PEAR-GLIO

TITLE OF THE PROTOCOL: Prospective Evaluation of AI R&D tool in adult Glioma and other primary brain tumours (PEAR-GLIO)

IRAS Reference: 318531

Sponsor:	Ourotech Limited t/a Pear Bio Representative of the Sponsor: Duleek Ranatunga Finsgate, 5-7 Cranwood Street London, England EC1V 9EE Tel: 07716558079 E-mail: duleek@pearbio.com
Chief Investigator(s):	Dr. Matt Williams Radiotherapy Dept Imperial College Healthcare NHS Trust Fulham Palace Rd W6 8RF EmaiL: matthew.williams@imperial.ac.uk Tel: 0203 3311 0733
Principal Investigator(s):	

STUDY CONTACTS

Funder(s):

Ourotech Limited (trading as Pear Bio), Finsgate, 5-7 Cranwood Street, London, England, EC1V 9EE

Statistical Advice:

Pear Bio, Imperial College White City Campus, 84 Wood Lane, London, W12 0BZ.
Contact: Dr. Francesco Iori, PhD
E: francesco@pearbio.com

Sponsor Laboratory:

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

Trial Coordination:

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

Participating Sites:

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

PROTOCOL SIGNATURE PAGE

Signature of the Chief Investigator:

The clinical study as detailed within this research protocol (Version 1.0, Dated 3 March 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.

Chief Investigator Name: Dr. Matthew Williams

Signature and Date: M.Williams 25 / May / 2023

Signature of Statistical Advisor:

The clinical study as detailed within this research protocol (Version 1.0, Dated 3 March 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: Francesco Iori

Signature and Date: Francesco Iori 22 / May / 2023

Signature of the Principal Investigator:

The clinical study as detailed within this research protocol (Version 1.0, Dated 3 March 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:

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

TABLE OF CONTENTS

List of Tables and Figures in the Text

Tables

Figures

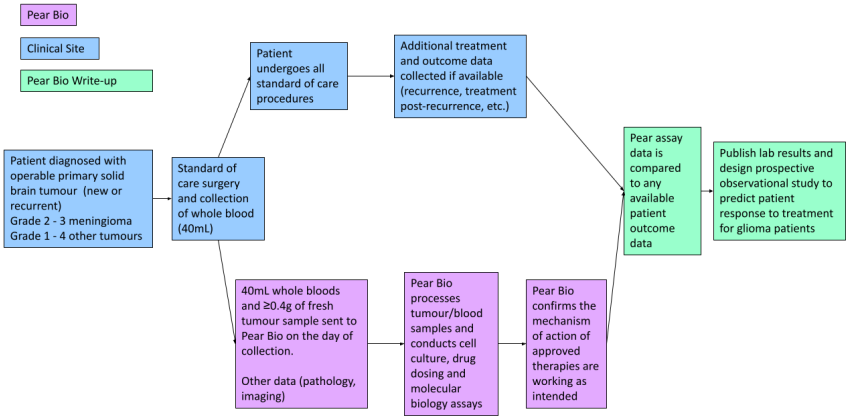
GLOSSARY OF TERMS AND ABBREVIATIONS

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-(L)1	Programmed death (ligand) 1
PFS	Progression free survival
PI	Principal Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee
RNASeq	RNA sequencing

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

STUDY SYNOPSIS

Title	Prospective Evaluation of AI R&D tool for Biology and Response in Adult Invasive Neuro-oncology (PEAR-GLIO)
Main Objectives	The study objectives are concerned with measurements and endpoints collected from laboratory testing on patient-derived primary brain tumours. The primary objective is to establish a functional dose of each FDA-approved therapy for primary solid brain tumours 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.) This objective is quantified using intra-patient and inter-patient statistics to evaluate differential *ex vivo* treatment efficacy for approved glioma therapies. Other objectives include measuring the correlation between multi-omic biomarkers and *ex vivo* treatment response, and exploring responses to other potential therapies for brain tumours. Our Patient & Public Involvement & Engagement (PPIE) workstream will run in parallel, but will be open to a wider group of people, who may or may not be patients who participate in PEAR-GLIO, and will be managed outside the study.
Phase	N/A
Design	This is a UK-based, observational study that aims to validate a diagnostic tool on its ability to test therapeutic sensitivity of brain tumours *ex vivo*. Patients diagnosed with brain tumours will undergo standard of care surgery, and have 40mL of whole blood collected. The blood and tissue material will be shipped fresh. The patient will go through routine post-surgical care and data collection. Any known treatments and events occurring post-surgery, such as the administration of adjuvant therapy or recurrence, will be recorded and communicated to Pear Bio. The samples and data sent to Pear Bio will be used to evaluate the study's objectives.

	
Sample Size	50
Inclusion Criteria	1. Patient diagnosed with operable brain cancer, thought likely to be primary solid brain tumour on imaging (grade 2 - 3 meningioma; grade 1 - 4 tumours otherwise) or with histologically proven primary malignant solid brain tumour; 2. Able to give written informed consent prior to admission to this study; 3. Female or male aged ≥18 years; 4. Patient consents to the use of their surgical sample and 40mL of whole blood for research purposes; 5. Surgical sample and yields ≥0.4g for the study; 6. Patient consents to providing histopathology data (e.g., confirmation of histological subtype as oligodendroglioma) and other pseudonymised health information including imaging, treatment and outcome data.
Exclusion Criteria	1. Inoperable or biopsy only; 2. Suspected lymphoma or myeloma, or grade 1 meningioma 3. Preoperative haemoglobin levels below 120g/L; 4. Patients who have already received chemotherapy, targeted therapy, immunotherapy, or radiotherapy less than 30 days before date of surgery, unless as part of a clinical trial (requires per-patient sponsor approval). 5. Recurrence of cancer originating from a site other than the brain; 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

Pear Bio have developed a 3D micro-tumour and computer vision platform to culture patient-derived tumour samples and predict sensitivity to various therapeutic agents. This study is intended to validate the mechanism of action of FDA-approved primary brain tumour therapies, such as Temozolomide, Lomustine and Bevacizumab, as well as therapies being assessed in clinical trials, on fresh brain tumours and autologous immune cells isolated from whole blood.

This is a proof of concept study to confirm that therapies used in primary brain tumours can be tested in Pear Bio's platform. Follow-on studies will be used to determine the sensitivity and specificity of Pear Bio's test in predicting patient response (OS and PFS). This study will acquire fresh patient tumour samples and 40 mL of whole blood per patient. Samples will be shipped fresh on the same day of collection to Pear Bio's lab. Histopathology reports will be provided to Pear Bio shortly after the sample shipment in order to confirm the tumour subtype (although samples will not be excluded from analysis based on tumour subtype). Other available patient data, including imaging and imaging reports, will also be provided in a pseudonymised form. Patients who have had previous tissue sampling (i.e. those undergoing treatment for relapsed disease) will also provide information on previous histology and treatment.

Pear Bio will isolate cells from the tumour samples and whole blood, co-culture them into 3D micro-tumours, and run live microscopy-based assays to determine *ex vivo* tumour response to treatments. Image data will be analysed using a proprietary computer vision pipeline to measure *ex vivo* tumour response metrics, such as tumour cell death, tumour cell migration and immune cell infiltration. Molecular biology assays will be conducted to determine changes in DNA features (tumour mutational burden and mis-match repair), gene expression (PCR or RNASeq) and protein distribution (immunofluorescence or spatial proteomics assays) for biomarkers due to *ex vivo* treatment exposure.

Various Pear Bio laboratory assays will be compared to validate cell culture models, computer vision algorithms and drug mechanisms of action. Any available patient data will also be used to determine whether Pear Bio's assays can predict patient outcomes, such as recurrence or eligibility for future targeted therapies. However, due to the variety of patients recruited, the low total sample size, and the lack of evaluable patients receiving non-surgical treatment, no formal statistical analysis will be conducted on patient outcomes.

At the end of this study, Pear Bio will determine whether the platform has potential for patient stratification in primary brain tumours. Future studies will aim to demonstrate the sensitivity/specificity of Pear Bio's platform for an eventual intended use in guiding treatment decision making for patients with primary brain tumours in order to increase their response rates.

Our Patient & Public Engagement & Involvement (PPIE) workstream will hold three meetings and use online questionnaires and consultations to help understand patient, carer, professional and public views on the Pear Bio platform and its use.

1.2 Background and rationale

Brain tumours are a large unmet need in the UK and worldwide. They are the leading cause of cancer death in the under-40's, with 12,500 new patients diagnosed annually in the UK and 88% dying within 5 years (1) . The US faces similar problems, with 79,000 new cases and nearly 14,000 deaths annually (2). Of the malignant brain tumours, glial tumours are the most common, making up 70% of all adult malignant brain tumour diagnoses.

1.2.1 Current management of malignant glioma

Malignant glioma includes grade 2-4 glioma, and forms the majority of primary brain tumours in adults. Glioma therapy depends on grade and subtype but broadly includes a combination of surgical resection, radiotherapy and chemotherapy.

Optimal treatment of glioblastoma (grade 4 glioma) consists of surgery, chemo-radiotherapy and chemotherapy with Temozolomide (3). For grade 3 astrocytoma, surgery, radiotherapy and adjuvant temozolomide are standard of care (4), and for oligodendroglioma, surgery, radiotherapy and PCV-based chemotherapy are standard of care. At relapse, therapy is typically further chemotherapy, either with the same agents, or previous unused chemotherapy. 3rd-line chemotherapy is poorly defined, but might include carboplatin or irinotecan, and may include bevacizumab.

Combined TMZ & Lomustine may be beneficial in MGMT methylated GBM, but apart from MGMT methylation, IDH mutation and 1p/19q co-deletion - which predict response to chemotherapy in general - there are no biomarkers to predict response to specific therapeutic agents.

1.2.2 Treatment response in malignant primary brain tumours

Primary brain tumours have a 5-year survival rate of only 12%, and GBM has a median survival of 15 months with aggressive multimodal treatment (5). However, there is some evidence to support use of combined chemotherapy in newly diagnosed patients with GBM, uncertainty about chemotherapy regimen choice in grade 2 and 3 gliomas, and uncertainty about therapy choice at relapse across all gliomas.

1.2.2 Other primary brain tumours

Outside of the gliomas, the commonest primary brain tumour is a grade 1 meningioma. We are not including these patients in the study, as the tumours have very good outcomes, and are unlikely to be possible to grow in our system. However, there are a range of other primary brain tumours, all of which are much rarer (e.g. ependymoma, grade 1 gliomas) which would all be eligible for inclusion in the study.

1.2.3 Predictive biomarkers of patient response

The only good predictive biomarkers for glioma are IDH mutation status, 1p/19q codeletion and MGMT methylation. However, these all generally predict for chemotherapy responsiveness, rather than which therapy is best.

Pear Bio's test provides a potential avenue to test single agents and combination therapies prior to treatment selection to guide decision-making.

1.3 Benefit/risk assessment

This is an observational study with patients receiving standard of care surgery. Samples collected from surgery will be used in Pear Bio's test, but they will not guide any treatment decisions. As such, there are no benefits to the patients taking part in this study.

This study will be used to conduct analytical validation of the patient stratification tool. The study data will be used to inform the design of future trials, which will be aimed at increasing patient response rates by using the test before systemic therapy starts to decide on the optimal treatment regimen(s) to use for an individual with glioma.

As surgery and blood collection are within routine care for resectable brain cancer, study-specific procedures do not pose significant risks to patients. A source of potential risk comes from collecting 40mL of matched whole blood per patient, which may go beyond routine care to satisfy study requirements. Possible risks associated with extra blood collection are a feeling of lightheadedness, dizziness and local bruising. However, this will be mitigated as patients with preoperative haemoglobin levels below 120g/L will not be recruited.

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 FDA-approved therapy for glioma 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 *ex vivo* treatment response across the therapies/combos tested on each patient's tumour sample (intra-patient sample comparison) 2. Observing differentiated *ex vivo* treatment response levels between the cohort of samples collected from patients on a per therapeutic/combo basis (inter-patient sample comparison)

2.2 Secondary objectives and endpoints

Secondary objectives	Endpoints
Assess the accuracy of Pear Bio's assay at correlating to patient progression free survival (PFS)	Tumour response will be measured as clinically indicated (typically every 3 months), 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 RANO 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 surgical samples	The percentage of cultures in which ≥70% of viable tumour cells encapsulated post-isolation on day 0 are still alive on day 3 in the control cultures (no treatment) compared to the number of tumour samples (≥0.4g) successfully accepted at Pear Bio's lab.
Determine the rate of successfully cultured immune cells	The percentage of cultures in which ≥70% of viable immune cells plated post-extraction on day 0 are still alive on day 3 in the control cultures (no treatment) compared to the number of blood samples (≥40mL) successfully accepted at Pear Bio's lab.
Find correlations between *ex vivo* tumour culture or multi-omic biomarkers and real-world patient outcomes	For any cases where patient outcomes are available or become available prospectively, such as recurrence post-surgery, potential predictive or prognostic biomarkers will be identified. As this data will be sparse, the analysis will only be used to generate hypotheses for future trials, if the data allows.
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 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

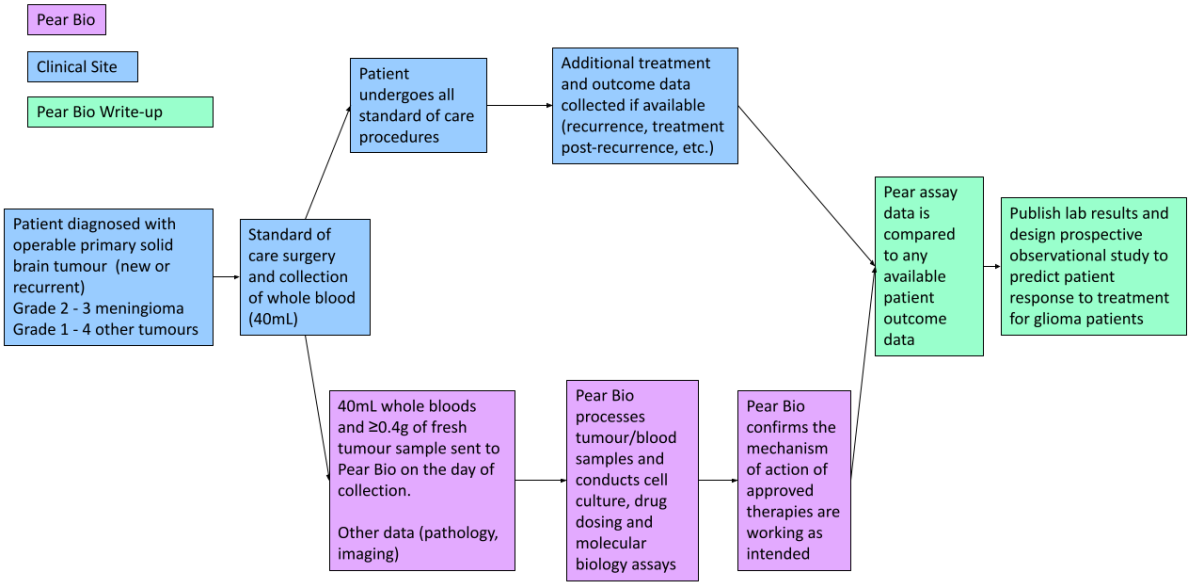
3: INVESTIGATIONAL PLAN

3.1 Overall design

This is a UK-based, observational study that aims to validate a diagnostic tool on its ability to test therapeutic sensitivity of primary solid brain tumours *ex vivo*. Patients diagnosed with a brain tumour on imaging, or who have had previous treatment for a brain tumour and are now undergoing further surgery as clinically indicated, will undergo standard of care surgery, and have 40mL of whole blood collected. The material available from the surgical sample for this study will be shipped fresh alongside whole blood to Pear Bio. The patient will go through routine post-surgical care and data collection. Any previous or subsequent treatments and events occurring post-surgery, such as the administration of adjuvant therapy or recurrence, will be recorded and communicated to Pear Bio. The samples and data sent to Pear Bio will be used to evaluate the study's objectives.

3.2 Trial schema

Figure 1: Trial schema



3.3 Data and tissue collected

	Biospecimens	Data
Required	1. At least 0.4g of fresh, unfixed, tumour tissue taken from surgery 2. 4 x 10mL EDTA vials of matched whole blood	1. Demographic data (pseudonymised) 2. Redacted pathology reports, including immunohistochemistry tests, molecular pathology and NGS (IDH and MGMT in particular) and further genomic data where available, including methylation profiling

		3. Radiology images and reports 4. Concomitant medications
Collected if available	N/A	1. Blood and liver/kidney function tests at baseline 2. Follow-up outcome data, including recurrences 3. Follow-up treatment data, including adjuvant therapy regimens

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 viable cells 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 various omics assays including looking at DNA alterations (tumour mutational burden), gene expression (PCR or RNASeq) and protein/receptor distribution (immunofluorescence or spatial proteomics assays for biomarkers such as PD-(L)1). Wherever sample size allows it, tissue chunks are cut out of the main tumour bulk prior to cell isolation for spatial analyses (GeoMX Nanostring).

The stained cells will be cultured in a biomimetic hydrogel within Pear Bio's 3D micro-tumour platform to provide a physiological environment for drug dosing experiments. Therapies will be administered to each 3D micro-tumour over multiple days (either as monotherapy or combination therapies as outlined below) .

Standard set of therapies tested	
3D cell culture 1	Control
3D cell culture 2	Temozolomide
3D cell culture 3	Lomustine
3D cell culture 4	Temozolomide + Lomustine

3D cell culture 5	Procarbazine + Lomustine
3D cell culture 6	Carboplatin
If enough cells are available, we will test additional therapies including Etoposide, Regorafenib, Pembrolizumab, Irinotecan, Avastin, Olaparib, Nariparib, Lapatinib, Entrectinib, Osimertinib and others, either alone or in combination.	

In parallel, PBMCs will be extracted from whole blood, characterised via flow cytometry and sorted via 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 3D system jointly with cells isolated from the matched tumour sample. Tumour-isolated cells will be co-cultured with immune cells. Cultures receiving immunotherapies may be tested for tumour mutational burden.

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 or snap-frozen for further 3D immunofluorescence analyses or used for embedding, sectioning and assessment of spatial proteomics. For targeted therapies, RNAseq, IF and proteomics data will be integrated to confirm drug MoA and identify other potential therapeutic targets. Concurrently, 3D image data will be processed through a computer vision pipeline to measure functional metrics of the *ex vivo* 3D cell cultures, including cell viability, tumour culture width and cell migration, both at a bulk tumour level and at a single-cell resolution. A patient report is generated outlining an individual patient sample's response to each therapy tested.

Potential additional analyses:

- DNA analyses:
    - Tumour mutational burden (TMB)
    - Microsatellite instability (MSI)
    - Fluorescent in-situ hybridisation (FISH)
    - Genome-wide methylation patterns
    - Whole genome sequencing
    - Whole exome sequencing

- RNA analyses:
    - RNASeq
    - Microarrays
    - Quantitative reverse transcription PCR (RT-qPCR)
    - Spatial analyses (Nanostring, ResolveBiosciences)
- Protein analyses:
    - Flow cytometry (FC)
    - Immunofluorescence (IF)
    - Analysis of secreted factors

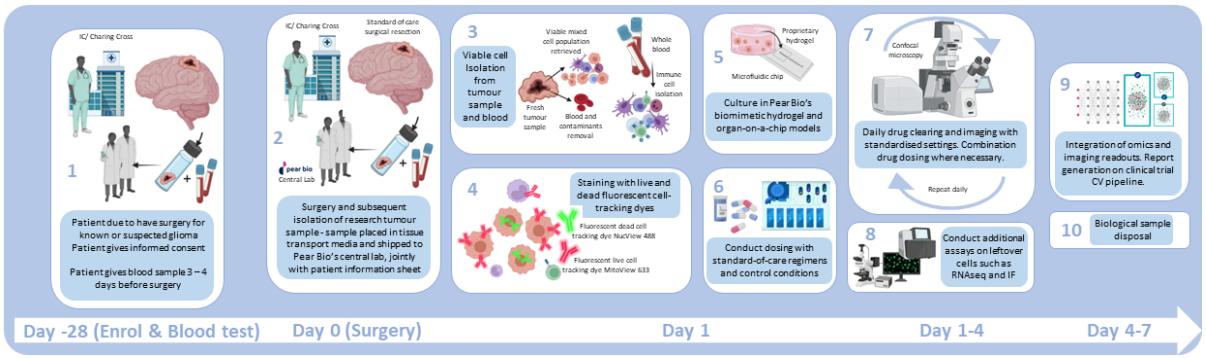


Figure 2: PEAR-GLIO laboratory workflow. (1) Patient gives a pre-op blood sample (2) Patient undergoes surgery as part of standard of care (3) Viable cells are isolated from the tumour and immune cells are isolated from blood samples (4) Tumour-dissociated cells and immune cells are stained with live-cell and dead-cell fluorescent dyes. (5) The cells are cultured simultaneously in multiple Pear Bio hydrogels contained in a microfluidics device. (6) Standard-of-care chemotherapy and targeted treatments are dosed within the device whilst (7) daily imaging allows for live tracking of cell viability and migration. (8) Wherever additional cells are available, omics assays (e.g. RNA sequencing and immunofluorescence) will be run in parallel to check expression of common biomarkers and validate drug mode of action. (9) 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 prediction of differential treatment efficacies.

3.5 Patient evaluability

In order to be considered evaluable, patients must meet the eligibility criteria, consent to the use of their surgical sample and blood for the study, and yield a research sample with sufficient weight and tumour cell content resulting in the establishment of a successful cell culture in the Pear Bio laboratory.

3.6 Replacement of patients

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

3.7 Target accrual

A maximum of 50 evaluable patients will be recruited in this study. On recruitment of the first 10 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 50 patients.

4: PATIENT SELECTION

4.1 Inclusion criteria

1. Patient diagnosed with operable brain tumour, thought likely to be primary solid brain tumour on imaging (grade 2 - 3 meningioma or grade 1 - 4 for other tumours) or with histologically proven primary malignant solid brain tumour and due to undergo surgery as clinically indicated
2. Able to give written informed consent prior to admission to this study;
3. Female or male aged ≥18 years;
4. Patient consents to the use of their surgical sample and 40mL of whole blood for research purposes;
5. Surgical sample yields ≥0.4g for the study;
6. Patient consents to providing histopathology data (e.g., confirmation of histological subtype as oligodendroglioma) and other pseudonymised health information including imaging, treatment and outcome data.

4.2 Exclusion criteria

1. Inoperable or biopsy only
2. Suspected lymphoma or myeloma, or grade 1 meningioma;
3. Preoperative haemoglobin levels below 120g/L;
4. Patients who have already received chemotherapy, targeted therapy, immunotherapy, or radiotherapy less than 30 days before date of surgery, unless delivered as part of a clinical trial (must be discussed with sponsor on a per-patient basis)
5. Recurrence of cancer originating from a site other than the brain;
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 outpatient clinics by their clinical care team.

Only individuals with legitimate access to medical records (such as the patient's clinical care team) will be accessing these records for the purpose of identifying participants.

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 taking part in 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 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 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) 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-GLIO eCRF can be found in the eCRF completion guidance document.

5.4 Schedule of assessments

Patients may not need to make any additional site visits as the samples collected for the study can be taken from standard of care procedures. 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.

	Baseline (Before surgery)	**At or shortly after surgery**	**Standard of care follow-ups**	**If cancer recurs**
Informed consent and eligibility checks	B			
Demographics and medical history	A			
Height, weight ECOG	A			
Concomitant medication	A			
Results from standard of care haematology, biochemistry assessments	A			
Standard of care imaging	A			
Tumour size evaluation	A			
Standard of care surgery		A		
Histopathology[1]		A		A
Collection of blood for the study[2]		B (40mL whole blood)		
Collection of tissue and data for the study[2]		B		

Transfer of data for the study[2]		B	B	B
Adverse Events by CTCAE v5.0[3]		B		
Treatment and follow-up as clinically indicated			A	
Follow-up assessments for recurrence[4]			A	
Further therapy[5]				A
A = Standard of care assessment **B = Study-specific assessment or data collection**				

Table 1: Schedule of assessments

Table notes:

1. A redacted histopathology report will be sent to Pear Bio when it is ready (i.e., it does not have to be sent alongside the tissue, blood and other available data from the day of the surgery).
2. Available data is transferred alongside the tumour sample within 24 hours of surgery to Pear Bio's laboratory. Any remaining data will be transferred as soon as feasible, and we will transfer any further imaging at a single timepoint at 12 months after surgery
3. Relating only to research procedures (e.g., taking a volume of blood that is beyond standard care for a given patient). This can be conducted by telephone – physical examination to be done only if clinically indicated.
4. Collected only if available for consenting patients within the study period.
5. Collected only if available for consenting patients within the study period.

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 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, and tumour size.

5.5.2 Height, weight and performance status

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

Performance status data will be collected at baseline using the ECOG performance score according to Table 2. This information 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 surgery as per standard of care. Consenting patients will have a portion of their tumour and blood used for this study.

For patients who have adjuvant therapy within the study period, 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, or details of radiotherapy dose, fractionation, start and end dates, and for surgery, dates and times of surgery and post-operative stay.

5.5.6 Tumour size evaluation

Measurements will be made by contrast-enhanced MRI. Imaging reports and pseudonymised images will be included as study data collected and transferred to Pear Bio, provided the imaging was done within the period of 3 months prior to surgery or during the follow-up period. Sites will transfer initial imaging shortly after surgery, and again at a single timepoint 12 months after surgery.

5.5.7 Adverse events

Adverse events will be restricted to those resulting from study-related procedures (e.g., a volume of blood collected beyond standard care to satisfy study requirements). 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 Surgical biopsy and blood collection

40mL of whole blood will be collected in 4x 10mL EDTA tubes on the day of surgery.

At the time of surgery, a portion of the tumour resected from surgery will be provided to Pear Bio (≥0.4g). Samples must be placed in tissue transport medium to be supplied by Pear Bio.

Each sample should be stored at 4°C before being transported by express courier to Pear Bio so that samples arrive within 24 hours of collection; blood and tumour samples may be sent separately because they may be collected up to 4 days apart.

5.5.9 Exposure to Ionizing radiation

There are no expected research exposures to ionising radiation. Although patients may undergo imaging with ionising radiation, and many may undergo radiotherapy, all of these will be delivered as part of routine care, and not as part of the trial. In addition, disease assessments are routinely carried out using MRI, which is a non-ionising modality.

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 their research sample has insufficient weight (<0.4g) or tumour content (<100 000 viable cells), or the sample fails to establish a culture in the laboratory.

5.8 PPIE workstream

The PPIE workstream will run separately from the trial: patients enrolled in the trial will be invited to participate in the PPIE work, but there will not be automatic overlap (i.e. patients will be invited to PPIE, but enrollment in the trial is independent of PPIE engagement, and PPIE engagement does not count towards trial enrollment).

We will engage with brain tumour patients and carers, as well as professionals and the wider public by working with the local neuro-oncology PPI team. We will work with the local clinical team to identify participants, and participation will be by invitation and voluntary participation. All data collected will be anonymised and summarised for thematic analysis and discussion, and to inform the development of the next round of clinical trials.

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 throughout the study, from informed consent through post-surgical care. 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-GLIO 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 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 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 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 REC (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

A maximum of fifty (50) 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 broad inclusion criteria. However, the number of samples acquired in this study will enable Pear Bio to conduct analytical validation on the robustness of its precision medicine platform in brain cancer.

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 analytical comparisons, they are tied to laboratory results of the Pear Bio test on patient samples. No correlations are made between Pear Bio 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 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 at multiple timepoints, which yields multiple phenotypic metrics of *ex vivo* tumour response, including:
    a. Cell death
    b. Cell migration distance/speed (mean, median, 5% most aggressive cells, etc.)
    c. Immune cell infiltration into the tumour

These analytical measurements are used to conduct the following comparisons:

1. Observing differentiated *ex vivo* treatment response across the therapies/combos tested on each patient's tumour sample (intra-patient sample comparison)
    a. Ranking of regimen efficacy will be done for each assay metric on a per patient basis to determine agreement/disagreement between assay metrics
    b. Calculating the range of response to all tested regimens 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/combo basis (inter-patient sample comparison)
    a. Grouping of patient samples based on change in biomarker levels on a per regimen basis
    b. Grouping of patient samples based on *ex vivo* response or resistance at a phenotypic level (quantified by computer vision) on a per regimen basis
    c. Comparing the general efficacy of the regimens 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 regimens tested across patient samples using regimen efficacy ranks on each sample and using Kendall's W and Spearman ranked correlation tests to determine whether some regimens consistently outperform others *ex vivo* (on a given metric of interest)

e. Comparing the general efficacy of the regimens 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 regimens 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 sample responses. Due to the heterogeneity of glioma treatment response in the clinic, one regimen is not expected to consistently outperform other options across all patient samples in the laboratory. That could indicate *ex vivo* overperformance due to assay conditions that require adjustment, either in the biology workflow or response vs resistance thresholds, before the assay is used in interventional trials to guide treatment decisions.

7.2.2 Secondary efficacy analysis

Correlating omics biomarkers to *ex vivo* tumour response

Biomarker expression tied to targeted therapy response (e.g., EGFR to EGFR inhibitors) will be correlated to *ex vivo* tumour response (3D micro-tumour and computer vision platform).

Correlations will be done with various regression models, including:

1. Impact of MGMT methylation on response to treatment
2. Linear regression between continuous biomarkers and *ex vivo* tumour response metrics (e.g., baseline gene expression vs cell viability after treatment with a targeted drug)
3. (Ordinal) Logistic regression correlating low-high, and potentially low-medium-high, biomarker levels against *ex vivo* treatment sensitivity/resistance based on a range of image-based metrics, such as
    a. Cell viability
    b. Cell migration speed
    c. Immune cell infiltration into the tumour

This analysis will be used to establish the concordance/discordance between *ex vivo* responses and biomarkers. However, neither the *ex vivo* responses nor biomarkers will act as a ground truth due to the poor correlation between biomarkers and patient response in patients with malignant brain tumours.. Discordance ($0 < r < 0.7$) will be used to identify patient populations where Pear Bio may be able to predict response differently from known biomarkers. These populations will be considered for future studies to determine whether Pear Bio can accurately predict real-world patient response.

7.2.3 Exploratory analyses

Successful tumour cell culture rate from surgical samples

The successful cell culture rate is the percentage of cultures in which ≥70% of viable tumour cells plated post-isolation on day 0 are still alive on day 3 in the control hydrogels (cultures with no treatment) compared to the number of tumour samples (≥0.4g) arriving uncompromised within 24 hours of collection to the Pear Bio laboratory.

Successful immune cell culture rate from blood samples

The successful cell culture rate is the percentage of cultures in which ≥70% of viable immune cells plated post-extraction on day 0 are still alive on day 3 in the control well (no treatment) compared to the number of blood samples (≥40mL) arriving uncompromised within 24 hours of collection to the Pear Bio laboratory.

Correlating *ex vivo* tumour culture or multi-omic biomarkers to real-world patient outcomes

For any cases where patient outcomes are available or become available prospectively, such as recurrence post-surgery, potential predictive or prognostic biomarkers will be identified. As this data will be sparse, the analysis will only be used to generate hypotheses for future trials, if they present themselves.

Assessing response to experimental therapies

Observing differentiated *ex vivo* treatment response across the therapies/combos tested on each patient's tumour sample (intra-patient sample comparison)

a. Ranking of regimen efficacy will be done for each assay metric on a per patient basis to determine agreement/disagreement between assay metrics
b. Calculating the range of response to all tested regimens for each assay metric on a per patient basis

7.3 Interim analysis and study termination

Interim analysis will be done on laboratory research milestones, such as confirmation of a successful hydrogel formulation to culture brain tumour cells after testing the first 5 patient samples. This interim analysis will be used to allocate samples for research based on the most pressing requirements for *ex vivo* model validation (e.g., hydrogel formulation, drug dosing, etc.).

On recruitment of the first 10 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 50 patients.

7.4 End of study definition

The end of the trial is defined as the last patient's last data at a maximum of 12 months post-surgery (outstanding data sent to Pear Bio or final laboratory readout, whichever happens later). In cases of early termination of the trial (e.g., due to slow accrual) 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. 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.

PPIE work will take place within the host NHS Trust, and any notes will be kept securely by the clinical team. Any results will be provided in an anonymised, summarised format only. Staff from the Sponsor may be invited to take part in these sessions in order to explain the product in detail, but will not be allowed to collect identifiable data on any participants.

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 clinical coordinating centre (CI, Trial Coordinator, Clinical Research Fellow) 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 twice 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.

Participation in the PPIE stream will be by invitation, and participants will be provided with written information on background, purpose and use of data from the sessions.

10.2 Summary of monitoring plan

Refer to the PEAR-GLIO 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.

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. Matthew Williams 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 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 TMG and the Sponsor. The publication policy will adhere to the contractual agreement between the Sponsor and its collaborators.

14: REFERENCES

1. Cancer Research UK: Brain Tumour Statistics [Internet]. [cited 2023 Mar 3]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/brain-other-cns-and-intracranial-tumours

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022 Jan;72(1):7–33.

3. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: State of the art and future directions. CA Cancer J Clin. 2020 Jul;70(4):299–312.

4. van den Bent MJ, Tesileanu CMS, Wick W, Sanson M, Brandes AA, Clement PM, et al. Adjuvant and concurrent temozolomide for 1p/19q non-co-deleted anaplastic glioma (CATNON; EORTC study 26053-22054): second interim analysis of a randomised, open-label, phase 3 study. Lancet Oncol. 2021 Jun;22(6):813–23.

5. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009 May;10(5):459–66.