022), 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.

Chief Investigator Name: Dr Peter Hall

Signature and Date: _____ _____ _12 __ / _May_/_2023_

Signature of Statistical Advisor:

The clinical study as detailed within this research protocol (Version 3.0, Dated 11 November 2022), 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:  2 3 / M A Y / 2 0 2 3

Signature of the Principal Investigator:

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Principal Investigator Site: _____

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

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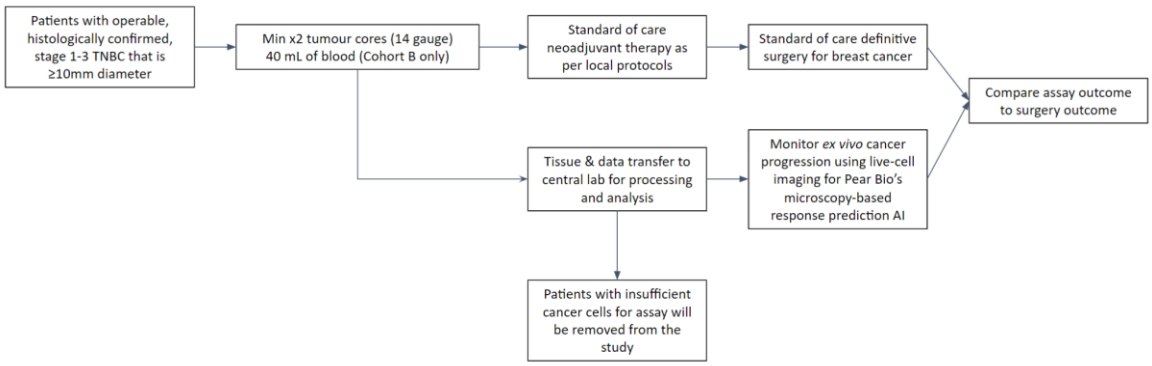
GLOSSARY OF TERMS AND ABBREVIATIONS

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
ISF	Investigator Site File
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria For Adverse Events
NRES	National Research Ethics Service
PI	Principal Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee

SAE	Serious adverse event
SAR	Serious adverse reaction
SDV	Source data verification
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
US	Ultrasound
WBC	White blood cell count

STUDY SYNOPSIS

Title	PEAR_TNBC: **P**rospective **E**valuation of image-based **A**rtificial intelligence **R**esearch and development tool for precision neoadjuvant **T**riple-**N**egative **B**reast **C**ancer treatment
Main Objectives	**Primary:** The primary objective is to assess the accuracy of the Pear Bio tool in predicting a non-pCR vs pCR in patients with early TNBC receiving neoadjuvant chemotherapy with or without immunotherapy, in two cohorts of patients (cohort A and cohort B). **Secondary:** ● To assess the sensitivity of the Pear Bio tool in predicting a non-pCR vs pCR in patients with early TNBC receiving neoadjuvant chemotherapy +/- immunotherapy ● To assess the positive predictive value of the Pear Bio tool in predicting a pCR in patients with early TNBC receiving neoadjuvant chemotherapy +/- immunotherapy ● To assess the negative predictive value of the Pear Bio tool in predicting a non pCR in patients with early TNBC receiving neoadjuvant chemotherapy +/- immunotherapy **Exploratory**: ● To determine the frequency of successfully established cultures from core needle biopsy samples. ● Exploratory subgroup analyses to determine their impact on the ability of the Pear Bio tool to predict a non-pCR vs pCR. ● Determine correlation between proteins and pCR vs non-pCR patients using immunofluorescence or immunohistochemistry measurements ● Explore protein, RNA and DNA biomarkers related to pathological complete response ● Explore immune cell activation in vitro (Cohort B only)
Phase	Not applicable
Design	This is a multicentre, UK-based, observational pilot study that aims to determine the accuracy of a new assay, the Pear Bio tool, in predicting a pCR in patients receiving neoadjuvant chemotherapy with or without immunotherapy for early TNBC. Patients will undergo an additional, mandatory biopsy of the breast tumour before commencing neoadjuvant chemotherapy +/- immunotherapy. For Cohort A, the biopsy sample alone will be run on the Pear Bio tool whilst the patient receives their standard of care neoadjuvant chemotherapy +/- immunotherapy. For Cohort B, the biopsy sample plus peripheral immune cells from the blood sample will be run on the Pear Bio tool whilst the patient receives their standard of care neoadjuvant chemotherapy +/- immunotherapy. As such, for this study, the result from the Pear Bio tool will not be used to inform the choice of neoadjuvant chemotherapy and the treating oncologist will be blinded to the

	assay results. The pathological outcome from surgery (pCR vs non-pCR) will be collected and used to calculate the specificity of the assay as the primary endpoint of the study. 
Sample Size	Up to sixty (60) evaluable patients will be recruited to this study (30 in each cohort)
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 invasive primary breast cancer which is triple-negative by the most recent ASCO/College of American Pathologists (CAP) guidelines. 4. Stage I-III breast cancer planned for neoadjuvant chemotherapy with or without immunotherapy followed by surgery. 5. Primary breast tumour size ≥10 mm. For patients with bilateral tumours both of the breast tumours have to be TNBC and at least one has to be ≥10 mm. 6. Willing to undergo a mandatory additional core needle biopsy from the primary breast mass prior to starting neoadjuvant chemotherapy. Patients with bilateral breast cancer only need to have one tumour biopsied if both tumours are ≥10 mm. 7. Willing to donate 40mL of whole blood (cohort B only)
Exclusion Criteria	1. Inflammatory breast cancer. 2. Inoperable or metastatic TNBC. 3. Patients who have already commenced neoadjuvant chemotherapy. 4. Treatment concurrently or within 4 weeks of commencing neoadjuvant chemotherapy with any experimental therapies. Patients who are due to receive standard of care neoadjuvant chemotherapy on the control arm of a trial may be eligible after discussion with the medical monitor. 5. Secretory or adenoid cystic histological subtypes of triple-negative breast cancer. 6. 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 outcomes of all the subtypes, with a recurrence rate of around 30% at five years in the early setting (1). Pathological complete response (pCR) rate has been shown to be prognostic with the risk of recurrence and death reducing by 76% and 81%, respectively, in those patients who achieve a pCR following neoadjuvant chemotherapy compared to those who do not (2). Recent data have shown that the addition of immunotherapy (pembrolizumab) to neoadjuvant chemotherapy significantly improves pCR and event-free survival rates in patients with early TNBC (N Engl J Med 2020; 382:810-821; N Engl J Med 2022; 386:556-567). As such, the use of pembrolizumab together with chemotherapy has recently been approved by the National Institute for Health and Care Excellence (NICE) for patients with early TNBC who are undergoing neoadjuvant treatment. This combination is likely to be used in routine practice in approximately 50% of all patients with early TNBC.

There are multiple neoadjuvant chemotherapy regimens available for patients with TNBC (3). However, currently, there are no predictive biomarkers as to which the regimen is suitable for an individual in order to maximise the chance of achieving a pCR. This potentially adversely impacts on the prognosis and toxicity (short- and long-term) for the patient.

Pear Bio have developed an organ-on-a-chip device together with a computer vision pipeline through which the response of an individual patient's tumour to different treatment regimens can be tested simultaneously. Initial development has used retrospective biobank samples, but the aim now is to assess the tool's accuracy in predicting a pCR in a prospective, observational study. This study will therefore recruit patients with early TNBC who are planned for neoadjuvant chemotherapy followed by surgery. For this study, the oncologist will be blinded to the response on the Pear Bio tool and the assay will be run in parallel with the patient's neoadjuvant treatment. The aim for future trials will be to run the test prior to starting neoadjuvant treatment to guide the choice of regimen to see whether this can increase the pCR rate.

1.2 BACKGROUND AND RATIONALE

Breast cancer is the second most common cancer worldwide with nearly 2.1 million new cases in 2018, accounting for 11.6% of all cancers and 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 the overexpression or amplification of the human epidermal growth factor receptor 2 (HER2; (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-American race, younger age at diagnosis and *BRCA1* mutations (7). Triple-negative breast cancer has a more aggressive phenotype, shorter time to recurrence and a worse overall survival, regardless of stage, compared to patients with non-TNBC (8-10).

1.2.1 *Current management of early triple-negative breast cancer*

The current treatment of early TNBC usually involves systemic therapy, surgery and radiotherapy. Chemotherapy has been the mainstay of systemic treatment for early TNBC owing to the lack of existing targeted therapy options by definition (3, 6). However, the distant metastatic recurrence rate for patients with early TNBC remains around 30%, nearly all of which occur within five years of diagnosis (8).

A role for chemotherapy in early breast cancer was established in the 1970s when a study showed that adjuvant cyclophosphamide, methotrexate and 5-fluorouracil (5-FU) in combination (CMF) led to a reduction in recurrence rates (11). A trial by the National Surgical Adjuvant Breast and Bowel Project (NSABP) demonstrated that doxorubicin in combination with cyclophosphamide (AC) had the same efficacy as CMF, but had a shorter treatment duration and lower side-effect profile, leading to AC becoming the standard of care (12, 13). The addition of taxanes (docetaxel or paclitaxel) to anthracycline-based regimens led to an improvement in efficacy, with relative reductions in the risk of recurrence, breast cancer specific mortality and overall mortality of 14-16% (13). Sequential administration of anthracycline-taxane provides significant disease-free survival (DFS) and overall survival (OS) benefits compared to concurrent administration (14). Dose-dense (2-weekly) anthracycline/cyclophosphamide schedules with growth factor support should be considered based on evidence showing a significant improvement in survival compared to conventional, 3-weekly schedules (15, 16).

Chemotherapy is recommended for all patients with early TNBC with the possible exceptions of T1aN0 tumours and low risk subtypes such as secretory and adenoid cystic carcinomas (3, 17). Sequential anthracycline/cyclophosphamide-taxane chemotherapy is the current standard of care, but disease-free survival (DFS) at 5 years remains around only 70% (3, 17).

The recent KEYNOTE-522 trial randomly assigned patients to standard neoadjuvant chemotherapy or neoadjuvant chemotherapy plus pembrolizumab, and demonstrated an improvement in event-free survival at 3 years post-randomisation (N Engl J Med 2022; 386:556-567). Based on this, NICE approved the addition of pembrolizumab to neoadjuvant chemotherapy in November 2022.

1.2.2 *Pathological complete response rate in early triple-negative breast cancer*

Neoadjuvant chemotherapy has the potential benefits over adjuvant chemotherapy of downstaging a tumour to facilitate breast conserving surgery as well as allowing the response to chemotherapy to be monitored. Achieving a pathological complete response (pCR), defined as no residual invasive tumour present in the breast or axillary lymph nodes in the surgical resection specimen after neoadjuvant chemotherapy (ypT0/is ypN0), carries a significant prognostic advantage (2, 18). The strongest correlation is seen in patients with TNBC, where achieving a pCR leads to a 76% and 81-84% reduction in the risk of recurrence and death, respectively (2, 18). The event-free survival (EFS) rate at five years increases from 50% to 86% in those patients who have a pCR compared to those who do not. Similarly, overall survival (OS) increases from 58% to 92%. On the basis of these prognostic features, pCR has been approved by the US Food and Drug Administration (FDA) as an endpoint for neoadjuvant trials in early TNBC (19).

When given in the neoadjuvant setting, the pCR rate for anthracycline/cyclophosphamide-taxane chemotherapy is only around 35% (18, 20, 21). Two approaches have been adopted to mitigate this low rate. Firstly, additional systemic therapy can be given in the adjuvant setting to those patients who do not have a

pCR, for example, capecitabine based on the CREATE-X trial (22). The second approach is to add agents to the anthracycline-taxane backbone in order to increase the pCR rate. The addition of carboplatin during neoadjuvant chemotherapy has been demonstrated to increase the pCR from 43% to 53% in a phase II trial and by two-fold in a meta-analysis (21, 23). Furthermore, the addition of the programmed death-1 (PD-1) immune checkpoint inhibitor, pembrolizumab, or the programmed death-ligand 1 (PD-L1) inhibitor, atezolizumab, to neoadjuvant chemotherapy has been shown to increase the pCR rate to around 60-65% (24, 25). However, the increase in pCR rate through the addition of extra agents to the anthracycline-taxane backbone is at the expense of increased toxicity, both haematological and non-haematological, and a higher discontinuation rate (21, 24, 25).

1.2.3 *Predictive biomarkers of pathological complete response*

Currently, there are no predictive biomarkers for pCR, including for immune checkpoint inhibitors (24, 25). This means that some patients are being undertreated, impacting on their survival, and some are being overtreated, exposing them to unnecessary toxicity. Therefore, an assay which can determine the optimal chemotherapy combination to achieve a pCR in an individual patient will be of clinical benefit.

Pear Bio have developed an organ-on-a-chip device together with a computer vision pipeline through which the response of an individual patient's tumour to different chemotherapy regimens can be tested simultaneously. A patient tumour biopsy sample is dissociated into a single cell suspension, stained and cultured in six chips. The characteristics of the cancer cells within the chip, for example, cell migration and viability, are recorded by time-lapse microscopy. The first chip acts as a baseline with no chemotherapy agents added. A second chip is used to test the neoadjuvant therapy combination that is given to the patient, and four other chips test alternative treatment combinations (either escalation or de-escalation). The assay then uses artificial intelligence to analyse the different responses between the wells and determine the probability of achieving a pCR in the patient with a particular chemotherapy combination. Initial development has used retrospective biobank samples and cell lines. Olaparib was tested on 8 responsive (*BRCA1/2* mutated) and 8 non-responsive (*BRCA1/2* wild-type) samples, with cancer cell viability being 41% lower on responsive samples ($p = 0.0003$; Pear Bio, unpublished data). Further studies have been conducted to optimise drug dosing for AC-Paclitaxel and AC-Carboplatin/Paclitaxel on cell lines and patient-derived tumour samples. The aim now is to assess the tool's accuracy in predicting a pCR in a prospective, observational study. This study will therefore recruit patients with early TNBC who are planned for neoadjuvant chemotherapy followed by surgery. For this study, the oncologist will be blinded to the response on the Pear Bio tool and the assay will be run in parallel with the patient's neoadjuvant chemotherapy. The aim for future trials will be to run the test prior to starting neoadjuvant chemotherapy to guide the choice of regimen to see whether this can increase the pCR rate.

1.3 Benefit/risk assessment

This is an observational study with patients receiving standard of care neoadjuvant chemotherapy followed by surgery. The Pear Bio tool will be 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 the pCR rate by using the tool before neoadjuvant chemotherapy starts to decide on the optimal combination of agents for an individual patient.

The main risk to the patient comes from the additional core needle biopsy that is required as fresh tissue (rather than FFPE preserved tissue) is needed for the assay. This would be done separately from the diagnostic core needle biopsy as only 10-20% of cases will be TNBC, leading to 80-90% of patients having

unnecessary extra cores taken if done concurrently. The risks to the patient 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%) (26). In order to assess the impact of immunotherapy on peripheral blood immune cells, 40mL of whole blood will be taken from patients enrolled in Cohort B. The taking of a blood sample may cause some discomfort such as pain at the site where the blood is drawn, bruising, occasional light-headedness and, rarely, fainting.

There is a risk that the biopsy sample will not establish a culture in the laboratory and therefore it would not be possible to run the Pear Bio tool. During initial development, the culture failure rate was 16.67%. 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 sample under specified conditions.

2 STUDY AIMS AND OBJECTIVES

2.1 Primary Objectives and Endpoints

Primary Objective	Endpoints
The primary objective is to assess the accuracy of the Pear Bio tool in predicting a non-pCR vs pCR in patients with early TNBC receiving neoadjuvant chemotherapy with or without immunotherapy	Specificity, defined as the true negative predictions from the Pear Bio tool based on the overall negative (non-pCR) population determined from the surgical resection specimen, is the primary endpoint. pCR is defined as ypT0/is ypN0.

2.2 Secondary Objectives and Endpoints

Secondary objectives	Endpoints
To assess the sensitivity of the Pear Bio tool in predicting a non-pCR vs pCR in patients with early TNBC receiving neoadjuvant chemotherapy with or without immunotherapy	Sensitivity is defined as the percentage of patients that Pear Bio's tool correctly identified would achieve a pCR from the total population of patients who did achieve a pCR (i.e., sensitivity is the true positive percentage).
To assess the positive predictive value of the Pear Bio tool in predicting a pCR in patients with early TNBC receiving neoadjuvant chemotherapy with or without immunotherapy.	Positive predictive value is defined as the percentage of patients for whom the Pear Bio tool correctly predicts a pCR out of all of the Pear Bio tool pCR predictions.
To assess the negative predictive value of the Pear Bio tool in predicting a non-pCR in patients with early TNBC receiving neoadjuvant chemotherapy with or without immunotherapy.	Negative predictive value is defined as the percentage of patients for whom the Pear Bio tool correctly predicts a non pCR out of all of the Pear Bio tool non-pCR predictions.

2.3 Exploratory Objectives and Endpoints

Tertiary objectives	Endpoints
To determine the frequency of successfully established cultures from core needle biopsy samples.	The percentage of cultures in which 70% of viable cells plated post-isolation on day 1 are still alive on day 4 in the control well (no chemotherapy) compared to the number of biopsies taken and successfully arriving at Pear Bio's lab.

Exploratory subgroup analyses to determine their impact on the ability of the Pear Bio tool to predict a non-pCR vs pCR.	Specificity, sensitivity, positive predictive value, negative predictive value as defined above.
Determine correlation between proteins and pCR vs non-pCR patients using immunofluorescence or immunohistochemistry measurements	Specificity, sensitivity, positive predictive value, negative predictive value as defined above for the presence/absence/overexpression of key proteins, such as CD44 and CD24
Determine correlation between RNA expression levels (or DNA biomarkers) and pCR vs non-pCR patients	Log 2-fold change and adjusted p-value, visualised through volcano plots of RNASeq data on pCR vs non-PCR patients
Determine immune cell activation with the addition of immunotherapy to chemotherapy	Immune cell activity and infiltration, measured as the immune cell count and density (cells/mm3) inside the tumour culture and immune cell movement (microns/min).

3 INVESTIGATIONAL PLAN

3.1 Overall design

This is a multicentre, UK-based, observational pilot study that aims to determine the accuracy of a new assay, the Pear Bio tool, in predicting a pCR in patients receiving neoadjuvant chemotherapy for early TNBC. Patients will undergo an additional, mandatory biopsy of the breast tumour before commencing neoadjuvant chemotherapy. The biopsy sample will be run on the Pear Bio tool whilst the patient receives their standard of care neoadjuvant chemotherapy, with or without immunotherapy. For Cohort A, the biopsy sample alone will be run on the Pear Bio tool whilst the patient receives their standard of care neoadjuvant chemotherapy +/- immunotherapy. For Cohort B, the biopsy sample plus peripheral immune cells from the blood sample will be run on the Pear Bio tool whilst the patient receives their standard of care neoadjuvant chemotherapy +/- immunotherapy. As such, for this study, the result from the Pear Bio tool will not be used to inform the choice of neoadjuvant chemotherapy and/or immunotherapy, and the treating oncologist will be blinded to the outcome. The pathological outcome from surgery (pCR vs non-pCR) will be collected and used to calculate the specificity of the assay as the primary endpoint of the study.

3.2 Trial Schema

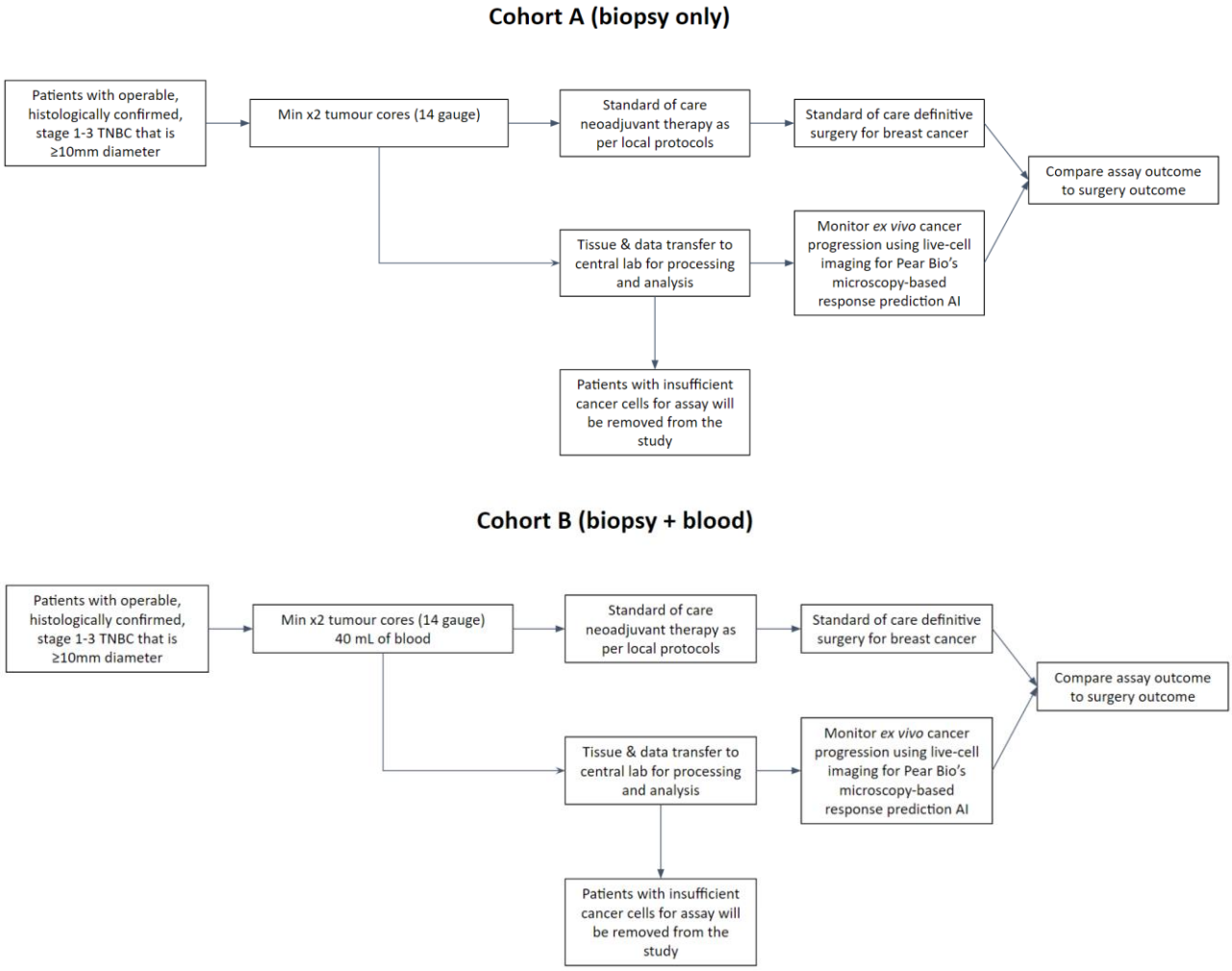


Figure 1: Trial Schema

3.3 Patient Evaluability

All patients, who meet the eligibility criteria, have a baseline biopsy which establishes a culture in the laboratory, complete at least four cycles of neoadjuvant chemotherapy and undergo surgery will be considered evaluable.

3.4 Replacement of Patients

Patients who do not meet the evaluability criteria set out in section 3.4 will be replaced.

3.5 Target Accrual

A maximum of 60 patients will be recruited in this trial. 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 60 patients, with a maximum of 30 patients in cohort A and a maximum of 30 patients in cohort B.

4 PATIENT SELECTION

4.1 Inclusion Criteria

Each patient must meet **all of the following inclusion criteria** to be enrolled in the study:

1. Able to give written informed consent prior to admission to this study.
2. Female or male aged ≥18 years.
3. Histologically confirmed invasive primary breast cancer which is triple-negative by the most recent ASCO/College of American Pathologists (CAP) guidelines.
4. Stage I-III breast cancer planned for neoadjuvant chemotherapy followed by surgery.
5. Primary breast tumour size ≥10 mm. For patients with bilateral tumours both of the breast tumours have to be TNBC and at least one has to be ≥10 mm.
6. Willing to undergo a mandatory additional core needle biopsy from the primary breast mass prior to starting neoadjuvant chemotherapy. Patients with bilateral breast cancer only need to have one tumour biopsied if both tumours are ≥10 mm.
7. Willing to undergo venous sampling for 40mL of blood (Cohort B only)

4.2 Exclusion Criteria

Patients meeting **any of the following exclusion criteria** are not to be enrolled in the study:

1. Inflammatory breast cancer.
2. Inoperable or metastatic TNBC.
3. Patients who have already commenced neoadjuvant chemotherapy.
4. Treatment concurrently or within 4 weeks of commencing neoadjuvant chemotherapy with any experimental therapies. Patients who are due to receive standard of care neoadjuvant chemotherapy on the control arm of a trial may be eligible after discussion with the medical monitor.
5. Secretory or adenoid cystic histological subtypes of triple-negative breast cancer.
6. 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 out-patient clinics by their clinical care team.

5.2 Informed consent procedure

It is the responsibility of the Investigator, or a medically trained person 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. Attempts will be made to arrange for an official hospital translator 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 they have understood the implications of participation in the study. Patients with mental capacity issues 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 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 number of assessments / visits required.

5.3 Patient Enrolment

Principal Investigator(s) (PIs) 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 PEAR-TNBC Trial Coordinator will assign patients with a unique study ID for use in all correspondence. To ensure patient confidentiality, patients will only be identified on eCRFs, other study specific forms and all communication to Pear Bio using their assigned study ID. 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-TNBC 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 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		**Prior to each cycle of neoadjuvant chemotherapy**	**Within 28 days of the final surgery**
	Up to 28 days prior to starting neoadjuvant chemotherapy	**Up to 3 days post biopsy**		
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	X[1]			
Tumour size evaluation [2]	X *(up to 2 months prior to starting neoadjuvant chemotherapy)*			
Adverse Events by CTCAE v5.0 [3]		X		
US/MRI guided breast tumour research biopsy [4]	X			
Research blood sample (cohort B only)	X			
Neoadjuvant chemotherapy details [5]	X		X	X

Surgical outcome				X [6]

Table 1: Schedule of Assessments

[1] Copies of anonymised histology reports from the patient's diagnostic biopsy will be collected. [2] The size of the breast tumour will be collected from any standard of care imaging tumour assessments carried out up to 2 months prior to starting neoadjuvant chemotherapy (Mammogram, US, MRI) as well as during treatment. [3] Relating to biopsy only. Can be conducted by telephone – physical examination to be done only if clinically indicated. [4] Patients must be willing to undergo a new US/MRI-guided biopsy in order to obtain fresh tissue. This biopsy must be carried out after the chemotherapy regimen and dosing has been chosen. The standard of care diagnostic biopsy ideally should have been done within two months of consenting to the trial.
[5] To include regimen (chemotherapy drugs, doses, schedule) and any changes (chemotherapy drugs, doses, schedule/delays) during treatment.
[6] Copies of anonymised histology reports from the patient's definitive breast cancer surgery will be collected. All histology reports must be sent to the PEAR-TNBC coordinating centre, if more than one operation occurs.

5.5 Procedures and Measurements

5.5.1 *Demographics and medical history*

Demographic data collected will include date of birth, sex and race/ethnicity. Details of standard medical history obtained as part of standard of 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, date of diagnosis, pathological and/or physical tumour size, 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. The date and result for each test must be recorded in the appropriate eCRF.

5.5.5 *Treatment details*

Patients will receive neoadjuvant treatment 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 measuring ≥ 10 mm in the longest diameter 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 etc) carried out as per the patient's standard care up to 2 months prior to the start of neoadjuvant chemotherapy. For bilateral cancers, only one of the two breast tumour sites has to measure ≥ 10 mm in the longest diameter. Measurements will be made by clinical imaging with the modality used dependent on local institutional guidelines.

5.5.7 *Adverse events*

Adverse events will be restricted to those resulting from the study-mandated breast tumour biopsy and will be collected up to 3 days post biopsy. 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*

One additional US/MRI-guided core needle biopsy (minimum 14-gauge) from the primary breast tumour will be required from which at least two cores are taken. This biopsy must be carried out after the chemotherapy regimen and dosing has been chosen. For patients with bilateral TNBC, core biopsies only have to be collected from one primary breast tumour and placed in a container clearly indicating the collection site (left / right breast). Samples must be placed in tissue transport medium to be supplied by Pear Bio's laboratory. The sample can then be stored at 4^{o}C before being transported by courier to Pear Bio's laboratory so that it arrives within 24 hours of collection.

5.5.9 *Research blood sample (Cohort B only)*

One additional venous blood sample (40ml) will be taken on the same day as the biopsy. This should be transported along with the research tissue sample.

5.6 Exploratory research

All patients will be consented for the collection and use of their tissue and blood samples. All samples will be linked anonymised and only identified by the study ID and unique sample number allocated by the Pear-TNBC Coordinator. 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. Ourotech Limited (trading as Pear Bio) as the Sponsor will keep overall oversight of the entire lifecycle through internal procedures and monitoring of the study site, 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 all 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 of the breast tumour for any reason, the biopsy sample fails to establish a culture in the laboratory, the patient completes fewer than four cycles of neoadjuvant chemotherapy or the patient does not proceed to surgery.

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 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 Adverse Events

AEs will be collected throughout the study, from informed consent until 3 days post biopsy; 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 Adverse Events

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 Adverse Events

The Investigator will assess causal relationship 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[1]
- Requires inpatient hospitalisation or prolongation of existing hospitalisation[2]
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other important medical events[3]

[1] 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.
[2] "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 cancer surgery are also excluded.
[3] 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, within 24 hours of the PI or designee becoming aware of the event, of all SAEs occurring from consent until 3 days post biopsy, must be performed as detailed in the "SAE reporting instructions". 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 the PEAR-TNBC coordinator using the SAE form and will be reviewed by the CI or designated representative to confirm relatedness and expectedness. Following documented assessment by the CI, the completed SAE form will be forwarded to the Sponsor by the PEAR-TNBC coordinator within the pre-specified timelines.

All SAEs must be reported to the PEAR-TNBC coordinator using the PEAR-TNBC 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 and surgery for treatment of cancer or its complications.
- Prolonged hospitalisation for post-surgical complications or 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 treatment

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 administration of any of the research procedures associated. 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 PEAR-TNBC coordinator, who are responsible for rapid reporting to the Sponsor. It is the CI'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 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 and the Research Ethics Committee (REC) (via telephone for discussion with the medical assessor at the clinical trials unit) of this event immediately.

The CI 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 sixty patients will be recruited to this study (2 cohorts of up 30 patients each). This study is not formally powered due to the lack of comparable historical data, but the patient numbers will allow for a Receiver Operator Curve (ROC) analysis to be performed. Each cohort will be assessed under the statistical analysis plan independently (except for an exploratory analysis of the pooled patient population).

7.2 Statistical Analysis

7.2.1 *Primary Efficacy Analysis*

The primary statistical analysis will be conducted using Receiver Operating Characteristic (ROC) curves for tumour response metrics extracted from the Pear Bio tool for each patient sample. These metrics include: -
Cell viability

- Mean migration distance
- Speed of cell migration
- Migration distance of the most aggressive single cell or subset of cells

These analytical metrics from the Pear Bio tool will be compared with patient pathological outcomes at surgery to determine which metrics are the most accurate at predicting pCR, defined as ypT0/is ypN0, or non-pCR, based on the pathology report from definitive surgery for each patient.

Endpoints:

	Number of patients who achieve pCR	Number of patient who do not achieve pCR
Pear predicts pCR	A	B
Pear predicts non-pCR	C	D

Specificity: measured as the percentage of non-pCR patients identified by the Pear Bio tool from the total number of patients who did not achieve pCR (true negatives). Specificity = D/(B+D)

7.2.2 *Secondary Efficacy Analysis*

Sensitivity: measured as the percentage of patients that the Pear Bio tool identified would achieve a pCR from the total population of patients who achieved pCR (true positives). Sensitivity = A/(A+C)

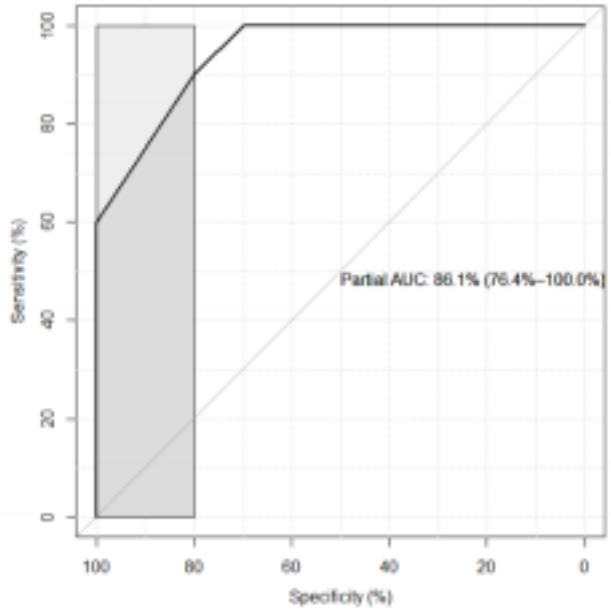
Positive predictive value: the percentage of patients that the Pear Bio tool correctly predicts as pCR out of all pCR predictions. PPV = A/(A+B)

Negative predictive value: the percentage of patients that the Pear Bio tool correctly predicts as non-pCR out of all non-pCR predictions. 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 ≥80% specificity, while retaining adequate sensitivity and an area under the curve (AUC) ≥70%. The ROC will be generated using the pROC package on R and using input metrics of cell behaviour collected by Pear Bio's computer vision algorithms, which extract single cell and bulk tumour metrics like cell viability and cell migration distance of patient tumour samples exposed to a given treatment regimen. A specificity zone will be set at 80-100% specificity to determine the analytical thresholds at which each assay metric is able to achieve 80% or greater specificity. A partial AUC is calculated with 95% confidence intervals. Coordinates of the analytical threshold at which ≥80% specificity is met, and the maximum sensitivity, PPV and NPV at that point are returned.

A p-value can be calculated between any 2 curves (ie. cell viability curve vs migration distance curve). A control curve is set with an AUC of 50% to compare the Pear Bio tool predictions to random guesses of pCR/non-pCR.

An example ROC shows the sensitivity, specificity, and AUC of cell viability (1 of the assay metrics) at different classification (pCR vs non-pCR) thresholds:



Other analysis methods:

A Fisher Exact test will be used on a 2x2 contingency table of patients who achieved pCR vs non-pCR to measure sensitivity and specificity. A specificity ≥80% must be obtained with adequate sensitivity and $p<0.05$.

As part of the exploratory analysis, more complex AI models based on decision trees, such as random forest classifiers, will also be used to differentiate patients who achieved pCR and those who did not.

7.2.3 *Exploratory Analysis*

Culture success rate analysis

The successful culture rate is defined as the percentage of cultures in which 70% of viable cells plated post isolation on day 1 are still alive on day 4 in the control well (no chemotherapy) compared to the number of biopsies taken and successfully arriving at Pear Bio's lab.

Patient subgroup analysis

For all exploratory and subgroup analyses, the endpoints will remain specificity, sensitivity, PPV and NPV. Patient subgroups will be compared to determine which groups have higher pCR rates, and to determine the Pear Bio tool's specificity, sensitivity, PPV and NPV on each of those subgroups.

Logistic regression will be used to compare sub-groups of patients based on whether they achieved pCR. The results of these classifiers will also be validated using a Fisher Exact test. Patient sub-groups can be created out of any available patient information, including but not limited to:

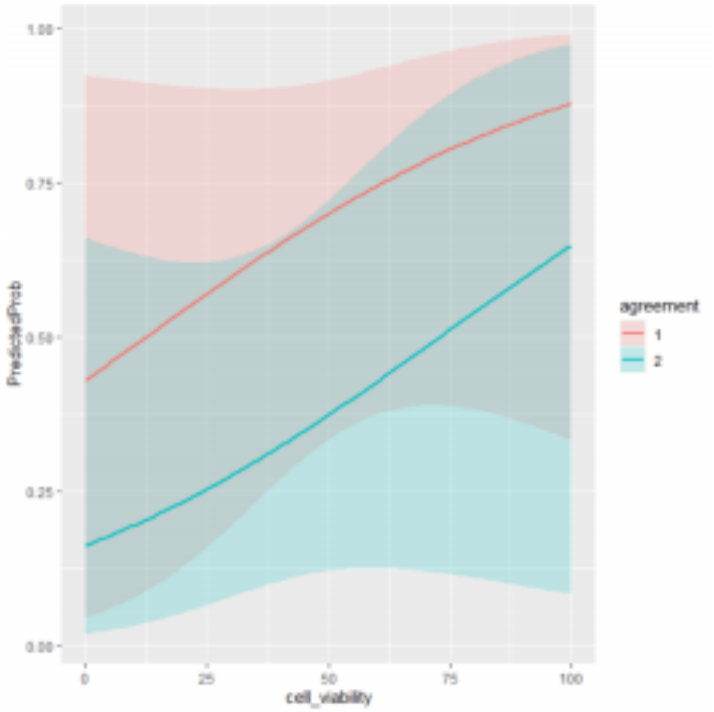
- Age groups
- Ethnicity
- The use of other medication(s)
- Pre-existing medical conditions
- Baseline blood test results

Due to the limited number of patients, many sub-groups will not be simultaneously compared. It is planned that sub-groups will not exceed 3 to allocate approximately 10 (or more) patients per sub-group from the total of 30 patients recruited per cohort. For example, age groups are split into patients above and below a certain age for comparison, or patients with pre-existing conditions are compared to patients without pre-existing conditions.

Patients are compared on the basis of achieving pCR. Patients who achieve pCR are given an outcome value of 1, while patients who do not achieve pCR are given an outcome value of 0. The aod package on R is used to conduct the logistic regression. Each assay metric from the ROC, such as cell viability, can be used again in the logistic regression to determine the probability of correctly predicting pCR at various analytical thresholds. Sub-groups can be compared by splitting patients based on a defining factor, such as age, into 2 or more categories.

If patient data is missing for the purpose of allocating them to a particular sub-group for analysis, they will be excluded from the logistic regression on that comparison (ie. a patient whose age is not recorded will not be included in the comparison of 2 age groups).

To illustrate this analysis method, an example logistic regression with 95% confidence intervals is shown with 2 groups of patients plotted against the predicted probability of achieving pCR at different cell viability values from Pear's assay:



Protein biomarker analysis

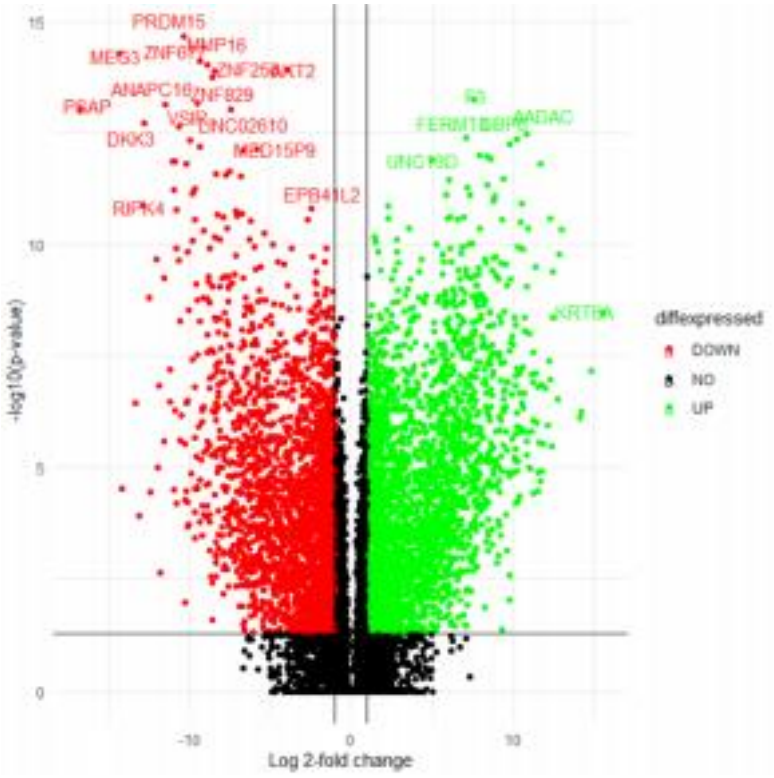
Cell cultures processed through Pear Bio's assay are fixed prior to drug dosing (including the control culture receiving no drug). Immunofluorescence (or immunohistochemistry) is used to measure the expression of proteins such as CD44 and CD24.

The same endpoints of sensitivity, specificity, positive predictive value and negative predictive value are used to determine prediction accuracy for pCR vs non-pCR. The presence or absence of a protein, such as CD44, is used to predict the pCR status of a patient. A 2x2 contingency table and Fisher Exact test similar to the one described in the primary and secondary analysis are used to compare Pear's prediction with patient outcomes.

RNA/DNA biomarker analysis

From biopsy samples that yield an excess of live cells, RNA and DNA are extracted. The RNA is used to run RNASeq tests to measure expression levels. Patients who achieve a pCR are compared to patients with a non-pCR based on these expression levels. Processing of RNASeq data is done using Python. The "pandas" Python library and a t-test are used to compare the patient population with pCR against the population with non-pCR for each RNA biomarker. Biomarkers with a log 2-fold change greater than 1 and an adjusted p value less than 0.05 will be considered as upregulated in patients with pCR. Biomarkers with a log 2-fold change less than -1 (negative 1) and an adjusted p-value less than 0.05 will be considered as downregulated in patients with pCR. All other biomarkers will be considered not significant for a pCR outcome. The R packages "ggplot" and "ggrepel" will be used to visualise biomarkers based on log 2-fold change in expression levels and $-\log_{10}$(p-value), with the most significant biomarkers being labelled in the plot.

An example volcano plot with RNASeq data is shown below:



Biomarkers to the top right have a strong positive association with patients who achieve pCR, while biomarkers in the top left will have a strong negative association with patients who achieve pCR.

Explore immune cell activation in vitro (Cohort B only)

Phenotypic and omic readouts will be collected on the peripheral blood immune cells extracted from patient blood. These biomarkers will be correlated to patient data, including their pCR status at the end of neoadjuvant therapy. Readouts will include immune cell infiltration and activity. This analysis will only apply to patients in Cohort B as it requires blood.

Pooled cohort analysis

The primary, secondary and exploratory analyses will be repeated on the combined cohorts (up to 60 total patients) to determine whether consistent biomarkers appear regardless of the presence of peripheral blood mononuclear cells from patient blood being added to the laboratory testing. Inter-cohort and intra-cohort variation will also be analysed.

7.3 Interim analysis and study termination

As patients are recruited in close proximity to each other, and pathological response data is collected ~6 months from the first administration of their neoadjuvant therapy and the conducting of the Pear Bio assay, there is no designated interim analysis necessary for the earliest patients. 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 laboratory. The TMG will use the results to determine whether to increase accrual up to a maximum of 30 patients.

Patients can be analysed on a rolling basis or at once after 30 patients have completed trial participation. If a ≥80% AUC can be achieved alongside ≥80% specificity and adequate sensitivity on the ROC analysis before all 30 patients have reported outcomes, the analysis can be stopped, and the study terminated for publication of results.

7.4 End of Study Definition

The end of the trial is defined as last patient last data collection, which is estimated to take place within 3 months of the last patient's surgery. In cases of early termination of the trial (e.g., due to slow accrual) or a temporary halt, the coordinating centre will notify the main 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. 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 of 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 accessed 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 PEAR-TNBC coordinator. The PEAR-TNBC coordinator will inform the PI, and their staff, of any updates and forward on 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 CRF completion. CRFs 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 CRFs should be completed by designated, trained examining personnel or the study coordinator as appropriate. The CRF 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 in a readable format on a compact disc that must be kept with the study records.

The PEAR-TNBC coordinator reserves the right to amend or add to the CRFs as appropriate Revised or additional forms should be used by centres in accordance with the guidelines provided by the Sponsor.

PEAR-TNBC coordinator will be responsible for monitoring the transfer and receipt of biological specimens. Tracking forms will accompany all sample transfers to the central lab. The PEAR-TNBC coordinator 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 PEAR-TNBC coordinator and the Sponsor.

The PEAR-TNBC coordinator 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.

These 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 CRFs, 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 coordinating centre (CI, Trial Coordinator, Project Lead, Statistician and Sponsor's representatives and scientists). 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 three times a year.

Final decisions about 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 PEAR-TNBC coordinator 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 CI 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 PEAR-TNBC 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 sites 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 PEAR-TNBC coordinator and 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 allocate his/her time and the time of his/her staff to the auditor to discuss any findings or issues. Audit 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 PEAR-TNBC coordinator 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 PEAR-TNBC coordinator is responsible for notifying the main REC in writing of any serious breach of:

- The conditions and principles of GCP in connection with that trial; or
- The protocol relating to that trial, as amended from time to time in accordance with regulations 22 to 25, within 7 days of becoming aware of that breach.
- A “serious breach” is a breach which is likely to affect to a significant degree:
    - The safety or physical or mental integrity of the patients of the trial; or
    - The scientific value of the trial.

Participating centres should contact the PEAR-TNBC coordinator or CI for further information.

11 STUDY FINANCES

11.1 Funding Sources

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

11.2 Patient expenses / payments

Study participants will not be compensated for participating in this trial.

12 SPONSORSHIP AND INDEMNITY

Dr Peter Hall is the Chief Investigator. Ourotech Limited (trading as Pear Bio) is sponsoring the study. Indemnity for participating sites is provided by the Sponsor.

13 PUBLICATION POLICY

This is an investigator-led study sponsored by Ourotech Limited (trading as Pear Bio). The data collected 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 the statistical design, TMG, accrual of eligible patients and statistical analysis. Contributing centres (and participating investigators) will be acknowledged in the final manuscript. Representatives for the Sponsor will be added, as appropriate, as co-authors. No participant may present data from his/her centre separately from the rest of the study results unless approved by the TMG and the Sponsor. The publication policy will adhere to the contractual agreement between the Sponsor and its collaborators.

14 REFERENCES

1. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674-90.
2. Huang M, O'Shaughnessy J, Zhao J, Haiderali A, Cortés J, Ramsey SD, et al. Association of Pathologic Complete Response with Long-Term Survival Outcomes in Triple-Negative Breast Cancer: A Meta-Analysis. Cancer Res. 2020;80(24):5427-34.
3. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019;30(8):1194-220.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
5. Observatory GC. Cancer Tomorrow. 2018.
6. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4)†. Ann Oncol. 2018;29(8):1634-57.
7. Boyle P. Triple-negative breast cancer: epidemiological considerations and recommendations. Ann Oncol. 2012;23 Suppl 6:vi7-12.
8. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol. 2010;28(20):3271-7.
9. Metzger-Filho O, Sun Z, Viale G, Price KN, Crivellari D, Snyder RD, et al. 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. J Clin Oncol. 2013;31(25):3083-90.
10. Li X, Yang J, Peng L, Sahin AA, Huo L, Ward KC, et al. Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer. Breast cancer research and treatment. 2017;161(2):279-87.
11. Bonadonna G, Brusamolino E, Valagussa P, Rossi A, Brugnatelli L, Brambilla C, et al. Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med. 1976;294(8):405-10.
12. Fisher B, Brown AM, Dimitrov NV, Poisson R, Redmond C, Margolese RG, et al. Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel Project B-15. J Clin Oncol. 1990;8(9):1483-96.
13. Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379(9814):432-44.
14. Shao N, Wang S, Yao C, Xu X, Zhang Y, Zhang Y, et al. Sequential versus concurrent anthracyclines and taxanes as adjuvant chemotherapy of early breast cancer: a meta-analysis of phase III randomized control trials. Breast (Edinburgh, Scotland). 2012;21(3):389-93.
15. Citron ML, Berry DA, Cirrincione C, Hudis C, Winer EP, Gradishar WJ, et al. Randomized trial of dose dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol. 2003;21(8):1431-9.
16. Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. Lancet. 2019;393(10179):1440-52.

17. NCCN. NCCN Guidelines Breast Cancer. 2020.
18. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384(9938):164-72.
19. FDA. Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics Guidance for Industry. 2018.
20. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol. 2018;19(4):497-509.
21. G von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol. 2014;15(7):747-56.
22. Masuda N, Lee SJ, Ohtani S, Im YH, Lee ES, Yokota I, et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. N Engl J Med. 2017;376(22):2147-59.
23. Wang D, Feng J, Xu B. A meta-analysis of platinum-based neoadjuvant chemotherapy versus standard neoadjuvant chemotherapy for triple-negative breast cancer. Future oncology (London, England). 2019;15(23):2779-90.
24. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, et al. Pembrolizumab for Early Triple Negative Breast Cancer. N Engl J Med. 2020;382(9):810-21.
25. Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet. 2020;396(10257):1090-100.
26. Bruening W, Fontanarosa J, Tipton K, Treadwell JR, Launders J, Schoelles K. Systematic review: comparative effectiveness of core-needle and open surgical biopsy to diagnose breast lesions. Annals of internal medicine. 2010;152(4):238-46.