Refining Ovarian Cancer Test Accuracy Scores:

A test accuracy study to validate new risk scores in postmenopausal women with symptoms of suspected ovarian cancer protocol

ROCkeTS GEN V2 Study

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The Funders of the ROCkeTS GEN V2 trials have had no role in the trial design, data collection, data analysis or data interpretation. The views expressed herein are those of the authors and not necessarily those of the Cancer Research UK.

Compliance statement

This protocol describes the ROCkeTS GEN V2 study only. The protocol should not be used as a guide for the treatment of participants not taking part in the ROCkeTS study.

The trial will be conducted in compliance with the approved protocol, UK Policy Framework for Health and Social Care Research 2023, the Data Protection Act 2018, and the principles of Good Clinical Practice as defined by the European Good Clinical Practice (GCP) Directive. Every care has been taken in the drafting of this protocol, but future amendments may be necessary, which will receive the required approvals prior to implementation.

Previous Protocol Versions

Sponsor and Sponsor Roles

University of Birmingham is the sponsor. Prof Sudha Sundar is the Chief Investigator.

University of Birmingham is responsible for obtaining necessary approvals, the Project Management Group is jointly responsible for overseeing good clinical practice and the investigators are responsible for obtaining informed consent and care of the participants.

Signatures

The investigators and the sponsor have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol except in case of medical emergency or where departures from it are mutually agreed in writing.

Chief investigator

Organisation	Signature	Date

Sponsor

For UoB sponsored trials, the sponsor will confirm approval of the protocol by signing the IRAS form and therefore a signature on the protocol is not required.

Protocol amendments

Amendment number	Relevant Documents	Changes summary
Original application	Protocol, PIS, Consent v1.0	n/a

Abbreviations

AE	Adverse Event
AUC	Area Under the Curve
CI	Chief Investigator
CMDL	Cancer Molecular Diagnostic Laboratory
CRF	Case Report Form
ctDNA	Circulating tumour DNA
DH	Department of Health
GCP	Good Clinical Practice
GP	General Practitioner
HGSOC	High-Grade Serous Ovarian Cancer
IOTA	International Ovarian Tumour Analysis
ISRCTN	International Standard Randomised Controlled Trial Number
NGS	Next Generation Sequencing techniques
NICE	National Institute for Health and Care Excellence
NPV	Negative Predictive Value
OC	Ovarian Cancer
PI	Principal Investigator
PIS	Participant Information Sheet
PMG	Project Management Group
PPV	Positive Predictive Value
QMUL	Queen Mary University of London
RCOG	Royal College of Obstetricians and Gynaecologists
RMI	Risk of Malignancy Index
ROC	Receiver Operating Curve
ROCkeTS	Refining Ovarian Cancer Test Accuracy Scores
ROMA	Risk of Ovarian Malignancy Algorithm
RR	Relative Risk
SAE	Serious Adverse Event
sWGS	Shallow Whole Genome Sequencing
SOP	Standard Operating Procedure
UoB	University of Birmingham
USG	Ultrasound

CONTENTS

1 INTRODUCTION

1.1 Ovarian Cancer background

Ovarian cancer (OC) has an annual incidence of 7500 women and causes 4100 deaths annually in the UK; the lifetime risk of developing OC is 1 in 56.[1] 70% of patients will present at advanced stage and all stage, 5 year survival rate is around 45%. OC is predominantly a disease of older, post-menopausal women, however 1000 women under 50 will be diagnosed with OC annually.[1,17] An international cancer bench marking project shows OC survival in the UK is significantly lower than other western countries; it is unclear as to whether this could be attributed to delay in diagnosis or differences in treatment received.[1,2]

1.2 Current diagnosis of Ovarian Cancer

National Institute for Health and Care Excellence (NICE) guidelines in 2011 (last updated in 2023) recommended sequential testing using serum CA125 followed by pelvic ultrasound (USG) in women (particularly aged $\geq$50) presenting to primary care with symptoms on a persistent or frequent basis: persistent abdominal distension/'bloating', feeling full and/or loss of appetite, pelvic/abdominal pain, increased urinary urgency and/or frequency, unexplained weight loss, fatigue or changes in bowel habit[28]. However these symptoms are very common[3-6,11,16,20] with abdominal bloating alone being documented in 16-30% of women presenting to General Practitioners (GPs)[7]. Diagnostic challenges are considerable given (1) the low incidence of OC (a GP sees a woman with OC once in 3-5 years) (2) the low positive predictive value (PPV) of symptoms (only 1 in 400-600 symptomatic women have OC)[8,9] and (3) the lack of clear diagnostic pathways. Furthermore, NICE guidelines do not detail what USG abnormalities should prompt referral[29].

Use of this NICE guidance is extremely variable. A survey of 258 GPs report that the majority would refer patients on the basis of raised CA125 even if the USG was normal[10]. Referrals were heterogeneous in symptoms and in what the GPs considered were abnormal levels of CA125 or abnormal USG. Two thirds of women referred were premenopausal. Women with complex masses considered benign can undergo laparoscopic or conservative management, whereas women with malignancy who undergo thorough surgery by gynaecological oncologist have the best outcomes[12,13]. Therefore, there are compelling health needs to improve early detection[19,20,25-27] and reduce cancer mortality whilst minimising unnecessary interventions in women.

1.3 The need for a new algorithm to test for OC

Earlier diagnosis of cancer has great potential to reduce mortality and morbidity. Recent technological advances allow tiny amounts of cancer signals including tumour-derived DNA and protein markers to be detected in blood samples. These methodologies have been utilised in recent years to develop cancer screening tests that are designed for the general population, driven by large commercial investments. There is a need to expand our understanding of the relationship between early cancer development and its representation in circulating tumour DNA (ctDNA) in blood plasma, to allow us to develop better analysis and detection tools for the future.

Routes to diagnosis data shows that currently OC diagnosis is made in women presenting through diverse routes – 2 week referrals, routine GP referrals, cross specialty referrals, with

a third of patients presenting as emergency presentations[6,53]. Thus, risk prediction models must be assessed in a heterogeneous study population in all these settings.

1.4 The ROCkeTS GEN V2 project

ROCkeTS GEN validation study 2 evaluates ctDNA as a diagnostic test for ovarian cancer in women with non-specific abdominal symptoms. **This protocol refers to the ROCkeTS-GEN V2 study only.**

1.4.1 ROCkeTS GEN V2 prospective study

The ROCkeTS GEN V2 study: patients entering the study will donate a sample of blood – dried blood spot (~50-100ul), plasma (~9ml of blood) and serum samples. Patients who undergo surgery will have their histology details recorded for the study and representative tissue block will be collected for DNA isolation, for patients who do not undergo surgery wellbeing will be ascertained at 12 months follow-up after presentation by a clinic visit, a telephone call or by contacting GP. These data will be used at the end of the study.

FOR THE PURPOSE OF THIS PROTOCOL, THE TERM OVARIAN CANCER INCLUDES FALLOPIAN TUBE CANCER AND PRIMARY PERITONEAL CANCER.

1.5 Plasma circulating tumour DNA as a diagnostic biomarker for ovarian cancer

Using next generation sequencing (NGS) techniques it is now possible to amplify small amounts of free circulating DNA in the blood to identify molecular signals derived from the tumour. These tumour derived signals can include both genetic and epigenetic marks. Somatic mutations such as SNVs, SVs and copy number changes are highly specific biomarkers of cancer which can be used to detect circulating tumour DNA (ctDNA). The most commonly analysed epigenetic signal is DNA methylation which can be a reflection of tumour specific epigenetic events and will also reflect differences in representation of cell types in the blood which will change during disease processes such as carcinogenesis. Together these could provide a powerful non-invasive method for earlier cancer diagnosis [24,30,31,32]. Other potential blood derived biomarkers would include the proteome.

The most common subtype of OC is high-grade serous ovarian cancer (HGSOC) which accounts for 70% of primary invasive epithelial ovarian cancers and the majority of mortality[33]. To be useful as a diagnostic test a biomarker needs to be highly specific for the disease of interest and ubiquitous in the target population. The gene TP53 encodes the tumour suppressor protein p53, a transcription factor that regulates the expression of proteins involved in apoptosis and genomic integrity[18]. We have previously shown that mutations in TP53 occur in at least 99% of HGSOC cases[34,35] making TP53 mutations ubiquitous in HGSOC. In HGSOC TP53 mutations are located throughout the gene in all 10 coding exons (exons 2 to 11) therefore a NGS method that is not limited to assaying hotspot mutations is required. To achieve a high level of sensitivity it is necessary to use as much blood as possible or to interrogate as many genomic positions as possible. TP53 mutation will only represent a single locus therefore limiting sensitivity, therefore we are making use of genome wide approaches these include the analysis of copy numbers HGSOC is also a tumour characterized by complex copy number changes[36] that can be detected by shallow whole genome sequencing (sWGS) which is a relatively inexpensive and easy to apply

methodology. sWGS data is also very appropriate data for the new and exciting field of fragmentomics. Fragmentomic analysis relies on the fundamental processes whereby cfDNA and ctDNA are generated by cleavage with specific enzymes and the accessibility of the DNA to these enzymes is dependent on the chromatin conformation. Therefore, there are valuable information contained within the cfDNA fragment ends, lengths and representation which can be used to enhance sensitivity of detection. Analysis of DNA methylation allows the interrogation of multiple loci genome wide or at higher depth restricted to a panel of selected loci these approaches have been demonstrated to enable the detection of cancer[51] [*for example GRAIL studies*][52].

In preliminary analysis using samples from our previously conducted ROCkeTS-GEN study we have shown that a methylation based classifier developed by us can, even in women for whom there was no suspicion of ovarian cancer based on Risk of Malignancy Index (RMI, the standard of care test combining ultrasound and CA-125), with samples and data blindly analysed, 89% of women with stage III-IV ovarian cancer were correctly identified as cancer, while fewer than 20% of women without ovarian cancer were incorrectly identified as cancer, an error that could trigger further imaging by CT or MRI prior to surgery (unpublished data). This evaluation performance is based on blinded and statistically unbiased assessment of a subset of cases and controls from the ROCkeTS-GEN study. This includes approximately 30% of the cancers from the multi-centre study which recruited an unselected cohort of women immediately prior to surgery, where surgery was used to establish ovarian cancer status and disease staging. (Exact numbers and 95% confidence intervals are not reported here to protect the blinding of the main ROCkeTS-GEN trial analysis which is pending).

1.6. Other novel markers

Currently there is multiple promising biomarkers for early cancer detection including circulating tumor DNA (ctDNA)[24,30,31], microRNAs (miRNAs)[39], cancer glycomics[48,49,50] and protein biomarkers[40,41,44-46], with emerging research exploring metabolites[42,47] and epigenetic markers like DNA methylation[43]. In ROCkeTS GEN V2, based on our developing understanding of technologies, we will analyse samples collected with some/all these technologies to try and identify the best biomarkers for OC.

1.6.1 ROCkeTS-GEN V2 Project

We propose to evaluate plasma ctDNA and collect additional samples suited for evaluation in the ROCkeTS-GEN V2 study by:

1) Collecting plasma samples and analysing ctDNA in a prospective cohort study (ROCkeTS-GEN V2) that will recruit **post**menopausal participants

2) Collecting representative tissue blocks from resected tissue from patients undergoing surgery or biopsy so that the mutational profiles of plasma ctDNA can be verified against the mutational profile of tumours.

4) Collect additional samples of dried blood spot and serum which may be used in the future to improve diagnostic testing

1.7. Risks and Benefits

There are no vulnerable groups or risks associated with this project. There is no intervention, and all participants follow their normal care pathway.

2 ROCKETS GEN V2 STUDY DESIGN

2.1 Aim of the study

- To evaluate plasma ctDNA as a diagnostic test with increased sensitivity and specificity compared to serum CA125 for earlier diagnosis of ovarian cancer in postmenopausal women.
- To collect representative blocks from resected tumour tissue to establish a translational resource for future early detection research.
- To collect dried blood spots and serum for analysis of circulating biomarkers and blood ctDNA in cohorts of women at high risk of ovarian cancer, to establish improved diagnostic test for postmenopausal women.

2.2 Design

ROCkeTS GEN V2 study is a prospective single arm cohort diagnostic accuracy study where all patients receive all tests and accuracy of tests is evaluated against a gold standard of histology or 12-month outcome.

A test accuracy study compares measurements obtained by index tests with those obtained by a reference standard. In this way the accuracy of index tests can be estimated. A reference standard is a test (or combination of tests) that confirms or refutes the presence or absence of disease beyond reasonable doubt.

Here, the reference standard will be histology or cytology of tissues taken from patients who proceed to surgery or biopsy, or in patients who do not undergo surgery or biopsy assessment of cancer/non cancer outcomes determined from hospital records at 12 months. The diagnostic performance of the index test will be compared against that of the comparator test – the existing standard risk prediction score RMI 1. RMI combines CA125 and limited ultrasound features to provide a score that is used to determine patient management.[3,37]

ROCkeTS GEN V2 will recruit postmenopausal women at 'low' or unknown genetic risk of ovarian cancer i.e. not women known to have a BRCA1/2 or other germline mutation predisposing to ovarian cancer.

Recruited women will have the following tests and follow up recorded:

- CA125 (if not performed already as part of standard care).
- IOTA transvaginal ultrasound and additional abdominal scan if performed as part of standard care
- Histology result where biopsy or surgery is clinically indicated
- 12 month follow up status ascertained by research team using hospital records, a telephone call or by contacting GP.
- Women entering ROCkeTS-GEN V2 will donate dried blood spots, blood samples for plasma and serum analysis at recruitment. Women undergoing surgery for ovarian pathology will also be consented to donate tissue block/s of representative tissue for research.

Representative tissue block/s and slide will be retrieved from pathology labs and proceeding to undergo surgery as part of standard care. These blocks will first undergo specialist pathology review by an expert gynaecological pathologist. DNA will then be extracted from cancer tissues for additional analyses including shallow whole genome sequencing.

In ROCkeTS GEN V2, the index tests (ctDNA analysis) will be externally validated at the end of the study. Therefore, we will collect dried blood spots, serum and plasma in the study to be analysed and validated at the end of the study.

2.3 Setting

Recruiting from NHS sites within the UK. Secondary care outpatients: 2 week referrals, USG clinics, routine GP referrals, cross specialty referrals, preadmission clinics. Inpatients: emergency presentations to secondary care.

2.4 Target Population

Postmenopausal women who have been referred to secondary care with symptoms of suspected OC.

Symptoms are as defined by NICE which include but are not restricted to persistent or frequent abdominal distension, feeling full (early satiety) and/or loss of appetite, pelvic or abdominal pain, increased urinary urgency and/or frequency. Symptoms listed here are not an exhaustive list.

2.5 Comparator for ROCkeTS GEN V2

RMI at cut off 200[37].

2.6 Analysis

Plasma ctDNA analysis at Cancer Molecular Diagnostic Laboratory (CMDL) will be performed blind from tissue DNA analysis. Sensitivity and specificity of plasma ctDNA will be established by statistical analysis. Blood and dried blood spot samples will be stored and analysed for multiple analytes such as proteins, by CMDL and QMUL. The genomic methodology will be used, including whole genome sequencing. ROCkeTS-GEN v2 uses a rapidly changing technology for analysis and therefore exact detail on how the analysis will be performed will not be detailed in the protocol but will be detailed in full in the final report and any publications.

2.7 Reference standard

Reference standard for the study will be histology of tissue taken at surgery or biopsy or cytology in postmenopausal women who are managed surgically following study enrolment or assessment of wellbeing at 12 months after presentation for patients who do not undergo surgery.

Study data collection will be undertaken prospectively for all participants in order to inform the costs for each pathway. Following giving consent at baseline, the participants and the local study team will complete a series of CRFs (see table 1) including:

1) Participant Baseline CRF

2) Registration form

3) A surgery CRF will be completed where histology is attempted from tissue taken at surgery or biopsy or cytology. Should histology from one of these procedures come back as unknown but is identified at a later date from a subsequent surgery/histology, the surgery CRF should be updated. The surgery CRF will be completed by the research team.

If no surgery CRF has been completed by 12 months post study entry, a 12 month Clinical CRF will be completed by the research team after either a clinic visit or a telephone call to the participant or from medical records.

If insufficient information is available through the medical records, the GP will be contacted to ensure that all available data are collected.

Participation in the ROCkeTS GEN V2 study is completed on receipt of a completed surgery CRF. If the participant does not have surgery, then completed 12 month Clinical CRFs will indicate completion.

2.8 Sample size

Sample size will be 500 newly presenting postmenopausal women blood samples, allowing 5% for loss to follow up.

The Cancer Molecular Diagnostics Laboratory (CMDL) and Queen Mary University of London (QMUL) teams performing plasma ctDNA analysis and additional biomarker analysis will be blinded to the clinical dataset held at UoB. This is to eliminate bias in analysis of the samples and to test the validity of the biomarker. To ensure this the clinical dataset will be maintained in UoB and the team at UoB will supply research teams with the samples anonymised with participant study numbers only. Research teams will send UoB the results of the analysis and the UoB and statistical team will then check that against outcome data to analyse trial outcomes.

2.9 ROCkeTS-GEN V2 Outcome measures

Sensitivity and specificity of plasma ctDNA analysis in the diagnosis of OC. See 2.6 ROCkeTS GEN V2 analysis.

2.10 Schedule of Events & CRF completion for ROCkeTS-GEN V2

Table 1: Schedule of events and CRF completion

	Screening	Baseline	≤ 12 months
Eligibility Check	x[1]		
Valid Informed Consent	x[1]		
Registration Form CRF		x	
Online registration		x[2]	
GP Letter		x	

Blood sample		x[4]	
IOTA USG		Optional	
Ultrasound CRF		Optional	
Participant Baseline CRF		x[3]	
Surgery CRF			Post-surgery/biopsy
Representative tissue block/s			X[5]
Outcome CRF			12 months – *only required if histology/cytology not obtained*

[1] See section 'Approaching Potential Participants to Consent'.

[2] It is acceptable to delay registration until blood sample, IOTA USG & Participant Baseline CRF are complete.

[3] Blood sample, IOTA USG and Participant Baseline CRF should all be captured within 3 months of whichever of the following occurred first: presentation, IOTA USG (see section 5.1).

[4] Blood samples including dried blood spots, serum and plasma, send immediately to CMDL

[5] Tissue sample (if participant proceeds to surgery) or biopsy send to Central study lab (CMDL or QMUL).

3 SELECTION OF PARTICIPANTS

3.1 Source of potential participants

Patients referred as Outpatients; either as 2 week or routine referrals, USG clinics, inpatient and emergency presentation to secondary care. Patients proceeding to surgery attending preadmission clinics. Patients proceeding to biopsy. Only postmenopausal women with suspected OC should be considered for ROCkeTS GEN V2. Participants can also be recruited into the study on the morning of surgery if there has not been a previous opportunity to recruit.

These should be new patients only, i.e. first presentation to the service; patients who are on routine follow up in the secondary care service as part of standard practice should not be approached for recruitment.

ROCkeTS GEN V2 will only recruit postmenopausal women.

3.2 Inclusion and Exclusion Criteria

3.2.1 Inclusion criteria

- Women referred with symptoms of suspected OC (typical referral symptoms are defined in section 2.5 of the protocol).
- Only postmenopausal women are included.
  Menopause is defined as >12 months without menstruation. Those no longer menstruating >12 months for reasons such as contraception or hysterectomy should

have their menopausal status categorised according to age; <50 years premenopausal, 51+ years postmenopausal.

- In addition, women must have test results from one of the following:
  1) A raised CA125 test result (even if imaging has not been done yet)
  2) Abnormal imaging result showing a lesion (even if CA125 test is not raised).
  3) Both a raised CA125 test and an abnormal imaging result showing a lesion
- Patients able to provide informed consent.

FOR THIS STUDY WE FOLLOW THE IOTA DEFINITION OF A LESION: A LESION IS PART OF AN OVARY OR AN ADNEXAL MASS THAT IS JUDGED TO BE INCONSISTENT WITH NORMAL PHYSIOLOGICAL FUNCTION.

3.2.2 Exclusion criteria

- Premenopausal women
- USG reveals simple ovarian cysts <5cm in size (very low risk of malignancy) and patient does not have a raised CA125.
- Previous ovarian malignancy.
- Active non ovarian malignancy – Women with a past history of cancer are only eligible if there are no documented persistent or recurrent disease and they have not received treatment for this in the last 12 months. This exclusion does not apply to patients with premalignant disease e.g. cervical intra-epithelial neoplasia or patients receiving Tamoxifen/other drugs to prevent breast cancer recurrence.

3.3 Approaching potential participants for consent

Potential participants can be approached ahead of their clinic appointment or at their clinic appointment or whilst they are inpatients in hospital. Eligible patients may also be identified at scan departments. If potential participants cannot be approached at this time, then remote or virtual consent can be obtained as documented in 3.4. A member of the research team or usual care team will approach patients via phone or remotely. The approach process will parallel the in-person process.

If they are interested, they will be supplied with the participant information sheet (PIS) and given the opportunity to ask any questions.

Patients who are admitted into hospital as emergencies and undergoing investigations for OC will be approached to give informed consent. In our experience, these patients are unwell enough to need hospital stay but are not critically unwell to the extent that they cannot fully understand the implications of consent.

This study can be led by nursing or allied health care professional staff. Consent should be sought under unhurried circumstances – however consent can be obtained on the same day the potential participant is approached (i.e. the potential participant may choose to consent on the day that they receive their PIS or take time to reflect and consent at a later date) when entry criteria are fulfilled. Patients can also be approached on day of surgery or biopsy as study participation has no change in clinical pathway and involves sample collection only.

Consent will be sought as follows:

- A PIS will be given to all women referred through rapid access 2 week wait clinics, USG and outpatient clinics and pre-admission clinics for suspected OC. This PIS and posters on the study will also be made generally available and prominently displayed in various areas within the participating hospitals and their primary care practices – including clinics, corridors, MDT meeting rooms, ultrasound rooms, offices. PIS are also available via the trial website. All women presenting as acute admissions to hospital will be offered the PIS and the option of study participation, unless deemed inappropriate by the attending clinical team for clinical reasons. However, wherever possible the patient should make the decision on whether to receive the study information or not.
- A potential participant can also be approached over the telephone/video call. Once the potential participant has shown interest in the study the PIS, Informed Consent Form (ICF) and baseline participant booklet can also be sent out for them to read at home. The site staff will then follow this up with remote consent as documented in 3.4.
- A potential participant can also be approached on the morning of surgery if it is not possible to approach in clinic or by remote consent before this time. They will be given the PIS and enough time to read the information before consent takes place.
- Patients admitted for investigations or as emergency admissions will be approached. They will be given the PIS and enough time to read the information before consent takes place.
- Where necessary, appropriate trust interpreters will be asked to aid discussion relating to study participation. Patients who do not understand English are eligible to enter the study provided an interpreter can fully explain the ICF and PIS to them.
- The initial approach to the potential participant will be through their clinician or appropriately trained person delegated the responsibility to approach patients to discuss the study. The consent form will be signed by the patient prior to any dried blood spot, serum or plasma samples being taken and counter signed by the person delegated the responsibility of taking consent.
  - If the scan is part of the standard care pathway, participants may receive an USG prior to consent as part of usual care. Data from these scans can be collected retrospectively into the study. As the British Gynaecological cancer society has recommended IOTA ADNEX ultrasound in their guidance on management of women with cysts, it is likely that at some sites patients will have the scan performed prior to study entry[38]. For ROCkeTS-GEN V2 the IOTA scan is optional.
- For patients who have already attended a hospital visit and who have not been given a PIS, the research team member can approach them by phone to provide information about the study.

3.4 Obtaining consent

The participant's written informed consent to participate in the study will be obtained before entry and after a full explanation has been given of the study. PIS and ICF will be provided so that patients can find out more about the study before deciding whether or not to participate.

- Visits throughout the informed consent process will take place in person at site or by telephone/video call as per local practice where patient and/or public health circumstances dictate. Signed informed consent forms can be completed in person or remotely if the circumstances dictate.

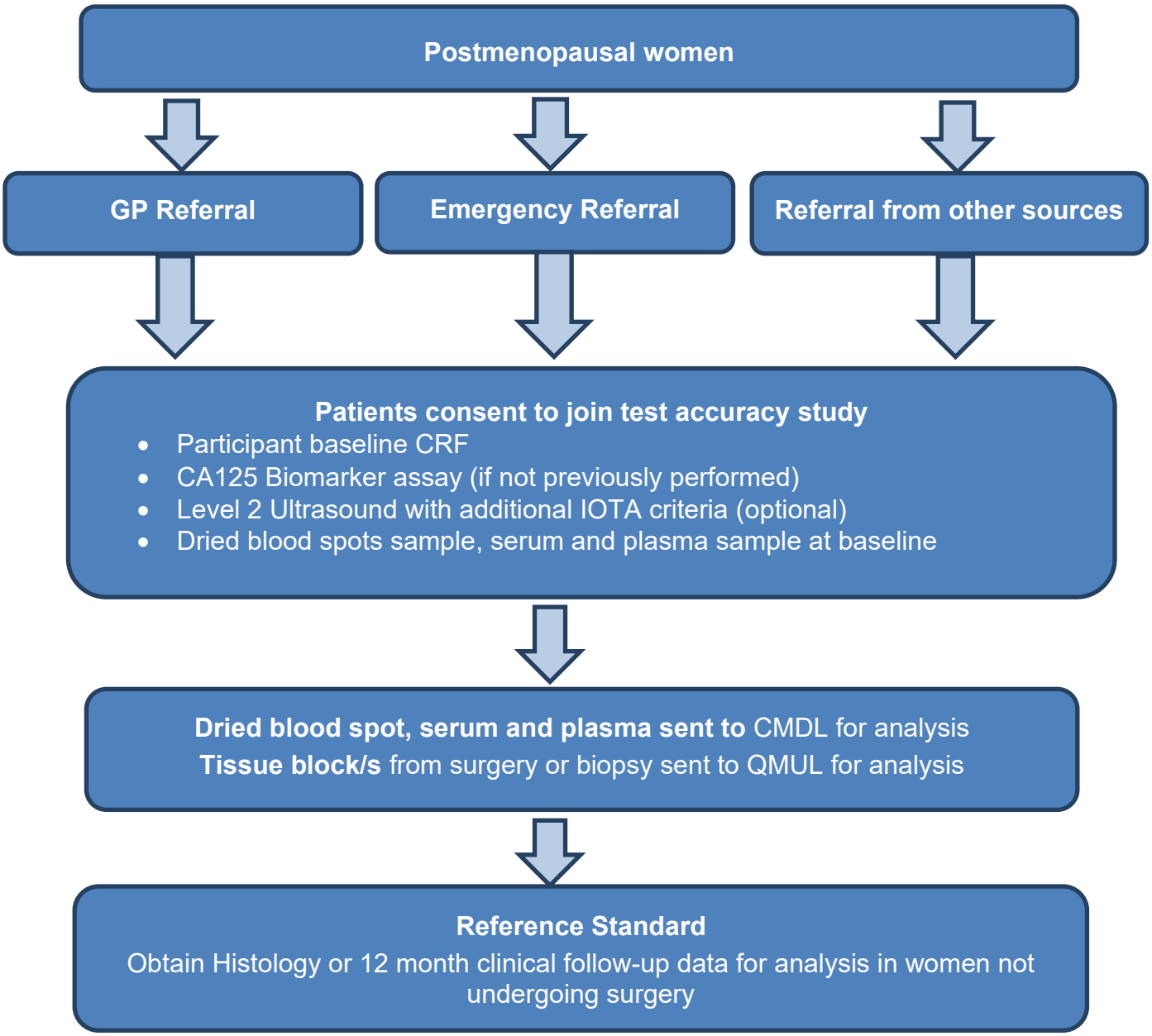
- If consent is not being taken in person, then the patient should be sent a PIS and ICF if not already supplied; then the clinical staff or delegated person will talk through each statement of the ICF with the patient, who will be asked to initial the boxes on the ICF and sign and date the form whilst on the call. This will be documented in the clinical notes. The ICF will then be returned to the clinical site for countersignature by the person who took consent remotely and the date the ICF was countersigned will be documented. Once a fully signed ICF has been completed it will be stored in the Investigator Site File and a copy will be sent to the Study Office and to the participant for their records. The participant baseline booklet can also be returned with the ICF.
- Participants will be assigned a study trial number after registration.

3.4.1 Informing the participant's GP

The participant's GP should be notified with the participant's consent and a specimen being sent to QMUL and CMDL. "GP Letter" is supplied.

4 RECRUITMENT

4.1 Flow chart for ROCkeTS GEN V2



4.2 Recruitment for ROCkeTS GEN V2

To obtain the large number of participants necessary for the reliable evaluation of the index tests, the study will need the participation of more than one centre. Study procedures therefore need to be kept simple, with minimal extra workload placed on participating clinicians, beyond that required to manage their patients. This will be achieved by simple entry procedures, early consent of women, the use of standard local testing regimens, minimising documentation and streamlining data collected procedures. Regular newsletters will keep collaborators informed of study progress, and regular meetings will be held to report progress of the study and to address any problems encountered in the conduct of the study.

4.3 Organisation of recruitment

Recruitment will be organised and supported by dedicated research team members who will work with local lead investigators. We believe that that the following strategy is likely to be successful in achieving maximum recruitment.

- **Whilst some participants in ROCkeTS GEN V2 will receive an IOTA ultrasound, the relevant scanner does not need to be IOTA certified.**
- **Eligibility for the study will be confirmed by clinical team members. Recruitment and study procedures can be performed by the research team.**
- Identification of appropriate research staff (doctors, clinical nurse specialists, research nurses, sonographers).
- A research team member at each site with responsibility for consent and study specific procedures.
- As part of the standard care pathway participants may receive an USG prior to consent and the data from these scans can be collected retrospectively.
- For participants in ROCkeTS GEN V2 the IOTA scan is not mandatory. If an IOTA ultrasound is performed as part of routine care, then we will collect that data as part of ROCkeTS GEN v2.
- Appointment of a trial manager at University of Birmingham, who will liaise with all the coordinating research nurses at each site and coordinate the screening at this hospital, provide training and trouble-shoot recruitment and follow-up problems.
- Provision of simple written study information, supported by face-to-face discussion with clinical staff.
- Provision of regular feedback on progress in study recruitment, including individual hospital teams' performance and progress against targets.
- Regular newsletters to all relevant staff involved in the study.

4.4 Other management at discretion of local doctors

Apart from the study tests, all other aspects of participant management are entirely at the discretion of the local doctors and as per the RCOG/BGCS guidelines for management of these participants.[9,19] Treating clinicians will be asked to record their treatment recommendations as per standard care.

4.5 Withdrawal

Participants will be free to withdraw from the study at any time without any effect on standard of care; data and samples provided up to the point a participant withdraws will be retained.

5 STUDY PROCEDURES AND TESTS

5.1 Assessments for ROCkeTS GEN V2

Whilst symptoms are routinely elicited and recorded as part of a clinical assessment at presentation to secondary care, this is not standardised and involves the doctor transcribing elicited symptoms from the participant. Study participants entering the study will complete a baseline CRF.

A transabdominal and transvaginal USG is always performed for all patients suspected of OC as part of NICE guidelines. This is usually performed by trained ultrasonographers who report the scan as per routine. **For ROCkeTS-GEN V2 a transvaginal scan recording IOTA variables is not mandatory, but desirable if data available as part of standard of care.**

Participants will have an additional dried blood spot, serum and plasma sample taken at baseline for ctDNA and proteomics analysis. Details of sample collection will be provided in the lab manual.

5.2 Reference standard/Follow-up schedule

Reference standard for the study will be histology of tissue taken at surgery or biopsy or cytology in women who are managed surgically following study enrolment. Outcome of participants referred for suspected OC that do not undergo surgery will be assessed by a follow-up visit at 12 months or by a telephone call from the research team at 12 months, as per the local investigators' discretion and clinical assessment. Wellbeing will be ascertained at this follow-up.

5.3 Study duration

For **ROCkeTS GEN V2 participants** we anticipate recruitment of 500 participants. The current recruitment end date for ROCkeTS is 3 years from when the study is open at sites. It is anticipated that the recruitment will take 2 years. The follow-up will be 12 months from recruitment of last patient.

As per protocol, where histology is unavailable, participants should be followed up for the full 12 months, however the study recognises that for participants recruited within the last 12 months of the recruitment period the full 12 month follow-up will not be possible; instead, these participants should only be followed up until the end of study period.

The end of the ROCkeTS GEN V2 study, will be the date of the last data capture including sample analysis and resolution of all data clarification forms. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The Trial Office will notify the REC and the Sponsor within 90 days of the end of trial. Where the trial has terminated early, the Trial Office will notify the REC within 15 days of the end of trial. The Trial Office will provide the REC and the Sponsor with a summary of the clinical trial report within 12 months of the end of trial.

5.4 Sample acquisition, storage and transport

Please refer to the laboratory manual.

Dried blood spot, serum, plasma and tissue blocks collection and initial processing (labelling, handling, completing sample tracking log) will be performed at trial sites and will then be transferred for further processing in CMDL or QMUL.

Remining tissue blocks, after study is ended, will be transported for further storage to the biorepository.

All samples will be fully tracked from site, to laboratory, to biorepository using sample tracking logs.

5.5 Data collection

All information will be collected on standard proformas (Table 1) and identified by study number, initials and date of birth. Registration Form, Participant Baseline CRF, Surgery CRF, and Outcome CRF will be entered by the relevant site directly into the study database in REDCAP via a web interface. Sites are also permitted to enter study data into paper CRFs when needed.

We aim to collect a minimal demographic dataset including age, ethnicity, parity, GP details and significant medical/surgical history. We aim to use the NHS number as the primary identifier when linking to national registries and to track individuals throughout the NHS. Some additional data will be collected at follow-up.

Data will be collected on relevant medical, obstetric and gynaecological, surgical history, emotional impact as well as information on the symptoms that prompted GP referral or investigation. USG information will be collected. Data on the reference diagnosis will be obtained from the histopathology form and a structured template to assess wellbeing for participants who do not undergo surgery will be developed in association with the participating sites.

5.6 Death

If a participant dies prior to histology data being provided (if available), inform Study Office immediately via a Change of Status form.

5.7 Analysis

5.7.1 Test accuracy

We will compare ctDNA to Risk of malignancy index 1 (RMI 1) as an alternative test used at this point in the patient pathway in clinical practice as specified in primary outcome. All test comparisons will be in relation to the reference test results, so the comparative accuracy of tests to detect ovarian cancers can be calculated. We will report estimates of sensitivity, specificity, PPV and NPV. Imputation will be used to account for missing data and imperfect reference data.

5.7.2 Primary outcome

Comparison of sensitivity and specificity of ctDNA to RMI 1 at threshold of 250 for early stage (stage I/II) high grade serous ovarian cancer (HGSOC) in postmenopausal women.

- Of 500 women, with an expected 5% loss of follow up, and 10 - 15% not referred for surgery or biopsy we expect to analyse approximately 406 cases. Given a prevalence (from our experience in ROCkETS-GEN) of 27% we expect to observe 110 ovarian cancers, ~50% of which will be Stage 1-2 and ~50% stage 3-4. Of the 110 ovarian cancers, 70% would be expected to be HGSOC, of these 25% to be early stage. Blood packs will be posted for central processing.

5.7.3 Reduction of bias: blinding of test interpretations

- Index test interpretations (ctDNA/ other biomarkers) will be blinded from other index tests (RMI, CA125, USG). Some of the index tests in current pathways will be interpreted as part of current practice and blinding between these test interpretations will be maximized whilst maintaining patient best care.
- Index test interpretations will be blinded where possible from rest of diagnostic pathway – i.e. only using relevant clinical information known at time in clinical pathway.
- Index test interpretations will be blinded from reference standard interpretation (histology and clinical follow up).
- As part of the blinding, the analysis algorithm for ctDNA from plasma will be analysed using auditable analysis code which will be locked down prior to comparison to surgical tumour mutation comparison.

6 ADVERSE EVENT REPORTING

There are no foreseeable risks of mortality or significant morbidity associated with testing. Every effort will be made to minimise any risk through training. **Therefore, only serious adverse events* (SAEs) believed to be associated with any study procedures should be reported**. SAEs should be reported via email to the study email address.

The collection and reporting Serious Adverse Events (SAEs) will be in accordance with Good Clinical Practice (GCP) and the Research Governance Framework 2005.

Safety will be assessed continuously throughout the study. There are no Investigational Medicinal Products being used as part of the ROCkeTS-GEN V2 study and the tests evaluated in the study are not being used to determine patient management. A risk assessment of the ROCkeTS GEN V2 study has been performed with all testing considered to be of low risk.

6.1 Definition of a Serious Adverse Event

The definition of an SAE is an untoward event that:

- results in death
- is life-threatening*

*For the purposes of this study, "**serious**" adverse events are those occurring in participants which are fatal, life-threatening, disabling or require some form of medical or surgical treatment.

- requires hospitalisation** or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- Consists of a congenital anomaly/ birth defect
- or, is otherwise considered medically significant by the Investigator

*The term “life-threatening” 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 severe.

** Patients must be formally admitted – waiting in out-patients or A&E does not constitute an SAE (even though this can sometimes be overnight). Similarly, planned hospitalisations that clearly are not related to the condition under investigation or hospitalisations/prolongation of hospitalisation due to social reasons should not be considered as SAEs.

6.2 Reporting period

The main theoretically possible recognised reportable SAEs associated with this study relate to the dried blood spot, serum and plasma sample being taken, USG conducted or distress following answering questions in baseline CRF.sSAEs occurring within 24 hours of one of these events should be reported immediately upon awareness to the study office on an SAE form. The assessment of relatedness and expectedness is a clinical decision based on all available information at the time.

SAEs outside of this timeframe can also be reported if it is the opinion of the Investigator that there is a possible causal relationship to another aspect of the study. An assessment of relatedness and expectedness will also be undertaken by the Chief Investigator (or designee).

6.3 Reporting procedure – at Site

SAEs believed associated with any study procedures will be notifiable to Study Office **immediately and within 24 hours of becoming aware of the event**. On becoming aware that a participant has experienced said SAE, the Investigator (or delegate) must complete, date and sign an SAE Form. The form should be sent to Study Office using the study email address. The Investigator will also be asked to provide a categorisation of seriousness and causality (continue reading for further details).

For contact details, refer to the ‘ROCkeTS GEN V2 Study Office’ section at the front of this protocol.

For SAE Forms completed by a member of the site trial team other than the Principal Investigator (PI), the PI will be required to countersign the original SAE Form to confirm agreement with the causality and seriousness/severity assessments. The form should then be returned to Study Office and a copy kept in the Site File.

Investigators should also report SAEs to their own Trust in accordance with local practice.

6.4 Causality assessment

AEs defined as serious, and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the PI (or delegate) will be asked to define the causality and the severity of the AE.

Causality (relatedness) will be categorised according to the following Table 2.

Table 2: Definitions of relatedness

Category	Definition	Causality
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out	Related
Probably	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely	
Possibly	There is some evidence to suggest a causal relationship. However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant events or medication)	
Unlikely	There is little evidence to suggest there is a causal relationship. There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant events or medication)	Unrelated
Unrelated	There is no evidence of any causal relationship	

6.5 Assessment of Expectedness

Expectedness will be assessed by the CI or designee using this study protocol as the reference document. Table 3 gives definitions of expectedness with respect to SAEs.

Table 3: Definitions of expectedness

Category	Definition
Expected	An adverse event that is consistent with known information about the study related procedures.
Unexpected	An adverse event that is not consistent with known information about the study related procedures

6.6 Provision of follow-up information

Participants should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form, making sure to include the SAE reference number, provided by the Trials Unit upon receipt of the initial SAE.

6.7 Reporting procedure – ROCkeTS GEN V2 Study Office

On receipt the Study Office will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt within 1 working day. The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE.

On receipt of an SAE Form, seriousness and causality (relatedness to the study intervention) will be assessed independently by the CI. Further information may be immediately requested from the clinical team at site. The CI will not overrule the causality or seriousness assessment given by the site PI but may add additional comment on these.

An SAE judged to have a reasonable causal relationship with study processes will be regarded as a related SAE. The CI or delegate will assess all related SAEs for expectedness. If the event is assessed as unexpected it will be classified as **an unexpected and related SAE**.

6.8 Reporting procedure to Research Ethics Committee (REC)

SAEs categorised by the CI as unexpected and related will be subject to expedited reporting to the REC by the Study Office within 15 days after the Study Office has been notified. A copy will also be sent to the University of Birmingham Research Governance Team at the same time.

The Study Office (on behalf of the CI) will inform all PIs of relevant information about SAEs that could adversely affect the safety of participants.

The REC will be notified immediately if a significant safety issue is identified during the course of the study. The University of Birmingham Research Governance Team will also be informed at the time that the REC is informed.

7 DATA ACCESS AND QUALITY ASSURANCE

7.1 Confidentiality of personal data

Individual participant information obtained as a result of this study is considered confidential. Each participant will be allocated a unique study number at recruitment.

Personal data and sensitive information required for ROCkeTS GEN V2 will be collected directly from study participants and hospital notes. Participants will be informed about the transfer of this information to University of Birmingham and asked for their consent. The data will be entered onto a secure computer database, either directly via the internet using secure socket layer encryption technology or indirectly from paper by ROCkeTS GEN V2 member of staff. Study database will be held in a secure internet facility (REDCap). REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources. Data will be stored on a secure server under the provisions of the Data Protection Act and/or applicable laws and regulations. Access to data will be restricted by usernames and

passwords. The necessary study data will be encrypted. No study data will be held in handheld media, laptops, personal computers, or other similar media.

The online database will be maintained according to prescribed security policies of University of Birmingham. These cover assignment of passwords, encryption, database immediate back-up, offsite back-up and disaster recovery processes. Electronic data will be backed up every 24 hours to both local and remote media in encrypted format. Paper-based data (e.g. signed consent forms) or any paper based Case Report forms will be kept in locked filing cabinets.

Participants will also be informed that, and consent to, their samples, being transferred from local centres to the central laboratory. Samples will only be identified by study number. Central laboratory staff will not have access to personal data.

All personal information received in paper format for the study will be held securely and treated as strictly confidential according to University of Birmingham policies. All staff involved in the study (clinical and academic) share the same duty of care to prevent unauthorised disclosure of personal information. No data that could be used to identify an individual will be published.

7.2 Monitoring and audit

Investigators and their host Trusts will be required to permit study-related monitoring and audits to take place by the Study Office or Sponsor, providing direct access to source data and documents as requested. Trusts may also be subject to inspection by the Research and Development Manager of their own Trust and should do everything requested by the CI to prepare and contribute to any inspection or audit. Study participants will be made aware of the possibility of external audit of data they provide in the PIS.

7.3 Statistical monitoring throughout the study

The prevalence of OC in the study will be constantly monitored and sample size calculations will be reviewed to check if the study has accrued enough samples and data to report.

7.4 Long-term storage of data

After the end of the study, the site files from each centre should be archived by the NHS Trust as per regulations for a non-CTIMP.

All data will be stored for at least 10 years. Any queries or concerns about the data, conduct or conclusions of the study can also be resolved in this time.

Study data will be stored within the University of Birmingham under controlled conditions for at least 3 years after closure. Long-term offsite data archiving facilities will be considered for storage after this time. The University of Birmingham has standard processes for both hard copy and computer database legacy archiving.

7.4.1 Data sharing

There are data sharing agreements in place between the CMDL, Barts groups and with the ROCkeTS Trial Team. Over the duration of the study data, will be shared between these groups. All data shared between groups will be fully anonymised and has been clearly explained in the PIS and consent form.

8 ORGANISATION AND RESPONSIBILITIES

To ensure the smooth running of the study and to minimise the overall procedural workload, it is proposed that each participating centre should designate individuals who would be chiefly responsible for local co-ordination of clinical and administrative aspects of the study.

All investigators are responsible for ensuring that any research they undertake follows the agreed protocol, for helping care professionals to ensure that participants receive appropriate care while involved in research, for protecting the integrity and confidentiality of clinical and other records and data generated by the research, and for reporting any failures in these respects, adverse drug reactions and other events or suspected misconduct through the appropriate systems.

8.1 Local Co-ordinator at each centre

Each Centre should nominate a clinical lead Doctor or research team member who will act as the local PI and bear responsibility for the conduct of research at their centre. The responsibilities of the local PI will be to ensure that all medical and nursing staff involved in the care of participants are well informed about the study and trained in study procedures, including obtaining informed consent. The local PI should liaise with the Trial Manager on logistic and administrative matters connected with the study. We encourage trainees to participate in the NIHR associate PI scheme and the Trial manager will support this

8.2 Research study Co-ordinator at each centre

Each participating centre should also designate one team member as local study Coordinator. This person would be responsible for ensuring that all eligible participants are considered for the study, that patients are provided with study information sheets, and have an opportunity to discuss the study if required. The research team member may be responsible for collecting the baseline participant data and for administering the follow-up evaluations. Again, this person would be sent updates and would be invited to progress meetings.

8.3 The Study Office

The Study Office is responsible for providing all study materials, including the study folders containing printed materials and the update slides. These will be supplied to each collaborating centre, after relevant ethics committee approval has been obtained. Additional supplies of any printed material can be obtained on request. The Study Office is responsible for collection and checking of data (including reports of SAEs thought to be due to study investigations), for reporting of serious and unexpected AEs to the sponsor and/ or regulatory authorities and for analyses. The Study Office will help resolve any local problems that may be encountered in study participation.

8.4 Research Governance

The conduct of the study will be according to the Research Governance Framework for Health and Social Care and/or the Research Governance Framework for Health and Community Care.

All centres will be required to sign an Investigator's Agreement, detailing their commitment to accrual, compliance, Good Clinical Practice (GCP), confidentiality and publication. Deviations from the agreement will be monitored and the Project management group will decide whether any action needs to be taken, e.g. withdrawal of funding, suspension of centre.

The Study Office will ensure researchers not employed by an NHS organisation hold an NHS honorary contract for that organisation.

8.5 Ethical and Trust management approval

Trust R&D departments will conduct local governance checks and assess the facilities and resources needed to run the study, in order to give host site permission. The Study Office is able to help the local PI in the process of the site specific assessment by completing much of Site Specific Information section of the standard IRAS form as possible. The local PI will be responsible for liaison with the Trust management with respect to locality issues and obtaining the necessary signatures at their Trust.

As soon as Trust approval has been obtained, the Study Office will send a folder containing all study materials to the local PI. Potential study participants can then start to be approached.

8.6 Funding and cost implications

The research costs of the study are funded by a grant from Cancer Research UK awarded to the Barts (Cambridge and Bham coapplicants)

The study has been designed to minimise extra 'service support' costs for participating hospitals, with no extra visits to hospital and no extra tests. Additional NHS service support costs associated with the study, e.g. gaining consent, aliquoting extra blood samples etc, are estimated in the Site Specific Information section of the standard IRAS form.

8.7 Indemnity

There are no special arrangements for compensation for non-negligent harm suffered by participants as a result of participating in the study. The study is not an industry-sponsored study and so ABPI/ABHI guidelines on indemnity do not apply. The normal NHS indemnity liability arrangements for research detailed in HSG96(48) will operate in this case.

However, it should be stressed that in terms of negligent liability, NHS Trust hospitals have a duty of care to a patient being treated within their hospital, whether or not that patient is participating in a clinical study. Apart from defective products, legal liability does not arise where there is non-negligent harm. NHS Trusts may not offer advance indemnities or take out commercial insurance for non-negligent harm.

As Sponsor, the University is responsible for the general conduct of the study and shall indemnify the Clinical Centre against any claims arising from any negligent act or omission by the University in fulfilling the Sponsor role in respect of the Study.

8.8 Publication

A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of many doctors, nurses and others. Manuscripts published from this research will give due credit to the collaborators who participated in the study

8.9 Ancillary studies

It is requested that any proposals for formal additional studies of the effects of the study treatments on some participants (e.g. special investigations in selected hospitals) be referred to the Project Management Group for consideration. In general, it would be preferable for the study to be kept as simple as possible, and add-on studies will need to be fully justified.

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