GeCIP Detailed Research Plan Form

Background

The Genomics England Clinical Interpretation Partnership (GeCIP) brings together researchers, clinicians and trainees from both academia and the NHS to analyse, refine and make new discoveries from the data from the 100,000 Genomes Project.

The aims of the partnerships are:

1. To optimise:

- clinical data and sample collection
- clinical reporting
- data validation and interpretation.

2. To improve understanding of the implications of genomic findings and improve the accuracy and reliability of information fed back to patients. To add to knowledge of the genetic basis of disease.

3. To provide a sustainable thriving training environment.

The initial wave of GeCIP domains was announced in June 2015 following a first round of applications with expressions of interest in January 2015. In April 2016 we invited the inaugurated Cancer GeCIP domains to develop research plans for 'Gear 2' working closely with Genomics England. Within the Cancer Main Programme, the 'Gear 2' phase of the project refers to recruitment of specific cohorts of patients, inclusion of biopsy tissue (diagnostic/recurrence) and ctDNA in selected cohorts and the initiation of clinical trials in early stage (adjuvant/consolidation) setting. These will be used to ensure that the plans are complimentary and add real value across the GeCIP portfolio and address the aims and objectives of the 100,000 Genomes Project. They will be shared with the MRC, Wellcome Trust, NIHR and Cancer Research UK as existing members of the GeCIP Board to give advance warning and manage funding requests to maximise the funds available to each domain. However, formal applications will then be required to be submitted to individual funders. They will allow Genomics England to plan shared core analyses and the required research and computing infrastructure to support the proposed research. They will also form the basis of assessment by the Project's Access Review Committee, to permit access to data.

Domain leads are asked to complete all relevant sections of the GeCIP Detailed Research Plan Form, ensuring that you provide names of domain members involved in each aspect so we or funders can see who to approach if there are specific questions or feedback and that you provide details if your plan relies on a third party or commercial entity. You may also attach additional supporting documents as relevant (optional).

Additional members can apply to join the GeCIP domain by completing the form on our website found here: http://www.genomicsengland.co.uk/join-a-gecip-domain/.

Genomics England Clinical Interpretation Partnership (GeCIP) Detailed Research Plan Form

Application Summary	
GeCIP domain name	**Ovarian and Endometrial cancer**
Project title *(max 150 characters)*	**Ovarian and Endometrial cancer research in the 100,000 Genomes Project**

Objectives. *Set out the key objectives of your research. (max 200 words)*

The Ovarian Cancer Genomic England Clinical Implementation Partnership (GeCIP) is forming a consortium of clinicians, trainees, translational scientists, bioinformaticians and patient representatives. We will enhance the Genomics England Programme by providing strong expertise in genomics and clinical trials to aid clinical interpretation and implementation, help develop new disease classifications, candidate biomarkers and proposals for future "basket" trials in ovarian cancer.

Lay summary. *Information from this summary may be displayed on a public facing website. Provide a brief lay summary of your planned research. (max 200 words)*

Over the past five years, new insights into the molecular basis of ovarian cancer have brought about rapid change in our understanding of the disease. We now recognize that different histological subtypes of ovarian cancer are fundamentally different diseases. The Ovarian Cancer GeCIP will focus on the study of the commonest subtype, high grade serous ovarian carcinoma (HGSOC), as it accounts for the majority of deaths from ovarian cancer and shown no improvement in overall survival. HGSOC is genomically characterised by ubiquitous TP53 mutation, high-frequency somatic copy number alterations (CNAs), and whole genome duplications. Oncogenic mutations are rare, and recurrent non-synonymous changes are seen in tumour suppressor genes, including somatic mutations in TP53, BRCA1, BRCA2, RB1, and NF1. Whole genome sequencing (WGS) of 92 HGSOC patients by the ICGC (submitted; Gabra, Grimmond co- senior author) shows that complex gene rearrangements frequently involve NF1 and RB1, making these the commonest involved genes after TP53 (20% and 24% respectively). Germline mutations in BRCA1/BRCA2 are found in 15.5% of women with serous or endometrioid ovarian cancer. However, at present genetic testing is not systematically offered to this patient group and many women with inherited mutations are missed because of concerns regarding the cost of sequencing and acceptability. Intra-tumour heterogeneity is common in HGSOC and may contribute to acquired resistance. Unpublished results from ICGC confirm previous data showing that secondary mutations at BRCA1 and BRCA2 can during treatment in the tumours of germline carriers. These mutations are predicted to restore BRCA1 and BRCA2 function and increase resistance to platinum and PARP inhibitors.

It is clear that women with HGSOC have failed to benefit from current clinical sequencing efforts because targeted or panel sequencing of "actionable" genes do not detect the complex rearrangements that are driving the disease. HGSOC is the archetypal cancer for which WGS may transform disease classification and personalization of therapy. The major opportunity is to use high quality WGS from the UK 100,000 Genomes Project to identify drivers across approximately 2000 patients, to use this to develop accessible WGS assays for the clinic and to define the effects of intratumoural heterogeneity using longitudinal samples.

Expected start date	**Q2 2017**
Expected end date	**Q2 2020**

Lead Applicant(s)	
Name	David Church
Post	Group Head / PI and Consultant Physician
Department	Wellcome Trust Centre for Human Genetics
Institution	University of Oxford
Current commercial links	

Lead Applicant(s)	
Name	James Brenton
Post	Senior Group Leader and Honorary Consultant in Medical Oncology
Department	Cancer Research UK Cambridge Institute
Institution	University of Cambridge
Current commercial links	

Gear 2 Substudies
EC01: Identifying actionable mutations in endometrial cancer
EC02: Biology of uterine carcinosarcomas (metaplastic endometrial carcinomas)
EC03: Biology of somatic copy number-high (serous-like) endometrial cancers
EC04: Mechanisms of hypermutation in endometrial cancer
EC05: Determinants of the immune response in endometrial cancer
EC06: Intratumoural heterogeneity and endometrial cancer evolution
EC07: Predicting toxicity of radiotherapy for women with endometrial cancer
EC08: Impact of risk variants on endometrial cancer genomes

GeCIP domain - Expression of interest

Full proposal

Title *(max 150 characters)*	**Ovarian and Endometrial cancer**

Research plans. *Give details of the analyses and experimental approaches, study designs and techniques that will be used and timelines for your analysis. Describe the major challenges of the research and the steps required to mitigate these.*

Proposed research

The main aim of the research will be to develop new, robust molecular classifications of HGSOC in order to develop molecularly stratified clinical trials. As detailed, the participants in the proposed Ovarian and Endometrial Cancer GeCIP are already participating in major cancer genomic efforts, including the ICGC ovarian themes and ongoing collections in national trials and studies (e.g. BRITROC, TRICON8, SEARCH). We will be able to augment the GEL-enrolled collections of cancer cases and increase their value because: (i) our translational programmes have strong clinical integration and will provide additional orthogonal data from existing funding sources; (ii) our centres include strong bioinformatics expertise which will be able to compare the GEL-generated WGS data sets to existing data allowing more rapid interpretation and clinical translation; (iii) we will construct tissue microarrays for all ovarian cancer cases (for immunohistochemistry classification and validation of the genomic data) and will develop image analysis to correlate genomic data with cellular and stromal phenotypes, including new immunohistochemical signatures of immune infiltration and iv) correlate findings with related prospective studies across the partner sites (including blood biomarkers such as circulating tumour DNA and RNA, plasma/serum proteins and other tissue profiling). This will maximize the value of the associated research and the clinical annotation of the GEL samples.

Research involving possible multi-omic endpoints

We have considerable expertise in developing circulating tumour DNA assays in clinical studies. All sites are collecting ctDNA as part of the MRC/CR-UK ICON8 trial. Brenton is leading clinical implementation of ctDNA in a Clinical Pathological Accredited environment in the Cambridge BRC Cancer Molecular Diagnostics Laboratory. Given our joint experience in BriTROC for relapsed tumour collection and ctDNA assays, we will focus on developing clinical interpretation of longitudinal samples collected in GEL.

Training

Professor Richard Edmondson (training director) brings extensive experience in developing the careers of academic gynaecological oncology trainees to the Ovarian and Endometrial Cancer GeCIP. The aims of the training theme will be to identify, enthuse and develop the academic careers of potential future Ovarian and Endometrial cancer researchers from gynaecology, pathology and oncology. We will primarily engage with NIHR Academic Clinical Fellows (ACF) and Academic Clinical Lecturers (ACL) by organizing three regional study days each year which will offer high level introduction to tools and concepts for genomic analysis of HGSOC. Secondly, we will offer a four small, focused whole genome sequencing analysis projects to ACF and ACLs across the GeCIP network. These projects will focus on practical use of R and Bioconductor for exploration of summary data from the Genomics England Knowledge Base with the aim of developing deeper skills and questions for competitive funding. For these short-term (3−6 month) projects the fellows will use existing NIHR funding for their time, but will be supported by bioinformatics support from the GMC. We will also seek to obtain external funding for study days for nurse specialists and will use local resources to engage with patients and the public via Biomedical Research Centre and other local Cancer Centre funding.

Funding
A significant strength of this application is that we can leverage significant programmatic funding in Ovarian and Endometrial cancer research and can utilize leadership from tenured senior investigators. Ovarian Cancer Action provides £380,834 for the national BriTROC-1 study. We will also be strongly placed for other international collaborative funding efforts to augment the GEL results.

Mechanisms for pre-competitive interaction with industry
Through the SGCTG and NCRI we have close links with the pharmaceutical industry and are developing translational trials designs which use common control arms and sequential phase 2 designs with rich biomarker collection (e.g. Octopus using AZD2014). The main aim will be to use GEL data to identify a short list of genomic classifiers that can be developed into a basket style design similar to the development of the MATRIX trial in lung cancer. AstraZeneca's global centre is moving to the Cambridge biomedical campus in 2016. AZ has identified therapies for ovarian cancer as a key strategy and we will seek to closely align this GeCIP with trials opportunities in the DNA repair space.

Collaborations including with other GeCIPs. *Outline your major planned academic, healthcare, patient and industrial collaborations. This should include collaborations and data sharing with other GeCIPs. Please attach letters of support.*

Group Members
James D. Brenton (Lead; University of Cambridge; East of England NHS GMC)
Ahmed Ashour Ahmed (University of Oxford; Oxford NHS GMC)
Susana Banerjee (Royal Marsden Hospital; Imperial College Health Partners NHS GMC)
Richard Edmondson (Training Director, University of Manchester; Greater Manchester NHS GMC)
Christina Fotopoulou (Imperial College London; Imperial College Health Partners NHS GMC)
Hani Gabra (Imperial College, London; Imperial College Health Partners NHS GMC)
Charlie Gourley (University of Edinburgh)
Sean Grimmond (University of Glasgow)
Geoff Lane (Guys and St Thomas' NHS Trust; South London NHS GMC)
Jonathan A. Ledermann (University College London; University College London Partners NHS GMC)
Glenn Mccluggage (Royal Group of Hospitals Trust, Belfast)
Iain McNeish (University of Glasgow)
Usha Menon (University College London; University College London Partners NHS GMC)
Sudhar Sundar (University of Birmingham; West Midlands NHS GMC)
Sohrab Shah (University of British Columbia)
ACF/ACL representative in O&G/Gynaecological Oncology) ACF/ACL representative in Pathology ACF/ACL representative in Oncology Patient representative (2; to be identified) Nurse specialist (2; to be identified)
Paul Pharoah (University of Cambridge; East of England NHS GMC)
Marc Tischkowitz (University of Cambridge; East of England NHS GMC)

Expertise
We have taken the following strategy in choosing the initial members of the GeCIP. Firstly, we have represented the Genomics Medicine Centres (GMC) with significant programmatic support for translational ovarian cancer research. Secondly, we recognize that the major challenge of clinical interpretation will be the bioinformatic analysis of very complex genomes and have ensured that representatives from the Universities of Glasgow, Oxford, Imperial and Cambridge can lead or expertly interpret bioinformatic analyses at scale. Sohrab Shah has also agreed to collaborate and provides further international HGSOC bioinformatic input. Thirdly, the

constituency provides a balanced representation of key clinical themes in Ovarian and Endometrial cancer care including academic surgery, clinical trials, and screening and early diagnosis. We have a strong track record in collaborative working; including membership of the NCRI Gynaecological Clinical Studies Group (Banerjee, Edmondson, Fotopolou, McNeish [chair], Sundar), the Scottish Gynaecological Cancer Trials Group (SGCTG; McNeish, Gourley, Brenton) and the international Ovarian Cancer Association Consortium (OCAC; Pharoah, SGCTG, Gourley, Menon, Brenton) and the Ovarian Tumor Tissue Analysis (OTTA) Consortium (Pharoah, Brenton, SGCTG). Grimmond, Gourley and McNeish are leading on the cancer theme in the Scottish Genomics Partnership and we are keen to align the Genomics England efforts on ovarian cancer with those in Scotland.

We recognise the need for further representation from trainees (particularly in pathology), patient representatives and nurse specialists. We have discussed patient representatives with the charities Ovarian Cancer Action and Target Ovarian Cancer and will widen these discussions. It is important to note that, in contrast to other cancers such as breast cancer, there are fewer patient advocates with HGSOC owing to higher age at diagnosis, lack of long term survivors and short intervals between chemotherapy. We will develop defined sub-domains for the specific tasks of bioinformatics, training, phenotyping and patient engagement in the full application.

We have established a UK ovarian cancer translational collaborative (Brenton, Gabra, Edmondson, Gourley, McNeish) to facilitate translational genomic research in HGSOC (BriTROC). The BriTROC-1 study is funded by Ovarian Cancer Action and is obtaining sequential biopsies and matched circulating tumour DNA samples at relapse in women with HGSOC across 12 ovarian cancer centres within the UK. The objectives are to show safety and feasibility of collecting 300 biopsies and to define the clinical important of intratumoural heterogeneity in relapsed disease. Since December 2012, we have screened 307 patients and obtained 125 relapsed samples. We have developed the use of methanol- based fixation to protect RNA and DNA in small samples, to facilitate sample collection and to allow standard tissue processing using wax blocks. Our initial results from microdissection of HGSOC tumour from 69 needle biopsies show a median yield of 2.6 µg (IQR 0.68−3.6 µg) of high molecular weight DNA. Targeted NGS from these DNAs had a median depth of coverage of 705 (IQR 424−1070).

Collection of optimum samples and clinical data

This application includes leads from all the sites that are currently involved in the NIHR Health Informatics Collaborative (NIHR HIC; UCL, IC, Oxford, Cambridge, GSTT) and is therefore well placed to ensure high quality clinical data collection. The aim of the HIC project has been to test whether data collected in the course of routine patient care across the five NIHR biomedical research centres (BRCs) can be reused for collaborative, translational research. The outputs from the ovarian cancer theme have been to define the minimum cancer dataset and describe typical ovarian cancer pathways using model-based tools. We have tested the pathway representations with individual patient data and agreed these pathways across the participating BRCs. These models have been submitted for incorporation into the HIC Model Catalogue, which is being delivered from the HIC coordinating centre in Oxford (Davies). Our data modelling approach has been used to identify required items missing from the Cancer Outcomes and Services Dataset (COSD) and we are working with the Department of Health to develop SNOMED Clinical Terms to describe these extra data items. We have tested data sharing between the BRCs using existing COSD and Systemic Anti- Cancer Therapy data sets to show that we can collect the majority of data for women with ovarian cancer. We have developed XML definitions and extensible tools for the upload and aggregation of these data using OpenClinica. This work will inform strategies for augmenting NHS data collected by GEL and we will look to interact with cross-cutting GeCIPs focused on clinical informatics as well as the interpretation, validation and feedback domain.

The group has other important experience in collecting optimum samples. Ahmed (Oxford) is using innovative approaches to monitor response in minimal residual disease using laparoscopic surgery with repeated sampling of tissues during chemotherapy treatment. Relapsed tissue biopsies are being collected from the ARIEL2 translational study (McNeish, Banerjee, Brenton, Ledermann). Gabra leads the Ovarian Cancer Action Research Centre and has provided extensive samples for the ovarian cancer ICGC and TCGA projects.

Clinical reporting and data interpretation

The primary forum for planning cancer treatment planning for women with HGSOC is at multidisciplinary MDTs site. This includes discussion of the results of genomic tests (e.g. BRCA1 or BRCA2 mutation; see above) and referral for clinical trials. We will ensure that local MDTs at each Genomics Medicine Centre receive clinically actionable genomic information from GeL. Firstly, we will agree the key genes in HGSOC that have clinical relevance (this will include germline mutations in BRCA1, BRCA2 and diagnostically important somatic mutations in TP53, KRAS, BRAF, PIK3CA, PTEN, CTNN1). Interpretation of germline mutations will be carried out with close working with the Inherited Cancer GeCIP (lead: Turnbull). Pharoah and Tischkowitz have joint representation on both the Ovarian and the Inherited Cancer GeCIP. Tischkowitz leads the GTEOC study which is testing feasibility and acceptability of universal BRCA1/2 testing for women with HGSOC and Gourley, McNeish and Banerjee are leading similar universal testing efforts at their centres. Abstracted results for these genes will be summarized from the bioinformatic analyses of the 100,000 Genomes Knowledge base data for return to MDTs who will include this in addenda to pathology reports at each site. These reports will be discussed at local MDTs to ensure appropriate recognition and action. Secondly, to provide national interpretation of the much more complex genomic data, we will organize a monthly MDT across the GeCIP sites. CUH is collaborating with Memorial Sloane Kettering Cancer Center (MSKCC) to develop cbioportal (http://cbioportal.org) as a graphically rich interface for storage and analysis of genomic data. As part of the MSKCC- CUH collaboration, cbioportal is being extended to hold clinical results in a secure manner. We will explore the possibility of extracting summary detailed genomic data from GEL (e.g. candidate cancer gene mutation panels of 100- 200 genes, copy number aberrations, rearrangements) for inclusion into a shared cbioportal instance that will be used for the GeCIP molecular MDTs. The main output from this analysis is likely to be focussed on research studies, particularly those for improved molecular classification—however a small fraction of changes (e.g. rearrangements that cause loss of function in BRCA1 or BRCA2) may allow prioritization of patients for clinical trials or olaparib treatment.

Detailed research plan – Cancer Main Programme Gear 2 studies

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC01: Identifying actionable mutations in endometrial cancer**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Any endometrial cancer.
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	Single sample
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To determine the prevalence and type of potentially actionable variants in endometrial cancer
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Sequencing of several hundred endometrial cancers by TCGA and other groups has revealed that an appreciable fraction of tumours harbour mutations and other genomic alterations of prognostic or predictive significance. Confirmation of the prevalence of these alterations, and the discovery of other actionable variants using the large number of tumours planned for WGS in this project will provide unprecedented understanding of the possibilities for precision medicine in endometrial cancer.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	This analysis will be aligned with the endometrial clinical trials portfolio nationally, and local trials recruiting in Manchester, specifically: • PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) • STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) • PETALS (the proportion of endometrial tumours associated with Lynch syndrome) • PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) • MIRENA (Mirena for reduction of endometrial neoplastic abnormalities)
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC02: Biology of uterine carcinosarcomas (metaplastic endometrial carcinomas**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Uterine carcinosarcomas (metaplastic endometrial carcinomas)
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	single sample, copy number analysis and transcriptomic profiling would be particularly informative
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To use improved understanding of carcinoarcoma biology to inform novel therapeutic strategies
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Metaplastic endometrial carcinomas, or carcinosarcomas as they are more commonly referred to, are an uncommon subtype of uterine tumour with a dismal prognosis. Relatively little is known about the biology of carcinosarcomas, although studies have shown that that mutations in TP53, FBXW7, KRAS and chromatin remodelling genes appear common, and that both mismatch repair deficiency and POLE proofreading domain mutation occur in an appreciable fraction of cases (5- 15%), suggesting that a proportion of these tumours may be excellent candidates for immune checkpoint inhibitor therapy. Consequently, understanding the frequency and consequences of these and other potentially actionable alterations, particularly on the antitumour immune response, is likely to be essential to improving outcomes in this disease. In this substudy, we will integrate WGS with RNAseq, CNA analysis and immunohistochemistry to generate a detailed immunogenomic profile of carcinosarcomas. We will focus particularly on the reltationship between genomic instability, hypermutation and immune response, with the aim of informing novel therapeutics. We will combine the data from this study with those generated from an international collaboration we have initiated to examine the impact of

	hypermutation on immune response and clinical outcome in carcinosarcomas, within which we already have over 400 (FFPE) tumours available for analysis.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	This histology is outside the inclusion criteria of most clinical trials.
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC03: Biology of somatic copy number-high (serous-like) endometrial cancers**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Non-endometrioid (Type II) endometrial cancers Mixed histology endometrial cancers Grade III endometrioid endometrial cancers
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	Single sample
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To understand the biology of poor prognosis serous-like endometrial cancers
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Endometrial cancer has traditionally been dichotomised into endometrioid (type I) and non- endometrioid (type II) subtypes on the basis of epidemiology, tumour biology and prognosis. This classification has recently been refined, following the demonstration by TCGA that endometrial tumours cluster into four biologically distinct subtypes on the basis of mutation burden and somatic copy number alterations (SNCAs). Of these, the SNCA high subset, for which (with the exception of POLE-mutant cases), the presence of TP53 mutation appears to be a reasonable surrogate, best corresponds to the traditional category of non-endometrioid tumours, though it also includes a subset (roughly 10%) of endometrioid cancers. It is currently unclear whether the SNCA high cancers that display endometrioid features tend to occur in older women on a

	background of atrophic endometrium, as is typically the case for prototypical non-endometrioid endometrial cancers. As a group, SNCA endometrial cancers display near-universal mutations in TP53 and also harbour frequent mutations in FBXW7, PIK3CA, PP2R1A, CHD4, and chromatin remodelling genes – several of which represent potentially actionable alterations. Another potential targetable alteration is amplification of ERBB2, which occurs in approximately one-third of cases. The substantial overlap between the biology of SNCA high endometrial cancers, and that of high-grade serous ovarian cancers and basal-like breast cancers, has been noted by several investigators. BRCA mutations appear uncommon in SNCA-high endometrial cancers, and the BRCA mutation signature has not been detected in the modest number of tumours analysed to date. However, homozygous deletions of genes essential for homologous recombination repair (HRR), including, ATM, MRE11 and WRN have been detected, and while individually infreqeunt, it is plausible that collectively these could comprise a clinically relevant subgroup of tumours that display characteristics of BRCAness and may benefit from novel therapeutics such as PARPi. As SNCA high tumours have the worst prognosis of all endometrial cancer subtypes and respond poorly to systemic chemotherapy defining the clinical implications of these and other potentially actionable alterations is a required. By integrated analysis of this tumour subgroup we will aim to: (i) confirm the frequency and possible co-existence of known actionable mutations and copy number alterations in SNCA high endometrial cancer to inform future therapeutic investigation (ii) determine the frequency of novel actionable genomic alterations, including mutation or loss of HRR genes, and the presence of the HRR deficiency (BRCAness) signature (iii) determine the frequency and consequences of other mutational signatures,

	such as that caused by APOBEC overexpression, which has been reported to predict enhanced T cell response in some settings (iv) investigate the possible existence of SNCA alterations in genes involved in the anti-tumour immune response, such as cytokines and their receptors (iii) detemine the molecular pathways and events involved in the development of the subgroup of PTEN-mutant SNCA high tumours, which appear to evolve through distinct mechanisms from most SNCA-high tumours, and represent a high-risk molecular subset of cancers traditionally regarded as having a good prognosis (endometrioid endometrial cancers) These studies should substantially increase understanding of this poor prognosis tumour subgroup, and will potentially reveal novel therapeutic strategies that can be explored in prospective clinical trials.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	Within the limitations of their respective exlusion criteria, this collection will be aligned with the the portfolio of endometrial clinical trials nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities) TBC
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC04: Mechanisms of hypermutation in endometrial cancer**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Any endometrial cancer.

Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	Single sample
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To determine the prevalence, causes and consequences of hypermutation in endometrial cancer
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Between 30 and 40% of endometrial cancers are hypermutated as a consequence of mismatch repair deficiency (MMR-D) or mutation in the proofreading exonuclease domain of the DNA polymerase POLE. Another 5% of tumours without MMR-D or POLE mutation demonstrate increased mutation burden (≥100 somatic mutations per exome). Numerous studies in multiple tumour types have demonstrated that hypermutation is a key determinant of prognosis, and emerging evidence points to a causal association between the two (to be examined in a related substudy). Yet much regarding the mechanisms and consequences of hypermutation in endometrial cancer remains unknown. For example, does the mutation burden vary between Lynch syndrome- associated MMR-D tumours and those caused by somatic methylation of the MLH1 promoter? Which POLE proofreading domain variants are pathogenic (i.e. cause tumour ultramutation)? At what point in tumour development do mutator phenotypes caused by MMR-D or POLE mutation occur? Do hypermutated tumours acquire so-called "antimutator mutations" which serve to reduce the mutation rate? Determining the answers to these questions will substantially increase understanding of the biology of a tumour subgroup that accounts for nearly half of all endometrial cancers. In addition to informing the rational application of targeted therapeutics such as immune checkpoint
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study?	Within the limitations of their respective exlusion criteria, this collection will be aligned

Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	with the the portfolio of endometrial clinical trials nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities) TBC
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC05: Determinants of the immune response in endometrial cancer**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Any endometrial cancer. Adequate data on baseline demographic variables, comprehensive drug history.
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	single sample, though additional sample for transcriptomic analysis and availability of FFPE tumour blocks would be very helpful
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To determine the genomic factors that contribute to the antitumour immune response
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Numerous studies have indicated that strength of the antitumor immune response is an important determinant of prognosis and response to therapy in multiple tumour types, including endometrial cancer. Emerging evidence suggests that this immune response is highly dependent upon the genomic landscape of the tumour, and particularly the number and type of somatic mutations the cancer harbours. For example, the excellent prognosis of ultramutated POLE- mutant endometrial cancers appears at least partly due to an enhanced immune response against the multitude of antigenic neo-epitopes they are

	predicted to generate. However while there is clearly a strong positive correlation between tumour mutation burden and the density of tumour infiltration by cytotoxic lymphocytes (and other immune effectors), this correlation is far from perfect. And while increased mutation burden in mismatch repair deficient endometrial cancers is associated with enhanced immunogenicity, any effect of this on clinical outcome is at best variable. The opportunity to perform integrated immunoprofiling on a substantial number of tumours provides an outstanding opportunity to delineate the biological determinants, and clinical consequences of immunogenicity in endometrial cancer. Combining genome sequencing with transcriptomic, and immunohistochemical characterisation and our bioinformatic pipeline to predict antigenic mutations will allow us to answer several pressing questions, including: i) what is the relationship between the number of antigenic neoepitopes and cytotoxic immune response in non-hypermutated, hypermutated and ultramutated endometrial cancers ii) does the association of antigenic tumour mutation burden with immune response depend upon the clonality of the antigenic variants? ii) do non-hypermutated cancers harbour clonal antigenic neoepitopes that may impact on prognosis and response to therapy iii) do hypermutated and ultramutated cancers that lack evidence of a significant anti-tumour immune response harbour “escape mutations” in antigen presentation machinery or apoptotic pathway components In a small subset of cases in specialist centres these studies will hopefully be complemented by additional immunophenotyping, including extraction of tumour infiltrating lymphocytes and analysis of the T cell clonal repertoire and TCR. The insights from these studies are likely to improve the risk stratification for patients with early stage endometrial cancer, and should

	provide important data to underpin the rational use of novel immunotherapies for patients with advanced or recurrent disease.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	This analysis will be aligned with the endometrial clinical trials portfolio nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities)
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC06: Intratumoural heterogeneity and endometrial cancer evolution**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Endometrial cancer for which multiple tumour biopsies are feasible
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	Up to two samples including primary tumour and lymph nodes if involved
# cores per tumour (if multi-region biopsying proposed)	between two and five biopsies per tumour
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To determine the extent of intratumoral heterogeneity in the common endometrial cancer subtypes and use multiregion sequencing to gain therapeutically-relevant insights into endometrial cancer evolution.
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Intratumoral heterogeneity exists in many solid tumours, and has the potential to confound molecular analyses based on a single biopsy sample. For example, sub–clonal driver mutations may be absent from a particular tumour region, or present at an allelic fraction that is unrepresentative of the rest of the

	tumour. This is clearly of major clinical relevance to the application of molecular targeted therapies against driver mutations, and may also be important for predicting the efficacy of immunotherapies, given emerging evidence suggesting that the clonality of antigenic neoepitopes is an important determinant of response. Although methods to estimate tumour clonality from a single biopsy sample have been developed, multiple biopsies are required to capture the complexity of tumour clonal architecture and gain insights into tumour evolution. Despite the fact that it is the most common gynaecological malignancy, to date there has been no investigation of the existence of intratumoural heterogeneity in endometrial cancer. In this cohort, we aim to determine this, and its implications for management, for the four principal endometrial cancer subtypes: somatic copy number low; somatic copy number high; mismatch repair deficient; POLE proofreading domain mutant. We will employ a similar approach to that we have successfully used in colorectal cancer. We will combine WGS with SNP arrays to profile mutations, SNCAs and large-scale chromosomal rearrangements from multiple tumour biopsies, and combine the data to determine the extent of intratumoural heterogeneity and to estimate the clonal fraction of alterations. To capture subclonal events we will increase sequencing depth to 150x. By collecting a total of 20 -30 cancers we should obtain sufficient numbers of each molecular subtype to permit meaningful biologically and clinically relevant insights.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	Within the limitations of trial logistics, this analysis will be aligned with the endometrial clinical trials portfolio nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial

	tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities)
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC07: Predicting toxicity of radiotherapy for women with endometrial cancer**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Any endometrial cancer for which details of radiotherapy treatment and toxicity are available
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	Single sample
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To better predict which women are at risk from enhanced toxicity of post operative radiotherapy following endometrial cancer resection
Scientific case and insights that will be gained from this cohort (more details, as indicated)	Post-operative radiotherapy, delivered either as vaginal vault brachytherapy or external beam radiotherapy, is generally recommended following hysterectomy for all but low risk endometrial cancers. While recommendations for treatment are based on the risk of recurrence, the current inability to identify which patients are likely to benefit from radiotherapy, and which are likely to incur toxicity represents a major shortcoming. Improving this would enable patients and clinicians to make a more informed choice on whether to opt for radiotherapy. While germline defects in DNA repair that confer increased radiosensitivity are usually clinically obvious, other common variants in DNA repair genes that are not clinically apparent may also be important determinants of radiotherapy toxicity. While the sample size means that we will only be powered to detect relatively common variants with a moderate to large effect size,

	we will seek to combine our data with those from other cohorts with genome sequencing and radiotherapy toxicity data in meta analysis to identify novel risk variants.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	This analysis will be aligned with the endometrial clinical trials portfolio nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities)
Is this sub-study a new therapeutic trial?	No

Full proposal (total max 1500 words per Gear 2 Substudy)	
Title *(max 150 characters)*	**EC08: Impact of risk variants on endometrial cancer genomes**
Cohort details and scientific case	
Cohort eligibility definition (disease type, sub-type, presentation, stage, treatment, clinical characteristics, epidemiological characteristics)	Any endometrial cancer. Adequate data on baseline demographic variables, comprehensive drug history.
Samples per patient at first ascertainment (primary tumour, LNs, metastatic sites) *It is assumed that in addition there will be one germline sample per patient.*	single sample, though additional sample for transcriptomics or methylomics would be useful
# cores per tumour (if multi-region biopsying proposed)	NA
Follow-up samples following first ascertainment	None
Purpose of analysis WGS and clinical data from this cohort of patients (brief)	To determine the impact of genetic and non-genetic risk factors on the genomic landscape of endometrial cancer
Scientific case and insights that will be gained from this cohort (more details, as indicated)	The genomic landscape of cancers provides a record of the mutagenic and selection pressures that have operated during the development of that cancer. These factors are potentially subject to influence from both genetic and non-genetic risk factors. Mutational processes, such as defective mismatch repair, or APOBEC overexpression, cause distinctive patterns of

	mutation. Obesity-associated cytokine overexpression may reduce the requirement for driver mutations to activate cellular signalling pathways. While the mutational consequences of mismatch repair deficiency and APOBEC upregulation have been comprehensively described in other tumour types, their impact in endometrial cancer is less well described. Furthermore, very little is known about the impact of common risk factors such as obesity on the endometrial cancer genome. Comparison of mutation signatures and driver mutations between endometrial cancers with and without these risk factors may generate new insights into the mechanisms by which they promote tumourigenesis, and provide novel therapeutic targets for treatment or cancer prevention.
Alignment to clinical trials	
Is it proposed that this sub-study is aligned to an existing clinical trial/sample collection study? Study details (incl Phase, commercial partner, geographic recruitment, remuneration). Is co-recruitment to this trial/study optional/mandatory for recruitment to this cohort eligibility?	This analysis will be aligned with the endometrial clinical trials portfolio nationally, and local trials recruiting in Manchester, specifically: – PORTEC4 (Multicenter Randomised Phase III Trial Comparing Vaginal Brachytherapy (Two Dose Schedules) with Observation after Surgery) – STATEC (Selective Targeting of Adjuvant Therapy for Endometrial Cancer) – PETALS (the proportion of endometrial tumours associated with Lynch syndrome) – PREMIUM (RCT of pre surgical metformin vs placebo for type 1 EC) – MIRENA (Mirena for reduction of endometrial neoplastic abnormalities)
Is this sub-study a new therapeutic trial?	No

Data and informatics requirements
All GeCIP Domains have contributed to the construction of the data model for their tumour type and have contributed to ongoing efforts within Genomics England to develop the clinical and research informatics infrastructure.

Data access and security	
GeCIP domain name	**Ovarian and Endometrial cancer**
Project title *(max 150 characters)*	Gear 2 substudies

Applicable Acceptable Uses. *Tick all those relevant to the request and ensure that the justification for selecting each acceptable use is supported in the 'Importance' section (page 3).*

X Clinical care

X Clinical trials feasibility

X Deeper phenotyping

X Education and training of health and public health professionals

X Hypothesis driven research and development in health and social care - observational

X Hypothesis driven research and development in health and social care - interventional

X Interpretation and validation of the Genomics England Knowledge Base

X Non hypothesis driven R&D - health

X Non hypothesis driven R&D - non health

X Other health use - clinical audit

X Public health purposes

X Tool evaluation and improvement

Information Governance

X The lead for each domain will be responsible for validating and assuring the identity of the researchers. The lead may be required to support assurance and audit activities by Genomics England.

Any research requiring access to the embassy will be required to complete IG Training and read and sign a declaration form. Access will only be granted once these requirements have been met.