TITLE OF THE PROTOCOL: PEAR-TREE2: **P**rospective **E**valuation of **A**I **R**&D tool for patient stratification: a **T**rial for **R**enal immuno-oncology model **E**xperimental **E**valuation **2**

IRAS Reference: 328435

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Participating Sites

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PROTOCOL SIGNATURE PAGE

Signatures of the Chief Investigator:

The clinical study as detailed within this research protocol (Version1.0, Dated 28th July 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.

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

Signature of Statistical Advisor:

The clinical study as detailed within this research protocol (Version1.0, Dated 28th July 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: _____ __ __ / __ __ __ / __ __ __ __

Signature of the Principal Investigator:

The clinical study as detailed within this research protocol (Version1.0, Dated 28th July 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.

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

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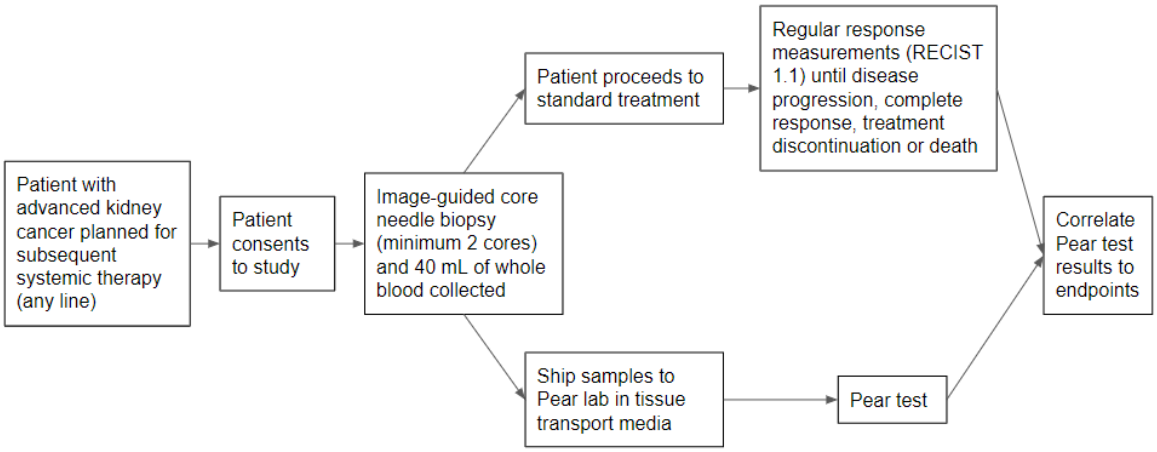
GLOSSARY OF TERMS AND ABBREVIATIONS

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-TREE2: **P**rospective **E**valuation of image-based **A**rtificial intelligence **R**esearch: a **T**rial for **R**enal immuno-oncology model **E**xperimental **E**valuation **2**
Main Objectives	**Primary:** The primary objective is to assess the accuracy of the Pear Bio test in predicting a response vs. non-response (based on RECIST 1.1 criteria) in patients with advanced clear cell RCC receiving systemic treatment. **Secondary:** ● Test sensitivity and specificity, positive and negative predictive values for complete response (CR) ● Test sensitivity and specificity, positive and negative predictive values for deep response ● Test sensitivity and specificity, positive and negative predictive values for 6 month, 1 year and 2-year durable response rate ● Test sensitivity and specificity, positive and negative predictive values for 6 month, 1 year and 2-year PFS ● Hazard ratio between low and high biomarker groups for progression free survival (PFS) ● **Exploratory**: ● Test sensitivity and specificity, positive and negative predictive values for overall survival (OS) ● To determine the frequency of successfully established cultures from core needle biopsy samples. ● Analysis of other patient subgroups against each primary and secondary endpoint, including the non clear cell RCC population ● Explore protein, RNA and DNA biomarkers related to outcomes ● Explore immune cell activation in vitro
Design	This is a multicentre, UK and US-based observational pilot study that aims to determine the accuracy of a new assay, the Pear Bio test, in predicting response to treatment in patients with advanced RCC. Patients with advanced RCC who are about to start new systemic therapy will undergo a mandatory biopsy before commencing clinically indicated therapy. As such, for this study, the result from the Pear Bio test will not be used to inform the choice of systemic therapy and the treating oncologist will be blinded to the assay results. The imaging-based response to treatment will be collected and used to calculate the sensitivity, specificity PPV and NPV of the assay to predict ORR as the primary endpoint of the study.

	
Sample Size	Up to two hundred (200) evaluable patients will be recruited to this study
Inclusion Criteria	1. Able to give written informed consent prior to admission to this study. 2. Female or male aged ≥18 years. 3. Evidence of advanced RCC with intention to receive systemic therapy, defined as: a. Clinical suspicion of advanced RCC with intention to undergo a clinically-mandated biopsy and subsequent systemic therapy OR b. Histological evidence of advanced RCC with intention to undergo subsequent systemic therapy and willing to undergo additional research biopsy 4. At least one lesion evaluable under RECIST 1.1 criteria 5. Willing to donate at least two additional core biopsy samples prior to starting subsequent systemic therapy. 6. Willing to undergo venous sampling for 40mL of blood
Exclusion Criteria	1. Early stage kidney cancer 2. Patients who do not have kidney cancer 3. Patients with RCC that do not intend to receive systemic therapy 4. Patients who have already commenced systemic therapy with no plans of changing the systemic therapy after the collection of the core needle biopsy. 5. 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. 6. Previous diagnosis of other cancer. Previously treated cancer may be acceptable in some circumstances (e.g. surgery for an unrelated cancer > 5 years ago) after discussion with the Sponsor. 7. No lesions are amenable to biopsy

1 INTRODUCTION

1.1 TRIAL OUTLINE

Pear Bio have developed a 3D microtumour and computer vision platform to culture patient-derived tumour samples and predict sensitivity to various therapeutic agents. This study is intended to assess the relationship between response rate seen in patients treated as part of routine clinical care and the response seen in the Pear Bio system, and to validate the mechanism of action of approved renal cell carcinoma therapies, such as checkpoint inhibitors and VEGFR inhibitors, on fresh kidney tumours and matched immune cells isolated from whole blood.

We are completing our initial study (PEAR-TREE) to confirm that therapies used in renal cell carcinoma (RCC) can be tested in Pear Bio's platform. PEAR-TREE2 is a follow-on study that will determine the sensitivity and specificity of Pear Bio's test in predicting patient response, as measured by overall response rate (ORR),progression free survival (PFS) and other measures of clinical response.

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 kidney cancer subtype (although samples will not be excluded from analysis based on cancer subtype). Other available patient data will also be provided in a pseudonymised form.

Pear Bio will isolate cells from the tumour samples and whole blood, co-culture them in a 3D cell culture platform, 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.

In cases where excess tissue, cells and/or blood are available, molecular biology assays will be conducted to determine DNA features (e.g., tumour mutational burden), gene expression (e.g., PCR or RNASeq) and protein distribution (e.g., immunofluorescence or spatial proteomics assays for biomarkers such as PD-(L)1), and to relate these to patient outcomes. These may be conducted in the whole trial cohort, or only in a subgroup, and may only be feasible in a subset of patients.

Available patient data will be used to determine whether Pear Bio's assays can predict patient outcomes, such as ORR or eligibility for future targeted therapies. At the end of this study, Pear Bio will determine whether the platform has potential for patient stratification in kidney cancer. Future interventional studies will aim to demonstrate the platform's intended use in guiding treatment decision making for patients with metastatic renal cell carcinoma in order to increase their response rates.

1.2 BACKGROUND AND RATIONALE

Kidney cancer is a large unmet need in the UK, with 13,000 new patients diagnosed annually, a 52% survival rate, and nearly 5,000 patients dying each year (1). The US faces similar problems, with 81,000 new cases and nearly 14,000 deaths annually (2). Of the kidney cancer subtypes, renal cell carcinoma (RCC) is the most common, with clear cell RCC (ccRCC) making up 75% of all kidney cancer diagnoses. The incidence of RCC has more than doubled in the last 50 years, and is increasing by 2-3% every year (2) . The risk factors for developing RCC are smoking, obesity and hypertension. Inherited causes comprise only 2-5% of cases (3). The rising

incidence is also due to the increased use of imaging as a detection technique. Metastatic RCC has a dismal 5-year survival rate of only 12%.

1.2.1 *Current management of advanced RCC*

Early-stage kidney cancer is primarily treated with surgery (4,5) .However, metastatic RCC requires systemic therapy, which can include immunotherapy and targeted therapy (6). Multiple approved therapies are available for metastatic RCC, such as nivolumab (immunotherapy targeting PD-1), cabozantinib (small molecule targeting c-Met, VEGFR2, AXL and RET), and belzutifan (small molecule targeting HIF-2α) (6,7).

Metastatic RCC is often treated with a range of single and multi-drug combinations. These include (but are not restricted to):

1. Immune checkpoint inhibitors (ipilimumab, nivolumab, pembrolizumab and avelumab)
2. Tyrosine kinase inhibitors (axitinib, cabozantinib, and lenvatinib)

Therapy choice is based on a combination of well defined risk factors and patient and clinician choice factors. Second-line therapy varies based on first-line therapy and duration of response, as well as patient fitness and choice, but regimens may include drugs such as Belzutifan and Cabozantinib, although this area is evolving relatively quickly.

1.2.2 Treatment response in advanced renal cell carcinoma

Metastatic RCC has a 5-year survival rate of only 12% (2,6) . The median progression free survival (PFS) of metastatic RCC varies based on the choice of first-line treatment, ranging from 8 months to 14 months, at which point tumours progress. However, there is ambiguity in terms of treatment choice due to heterogeneity in patient response, a lack of predictive biomarkers for patient stratification, and lack of randomised controlled trials.

1.2.3 Predictive biomarkers of patient response

While the number of therapies and prognostic biomarkers available for RCC have increased rapidly, there is a lack of predictive biomarkers that can help decide between the various therapeutic options and the commonly used regimens.[8] Pear Bio's test provides a potential avenue to test single agents and combination therapies prior to treatment selection to guide decision-making.

Pear Bio have developed a 3D microtumour and computer vision platform through which the response of an individual patient's tumour to different therapy regimens can be tested simultaneously. A patient tumour biopsy sample is dissociated into a single cell suspension, stained and cultured in parallel biomimetic hydrogels within Pear Bio's 3D micro-tumour platform. The characteristics of the cancer cells within each microtumour, such as cell migration and viability, are recorded by time-lapse microscopy. The first microtumour acts as a baseline with no systemic therapy agents added. A second is used to test the regimen that is given to the patient, and at least four other microtumours, each embedded in its own 3D hydrogel, are used to test alternative treatment regimens. The assay then uses artificial intelligence to analyse the different responses between the microtumours and determine the probability of achieving a response in the patient with a particular treatment regimen.

1.3 Benefit/risk assessment

This is an observational study with patients receiving standard of care systemic therapy. The Pear Bio test will be run at the start of the study (soon after study biopsy) and analysis will be conducted in parallel with the patient's treatment, with the treating oncologist 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 response rate and proportion of patients achieving durable response by using the test before systemic therapy starts to decide on the optimal regimen 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) for the assay. This can be done at the same time as the clinically-mandated core biopsy for diagnostic purposes, from the same site, or as a research-specific biopsy.

The risks are small, and depend on the area of the body biopsied, but may include pain, bleeding, pneumothorax and infection. In order to assess the impact of immunotherapy on peripheral blood immune cells, 40mL of whole blood will be taken from patients. 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 test. During initial development, the culture failure rate was 4%. However, these assays used both surgical and 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 98% by prospectively collecting the tumour samples under specified conditions.

2 STUDY AIMS AND OBJECTIVES

2.1 Primary Objectives and Endpoints

Primary Objective	Endpoints
The primary objective is to assess the accuracy of the Pear Bio test in predicting response (measured as overall response rate (ORR)) in patients with advanced clear cell RCC receiving systemic therapy	Test sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for overall response rate (ORR) in RCC having first-line, second or third-line+ systemic therapy. Sensitivity, specificity, PPV and NPV are used as the measurements of accuracy in predicting ORR. . Each subgroup of patients will be considered independently, with subgroups created based on: 1. Treatment line 2. Treatment regimen 3. IMDC risk score Subgroups will also undergo pooled and global analysis to determine whether predictive power holds across multiple subgroups and across the entire evaluable patient population.

2.2 Secondary Objectives and Endpoints

Secondary objectives	Endpoints
To assess the sensitivity of the Pear Bio test in predicting CR, deep response, PFS and durable response in patients with advanced clear cell RCC receiving systemic therapy	All secondary endpoints will use the same patient subgroups as the primary endpoint. **Secondary:** ● Test sensitivity and specificity, positive and negative predictive values for complete response (CR) ● Test sensitivity and specificity, positive and negative predictive values for deep response ● Test sensitivity and specificity, positive and negative predictive values for 6 month, 12 months and 2-year durable response rate ● Test sensitivity and specificity, positive and negative predictive values for 6 month, 12 month and 2-year PFS ● Hazard ratio between low and high biomarker groups for progression free survival (PFS)

2.3 Exploratory Objectives and Endpoints

Tertiary objectives	Endpoints
Determine the rate of successfully established cultures from the core needle biopsy samples.	The success of a cell isolation protocol is defined by obtaining a minimum of 100,000 viable cells per patient. The successful culture rate is the percentage of cultures in which ≥70% of viable cells cultured on day 0 are still alive on day 3 in the negative control culture (no treatment) compared to the total number of research samples taken and successfully arriving at Pear Bio's lab. Patients whose cell count after extraction is below 100,000 cells or are unable to maintain ≥70% cell viability in their negative control culture will be excluded from the trial (non-evaluable patients).
Overall Survival	Test sensitivity, specificity, PPV and NPV for overall survival (OS).
Exploratory subgroup analyses to determine their impact on the ability of the Pear Bio test to predict response.	1. Test sensitivity and specificity for complete response (CR) in patients who have had a prior nephrectomy 2. Test sensitivity and specificity for non-renal complete response (CR) 3. All primary and secondary endpoints applied to non-ccRCC kidney cancer patients 4. Biopsy site response (including comparisons where study cores were taken from the same lesion vs different lesions) 5. Impact of biopsy location on predictive accuracy against primary and secondary endpoints 6. Subgroup analysis by clinical trial site
Determine correlation between proteins and responsive vs non-responsive patients using i protein 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 (measurement assays include immunofluorescence and immunohistochemistry).

Determine correlation between RNA expression levels or DNA biomarkers and responsive vs non-responsive patients	Log 2-fold change and adjusted p-value, visualised through volcano plots of RNASeq data on responsive vs non-responsive patients. Comparison of mutations and other DNA biomarkers (microsatellite instability, tumour mutational burden, etc.) and patient response.
Determine immune cell activation due to immunotherapy exposure	Immune cell activity and infiltration, measured as the immune cell count and density (cells/mm3) inside the microtumour culture and immune cell movement (microns/min). A cytokine panel may also be used to measure immune activity.
Determine impact of biopsy location on culture metrics	Whether the success rate of culture varies by biopsy site

3 INVESTIGATIONAL PLAN

3.1 Overall design

This is a multicentre, UK and US-based observational study that aims to determine the accuracy of a new assay, the Pear Bio test, in predicting treatment response in metastatic RCC patients receiving systemic therapy. Patients will provide at least two additional core biopsy samples, either as a standalone procedure or taken at the same time as the standard, clinically mandated core biopsy, before commencing subsequent systemic treatment. 40mL of blood will take alongside the cores. The biopsy and blood samples will be run on the Pear Bio test whilst the patient receives their standard of care systemic therapy. As such, for this study, the result from the Pear Bio test will not be used to inform the choice of systemic therapy and the treating oncologist will be blinded to the outcome. The response rate will be collected and used to calculate the sensitivity, specificity, PPV and NPV of the assay as the primary endpoints of the study.

3.2 Trial Schema

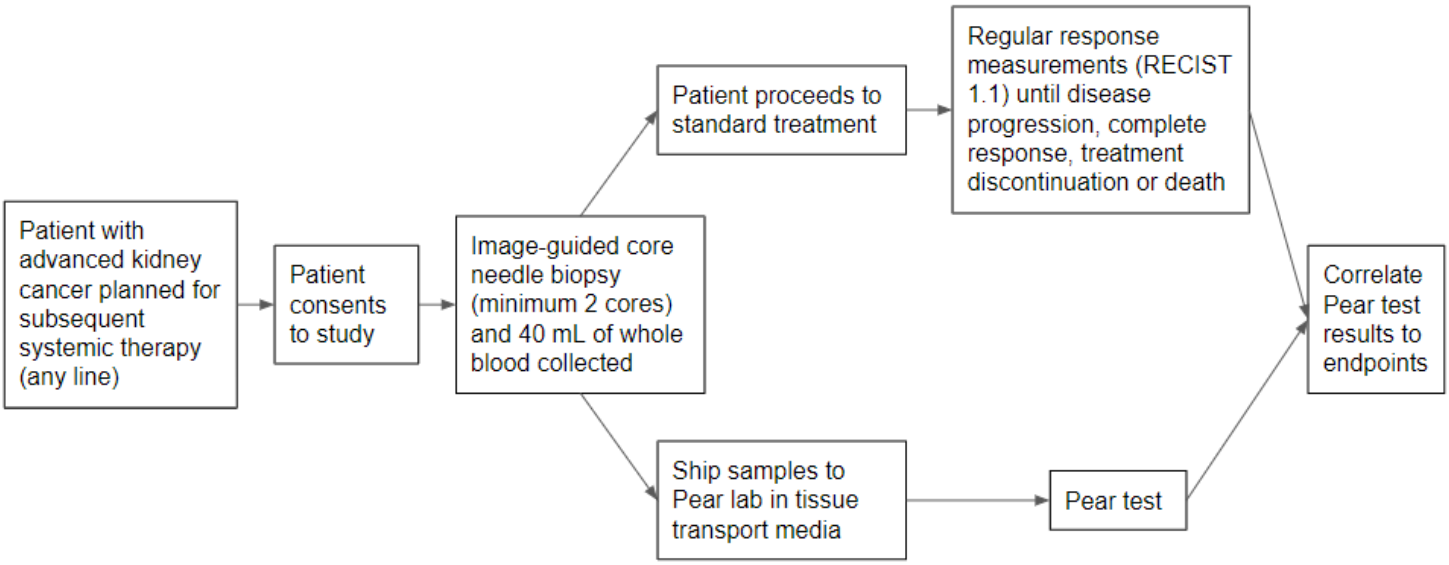


Figure 1: Trial Schema

3.3 Patient Evaluability

All patients, who meet the eligibility criteria, have a biopsy which establishes a successful culture in the laboratory, complete at least one cycle of subsequent systemic therapy and have RECIST evaluable subsequent imaging will be considered evaluable.

3.4 Replacement of Patients

Patients who do not meet the evaluability criteria set out in section 3.3 will be replaced.

3.5 Target Accrual

A maximum of 200 evaluable 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, and for every 50 patients after that. The TMG may limit recruitment of certain patient groups as the trial progresses in order to maximise information obtained (e.g. if we have recruited very many patients receiving a particular regimen, then we may stop recruiting further patients having that therapy, and if there are very few patients having another regimen, we may also limit recruitment of that group). Given the pace of development of RCC treatments, we expect to add new treatment regimens during the trial, and these will be added through submission of a trial protocol amendment.

The TMG will use the results to determine whether to increase accrual up to a maximum of 400 patients.

3.6 Study Duration

The initial study duration is expected to be 4 years and 6 months. This may be revised in light of recruitment, number of evaluable patients, and patient outcomes. Changes to the study timelines and duration will be made through discussion with the trial sponsor and Chief Investigator(s).

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. Evidence of advanced RCC with intention to receive systemic therapy, defined as:
    a. Clinical suspicion of advanced RCC with intention to undergo a clinically-mandated biopsy and subsequent systemic therapy OR
    b. Histological evidence of advanced RCC with intention to undergo subsequent systemic therapy and willing to undergo additional research biopsy
4. At least one lesion evaluable under RECIST 1.1 criteria
5. Willing to donate at least two additional core biopsy samples prior to starting subsequent systemic therapy.
6. Willing to undergo venous sampling for 40mL of blood

4.2 Exclusion Criteria

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

1. Early stage kidney cancer
2. Patients who do not have kidney cancer
3. Patients with RCC that do not intend to receive systemic therapy
4. Patients who have already commenced systemic therapy with no plans of changing the systemic therapy after the collection of the core needle biopsy.
5. 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.
6. Previous diagnosis of other cancer. Previously treated cancer may be acceptable in some circumstances (e.g. surgery for an unrelated cancer > 5 years ago) after discussion with the Sponsor.
7. No lesions are amenable to biopsy

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 Sponsor 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 the Sponsor 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-TREE2 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 systemic therapy**	**Disease Progression/ Death/ End of Study**
	Up to 28 days prior to biopsy	**Up to 3 days post biopsy**		
Informed consent and eligibility checks	X			
Demographics and medical history	X			
Height, weight, ECOG, IMDC risk score	X			
Concomitant medication	X			
Results from standard of care haematology, biochemistry assessments	X			
Cancer Diagnosis[1]	X			
Tumour size evaluation [2]	X		X (only if clinically indicated, normally every 8-12 weeks)	X *(Reporting of imaging obtained as clinically indicated)*
Image-guided research biopsy [3]	X			
Research blood sample	X			
Adverse Events by CTCAE v5.0 [4]		X		

Systemic therapy details [5]	X		X	X
Histopathology Data[6]	X			

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 primary tumour (if present) and any metastases will be collected from any standard of care imaging of the tumour, based on RECIST1.1 criteria, which may include the use of ionising radiation. Only imaging performed as part of standard of care will be used during this study.

[3] Patients must be willing to undergo a new image-guided biopsy, which can include ionising radiation, in order to obtain fresh tissue.

[4] Relating to research biopsy and blood only. Can be conducted by telephone – physical examination to be done only if clinically indicated.

[5] To include regimen (systemic therapy drugs, doses, schedule) and any changes (systemic therapy drugs, doses, schedule/delays) during treatment.

[6] Copies of anonymised histology reports from any and all tissue sampling will be collected. All histology reports must be pseudonymised and sent to the Sponsor, 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(s), date of diagnosis, pathological and/or physical tumour size, and tumour stage.

5.5.2 *Height, weight, ECOG and IMDC risk score*

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

IMDC Factor - each factors scores 1 point	
Time from diagnosis to treatment < 12 months	Corrected Calcium > Upper Limit of Normal
Karnofsky Performance Status < 80%	Neutrophils > Upper Limit of Normal
Hemoglobin < Lower Limit of Normal	Platelets > Upper Limit of Normal
Total Score	**Prognostic Group**
0	Favourable
1 - 2	Intermediate
3 -6	Poor

Table 3: IMDC Prognostic groups

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 systemic 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 *Disease evaluation and response*

Patients should have undergone standard of care imaging prior to study enrolment. Imaging should be repeated as clinically indicated throughout treatment, but must be reported to RECIST 1.1 criteria. Pseudonymised imaging and reports will be transferred to the Sponsor.

5.5.7 *Adverse events*

Adverse events will only be collected for events resulting from the study-mandated biopsy and blood, 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. Treatment-related AEs are not study-related because treatment decisions, administration and side-effects are all clinical standard of care.

5.5.8 *Tumour research biopsy*

One additional image-guided core needle biopsy (minimum 14-gauge) will be required from which at least two cores are taken. For patients with metastatic disease, samples should be taken from the same metastatic site if possible. 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*

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.

5.5.10 *Radiation dose and exposure considerations*

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

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

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

Expected trial research exposures therefore may consist of:

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

Tc-99m Bone scans: Delivered as part of routine care, which may contribute to measuring study outcomes

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

We would not expect radiotherapy to be a research exposure within this trial.

5.6 *Exploratory research*

All patients will be consented for the collection and use of their tissue and blood samples. All samples will be link-anonymised and only identified by the study ID and unique sample number allocated by the Sponsor. 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 relevant legislation (Human Tissue Act 2004 within the UK; HIPAA and FDA regulations in the USA).

5.7 Patient Withdrawal

Patients may voluntarily withdraw from the study at any time. Patients will also be withdrawn from the study if:

1. They are not able to undergo a biopsy of the kidney tumour or metastases for any reason;
2. The biopsy sample fails to establish a successful culture in the laboratory;
3. The patient completes fewer than one cycle of systemic therapy post-biopsy;
4. At least 1 set of imaging data for systemic therapy received post-biopsy is not available for RECIST 1.1 evaluation.

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 Adverse Events

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

6.3 Severity of 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 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-TREE2 SAE form via email and within 24 hours of the site becoming aware of the event.

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

6.7.1 Non-reportable events

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

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

6.8 Definition of an Adverse Reaction (AR)

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

6.9 Definition of Serious Adverse Reaction (SAR)

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

6.10 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

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

6.11 Reporting of SUSARs

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

6.12 Annual Reporting

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

6.13 Urgent Safety Measures

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

The Sponsor has an obligation to inform the REC/IRB 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 Definitions

For time to event analyses, evaluable patients who die without a reported event (and without start of subsequent anticancer therapy) will be considered to have that event on the date of their death. Patients who do not have an event or die will be censored on the date of their last evaluable tumour assessment on or before initiation of subsequent anticancer therapy. Patients who start a new anticancer therapy without a prior reported event will be censored on the date of their last evaluable tumour assessment on or before the initiation of subsequent anticancer therapy. PFS censoring rules will follow the FDA guidance of 2018 (replacing that of 2007)

Overall response rate (ORR) is defined as the proportion of subjects who have a best overall response of CR or PR using RECIST 1.1.

Progression-free survival (PFS) is defined as the time from the date of enrolment to the date of the first documentation of disease progression or death (whichever occurs first) as determined by RECIST 1.1.

Overall survival (OS) is defined as the time from the date of enrollment to the date of death from any cause. Subjects who are lost to follow-up and those who are alive at the date of data cut-off will be censored at the date the subject was last known alive, or date of data cut-off, whichever occurs first.

Duration of response (DOR) is defined as the time from the date a response was first documented until the date of the first documentation of disease progression or date of death from any cause.

Disease control rate is the proportion of subjects who have best overall response of CR or PR or SD. Stable disease must be achieved at ≥7 weeks after enrolment to be considered best overall response.

Durable stable disease rate is the proportion of subjects who have the duration of SD ≥ 26 weeks after randomisation.

Clinical benefit rate is the proportion of subjects who have best overall response or CR or PR or durable stable disease

Durable clinical benefit rate is the proportion of subjects who have best overall response or CR or PR or durable stable disease and have not experienced PD at ≥ 26 weeks after randomisation.

Depth of Response is defined as the maximum reduction in sum of diameters of target lesions (negative value means true reduction; positive value means increase only observed over time). A 30% reduction is consistent with a response according to RECIST version 1.1

Deep Response is defined as patients who achieve a PR, and not a CR, and where depth of response is 80% or more.

PFS on next-line therapy (PFS2) is defined as the time from starting subsequent therapy to disease progression on subsequent therapy, or death from any cause (whichever occurs first).

IMDC risk factors:
The IMDC prognostic model was derived and validated in previously untreated patients with metastatic RCC who received anti–vascular endothelial growth factor receptor (VEGFR) therapy. This model is composed of six clinical parameters that are used to categorise patients into favourable (zero risk factors), intermediate (1 or 2 risk factors), and poor (3 to 6 risk factors) prognosis groups. The individual risk

factors are a Karnofsky performance status score of <80%, a time from initial diagnosis to treatment of less than 1 year, a hemoglobin level below the lower limit of normal, a corrected calcium concentration above the upper limit of normal, a neutrophil count above the upper limit of normal, and a platelet count above the upper limit of normal.

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities. Minimal information includes date of consent, demography, screen failure details, eligibility criteria, and any serious AEs.

The Full Analysis Set (Enrolled Analysis Population, EnAP) is the group of all enrolled subjects regardless of tumour cell culture success or treatment actually received. This group will also be used for safety analyses.

The Evaluable Analysis Set (Evaluable Analysis Population, EvAP) is the group of all enrolled subjects who meet the definition of evaluability defined in section 3.

The QOL Analysis Set will consist of all evaluable subjects who have any QOL data.

7.2 Statistical Tests:

Overall Response Rate, Deep Response Rate and Complete Response Rate will be calculated with exact 95% confidence intervals using the method of Clopper and Pearson.

Median PFS and OS with 2-sided 95% CIs will be calculated using Kaplan-Meier product-limit estimates for each treatment arm and Kaplan-Meier curves of PFS and OS will be plotted over time.

Differences in time-to-event outcomes will be analysed using the stratified log rank test with each patient subgroup and/or pooled patient subgroups acting as strata.

Median DOR among responders for each treatment arm will be presented along with its corresponding 2-sided 95% CIs.

The difference between positive and negative response predictions will be tested using the Cochran-Mantel-Haenszel (CMH) test, stratified by each patient subgroup and/or pooled patient subgroup.

Depth of Response will be displayed using a Waterfall plot. Patient responses will be plotted on an individual patient basis, with change in the sum of the diameters (as per RECIST 1.1) on the y-axis and patients on the x-axis; we will use colour and schematics to superimpose the results of the Pear test and the RECIST 1.1 criteria.

Safety analyses will be based on the Full Analysis Set. Adverse events and serious adverse events will be summarised. Safety data will be summarised using descriptive statistics. Categorical variables will be summarised by number and percentage. Continuous variables will be summarised using n (number of subjects with available data), mean, standard deviation, median, interquartile range, and range (minimum and maximum) unless otherwise specified.

Multiplicity adjustments will be made for endpoint analysis involving multiple analysis subgroups for the primary endpoint (ie. not global analysis of the whole evaluable patient population), as well as for the analysis of the secondary endpoints. P value corrections will be done using a false-discovery rate adjustment approach based on the Benjamini–Hochberg approach with an acceptable FDR for secondary, and exploratory endpoints of 20%.

For final statistical testing, we will address PFS and durable response in a hierarchical fashion, starting with the 2 year rate and stepping backwards, but these hierarchies will not be used for interim analyses. We will only consider statistical testing in subgroups where there are >= 20 patients. We will use Forest plots to compare the AUC (with CIs) for larger patient subgroups.

7.3 Sample Size

Up to 200 evaluable patients, and a maximum of 400 patients, will be recruited to this study. 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 subgroup, pooled subgroup and the entire evaluable population will be assessed under the statistical analysis plan independently.

7.4 Statistical Analysis

7.4.1 *Grouping, subgroups and exploratory analyses*

Primary performance measures will split clear cell RCC patients into main subgroups based on:

1. Line of therapy
2. IMDC category
3. Therapy regimen

Many subgroups will not have enough evaluable patients for statistical analysis. Given the number of patients and subgroups, we expect to pool subgroups to provide interpretable results. We will do this based on clinical factors (e.g. pooling Intermediate and High IMDC risk groups; pooling similar IO + TKI treatments like nivolumab + cabozantinib, pembrolizumab + axitinib and pembrolizumab + lenvatinib) and also in a data-driven fashion, collapsing groups that have similar outcomes, with tests for heterogeneity across groups. Statistical analysis will also be done on the entire evaluable patient population (global analysis) to determine whether there is subgroup-agnostic accuracy for a given predictive/prognostic biomarker.

7.4.2 *Primary Efficacy Analysis*

Statistical analysis will be conducted using Receiver Operating Characteristic (ROC) curves for tumour response metrics extracted from the Pear Bio test for each patient sample, and 2x2 contingency tables.

These metrics include:

- Cell viability
- Speed of cell migration
- Immune cell infiltration (including subsets of cells like CD8+ cells)

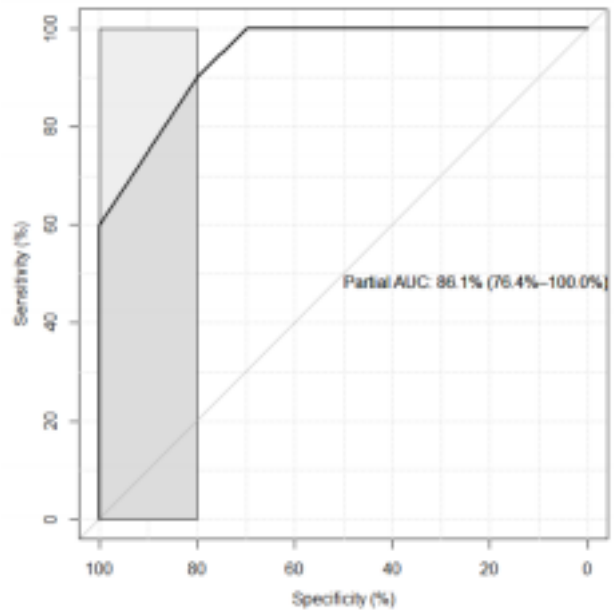
These analytical metrics from the Pear Bio test will be compared with patient response (defined as Overall Response: ORR) to determine which metrics are the most accurate at predicting ORR. These will be analysed separated by line of therapy, IMDC score and therapy regimen group, in clear-cell RCC (“Main subgroups”) and also analysed in a pooled and global fashion (as described above).

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 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 pipeline, 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. An optimal 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 test predictions to random guesses of response/non-response.

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



Other analysis methods:

A Fisher Exact test will be used on a 2x2 contingency table of patients who achieved response vs non-response to measure sensitivity and specificity. An AUC ≥70% must be obtained with $p<0.05$.

The data from the ROC curve will then be used to calculate and report the sensitFivity and specificity of the Pear-Bio test to predict ORR (and other binary response endpoints), as defined below.

Endpoints:

	Number of patients who achieve response (PR/CR)	Number of patient who do not achieve response

Pear predicts response	A	B
Pear predicts non-response	C	D

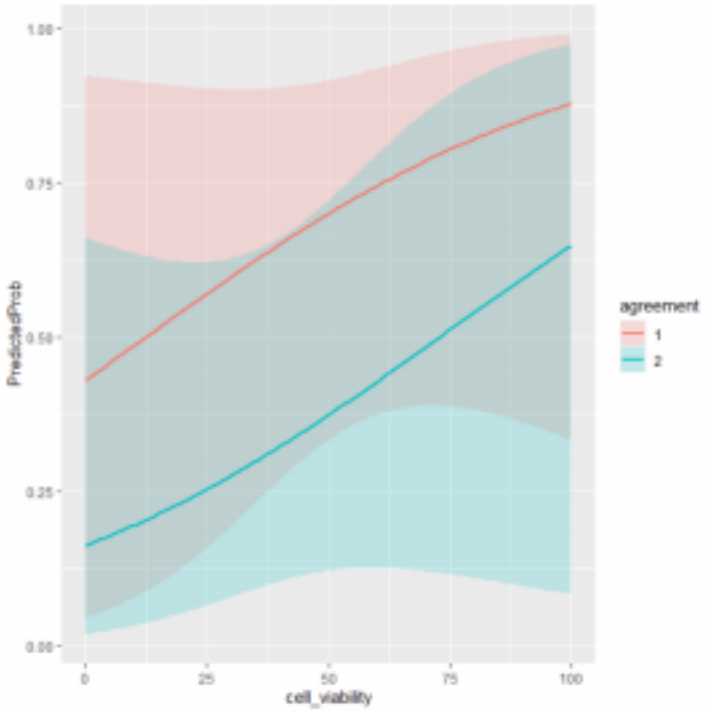
Specificity: measured as the percentage of non-responding patients identified by the Pear Bio test from the total number of patients who did not achieve a response. Specificity = D/(B+D)
Sensitivity: measured as the percentage of patients that the Pear Bio test identified would achieve a response from the total population of patients who achieved a response (true positives). Sensitivity = A/(A+C)
Positive predictive value: the percentage of patients that the Pear Bio test correctly predicts as OR out of all OR predictions. PPV = A/(A+B)
Negative predictive value: the percentage of patients that the Pear Bio test correctly predicts as non-OR out of all non-OR predictions. NPV = D/(C+D)

Logistic regression will be used to compare subgroups of patients (including pooled subgroups) based on whether they achieved a response. The results of these classifiers will also be validated using a Fisher Exact test. Patient subgroups are defined above.

Patients are compared on the basis of achieving response. Patients who achieve a response are given an outcome value of 1, while patients who do not achieve a response 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 response at various analytical thresholds.

If patient data is missing for the purpose of allocating them to a particular subgroup for analysis, that patient will be excluded from the logistic regression making that subgroup comparison.

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 response at different cell viability values from Pear's assay:



7.4.3 *Secondary Efficacy Analysis*

We will assess the ability of the Pear Bio test to accurately predict the following endpoints, reporting sensitivity, specificity, positive and negative predictive values for each outcome.

- Complete response
- Deep response
- 6-month, 1-year and 2-year durable response
- 6-month, 1-year and 2-year PFS
- Hazard ratio between low and high biomarker groups for progression free survival (PFS)

Performance measures will be done globally (all patient subgroups), on the main subgroups, exploratory subgroups, and pooled subgroups as described above.

7.4.4 *Exploratory Analysis*

Culture success rate analysis

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

The successful cell culture rate is the percentage of successful cell cultures established out of all tumour samples arriving uncompromised within 24 hours of collection to the Pear Bio laboratory.

Overall survival

Test accuracy for overall survival at 1, 2, 3 and 4 years will be reported using sensitivity, specificity, positive predictive value and negative predictive value with the same methods used for primary and secondary endpoint analysis.

Exploratory subgroup analysis

Exploratory analyses will include the following exploratory subgroups:

1. Biopsy location (e.g., kidney, liver, etc.)
2. Nephrectomy status
3. Trial site (e.g., Hospital A vs Hospital B, US vs UK, etc.)
4. Non-clear cell RCC

We will repeat all of the primary and secondary analyses in non-clear cell RCC.

We will measure test sensitivity and specificity for complete response (CR) in patients who have had a prior nephrectomy, test sensitivity and specificity for non-renal complete response (CR) and performance by biopsy site response (including comparisons where study cores were taken from the same lesion vs different lesions).

We will also determine whether the trial sites, including their country (US or UK), had an impact on any of the endpoints.

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 response and those who did not.

Protein biomarker analysis

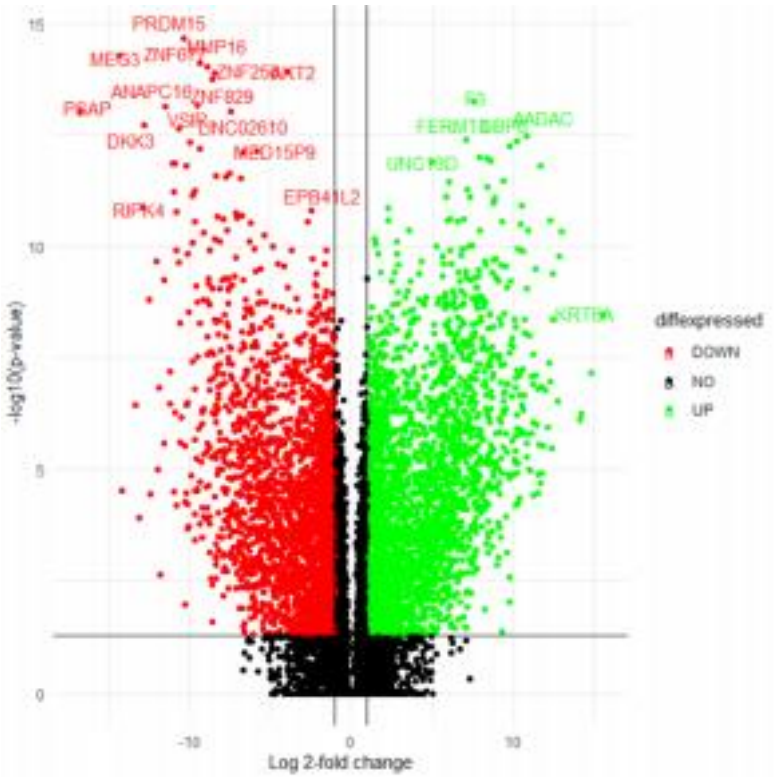
Patient samples can be fixed, including post-treatment in the Pear test. Immunofluorescence (or immunohistochemistry) is used to measure the expression of proteins such as CD44 and CD24.

The same endpoints of sensitivity, specificity, positive predictive value and negative predictive value are used to determine prediction accuracy for response vs non-response. The presence or absence of a protein, such as CD44, is used to predict the response 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. Where a staging system is established, it can be used instead of binary protein presence/absence to measure predictive accuracy. Where a staging system includes multiple levels, an NxM contingency table is used with a Chi Squared test. Provided enough data points are available, and ordinal logistic regression may also be used.

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 and/or spatial transcriptomics tests to measure expression levels. Patients who achieve a response are compared to patients with a non-response 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 responseR against the population with non-response 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 a response. 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 response. All other biomarkers will be considered not significant for a response 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 response, while biomarkers in the top left will have a strong negative association with patients who achieve response.

Explore immune cell activation in vitro

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 ORR status. Readouts will include immune cell infiltration and activity. Readouts may also include cytokine panels to measure immune activity.

7.5 Interim analysis and study termination

As patients are recruited in close proximity to each other, and ORR data is collected ~3-6 months from the first administration of treatment and the conducting of the Pear Bio assay, we will conduct interim assessments of ORR after the first 50 and 100 patients have been recruited. 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 continue to meet after every 50 patients. The TMG will use the results to determine whether to increase accrual up to a maximum of 400 patients.

If ≥80% AUC can be achieved alongside ≥70% sensitivity and specificity on the ROC analysis before all patients in each patient subgroup have reported outcomes, the analysis can be stopped. Should this occur across all major subgroups, the study can be terminated for publication of results.

7.6 End of Study Definition

The end of the trial is defined as last patient last data collection, which is estimated to take place within 2 years and 3 months of the last patient's enrolment. In cases of early termination of the trial (e.g., due to slow accrual) or a temporary halt, the Sponsor will notify the main REC/IRB within 15 days of the decision and a detailed written explanation for the termination/halt will be given.

7.7 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 relevant data protection regulations (e.g Data Protection Act 1998, GDPR, HIPAA) and the rights the patient has under these regulations. 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 treating institution, 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 Sponsor. The Sponsor 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 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 Sponsor) 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 relevant local regulations (e.g., Data Protection Act 1998, GDPR, HIPAA).

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.

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 lead clinical site (CI, Project Lead) and the Sponsor's representatives, scientists and statistician(s). The role of the TMG will be to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The TMG will meet at least three times a year.

Final decisions about continuation or termination of the trial are the responsibility of the TMG.

9.1 DISSEMINATION

Study results will be disseminated through the presentation of data and results and conferences and academic meetings, and the publication of results, including in peer-reviewed journals.

9.1.1 FEEDBACK TO PATIENTS

We are aware that patients may undergo an additional biopsy, and will donate a blood sample, in order to participate in this trial, and we are very grateful to them for doing so. At the same time, we cannot in good faith provide early readouts from the Pear assay, as we do not yet know the performance characteristics of the test.

Feedback will therefore take three forms:

1. When a patient undergoes a biopsy, we will provide feedback within 4 weeks to the treating centre as to whether we were able to establish a successful culture from that biopsy.
2. We will provide a summary of the Pear assay results to the treating physician, but with a time delay of a minimum of 3 months. We expect this delay to be longer initially, and for the first group of patients, we may not be able to provide any useful feedback. In that scenario, we will provide a feedback note that says whether we were able to establish a successful culture but that we cannot provide feedback on assay outcome. Feedback will not be used by physicians to alter treatment, but this is a way of sharing the results of our work with physicians and patients in an early fashion.
3. We will hold regular investigator meetings (every 4 - 8 weeks) where we feedback general results from the Pear assay, and how well it is performing, including technical challenges. This will allow investigators to place the results from (2) in context.

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) or Institutional Review Board (IRB) 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/IRB 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/IRB and will agree to share all such documents and reports with the CI.

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/IRB before distribution to patients.

10.2 Summary of Monitoring Plan

Refer to PEAR-TREE2 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 Sponsor. The investigator and institution will be informed of the audit outcome. Investigators are obliged to cooperate in any audit by allowing the auditor direct access to all relevant documents and by allocating 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 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 investigator 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/IRB in writing of any serious breach of:

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

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

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

Participating centres should contact the Sponsor for further information.

11 STUDY FINANCES

11.1 Funding Sources

This trial is Sponsor designed and 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' domestic travel expenses (ie. international travel will not be compensated).

12 SPONSORSHIP AND INDEMNITY

Dr. Ekaterini Boleti is the UK 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, laboratory analysis and statistical analysis.

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

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