Application Summary

Research project title

COLO-PREVENT; A platform for developing COLOrectal cancer PREVENTion therapies

Name of Applicant	Karen Brown
Host Institution	University of Leicester

People supporting this application

Name	Organisation	Role
Professor Mark Hull	University of Leeds	Joint Lead Applicant
Professor Anne Thomas	University of Leicester	Joint Lead Applicant
Dr Louise Brown	University College London	Co-Investigator
Mrs Rachel Hobson	University of Leicester	Senior Trials Unit Contact
Professor Phil Quirke	University of Leeds	Co-Investigator
Professor Colin Rees	South Tyneside Foundation NHS Trust	Co-Investigator
Dr Ruth Langley	Medical Research Council Clinical Trials Unit at UCL	Co-Investigator
Dr Hong Cai	University of Leicester	Named Research Staff
Professor Matthew Rutter	University Hospital of North Tees	Co-Investigator
Mrs Cassandra Brookes	University of Leicester	Co-Investigator
Mr Seid Mohammed	Leicester Clinical Trials Unit	Co-Investigator
Dr Barry Sandywell		Co-Investigator

Proposed Start Date	01/06/2020
Duration of Proposal (months)	120

Research Abstract

Research Abstract

Background Colorectal cancer (CRC) is the second most common cause of cancer death in the UK. Population aging and the increase in obesity and type 2 diabetes, both risk factors for CRC, are likely to magnify the problem in future decades. Prevention, through the use of safe and effective therapies offers a largely untapped strategy for reducing the incidence and mortality due to CRC. Such therapies could be employed for primary prevention, and in conjunction with screening programmes in patients considered 'high-risk' due to the detection of premalignant adenomas.

COLO-PREVENT is a unique multicentre phase 2/3 trial platform for evaluating the efficacy of cancer preventive therapies that is embedded within the Bowel Cancer Screening Programme. It will recruit patients deemed 'high-risk' for the development of metachronous colorectal polyps and/or cancer after clearing colonoscopy.

Aims: 1. To determine whether metformin plus aspirin is superior to aspirin alone. 2. To assess whether resveratrol has any effect on polyp recurrence and identify the most active dose in a signal-seeking trial.

Methods The design of COLO-PREVENT is based on the seAFOod trial, which was the first CTIMP carried out in the English BCSP and which established protocols and RCT infrastructure at screening sites. BCSP patients aged between 55–73.5 years, identified according to BCSP criteria as 'high-risk' at the first complete screening colonoscopy, will be randomised to aspirin (Standard of Care) or aspirin plus metformin for three years. For the parallel resveratrol signal-seeking phase 2 trial, participants either already on, or intolerant to aspirin or metformin, will be randomised to two doses of resveratrol or placebo for 12 months.

The primary endpoint is adenoma recurrence (MAP), secondary endpoints include adenoma detection rate (ADR), advanced adenomas, adenoma subtype and location, adverse events and compliance. Research endpoints will focus on the association between metabolic status and treatment efficacy, assessment of pharmacodynamic biomarkers and role of the gut microbiome in predicting and mediating the effects of the three therapies.

How the results of this research will be used. We anticipate our results will identify a number of safe effective therapies that can be used in conjunction with screening to prevent polyp recurrence and ultimately decrease CRC risk. Inclusion of the sub-trial is an innovative way to establish a pipeline for testing other promising therapies and interventions, generating the first evidence of clinical efficacy. COLOPREVENT will provide a long-term platform for testing therapies, other lifestyle/behavioural interventions and novel combinations.

Study Information

Proposed Study Details

Duration of exploratory development	0 months
Duration of pre-clinical development	0 months
Duration of trial set-up	10 months
Duration of trial recruitment	76 months
Duration of trial follow-up	36 months
What is the proposed trial sample size?	1458
Trial phase	Phase II/III

Type of Trial

Preventative
Adult
Combination
Small Molecule
Randomised
Pharmacodynamic
Pharmacokinetic
Mechanistic

Proposed Study Details

Why is the trial particularly important? Select all relevant statements.

Experimental arm may become control arm of future trials
May lead to change in clinical practice

Will contribute significantly to international knowledge base
Will lead to new guidelines
Largest trial in this group of patients

Please provide any additional information relating to the outcome or potential impact of the study.

The COLO-PREVENT platform will provide long-term infrastructure for testing candidate therapies and other intervention for the prevention of colorectal cancer. We anticipate a pipeline of therapies from members of the UKTCPN that can be fed into the platform as capacity becomes available.

Pilot/Feasibility Study

Was a pilot/feasibility study carried out?	Yes
If Yes, who funded the pilot/feasibility study?	Other

If Yes, briefly describe details of the number of patients, issues addressed (e.g., feasibility of treatment, toxicity) and key findings?

The recently completed SeAFOod Polyp Prevention trial of the omega-3 fatty acid EPA and aspirin, led by Mark Hull (joint lead on this application) is the direct precursor to COLO-PREVENT. SeAFOod was funded by the EME programme (MRC and NIHR) and recruited over 700 high-risk patients from 53 Bowel Cancer Screening Programme sites across England. SeAFOod has demonstrated feasibility in all aspects of our trial design (including recruitment targets, patient acceptability/compliance, and sample collection for biomarker analysis) and the full results (Hull et al The Lancet 2018) have provided robust numbers for powering our study. COLO-PREVENT will build on and extend the trial site infrastructure established by SeAFOod.

NCRI Clinical Studies Group Involvement

What has been the interaction with the NCRI Clinical Studies Group (CSG) in relation to this trial?

Developed by CSG	No
Presented to CSG for review	Yes
Discussed with CSG Chair	Yes

List CSG(s) approached and relevant discussions:

COLO-PREVENT has been presented and discussed at the March 2018 meeting of the Colorectal Cancer Screening and Prevention subgroup. A letter of support is included. Additionally, it was presented at the November 2018 meeting of the Colorectal Cancer CSG.

If there has been NO CSG input, explain why:

N/A

Clinical Trials Unit Study Association

Which Clinical Trial Unit is this application associated with?	Leicester
Clinical Trial Unit type	Other UKCRN Registered
Will core funding from the above Clinical Trial Unit be used to support this application?	No
Please could you estimate the amount of core funding (approximately)?	

Translational Research Study for Submission to BIDD

Are you planning to develop an associated translational research study for submission to BIDD or a prospective sample collection application to CTAAC?	Yes

If Yes, provide a summary

In the long-term we will seek funding from CRUK under the most appropriate scheme for the following translational studies associated with this trial: 1) The Identification of blood-based signatures that correlate with response to aspirin, metformin and resveratrol by conducting transcriptomic, proteomic and metabolomic profiling. The signatures would be used for personalising prevention. 2) Hypothesis-driven analysis of candidate predictive biomarkers of response, including those emanating from the ASCaP CRUK catalyst project. 3) Delineation of the associations between efficacy and colorectal adenoma subtype using new approaches developed (e.g. based on artificial intelligence) to enable the design of rational combinations of preventive therapies.

Additional Research Information

Animal Studies

Does the proposed research involve the use of animals?	No
Animal Species Used	
Other Animal Species	
Are any of these animals genetically modified?	
Status of license covering animal usage	

Human Studies

Does the proposed work involve human tissue samples?	Yes
Does the work require approval from the appropriate research ethics service?	Yes
Research Ethics Application Status	Pending

Other Regulatory Approvals (e.g. NHS, MHRA, National Information Governance Board)

Does the work require other regulatory approval?	Yes
If yes, please describe the type, status and the license number:	
MHRA - pending	

Human Stem Cell Research

Does the work involve the use of any human stem cells?	No

If yes select the Human stem cell types

Commercial Outputs

Do you anticipate that the proposed work will result in any output which can be translated to cancer patient benefit or otherwise commercialised?	Yes
If yes, briefly describe any commercial and/or translational opportunities from the proposed work:	
Since the therapies being studied are repurposed off-patent drugs and a dietary agent, opportunities to commercialise the interventions are unlikely. The translational work associated with the trial could present novel companion biomarkers or a custom panel which could be commercialised and further developed for use in subsequent trials, particularly those aimed at implementation of effective therapies in large populations.	

Biomarker Research

Biomarker Research

Does the proposed work involve the use of any biomarkers?	Yes
If yes, indicate the proportion of the proposed work which focuses on biomarker research	10

Biomarker Category Breakdown (if applicable)

Biomarker Type	**Percentage (%)**
Predictive	50
Pharmacological	50

Total:	100

Cancer Research UK Biomarker Roadmaps

Using Cancer Research UK Biomarker Roadmap, which biomarker category applies to the predominant biomarker identified above?	Biomarker Qualification
Using Cancer Research UK Biomarker Roadmap, which biomarker stage applies to your research?	Stage 1
Please provide a brief description of how the proposed research project aligns with the Biomarker Roadmap	
The biomarkers can be broadly divided into the following types: 1) Established biomarkers for metabolic health currently used clinically with existing routine assays conducted in the NHS. Here, the ability of these biomarkers to influence and predict efficacy will be explored – Biomarker qualification, stage 1. 2) Candidate pharmacodynamic/pharmacological biomarkers identified through our preclinical work or from the literature. Existing assays are available for all of these biomarkers, however, several of them (eg the ELISA assays) will need validation. These currently fall under discovery stage 1 or 2 but will advance to qualification stage 1 during the 2nd year of the project. Where existing validated assays are available (e.g for determination of resveratrol and metabolites in urine/plasma), these biomarkers come under qualification stage 1.	

Applicant Information

Are you applying for your own support?	No
Is your current position funded for the duration of the applied award?	Yes
What is the source of your current funding?	HEFCE

What is your primary profession?	Senior Scientist
Are you a clinician?	No

Number of hours per week in clinical sessions?	0
Number of hours per week contributing to this research project?	16
Total number of hours per week spent on all research projects?	30

Supporting Roles

Role Information

Name of Supporting Participant: Mark Hull

Role: Joint Lead Applicant

Number of hours per week contributing to this research project: 8

Total number of hours per week spent on all research projects: 30

Description of participation in this project, including any technologies, techniques or skills to be employed: Design and delivery of the trial platform using expertise gained from previous phase III randomised trials including the seAFOod Polyp Prevention Trial.

Name of Supporting Participant: Anne Thomas

Role: Joint Lead Applicant

Number of hours per week contributing to this research project: 10

Total number of hours per week spent on all research projects: 30

Description of participation in this project, including any technologies, techniques or skills to be employed: I will Co-lead this study with my colleagues. Specifically I will take responsibility for working with the Sponsor and CTU in capacity as Chief Investigator to facilitate all the regulatory and clinical governance procedures are robust. I will provide oversight to the running of the study, safety reviews, report writing, interpretation of data, and ultimately analysis review and manuscript writing.

Name of Supporting Participant: Louise Brown

Role: Co-Investigator

Number of hours per week contributing to this research project: 2

Total number of hours per week spent on all research projects: 37

Description of participation in this project, including any technologies, techniques or skills to be employed: Statistical advice

Name of Supporting Participant: Rachel Hobson

Role: Senior Trials Unit Contact

Number of hours per week contributing to this research project: 4

Total number of hours per week spent on all research projects: 30

Description of participation in this project, including any technologies, techniques or skills to be employed: Trial oversight and management of trial management staff responsible for the day to day delivery of the project.

Name of Supporting Participant: Phil Quirke

Role: Co-Investigator

Number of hours per week contributing to this research project: 2

Total number of hours per week spent on all research projects: 41.5

Description of participation in this project, including any technologies, techniques or skills to be employed: Microbiome expertise, bioinformatics, Pathology, molecular pathology, cancer biology Histopathology, digital pathology

Name of Supporting Participant: Colin Rees

Role: Co-Investigator

Number of hours per week contributing to this research project: 2

Total number of hours per week spent on all research projects: 30

Description of participation in this project, including any technologies, techniques or skills to be employed: BCSP and colonoscopy expertise
Delivery of large patient cohorts through COLO-SPEED platform

Name of Supporting Participant: Ruth Langley

Role: Co-Investigator

Number of hours per week contributing to this research project: 1

Total number of hours per week spent on all research projects: 32

Description of participation in this project, including any technologies, techniques or skills to be employed: Advice on trial design and clinical member of the Trial Development/Management Group. I have experience of designing and managing large multi-centre trialsin oncology including the use of re-purposed medicines including aspirin and metformin.

Name of Supporting Participant: Hong Cai

Role: Named Research Staff

Number of hours per week contributing to this research project: 18.75

Total number of hours per week spent on all research projects: 37.5

Description of participation in this project, including any technologies, techniques or skills to be employed: I will perform the analysis of resveratrol/metabolites and metformin in clinical samples using HPLC-UV and LC-MS/MS assays. I will be responsible for developing and validating new analytical assays, ELISA, IHC and immunofluorescence protocols and then conducting or supervising all the biomarker work in Leicester to GCP and GCLP standards.

Name of Supporting Participant: Matthew Rutter

Role: Co-Investigator

Number of hours per week contributing to this research project: 0.5

Total number of hours per week spent on all research projects: 12

Description of participation in this project, including any technologies, techniques or skills to be employed: As the Bowel Cancer Screening Programme (BCSP) Research Lead and Chair of the National Endoscopy Database, I will provide a link to the BCSP and advise re access to key BCSP data and strategy, to ensure we will be at the forefront of any changes occurring in screening and prevention practice and will be well equipped to respond in a timely manner.

Name of Supporting Participant: Cassandra Brookes

Role: Co-Investigator

Number of hours per week contributing to this research project: 1.5

Total number of hours per week spent on all research projects: 25

Description of participation in this project, including any technologies, techniques or skills to be employed: Principal Statistician for the Leicester Clinical trials Unit, I have contributed to the design of the study. I will be responsible for overseeing the trial statistician throughout the project and leading the biomarker statistical analysis.

Name of Supporting Participant: Seid Mohammed

Role: Co-Investigator

Number of hours per week contributing to this research project: 2

Total number of hours per week spent on all research projects: 37.5

Description of participation in this project, including any technologies, techniques or skills to be employed: I will provide statistical input into study design, managing and processing data as well as statistical analysis and preparing the report.

Name of Supporting Participant: Barry Sandywell

Role: Co-Investigator

Number of hours per week contributing to this research project: 0.5

Total number of hours per week spent on all research projects: 2

Description of participation in this project, including any technologies, techniques or skills to be employed: As lay member, having served for a number of years on the Bowel Cancer Screening Programme

Pharmaceutical Commercial Outputs

Is the CI/CTU in negotiations with any pharmaceutical company about this application?	No
Description (if applicable)	

Does the pharmaceutical company want to see the data?	No
Description (if applicable)	

Does the pharmaceutical company want to use the data?	No
Description (if applicable)	

Is the pharmaceutical company providing the material for the study for free?	No
Description (if applicable)	

Are inventions likely to arise from the study (e.g. novel assay or biomarker)	Yes
Description (if applicable)	
Novel biomarkers could arise from the microbiome study, for predicting efficacy or for monitoring effectiveness over time for the specific therapies. Other biomarker work proposed in the current application will use existing assays for established biomarkers, except in the case of Mlx and CIDEB proteins in plasma, which we have identified as candidate pharmacodynamic biomarkers of resveratrol in our preclinical studies. We hope to identify novel links between the biomarkers being analysed and preventive efficacy for these therapies. It is likely that future translational studies using the samples, beyond this application, will generate novel assays and biomarkers, since these will be more focused on discovery research.	

Data Sharing Plan

The data sharing plan for COLO-PREVENT will be in line with the CRUK data sharing Policy. All investigators and trial site staff will comply with the requirements of GDPR with regards to the collection, storage, processing and disclosure of personal information. Data will not be released prior to analyses for purposes that might detrimentally affect the progress of the trial.

The COLO-PREVENT collaborators are committed to furthering cancer research by sharing anonymised individual patient data from the study with others who wish to deliver advances in the field of therapeutic prevention. We recognise that these type of studies are long and complex to deliver; consequently all data collected is extremely valuable. We are happy to consider proposals from researchers and will share data subject to ethical approval and informed consent, and contractual and legal obligations including intellectual property protection. Data requests will be made via the Chief Investigator who will discuss the scientific merit of the proposals with the Trial Management Group and Trial Steering Committee. A data transfer agreement will be established for any group requesting access to the data who have been approved by the TMG/TSC. This agreement will require all third party use of the data to acknowledge the COLO-PREVENT Consortium and CRUK.

Uploads List

The following pages contain the following uploads provided by the applicant:

Upload Name
Research Proposal (CRC CTA Full) v3
Cover Letter
Trial Schema
Patient Information and Consent Forms
Patient Information and Consent Forms
Key Research Achievements
Disclosure of Potential Competing Interests
Gantt Chart
Letter of Support-CSG/Advisory/Strategic Grp Chair
Letter of Support - Industry Partner
SOP's
Computer Code
Application Appendices

RESEARCH PROPOSAL: CLINICAL TRIAL AWARD

Use single-line spaced text, in Calibri font, pt 11, black. Text boxes and tables can be expanded as required. Margins should be 2.5cm on all sides and total page count should not exceed 30 pages. Appendices can be used for additional supporting information where required.

1. STUDY DESIGN

1.1 Describe the background, rationale and aims of the study.

Please include the research question(s) to be addressed, why a study is needed and details of any background or supporting information, including pre-clinical data where applicable.

BACKGROUND AND RATIONALE

Colorectal cancer (CRC) is the second most common cause of cancer death in the UK, with 113 people diagnosed and 44 dying from the disease every day[1]. Furthermore, population aging and the increase in obesity and type 2 diabetes mellitus, which are both risk factors for CRC, are likely to magnify the problem in future decades[2-5]. It is estimated that the global burden of CRC will increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030[6]. Prevention, through the use of safe and effective therapies, offers a largely untapped strategy for reducing the incidence and mortality due to CRC. Such therapies could be employed for primary prevention, but also in conjunction with screening/surveillance programmes for patients considered high-risk due to the detection of premalignant colorectal adenomas; the latter constitutes secondary prevention and may also serve to reduce the need for, or frequency of, surveillance colonoscopy.

COLO-PREVENT is a **multicentre phase 2/3 trial platform** consisting of **a main study** and **integrated Signal-Seeking trial** for evaluating the efficacy of cancer preventive therapies that is embedded within the NHS Bowel Cancer Screening Programme (BCSP, see Trial Schema & Fig. 1). It will recruit patients deemed high-risk (defined according to new UK guidelines, which are likely to specify individuals with adenomas ≥20mm or high-grade dysplasia, or serrated lesions ≥10mm or any dysplasia, or ≥4 polyps) for the development of metachronous colorectal polyps and/or cancer after a clearance colonoscopy. As precursors to most CRCs, adenomatous polyps are the only validated surrogate outcome measure of cancer risk, and have been used as a primary colonoscopic outcome measure in multiple therapeutic prevention trials.

The first control intervention in COLO-PREVENT will be **aspirin**, which will be considered a standard of care comparator[8, 9], versus the combination of **metformin** plus aspirin, in an open-label study. There is compelling evidence from epidemiology data and randomised clinical trials that aspirin can prevent colorectal cancer and adenomas, and that this protection translates to reduced CRC-associated mortality[10-13]. However, aspirin does not work for everyone; therefore, we aim to identify effective adjuncts and/or alternatives to aspirin, to expand the proportion of patients who can experience a net benefit from preventive therapy. The weight of evidence for efficacy of metformin in this context, coupled with excellent safety profiles, places this drug at the top of the pipeline for testing in combination. It is striking that despite the strong case for using aspirin for CRC prevention in high-risk groups, such as those with Lynch syndrome[14], implementation has been challenging due to limited awareness among potential prescribers and concerns over the safety of higher doses[15]. We anticipate that **adopting aspirin as standard of care in COLO-PREVENT** will help raise awareness among healthcare professionals and the public, increase the acceptability and alleviate concerns over safety, which in turn will **aid wider implementation, leading to more routine use**.

Gaining proof of concept for clinical efficacy is a considerable barrier to translating promising preventive therapies to full phase 3 clinical evaluation. Therefore, a novel feature of the COLO-PREVENT platform is the incorporation of signal-seeking arms, which will allow earlier phase testing of interventions for which there is not yet sufficient evidence to justify a large phase 3 study.

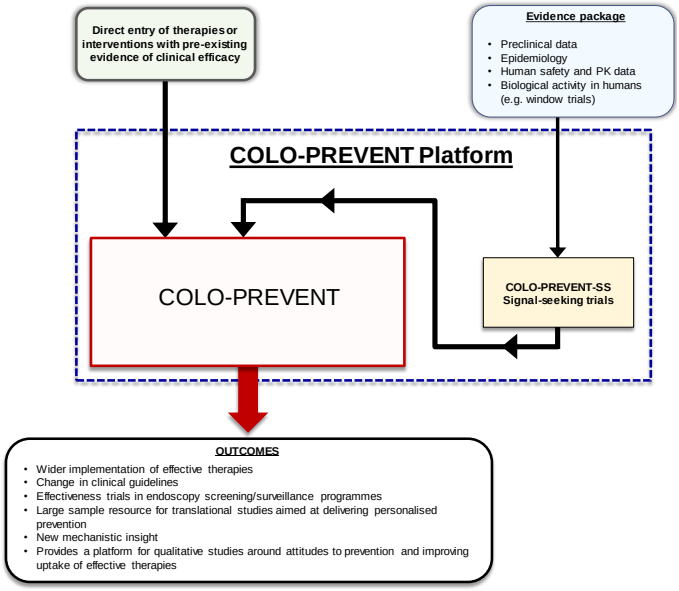

Figure 1. The COLO-PREVENT vision, illustrating how therapies and interventions can feed into the platform via two routes depending on the available evidence. Also shown are the anticipated outputs from the platform.

Efficacy will be evaluated in a smaller number of patients over a shorter period, at which stage a 'go/no-go' decision can be made regarding continued assessment within the COLO-PREVENT platform. The Signal-Seeking trial provides a conduit for linking laboratory work with phase 3 assessment, in a unique package.

Resveratrol is a polyphenolic compound contained in red grapes, peanuts, certain berries and red wine. Having developed resveratrol as a cancer preventive agent over the last ~12 years through CRUK programme funding it has become our prime candidate for evaluation in the Signal-Seeking sub-trial (COLO-PREVENT-SS). COLO-PREVENT represents the first platform prevention trial, extending the benefits of adaptive design, now commonplace in oncology, to the prevention arena. In the longer term, other promising interventions identified through our links with the UK Therapeutic Cancer Prevention Network can feed into the platform as capacity permits.

The platform will build on and extend the trial site infrastructure established by the recently completed seAFOod Polyp Prevention trial of eicosapentaenoic acid (EPA) and aspirin led by Mark Hull, which recruited over 700 patients from 53 sites across England[16, 17]. In COLO-PREVENT, patients identified as 'high-risk' at index colonoscopy will be recruited and randomly assigned to receive an intervention until first surveillance colonoscopy. 'High-risk' individuals have a high event rate over short periods of follow-up, with ~60% adenoma recurrence within 12 months, which reduces study size and duration[16]. Adenoma recurrence post polypectomy may be due to both 'missed' and new lesions[16, 18]. Evidence for *de novo* adenoma growth comes from tandem colonoscopy studies in which there is as much as a 30% difference in adenoma detection rate (ADR) at colonoscopy within three months of the index procedure compared with 12 months in high-risk individuals[16, 18]. Moreover, the occurrence of post-colonoscopy CRC, particularly right-sided tumours, is increasingly being recognised, even for those engaged in high quality surveillance programmes[19-21]. These facts highlight the opportunity for improvement by combining preventive therapies with endoscopic interventions in this population. By aligning with standard care and integrating COLO-PREVENT within a quality-assured national screening programme the trial will be delivered in a robust, efficient and cost-effective manner. An added benefit of embedding within the BCSP is that it will also be possible to obtain post-trial long-term colonoscopy outcomes.

AIMS

The Primary hypotheses of this study are that:

1) The combination of metformin and aspirin affords greater preventive efficacy in high-risk polyp patients compared to aspirin alone.
2) Resveratrol prevents colorectal polyp recurrence in high-risk individuals.

Primary aims:

1. To determine whether taking daily metformin plus aspirin for three years is superior to aspirin alone at preventing colorectal polyp recurrence in high-risk patients identified through the BCSP.
2. To assess whether daily resveratrol has any effect on polyp recurrence after one year and identify the most active dose in a Signal-Seeking trial.

The **primary outcome measure** will be adenomatous polyp recurrence, quantified as mean adenoma number per person (MAP); **secondary outcome measures** are adenoma detection rate (ADR), occurrence and number of advanced adenomas (expressed as ADR and MAP), and adenoma location and subtype (distinguishing the conventional dysplastic adenoma [driven by loss of *APC* function] from the serrated polyp [BRAF mutant, CIMP+, MSI, thought to lead to up to 30% of CRCs][22, 23], which may display differential sensitivity to prevention agents[17]). **Exploratory translational work** integral to interpreting the results of the trial will centre on the hypothesis that metabolic status and dietary factors influence the effectiveness of aspirin, metformin and resveratrol, such that individuals with poor metabolic health may experience greater benefit. **Further exploratory analyses** will investigate whether metformin and aspirin have additive effects on molecular targets associated with pathways modulated by both drugs and ascertain whether candidate pharmacodynamic biomarkers of resveratrol efficacy, previously identified in our preclinical studies, translate to the clinic. We will also examine whether correlations exist between plasma concentrations and/or metabolite profiles of resveratrol and metformin and efficacy and evaluate the role of the gut microbiome in predicting and mediating the effects of the three interventions.

INTERVENTIONS

Aspirin: A major part of the evidence base relating to aspirin and cancer has emerged from randomised controlled trials (RCTs) designed to evaluate the vascular effect of aspirin. Analysis of two such large trials with reliable post-trial follow-up for more than 20 years: the British Doctors Aspirin Trial and UK-TIA Aspirin Trial, demonstrated that high-dose aspirin (≥300mg/day) taken for at least 5 years is effective in primary prevention. Aspirin was associated with a 37% reduction in sporadic CRC incidence, with a latency of about 10 years, which is consistent with findings from observational studies[11]. In a further follow-up of these trials together with three other large studies, similar results were reported for lower doses of aspirin (≥75mg), and there appeared to be strong site specificity, with allocation to aspirin of 5 years or longer reducing the risk of proximal colon cancer by ~70%[13]. The magnitude of protection seems larger for mortality due to CRC, with an impressive 52% reduction following treatment for ≥5 years[12, 13].

Studies specifically designed to prevent cancer have echoed the long-term results of the vascular trials. In the Women's Health Study, 40,000 US female health professionals were randomised to aspirin or placebo to be taken every other day[24]. Colorectal cancer incidence was reduced in the aspirin group but the difference only became apparent after 10 years. Additionally, in the CAPP2 trial involving Lynch Syndrome patients, aspirin substantially reduced cancer incidence after 5 years[14].

Five RCTs including seAFOod, provide evidence that aspirin is effective for the prevention of colorectal adenomas in individuals with a history of these lesions[10]. For example, in the UK Colorectal Adenoma Prevention Trial, 3 years of aspirin led to a 21% relative risk (RR) reduction for recurrence of any adenoma and 37% reduction in advanced adenomas (defined as ≥10 mm diameter, high-grade dysplasia, or villous histology); additionally, the MAP was decreased by 34%[25]. In seAFOod, 12 months of aspirin (300mg/day) was associated with a 22% reduction in MAP with evidence of a preferential effect on serrated (IRR 0.46 [0.25-0.87]) and right sided (0.73 [0.61-0.88]) lesions[17]. ***Importantly, the reduction in adenoma recurrence observed across the aspirin polyp prevention RCTs[10] has predicted the longer-term effect of aspirin on CRC incidence[26] and***

mortality[13], confirming the utility of adenoma recurrence as a surrogate biomarker of CRC risk. Furthermore, the preferential effects of aspirin on right sided lesions observed in seAFOod[17], and also reported in a study examining correlations between lifestyle factors (including aspirin) and risk of adenomas in other prevention trials[27], mirrors the profound difference in effect of aspirin between proximal and distal colon cancers for both incidence and mortality, which supports adenoma recurrence as a surrogate for cancer prevention.

In polyp prevention trials, the largest benefit of aspirin on both total and advanced adenomas appeared during the first year after randomisation, with significant additional reductions over the subsequent two years[10]. ***This illustrates that short-term (both 1 and 3 year) intervention trials are sufficient for providing a read out of polyp prevention efficacy, which in turn is an excellent surrogate of the effects of aspirin on CRC risk and mortality.***

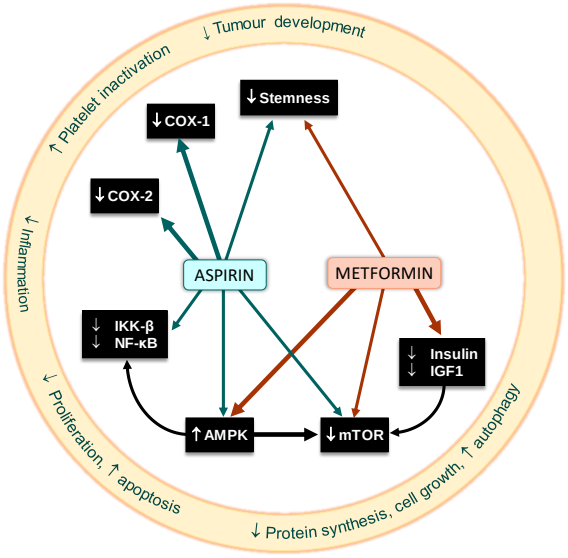

Figure 2. Overlapping and distinct mechanisms of action for aspirin and metformin. The key molecular targets and pathways are within the circle, and the resulting phenotypic outcomes are shown in the outer ring.

Aspirin has been recommended as a CRC preventive therapy in age-stratified groups by the US Preventive Services Task Force guidelines which advocates aspirin for primary prevention in individuals aged 50-59 years, who are at high-risk for cardiovascular disease[9]. A risk-benefit analysis has calculated that aspirin could prevent more deaths due to cancer than cardiovascular disease in the general population and proposed that prophylactic use of aspirin (75-325 mg/day) for a minimum of 5 years in the age range 55-65 would have a favourable risk-benefit ratio and a net 4% relative reduction in all deaths[8]. Therefore, we argue that the **use of aspirin as standard of care in high-risk patients within the BCSP is justified and should lead to greater awareness and wider acceptance of aspirin in this context**. In COLO-PREVENT, we will tailor the aspirin dose according to bodyweight, based on a recent analysis that advocates stratification, such that individuals weighing <70kg take low dose aspirin and those ≥70kg require higher doses for greater benefit and reduced risk[28]. We anticipate that ~85% of participants in COLO-PREVENT will receive the higher 300mg dose, based on body weight data for the high-risk population in the BCSP[17]; this dose was well tolerated in the recent seAFOod and AspECT[17, 29] trials.

Aspirin is not universally effective in all people; it appears to have a greater impact on right-sided and serrated adenomas and cancers arising in the proximal colon; there has also been some suggestion that aspirin confers greater survival advantage in patients with PIK3CA-mutated CRC[30]. Whilst these observations require further interrogation, it is clear that for primary or secondary prevention, during which the nature of any developing adenomas/cancers cannot currently be predicted, aspirin should be used in combination with an additional therapy to maximise efficacy.

Metformin: A strong mechanistic rationale underpins the focus on metformin, which is used for first-line treatment of type 2 diabetes mellitus (T2DM), as a cancer prevention agent. Hyperinsulinaemic states encompassing metabolic syndrome and T2DM are associated with increased risk of a number of cancers, including CRC. Multiple meta-analyses of observational studies predominantly comprising individuals with T2DM, have shown that metformin use is linked

with reduced overall cancer incidence of 10-40%, with a similar decrease in mortality[31]. Moreover, analyses addressing site-specific associations have demonstrated that metformin significantly decreases the risk of both colorectal adenomas[32] and cancers, and reduces CRC-related mortality[31].

A recent key randomised controlled trial in Japan has demonstrated that low dose (250mg daily) metformin can decrease adenoma recurrence by 40% in high-risk non-diabetic patients that had previously had polyps/adenomas endoscopically resected[33]. However, it is difficult to directly translate these results to Western populations as type 2 diabetes has a different phenotype in East Asians, where it is characterized primarily by β-cell dysfunction, and less adiposity and insulin resistance. The doses of metformin traditionally used to treat T2DM in Japan (maximum of 750 mg daily) are considerably lower than those routinely used in Europe and the US (typically >2g daily) and the high-risk population enrolled in the trial had a lower BMI (mean ~23-24kg/m^2)[33] than the equivalent patients in the English BCSP (82% of patients in the seAFOod trial were classed as overweight or obese with a BMI ≥ 25)[34]. Consequently, given the weight of epidemiological evidence and efficacy of metformin in an analogous, albeit phenotypically different high-risk population, it is now time to test it within the NHS health care system, as the first step in quantifying its value for the prevention of CRC.

Aspirin mechanisms of action: Both cyclooxygenase (COX)-dependent and -independent mechanisms have been proposed to explain the preventive effects of aspirin (Fig. 2). Inactivation of its primary target COX-1 through irreversible acetylation of Serine 529 near the catalytic site, and subsequent reduced biosynthesis of thromboxane A2 inhibits platelet function and activation, which is likely to contribute to its anticancer effects by moderating platelet-promoted cell proliferation, angiogenesis and metastasis[35]. Inhibition of inducible COX-2, which is often over-expressed in colorectal tumours, affords anti-inflammatory activity by decreasing concentrations of tumour-promoting prostaglandin E2 (PGE2)[36]. COX-independent mechanism of aspirin include prevention of NF-kB activation and inhibition of IkB kinase (IKK) β[37], activation of adenosine monophosphate-activated protein kinase (AMPK) and inhibition of mammalian target of rapamycin (mTOR) signalling[36]. The potential clinical relevance of AMPK and mTOR as targets has been demonstrated by analysis of repeated rectal biopsies taken from patients administered aspirin for 7 days; treatment was associated with reduced phosphorylation of S6 kinase 1 (S6K1) a downstream effector of mTOR in normal tissue, and its substrate ribosomal protein S6[38]. The transcription factor NF-κB has a central role in inflammation and is activated in response to growth factors, inflammatory stimuli, and pro-oxidants, leading to increased transcription of genes regulating proliferation, apoptosis, angiogenesis, invasion, inflammation, and metastasis[39]. CRC cells are particularly sensitive to the pro-apoptotic activity of aspirin mediated through IκBα degradation and modulation of NF-κB nuclear translocation[40]. Aspirin also reportedly inhibits tumour growth and 'stemness' in colorectal cancer cells by downregulating the transcription factor Nanog[41].

Metformin mechanisms of action: Metformin concentrates in human colonic tissue generating ~150-times higher levels than in plasma[42]. There is close correlation between plasma and tissue concentrations and importantly, the levels achieved in human colorectal tissue are in the range shown to produce a direct antitumor effect in various *in vivo* preclinical models. Mechanistically, metformin is thought to exert anticancer effects via both indirect and direct pathways[43] (Fig. 2). Systemically, reductions in insulin/insulin-like growth factor-1 (IGF1) signalling leads to reduced activation of mTOR and its targets 4EBP1 and S6K1, and decreased tumour growth. At a cellular level, metformin acts as a mitochondrial poison by inhibiting complex I in the electron transport chain; this impairs production of ATP, which activates AMPK resulting in mTOR inhibition[44]. AMPK activation can also inhibit NF-kB signalling[45] and suppress β-catenin-dependent Wnt signaling in CRC cells by cytoplasmic sequestering of β-catenin through AMPK, which further decreases cell proliferation. The effects of metformin on mitochondria also account for its ability to reduce

endogenous production of reactive oxygen species (ROS) and DNA damage, including ROS associated with oncogenic Ras expression[43, 46].

The most frequent side effects with metformin are gastrointestinal (GI); these are dose-related and can be minimised by gradual dose-escalation at the initiation of treatment[47]. Doses effective for the treatment of T2DM (1700 mg daily) have been used long-term within diabetes prevention trials in individuals with elevated glucose[48]. This dose is also being investigated in several cancer studies involving non-diabetics, including the CRUK-funded STAMPEDE trial, which aims to evaluate whether metformin improves survival of men with high-risk localised or metastatic prostate cancer[49]. A sub-study from the ongoing NCIC Clinical Trials Group (NCIC CTG) MA.32, investigating the effects of metformin (850mg BD) versus placebo on invasive disease-free survival and other outcomes in early breast cancer[50] has reported improvements in weight, BMI, and metabolic variables with no symptomatic episodes of hypoglycaemia or other evidence of adverse metabolic effects, including lactic acidosis.

Mechanistic rationale for combining aspirin and metformin

Findings from a recent population based study suggest that patients with T2DM who are taking both metformin and aspirin have higher five-year cancer-specific and relative survival for stage II and III CRC compared with diabetic patients not taking aspirin[51]; these results support the possibility of a favourable interaction for the anticancer activity of aspirin and metformin in humans.

*We anticipate that combining aspirin and metformin will lead to greater preventive efficacy as a result of **1**) the two drugs acting via the same targets or on converging pathways to afford greater potency against the same polyps and/or **2**) aspirin and metformin acting independently to prevent different subtypes of polyps or with different selectivity based on polyp location.* Several preclinical studies have demonstrated that combining the drugs leads to enhanced activity, with most focussing on energy homeostasis and metabolism plus apoptosis. For example, in CRC cell lines co-treatment with aspirin and metformin has a pronounced additive effect on AMPK activation and mTOR inhibition, significantly reducing S6K1 phosphorylation at concentrations where the single agents had no detectable effect on this endpoint of mTOR signalling[52]. Similarly, in breast cancer cells addition of aspirin to metformin significantly increased apoptosis and AMPK activation, whilst decreasing mTOR phosphorylation compared to metformin alone; these effects were associated with increased inhibition of complex I of the respiratory chain[53]. *In vivo*, aspirin enhanced the tumour growth inhibitory properties of metformin in an orthotopic model, but only in immuno-competent mice, suggesting the combination acts on both cancer cells and the microenvironment[53]. Analogous findings have been reported for pancreatic cancer cells *in vitro*[54] in which the combination inhibited phosphorylation of mTOR and STAT3, and induced apoptosis, accompanied by downregulation of the anti-apoptotic proteins Mcl-1 and Bcl-2, and upregulation of pro-apoptotic proteins Bim and Puma. The combination also acts synergistically to modulate the transcriptional profile in pancreatic cancer cells, with cholesterol biosynthesis and cell cycle:G1/S checkpoint regulation particularly affected[55]. Moreover, this additive activity translated to significantly greater *in vivo* efficacy in a xenograft model, with inhibition of pancreatic tumour growth and downregulation of Mcl-1 and Bcl-2 protein expression[54].

Resveratrol: There is a body of preclinical evidence supporting the potential of resveratrol as a preventive therapy for CRC aligned to promising pharmacodynamic biomarker changes in target tissues from pre-surgical window trials[7, 56, 57]. Efficacy testing in a clinical setting is the next logical step. Although there is no formalised route to phase 3 efficacy testing of preventive interventions for CRC, for the handful of therapies that have progressed this far, most were first screened in another high-risk group – patients with Familial Adenomatous Polyposis (FAP). These individuals have extensive adenomas at the outset, so the endpoint is a reduction, or smaller increase, over time compared to placebo. However, an adequate sample size for this rare disease can only be

achieved by conducting a multicentre and often a multinational study, which presents challenges as centres may have different practices, and standardizing colonoscopy and polyp scoring procedures in FAP trials is notoriously difficult[58, 59]. Furthermore, the FAP population is not appropriate for evaluating resveratrol because preclinical data and evidence from other disease states suggests it is most effective in animals or people with metabolic disturbances; this phenotype is better represented by the high-risk BCSP patients, who are characterised by high BMI[34]. In contrast, FAP patients participating in previous prevention trials typically had a much lower BMI[60].

A wealth of preclinical studies have described the cancer preventive activity of resveratrol in a variety of *in vivo* models across a spectrum of malignancies. It is also being pursued for the management of other chronic conditions including diabetes and metabolic syndrome, the pathophysiology of which share overlapping pathways with cancer. We have previously defined the safety and pharmacokinetics of resveratrol in phase 1 trials of healthy volunteers[61] and CRC patients[62, 63]. Others have since confirmed that resveratrol is well tolerated at once-daily doses of ≤1g, with the longest published trial in elderly Alzheimer's patients demonstrating an excellent safety profile at doses of up to 1g taken twice daily for one year[64, 65]. The high acceptability of resveratrol as a preventive therapy in healthy people is illustrated by the ongoing two-year RCT crossover Study RESHAW (ACTRN12616000679482), which is examining the effect of 12-month daily resveratrol supplementation on brain health in post-menopausal women. Of the 146 individuals originally randomised, 87% are still enrolled as the trial approaches completion next month (Peter Howe, University of Newcastle, Australia, personal communication).

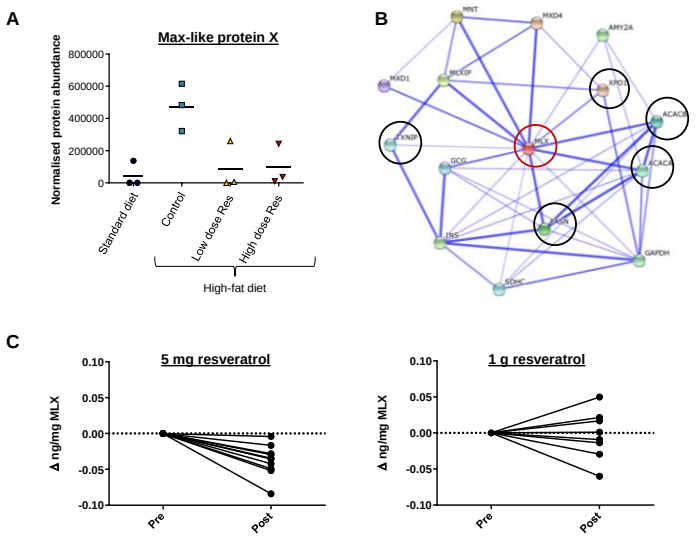

Figure 3. Max-like protein X (MLX) as a potential pharmacodynamic plasma biomarker of resveratrol in mice (A & B) and humans (C). MLX was identified using a novel dual proteomics strategy, which involved integrating protein expression data from a proteomics study of *Apc^{Min}* mice, with resveratrol protein binders identified using our in-house protein-pull down assay (**A** & **B**). Administration of a high fat diet (HFD) to the mice increased plasma MLX relative to levels in mice on a standard diet (SD). MLX was restored to baseline in mice that received resveratrol at both the low (0.7ppm) and high (143ppm) doses (**A**), illustrating the ability of resveratrol to abrogate the effects of a high fat diet at a molecular level. Plasma MLX levels were then measured in colorectal cancer patients that participated in our published [^{14}C]-resveratrol trial[7] and we found that daily intervention for one week with low dose (5mg) resveratrol decreased MLX levels in all patients, whereas a high dose (1g daily) had no apparent effect (**C**)**. This finding adds to our existing evidence that a low, dietary achievable dose of resveratrol can exert biological activity in humans.**

We have also reported that resveratrol is rapidly metabolised in humans, leading to low systemic bioavailability, however, we detected relatively high concentrations of parent resveratrol in colorectal tissue after doses of 0.5-1g daily and a significant reduction in the cellular proliferative fraction (%Ki67), supporting the GI tract as a target for cancer preventive effects of resveratrol[62].

The doses chosen for COLO-PREVENT-SS are primarily based on our previous CRUK-funded programme where we explored whether low dietary-achievable doses of resveratrol (equivalent to 5mg daily in humans, an amount contained in about two large glasses of certain red wines) can reach target tissues in patients, and compared efficacy with a 'pharmacological' dose 200-times higher (≡1g per day for humans)[7].

The 5mg dose of [^{14}C]-resveratrol generated detectable concentrations in colorectal mucosa of every patient, furnishing levels of ~0.2µM.

We consistently observed a non-linear dose response for the preventive effect of resveratrol in *Apc^{Min}* mice and human tissues, with the low dose preventing adenoma development, activating AMPK signalling, inducing autophagy and senescence in mouse intestinal cells to a greater extent than the high dose, but only when mice were maintained on a high fat diet[7]. This phenomenon translated to human explant cultures, with maximal effects on AMPK signalling and autophagy at lower concentrations. In patients, the lower dose (but not the high) significantly reduced markers of oxidative stress in normal colorectal mucosa (NQO1, protein carbonyls)[7] and decreased plasma levels of Max-like protein X (Fig. 3) a transcriptional regulator involved in the control of glucose-responsive genes[66]. Our ongoing biomarker discovery work has revealed that the high dose of resveratrol restores many features of the plasma proteome and metabolome perturbed by a high-fat diet (Fig. 4 and Appendix 1). This dose also has a profound protective effect on the survival of mice on a high fat diet with mutant ^{V600E}Braf conditionally expressed in the intestine, which represents a model of MSI/CIMP^{+} CRC[67, 68]. In contrast, the low dose was ineffective and both doses failed to affect survival of these mice on a standard diet (Fig. 5).

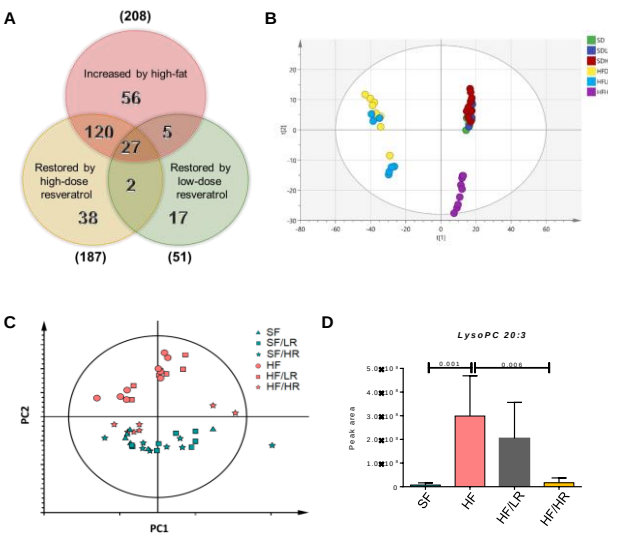

Figure 4. High dose resveratrol has a stronger systemic effect than low dose, and reverses changes caused by a high fat diet on the plasma proteome (A & B) and metabolome (C & D) in *Apc^{Min}* mice. (**A**) Venn diagrams showing plasma proteins increased by HFD, and decreased by either dose of resveratrol (0.7 or 143 ppm). (**B**) These dose-related differences are further illustrated by a Principal Component Analysis of the proteomic data. (**C**) LC-MS/MS-based metabolomics analysis of the same plasma samples suggests a similar pattern, with mice on HFD plus high dose resveratrol clustering towards the mice in the three standard fat groups (blue symbols). In contrast control mice on HFD, and those also supplemented with low dose resveratrol, are clearly separated (pink circles and squares). (**D**) Shows an example of a plasma lipid, putatively identified as lysophosphatidylcholine 20:3, which is significantly increased by HFD and then restored to baseline levels, comparable to those in mice on a standard fat diet, by high dose resveratrol.

Taken together, our data suggest that both a 5mg and 1g dose of resveratrol may have efficacy in humans, with the lower dose predominantly acting locally in colorectal tissue and the high dose having a greater systemic effect. Additionally, resveratrol is more effective on a background of poor metabolic health and different doses may be optimal for targeting different subtypes of adenoma; only the high dose had activity in ^{V600E}Braf mice, whereas the low dose was more effective than the high in mice harbouring *Apc* mutations, which develop conventional adenomas. Therefore, it is not currently possible to select a single dose to take forward and we will compare both doses against placebo in COLO-PREVENT-SS.

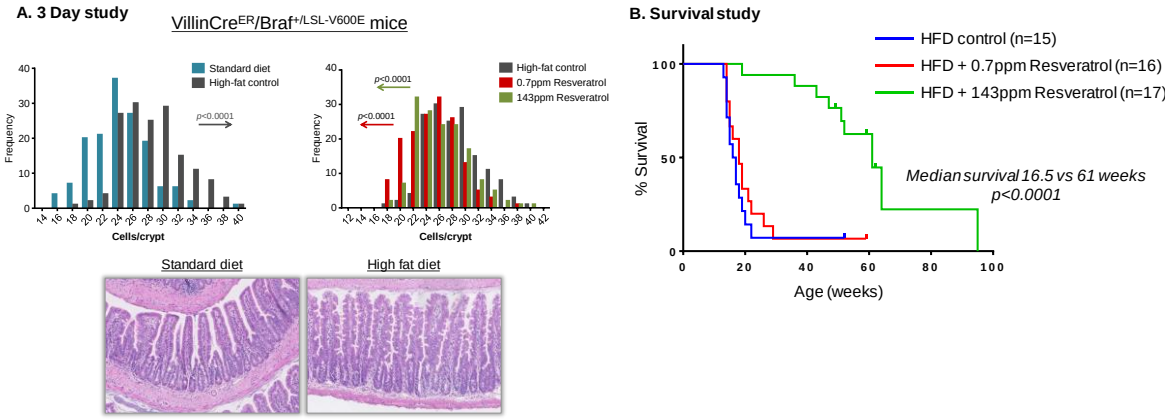

Figure 5. Effects of a high fat diet (HFD) and resveratrol in a ^{V600E}BRAF-mutant model of colorectal cancer. (**A**) Consumption of a HFD significantly enhances ^{V600E}Braf-induced serrated hyperplasia in VillinCre^{ER}/Braf$^{+/LSL-V600E}$ mice, but this effect is prevented by clinically achievable doses of resveratrol. Induction of mutant *Braf* initially triggers a burst of proliferation before senescence ensues. Consumption of a HFD significantly enhances the hyperplasia observed at 3 days post-tamoxifen induction (left hand graph and H&E images below), increasing the number of cells/crypt compared to mice on a standard diet. Administration of resveratrol at both a high and low dose reverses this response (right hand graph). The resveratrol doses used (0.7 and 143ppm) are the same as those described for Figure 3, and equate to 5mg and 1g per day in a human. (**B**) In a survival study using the same VillinCre^{ER}/Braf$^{+/LSL-V600E}$ model, HFD drastically reduced the survival of mice from a median of ~95 weeks (not shown) to 16.5 weeks. High dose resveratrol had a strong protective effect, improving survival to a median of 61 weeks. In comparison, the low dose of resveratrol had no effect on survival. An identical study in mice on a standard fat diet showed that neither dose of resveratrol affected survival, with the median for all mice being in the range ~85-95 weeks. This illustrates that different doses of resveratrol may be required for optimal preventive effects against different subtypes of colorectal adenomas and that resveratrol can counteract the effects of a HFD.

Therapy-induced microbiome changes as a potential mechanism of action

The microbiome is a metabolically active biomass in lifelong contact with the bowel mucosa. It varies widely between populations at differential risk of bowel cancer and between individuals within those populations. It is affected by a range of factors but one important contributor is drug intake[69]. We have previously shown[70] that the microbiome of healthy volunteers undergoes beneficial changes with the use of omega-3 polyunsaturated fatty acids supplements, a treatment linked to reduced adenoma formation[17]. There is also accumulating evidence that metformin alters the gut microbiome of humans to improve metabolic dysfunction and this may contribute to its therapeutic effect in T2DM[71, 72]. Similarly, it has been suggested, based on rodent models, that the beneficial effects of resveratrol across a range of conditions including heart failure, obesity and the metabolic syndrome may be mediated, at least partly through its interaction with gut microbiota[73, 74, 75]. Less is known about the potential effects of aspirin but several short-term ongoing pilot studies are investigating its influence on the gut microbial community in healthy volunteers (Clinicaltrials.gov).

We have demonstrated that we can use screening methods such as guaiac faecal occult blood testing (gFOBT) cards to monitor changes in the microbiome[76] and in unpublished studies on 1287 patients in the NHS BCSP we have found strong associations of cancers and adenomas with increases and decreases in specific bacterial populations (Fig. 6).

As part of a CRUK Grand Challenge, OPTIMISTICC, we are currently defining bacterial populations within the NHS bowel cancer screening programme and globally. We are also studying the Microbiome in the NINHR INTACT trial, CR07, PICCOLO and Quasar.

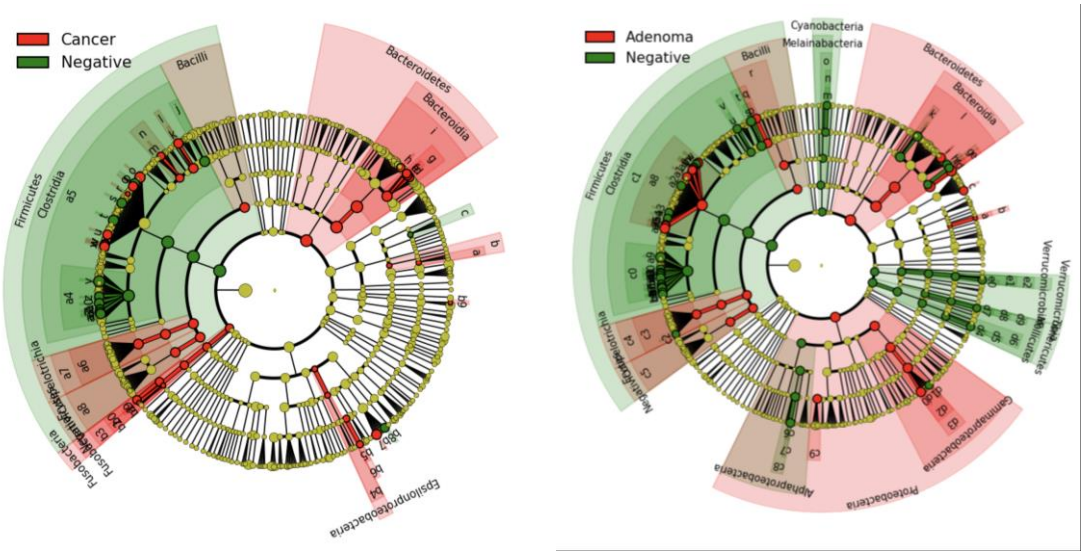

Figure 6. Increased (red) and decreased (green) bacterial populations in individuals with negative gFOBT screening tests and those gFOBT-positive individuals with confirmed cancers and confirmed adenomas in the NHS BCSP. Unpublished data, Young, Woods, Quirke.

1.2 Describe the expected clinical impact of the study and the anticipated future benefits for patients including both outcome and experience.

You should clearly articulate how your proposed study will impact on clinical practice and how it will lead to improvements for patients. It is also suggested that you indicate the timescale within which you anticipate any change in clinical practice will occur.

- There is evidence that aspirin should be the standard of care in high-risk populations and yet, to date, aspirin is not widely used for this indication. This trial provides a perfect opportunity to increase public and health care professionals' awareness of the benefits of aspirin for cancer prevention and facilitate service implementation into routine care, which is a significant attribute in its own right.
- Bring about a change in clinical practice by providing data to support revision of the NICE Clinical Guidelines on Colorectal Cancer Prevention (https://www.nice.org.uk/guidance/cg118) to include aspirin use in the high-risk BCSP patient population.
- We anticipate that our results will identify a number of safe effective therapies that can be used in conjunction with screening and surveillance to prevent polyp recurrence and ultimately decrease CRC risk.
- Ultimately, for effective therapies shown to be successful in these high-risk BCSP patients, COLO-PREVENT could be the first step towards use in a wider population at-risk for CRC, in the way aspirin is now being recommended in the US.
- Inclusion of the sub-trial is an innovative way to establish a pipeline for testing other promising therapies and interventions, providing the first evidence of clinical efficacy against 'sporadic' colorectal neoplasia and facilitating the establishment of a panel of preventive options that can eventually be tailored to individuals.
- COLO-PREVENT will provide a long-term platform for testing therapies, other lifestyle/behavioural interventions and novel combinations.

1.3 Describe how the proposed study aligns with the Cancer Research UK Research Strategy and Clinical Research Statement of Intent.

- COLO-PREVENT targets **early/premalignant disease**, thereby contributing **balance to the CRUK trials portfolio**, where the emphasis remains treatment of later stage disease.

- The overall platform, and in particular the signal-seeking sub-trial, is a **high impact innovative early phase study** that would not progress without CRUK support.
- We have adopted an **innovative cost-effective trial design**; COLO-PREVENT will be the first platform trial in prevention **worldwide**. It is cost-effective due to embedding in an existing national screening programme and having the ability to drop ineffective arms at the signal-seeking stage. Efficiency is maximised by offering COLO-PREVENT-SS to a group of patients who would otherwise be ineligible for the main trial. The platform is flexible, with the ability to add in lifestyle/behavioural interventions, in combination with preventive therapies in the future.
- The COLO-PREVENT platform fills a void in the development of preventive therapies, offering a route to **accelerating the translation** of promising candidates from the laboratory/early phase trials to efficacy evaluation in target populations.
- The associated translational work is centred on identification of who will benefit from each therapy/combination to allow **optimisation and personalised prevention**. The study will also generate a rich sample set for testing mechanistic hypotheses, identifying pharmacodynamic biomarkers plus the discovery and validation of short-term surrogate cancer biomarkers for predicting efficacy.
- This trial represents direct **translation of Cancer Research-UK funded preclinical research** since the evidence justifying inclusion of resveratrol in COLO-PREVENT-SS was generated through two successive Programme grants awarded by the Science Committee.
- Our **microbiome work** will link in with the **CRUK Grand Challenge OPTIMISTICC**, maximising the output from both projects and providing new opportunities to define how interventions might favourably modulate the microbiome to prevent colorectal cancer.

1.4 Provide a full description of the trial design.

Where applicable, please include details of the patient population including inclusion and exclusion criteria, study interventions, information about pharmacokinetics and pharmacodynamics, duration of treatment and follow up, primary and secondary endpoints and how these will be measured. Please also include justification for each chosen treatment and rationale for use of an agent(s) with the mechanism of action/target. If the study was reviewed at outline stage, please also comment on whether the design has changed since the outline application was submitted.

TRIAL DESIGN (see also Trial Schema and Schedule of Visits in Appendix 2 and 3)

The design of COLO-PREVENT is largely based on the seAFOod trial, which was the first CTIMP carried out in the English BCSP and established protocols and RCT infrastructure at screening sites[16]. Updates to the trial design submitted at outline stage have been made in response to feedback from the review panel and impending changes to the BCSP; these alterations are detailed in the accompanying letter to the panel.

***Patient population*:** BCSP patients between 55–71.5 years old (or 55–73.5 years for the Signal-Seeking trial), identified according to BCSP criteria as high-risk at the first complete screening colonoscopy. The upper age limit ensures patients can complete a 3 (or 1) year intervention and have an exit surveillance colonoscopy within the BCSP, for the main and Signal-Seeking trial, respectively.

General exclusion criteria for both trials:

Requirement for more than one repeat colonoscopy or flexible sigmoidoscopy within the BCSP 3-month screening window.
Malignant change in an adenoma.
Known clinical diagnosis or gene carrier of a hereditary CRC predisposition (FAP, hereditary non-polyposis CRC).
Previous or newly diagnosed inflammatory bowel disease.
Previous or planned colorectal resection.
Known bleeding diathesis or concomitant anti-coagulant or anti-platelet agent.
Severe liver or renal impairment.
Inability to comply with study procedures and agents.
Serious medical illness interfering with study participation.

Exclusion criteria for the main trial but not the resveratrol Signal-Seeking trial:
Regular (>3 doses per week) prescribed or 'over the counter' (OTC) aspirin or regular (>3 doses per week) prescribed or OTC non-aspirin NSAID use.
Allergic or intolerant to ibuprofen or naproxen, metformin, aspirin or salicylate.
Diabetic patients on drug treatment.
History of peptic ulcer disease.
History of vitamin B12 deficiency or megaloblastic anaemia.
History of lactic acidosis.
Prior use of NSAIDs is not an exclusion if they are self-prescribed and the patient is willing to stop use for the duration of the trial.
Additional exclusion criteria for the resveratrol Signal-Seeking trial only:
Unable to abstain from ingestion of OTC supplements containing resveratrol for the trial duration.

STUDY INTERVENTIONS: The main trial will be open label and patients will be randomised to aspirin alone or aspirin plus metformin. The aspirin dose will be adjusted according to bodyweight such that individuals weighing <70kg will take 75 mg daily and those ≥70kg will receive 300mg daily[28]. This higher dose of aspirin was well tolerated in the seAFOod trial with no safety concerns[17]. Despite the good safety profile of metformin, we are conscious that our target (healthy) population may have concerns over side effects, and experiencing GI symptoms within the trial could have an impact on compliance and retention. Indeed, dose reduction of metformin (from 1700 mg to 1000mg daily) has been necessary due to GI symptoms in the ongoing ASAMET (NCT03047837)[77] biomarker trial of aspirin and metformin in patients with previously resected CRC (DeCensi, personal Communication). We have taken this experience into account, along with the recommendations of our PPI members in selecting the dose of 500mg twice daily for COLO-PREVENT. If GI toxicities occur patients will be managed by dose reduction and/or a switch to a sustained release (SR) preparation according to the algorithm followed in the STAMPEDE trial. In the Signal-Seeking trial, patients will be randomised to resveratrol at a dose of either 5mg or 1g daily, or placebo.

PRIMARY ENDPOINT: Adenoma recurrence measured by MAP. One of the key conclusions from the seAFOod trial was that future polyp prevention studies should use the total number of adenomas per person (MAP) as the primary outcome since it offers greater sensitivity than ADR[34]. This is justified on the basis that colorectal adenoma number predicts future CRC incidence and mortality in observational studies, and that aspirin reduces adenoma multiplicity in all randomised controlled trials that used adenoma number as a secondary outcome[25, 78, 79].

SECONDARY ENDPOINTS:
Adenoma detection rate (ADR, proportion of individuals with one or more adenomas at surveillance), advanced adenomas (measured as MAP and ADR), adenoma subtype based on histopathology (conventional/serrated) and **location**. Differential preventive effects of aspirin have been described in the seAFOod trial with respect to adenoma location and subtype[34]. Our preclinical evidence also suggests that different doses of resveratrol may be needed to optimally target different adenoma subtypes[7] (Figs. 3-5). Defining and confirming this selectivity will be important for identifying the best combinations of therapies for future implementation at either an individual or population level.
Adverse events, including clinically significant bleeding episodes and GI tolerability.
Assessment of compliance. Compliance will be assessed in a proportion (20%) of patients using established HPLC-UV and HPLC-MS/MS assays for urinary resveratrol[63, 80] and plasma metformin concentrations[81, 82]; aspirin compliance will be examined using serum thromboxane B2 levels[83].
Accounting for dietary consumption of resveratrol. Although resveratrol is a dietary constituent, it is found in only a limited number of edible foods, predominantly grapes, grape juice and wine. Dark chocolate, peanuts, pistachios, certain beers and some varieties of berries also contain resveratrol but at extremely low concentrations, or they are rarely consumed, therefore have little contribution

to total intake. Indeed, it has been shown that wine, particularly red wine, accounts for over 92% of resveratrol and piceid (resveratrol glucoside) consumed and it is estimated that European dietary intake of these two compounds combined is in the region of 0.3-1 mg/day, with highest levels typically in traditional wine-producing countries[84]. Consequently, dietary consumption of resveratrol by patients in COLO-PREVENT is unlikely to have a significant impact compared to the doses used in the trial, which are at least ~5 and 1000-fold higher.

For COLO-PREVENT to be successful the trial design must be pragmatic and applicable to a real world setting; given the 12 month duration we, and our PPI representatives, do not consider it practical to restrict dietary consumption of foods/drinks containing resveratrol as it could have a major effect on recruitment and compliance. To ensure that dietary resveratrol is not a confounding factor and examine whether individuals change their behaviour as a result of participating in the study we will compare intake across trial arms and also at baseline and during the intervention. This will be achieved through the use of Phenol-Explorer, a database containing food composition information on all known polyphenols which also takes into account the effects of cooking and processing and can be used to estimate acute and habitual resveratrol intake from a 24 hour dietary recall or dietary questionnaire[85, 86], respectively. This work will be supported by Dr Augustin Scalbert (International Agency for Research on Cancer, Lyon), who established the database and has applied it to several resveratrol epidemiology studies (see accompanying letter of support).

COLO-PREVENT as a sustainable platform

As a team, we are experienced in the operational aspects and data management challenges associated with the addition of new arms to adaptive trials such as FOCUS4 and STAMPEDE[87, 88]. We will establish guidelines and an assessment matrix for selecting and prioritising new interventions, to ensure there is sufficient weight and standard of evidence to justify inclusion (subject to funding). In the future, we expect that promising interventions will be identified and proposed primarily through the UK Therapeutic Cancer Prevention Network, although we will also be open to dietary/exercise-based interventions (such as the low energy liquid diet proposed in the LEAP trial application under consideration by CRUK) and rational combinations. Furthermore, COLO-PREVENT is the ideal platform for testing the preventive efficacy of innovative interventions developed through the OPTIMISTICC Grand Challenge, aimed at altering the gut microbiome. We acknowledge that presently the multi-arm multi-stage (MAMS) methodology does not allow rate outcome measures such as MAP, but development of the software is in progress and would be available by the time we were in a position to drop or add arms to COLO-PREVENT.

1.5 Is age an inclusion/exclusion criterion?

If it is, please provide specific justification for both upper and lower age limits. Please note that if a lower age limit for studies involving adults is deemed essential it should normally be set at 16 rather than 18 years. Low incidence of patients aged 16 and 17 is not sufficient reason alone for selecting a lower age criterion of 18 years. Similar consideration should be given to the use of upper age boundaries for studies involving children.

Eligibility for inclusion is dictated by the age range covered by the Bowel Cancer Screening Programme, which is currently 55-75 years. To ensure that participants complete their surveillance colonoscopy at the end of a 3-year intervention within the BCSP, the upper age limit for entry into the main trial will be 71.5 years. In the signal-seeking trial the upper age limit can be increased to 73.5 years, since this involves a shorter, one year intervention.

1.6 Provide a full description of the statistical analysis plan for the trial.

Please include information about and justification of the power calculations, the sample size, stratification factors, randomisation ratio, interim analyses, go/no go criteria and early stopping rules. Statistical members of our Expert Review Panels will attempt to recreate your sample size calculations so we strongly recommend you provide sufficient information for them to be able to do so. Please note that computer code can be uploaded as an appendix. Please also describe other trial designs that were considered together with reasons why they were rejected during the development of the trial.

Sample Size: For all sample size calculations we have assumed a negative binomial distribution[17, 89], with over-dispersion of 2.0 and used equation (8) from Cundill & Alexander[90]. Replication of the sample size found was done using the PASS 16.1 software[91]. Detailed information can be found in the uploaded Stata 15.0 code. We have designed COLO-PREVENT with impending changes to the BCSP at the forefront. Specifically, we have factored in changes to the surveillance guidelines, which mean high-risk patients will have their first surveillance colonoscopy at 3 years instead of 12 months. Additionally, the risk classification is changing such that the new high-risk group will be composed of individuals that currently fall under this category, plus a proportion (~50%) of the current intermediate patients, who already have their first surveillance colonoscopy at 3 years. A consequence of these changes is that real data do not yet exist on MAP for the new high-risk group; therefore, we have made use of the most relevant data available and made a number of reasonable assumptions as described below.

For the main trial, The primary analysis aims to show whether the combination of aspirin with metformin leads to clinically meaningful reduction in the MAP as compared to aspirin alone. Data from the aspirin arms of the SeAFOod trial[17] and 1st surveillance in the BCSP (2006 -2016, 1 year MAP for high-risk patients and 3 year MAP for intermediate risk patients) have been provided (M Rutter, personal communication). Assuming a MAP at 3 years in the untreated population of 1.2 polyps, this leads to a control MAP of 0.94 in the standard of care arm, after accounting for a 22% reduction in total MAP due to aspirin use, based on the SeAFOod data[17]. This trial is powered to detect a reduction of 30% (0.66 vs 0.94) in the MAP of the combination arm, relative to aspirin alone. We estimate a sample size of 646 (323 per arm) would have 80% power at the 2-sided significance level of 5%. Allowing for 15% dropout at 3 years and crossover of 3% in each arm, the target sample size has been inflated to 646/0.85 = 760*~1.13 = 862 (431 per arm) individuals who will be randomised equally (1:1 ratio) to each treatment arm, with stratification for BCSP Centre. We have predicted the dropout rate based on the 10% value reported for the one-year seAFOod trial[17] and have increased this figure to 15% due to the longer intervention period in COLO-PREVENT; this rate is in line with previous aspirin polyp prevention trials that involved a ≥3 year follow-up[10]. Crossover accounts for participants who have to stop taking metformin completely due to side effects, and also patients who are diagnosed with T2DM during the course of the study and are prescribed metformin. Assuming a conservative but realistic acceptance and eligibility of 30% for the trial, a total of ~3200 high-risk individuals need to be identified at screening colonoscopy.

For the resveratrol signal-seeking phase II trial, comparing two doses of resveratrol against a placebo control group, the patients will be a combination of those taking aspirin and those not taking aspirin, so we have a used a pooled average MAP at 1 year from the aspirin groups in seAFOod and the untreated population from the BCSP (2006 -2016) data, and estimate the MAP in the control arm for this trial to be 1.2. As this is a signal-seeking study, we will aim for a 2-sided 10% significance level but half this to 5% to account for multiple testing of 2 doses against control. We aim for 80% power to ensure a high probability of detecting a larger Signal-Seeking reduction in MAP for this group of 35%, which will lead to a MAP of 0.78. We are aiming to recruit 596 (199 per arm) which includes a 10% drop out at 1 year. Therefore 596 individuals will be randomised equally (1:1:1 ratio) to the three treatment arms with stratification by BCSP Centre and according to aspirin or metformin use, ensuring an even distribution across arms of these factors. Assuming a 50% acceptance and eligibility rate for this trial, a total of ~1200 high-risk individuals will need to be identified at screening.

For both studies the total number of high-risk individuals needed for screening is 4400, of whom we anticipate 75% will not be taking aspirin or metformin and so will be eligible for the main trial. We predict that the remaining 25% will be intolerant or already taking aspirin/metformin and would be offered entry into COLO-PREVENT-SS.

Note that the overall predicted MAP value for the new HR group in the BCSP could be underestimated as we are using the one-year MAP for the current high-risk patients for three year MAP predictions, since data do not currently exist for recurrence at three years in this population. Furthermore, when factoring in the contribution from the current intermediate-risk patients we are using the average three-year MAP for the whole group, whereas only a proportion of these patients will be incorporated into the new high-risk classification. Once the new guidelines are in place, after three years we will apply to the BCSP Research Advisory Committee to access the emerging data from the control BCSP patients to see how the real MAP value compares with our predicted value and make any adjustments to our sample sizes; the findings will be made available to the IDMC. The tables in Appendix 4 illustrate how the trial would be overpowered if our predicted figure is lower than the true MAP value for this new group of patients.

Statistical Analysis: The trial will be analysed and reported following the CONSORT statement for RCTs[92] and intention-to-treat (ITT) will be the main approach of analysis. A statistical Analysis Plan (SAP) will be written at the outset. The primary outcome will be analysed using a generalised linear model to estimate incident rate ratios between randomised groups, assuming a negative binomial distribution, MAP as the outcome variable and randomisation group as the explanatory variable. Results of comparative analyses will present point estimates, 95% confidence interval and P-value.

All secondary end-points will be analysed using the ITT population, with the exception of adverse events, for which we will analyse the safety population, consisting of all participants who received at least one dose of trial medication. Data on adverse events will be tabulated by randomised group. Logistic regression will be used to estimate odds ratios between randomised groups for adenoma detection rate, adenoma subtype and location. The relative recurrence of 'advanced' adenoma detected at the first BCSP surveillance colonoscopy will be analysed using log-binomial regression model with robust standard errors to estimate the log relative risk. The region of the colorectum where adenomas are detected at the first BCSP surveillance colonoscopy will be explored, possibly using a negative binomial random effects model with bivariate response (corresponding to polyp counts in the left and right colon) in which treatment and a baseline polyp count will be independent variables together with the random intercepts corresponding to patient and BCSP centre.

A per-protocol analysis will be conducted as a sensitivity analysis, where the per-protocol population consists of all randomised participants who were not deemed to have a protocol violation. Additionally, some BCSP centres consist of multiple hospitals (sites), therefore an additional sensitivity analysis will be conducted in which both BCSP centre and site will be treated as random effects in a multi-level model. Drug adherence will be monitored and if adherence is considered to be poor, alternative analysis methods such as Complier Average Causal Effect (CACE) analysis will be considered.

There is no planned interim analysis for the main trial. For the Signal-Seeking trial colonoscopy at 1 year will be used as interim analysis of MAP for a 'stop/go' decision for each dose. The plan is to transfer the most effective dose to the main trial, subject to securing additional future funding. The result of the main trial will be analysed once the last patient has completed the 3 year colonoscopy (last visit).

1.7 Where applicable, describe the patient samples that you intend to use as part of this application.

Note that sample collection can only be included where it is either required to conduct the trial or where it directly underpins biomarker work included within this application (section 1.8). Please include details about and justification for the number and types of samples to be collected, the number of patients from whom the samples are to be collected, the time points at which samples are to be collected and evidence that they are fit for purpose. For imaging applications please comment on the type and number of scans you intend to collect. Please also describe how samples will be appropriately annotated to

ensure they can be used for future work. Relevant SOPs associated with sample collection, storage and processing should be included in the uploads section on eGMS.

Sample requirements mirror the blood and tissue that was successfully collected in the seAFOod trial[16, 34]. Established protocols are available for sample collection and processing and these will be reviewed and updated as necessary (see accompanying SOPs). In addition, we will collect three faecal samples from each patient using an gFOB test kit, at baseline (at least 2 weeks after the initial colonoscopy), part way through the trial (6 months or 1 year for the Signal-Seeking and main trial, respectively), and the end of each trial. Our validated protocols will be followed for faecal sample collection, storage and processing (see accompanying SOPs). Urine will also be provided from 20% of participants selected at random on three occasions in the Signal-Seeking Trial for analysis of resveratrol concentrations to assess compliance. **See Appendix 5 & 6 for information on sample numbers, evidence they are fit for purpose, and details of the assays that will be used.**

1.8 Where applicable, provide a full description of any biomarker work you will be conducting in association with this trial

Please include details of the research question(s), the experimental methods, techniques and analyses, including how the biomarkers will be measured and to what regulatory standard, and go/no-go decision points. You should also include details of the assay being used including the level of validation and provide relevant assay performance parameters (if available) such as sensitivity, specificity and positive predictive value, and intra- and inter-laboratory variability. For assay validation projects please provide a stepwise plan of validation. Please also provide details of any preliminary data supporting the approaches you plan to take.

EXPLORATORY ENDPOINTS: ***All the biomarker work proposed as part of this application relates to the following exploratory endpoints.***

1) We will test the hypothesis that metabolic status and dietary factors influence the effectiveness of aspirin, metformin and resveratrol, such that individuals with poor metabolic health may experience greater benefit. In breast cancer, changes in cell proliferation (measured by Ki67 labelling) in tissues obtained from pre-surgical window trials can be used as a predictive marker of efficacy for preventive therapies. In this context, metformin has been shown to have a heterogeneous effect on breast cancer cell proliferation in non-diabetic patients, depending on metabolic status. Metformin-associated significant reductions in Ki67 were only detected in subsets of women characterized by insulin resistance at baseline, defined by HOMA index>2.8, high C-peptide and IGFBP-3, and by low levels of free IGF-I and IGFBP-1. Studies in mice, including colorectal models, also suggest metformin has tumour suppressive effects where a metabolic phenotype of high caloric intake, metabolic syndrome, and diabetes exists, but offers no benefits under normal energy intake. This has similarities with resveratrol, particularly at low doses, where it protects against the tumour promoting effects of a high-fat diet in mice.

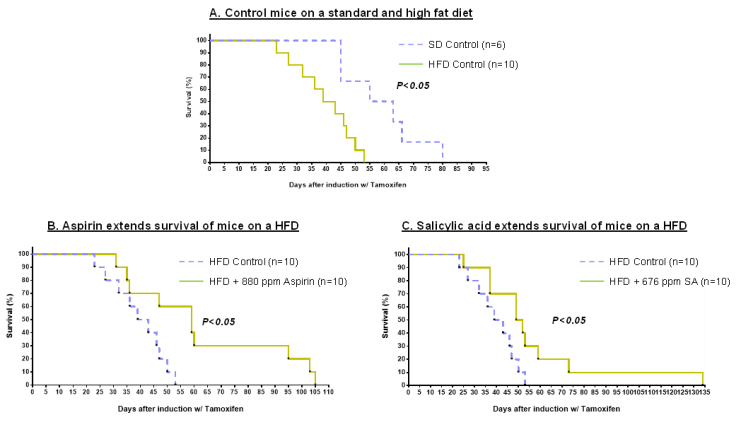

Figure 7. A high fat diet (HFD) reduces survival of Lgr5Cre^{ER}Apc$^{fl/fl}$ mice, but both aspirin and its metabolite salicylic acid protect against this effect. (**A**) HFD reduces survival of Lgr5Cre^{ER}Apc$^{fl/fl}$ mice, which represent a conditional model of Apc deficient CRC. However, when animals were given either aspirin (**B**) or an equimolar amount of its metabolite, salicylic acid (SA) (**C**), mixed in with the HFD, these drugs significantly extended their survival. Our results suggest an interaction between a HFD and the preventive efficacy of aspirin and that at least part of this activity is mediated through its metabolite SA.

Metabolic factors have also been implicated in influencing the CRC preventive effects of aspirin. Obesity is associated with substantially increased CRC risk in patients with Lynch Syndrome, and this risk is abrogated in those taking aspirin, but not placebo[93].

Furthermore, obesity was associated with ~4-fold greater CRC risk in patients MLH1 mutation, but no excess risk was observed in those with MSH2 or MSH6 mutation[93]. Our own studies in mice with *Apc* conditionally knocked out in the intestine have shown that aspirin and its metabolite salicylic acid extend the survival of animals on a high fat, but not standard diet (Fig. 7). Considering the potential for interaction between metabolic factors and/or diet and efficacy of the agents under investigation in the COLO-PREVENT platform, we intend to characterise the metabolic status and dietary patterns of participants at baseline, middle and end of both trials and examine associations with efficacy for each intervention. This will include determination of BMI, waist circumference and analysis of fasting glucose, insulin (HOMA), HbA1c, triglycerides, cholesterol, IGFBP-3, and free IGF-I, plus completion of EPIC Food Frequency Questionnaires (FFQs) and estimation of dietary fat content[94, 95].

2) We will examine the hypothesis that plasma drug concentrations and/or the metabolite profile correlate with efficacy for metformin and resveratrol. In the breast cancer window trial described above the heterogeneous response to metformin was associated with blood levels; for women with HOMA>2.8 metformin concentrations positively correlated with reduced anti-proliferative response[82]. Hence measurement of plasma drug concentrations may have value in predicting efficacy in cancer prevention trials or informing dose adjustments. Based on a clinical trial in patients with T2DM it has been suggested that metformin may interact with resveratrol metabolism, promoting generation of dihydroresveratrol by intestinal bacteria, which could influence efficacy[96]. Microbial metabolites represent an appreciable fraction of resveratrol species detected in humans[97] but current evidence, including our preliminary data, suggests they have considerably reduced anticancer activity compared to parent resveratrol (Appendix 7). Consequently, the plasma metabolite profile, including the proportion of human:bacteria-derived species may influence, and therefore correlate with, efficacy. Since a proportion of the patients in COLO-PREVENT-SS will be taking metformin there will be an opportunity to investigate the potential pharmacokinetic interaction between these agents and explore more widely the association between resveratrol metabolite profile and efficacy.

3) We will test the **hypothesis that combining aspirin and metformin leads to additive efficacy due to overlapping mechanisms of action and this can be detected through the measurement of pharmacodynamic biomarkers**. This is a pilot conducted with samples from 20% of randomly selected patients to ascertain if the drugs are modulating proposed targets. If the results suggest additive activity then the data will be used to inform the powering of a larger analysis in which correlations with preventive efficacy on polyp recurrence can also be explored (for which we will seek additional funding). We will focus on phosphorylated ribosomal protein S6 as the target of S6K1 and most robust downstream marker of AMPK activation and/or mTOR inhibition in normal rectal tissue of patients that received aspirin[38], as well as the NF-kB pathway. Apoptosis (cleaved PARP, measured by immunofluorescence) will be quantified as a functional endpoint for the effects of aspirin and metformin on these pathways. Activation of NF-kB is defined by nuclear translocation of the p65 subunit, therefore analysis will involve assessment of cytoplasmic and nuclear localisation of p65 by immunofluorescence. The ratio of phosphorylated:total S6 in normal tissue will also be determined using immunofluorescence. Therapy-associated differences will be investigated for the three biomarkers in normal tissue of all designated patients by comparing between study arms. Changes in p65, S6 phosphorylation status and cleaved PARP in adenomas will be determined for the proportion of patients (predicted to be $\leq$60%) presenting with a recurring polyp at 3 years by comparing results pre- and post-intervention and across groups.

Analysis of FFPE sections will be undertaken using a state-of-the-art Vectra Polaris multi-spectral imaging system, situated within the Leicester ECMC laboratories. This will allow simultaneous imaging and measurement of multiple overlapping biomarkers within a single tissue section via digital whole-slide scanning in both brightfield and fluorescence, revealing cell types, spatial context

and cell-to-cell interactions. This digital scanning capacity in conjunction with multi-colour Tyramide Signal Amplification (TSA) immunofluorescence, will allow us to quantitatively assess NF-kB activation, the degree of S6 phosphorylation and apoptosis in adenoma and normal tissue versus stromal areas by incorporating a pan-cytokeratin mask in conjunction with p65, pS6/S6 and cleaved PARP. Digital pathology outputs will be complemented via extensive use of R script, allowing data analysis and visualisation to be undertaken using multi-parameter approaches, engaging machine learning to effectively mine complex datasets.

4) We will test the hypothesis that resveratrol pharmacodynamic biomarkers identified in our preclinical studies as correlating with efficacy in mice, translate to the clinic. Specifically, NQO1 protein expression and protein carbonyl levels in normal tissue, plus Ki67 labelling index in both normal and adenoma tissue[7, 62], and plasma MLX and CIDEB protein concentrations (see Appendix 1 for preliminary supporting data on CIDEB) will be analysed across treatment groups and over time in COLO-PREVENT-SS and associations with efficacy examined. CIDEB is a lipid droplet-associated protein that regulates fat metabolism and has a role in apoptosis. Analysis methods are indicated in the Biomarker Tables in Appendix 5 and 6.

5) We propose that the **interventions used in both trials of the COLO-PREVENT platform favourably alter the gut microbiome in a way that might contribute to efficacy.** We will test this hypothesis by first comparing the baseline microbiome of the high-risk trial participants to our current data sets from BCSP patients, to confirm and refine our findings of a characteristic microbiome within these individuals. We will then ascertain the development of systematic changes in the microbiome caused by aspirin, metformin and resveratrol and explore the potential contribution of these therapy-induced microbiome changes to any efficacy observed, in terms of reducing adenoma recurrence.

The bacterial populations present will be assessed by next generation sequencing of the 16S rRNA V4 region using either our standard robust methodology on gFOBT cards or, if validated, on the new faecal immunochemical test (FIT) selected by NHS England for use in the English screening programme. Patients will provide baseline faecal samples after consenting at their post colonoscopy assessment visit which occurs ~2 weeks post colonoscopy; this is prior to starting their study medication, which will commence the next day. The interval has been selected as there is strong evidence the microbiome will have returned to baseline after the bowel preparation[98, 99]. Further samples will be donated at 1 and 3 years in COLO-PREVENT and at 6 months and 1 year in COLO-PREVENT-SS. Changes in the microbiome will be characterised by examining differences in alpha[100] and beta[101] diversity between sample groups. Bacterial taxa associated with phenotypic differences will be assessed using linear discriminant analysis[102]. Residual extracted DNA from the stool will be saved and subsequently tested for specific oncomicrobes or toxin genes should important new data emerge within the timeframe of the study.

1.9 Where applicable, provide a full description of the statistical analysis plan and bioinformatics plan for any biomarker work you will be conducting as part of this application.

Please include details about and justification of the power calculations, the sample size and any stratification factors used. It is also suggested you state the primary research question(s) that the main statistical analysis will address and include information on 1) the biomarker data that will be used to answer the question i.e. name the variables and describe the values 2) the clinical data that will be used to answer the question i.e. name the variables and describe the values and 3) the statistical analysis that will be undertaken using the variables specified. You should state the numbers of samples to be included in each of the analyses specified and describe what can be achieved with this number, including as appropriate the associated level of statistical power and any potential limitations. Please also provide other relevant details, either actual or expected, such as prevalence rates for the biomarkers, numbers of events in the clinical outcomes and length of follow-up for clinical outcomes. Statistical members of our Expert Review Panels will attempt to recreate your statistical calculations, so you must provide sufficient information for them to be able to do so. Please note that computer code for bioinformatics work can be provided as an appendix.

The main hypotheses underlying the biomarker analyses for both trials is that biomarkers of metabolic state will influence efficacy and there will be correlations between specific measures (or combinations of measures) and preventive efficacy (MAP) of the interventions, which may have potential as pharmacodynamic biomarkers that can be monitored over time as surrogates for efficacy.

The parameters to be explored are described in Section 1.8 above. For **hypothesis 1** these will be molecular (glucose, insulin (HOMA), HbA1c, triglycerides, cholesterol, IGFBP-3, free IGF-I) and physical (BMI, waist circumference). **Hypothesis 2** concerns measurement of plasma drug/metabolite concentrations for metformin and resveratrol. **Hypotheses 3** and **4** relate to pharmacodynamic biomarkers for the main and Signal-Seeking trial, respectively, namely p65, pS6/S6 and cleaved PARP for metformin/aspirin and NQO1, protein carbonyls,Ki67, MLX and CIDEB for resveratrol.

All the proposed biomarker work is exploratory and therefore no sample size estimates have been given. The type and number of samples collected as part of the biomarker work are shown in Figure 8, Section 2.5 and are described in detail in Appendix 5 & 6.

The broad aim of each of the research hypotheses is to investigate the prognostic and predictive value of each of the biomarkers with respect to the primary outcome of mean adenoma number per person (MAP).

The prognostic and predictive ability of each of the biomarkers will be explored univariately on the primary outcome measure (MAP), overall and in relation to each treatment comparison, as specified in the hypotheses. Generalised linear models, assuming a negative binomial distribution, will assess each biomarker as prognostic of MAP and then by inclusion of a treatment biomarker interaction term to assess differential treatment effects across the biomarkers. Inclusion of adjustments in the models for other demographic and clinical variables will be considered in line with the main trial analyses. Consideration of transformations using fractional polynomials or parametric modelling will be given to continuous measures. Graphical representations will be made for each biomarker in scatter plots exploring visually the correlation between biomarkers and MAP overall and by treatment arm. Exploration of categorisation of these variables into a dichotomous representation of the biomarker will enable forest plots of treatment effects within these groups and estimate heterogeneity between groups.

1.10 Do you plan to do any biomarker work associated with this trial that is not part of this funding application?

If yes, please briefly describe and state where you intend to obtain funding from for these studies.

Long-term plans for translational studies associated with this trial include: **1)** The Identification of blood-based signatures that correlate with response to aspirin, metformin and resveratrol by conducting transcriptomic, proteomic and metabolomic profiling of white blood cells/plasma and comparing patients that present with recurrent polyps at the end of the intervention period versus those that do not. The signatures would be used for personalising prevention by monitoring response over time and distinguishing at an early stage, individuals who are likely to benefit and should continue on therapy from those that should stop or switch to an alternative. **2**) We would also intend to perform hypothesis-driven analysis of candidate predictive biomarkers of response; for example, high expression of 15-hydroxyprostaglandin dehydrogenase (15-PDGH) in normal colorectal tissue reportedly predicts stronger preventive effects of aspirin[103] and metformin cellular uptake is influenced by gene polymorphisms in membrane transporters[44]. Other promising biomarkers emanating from the ongoing ASCaP catalyst project would also be incorporated. **3)** As part of the COLO-PREVENT application we will assess the effects of each intervention on different polyp subtypes, based on current histopathology classification. However, it is inevitable that in the

coming years the classification of colorectal adenomas will be refined with the use of more sophisticated models, artificial intelligence and inclusion of mutational information[30, 104]. We will therefore seek to further delineate the associations between efficacy and subtype using new approaches so that rational combinations of preventive therapies can be designed with broad efficacy across subtypes. **4)** We will also conduct long-term follow-up of participants within the BCSP as an exploratory analysis to investigate long-term effects of the interventions.
We would anticipate applying to CRUK for these areas of work and will seek advice on the most appropriate funding schemes.

1.11 Provide details of how the results of this study will be used.

For example, will there be a follow-on study? What further steps will be required after you have completed your proposed work prior to clinical implementation, or do you anticipate that the results would lead to a change in practice?

We anticipate that by conducting this trial we will minimise any uncertainty in implementing aspirin as the definitive therapy for polyp prevention in this patient population and will consolidate aspirin as standard of care. The data would justify a review of the NICE Clinical Guidelines for Colorectal Cancer Prevention and we would contact NICE to ascertain when the next update is due and/or contact the Guideline Development Group to make our findings available as part of this review.

If metformin was shown to provide additional benefit when combined with aspirin we would plan to implement its use clinically, but with ongoing toxicity review, which could be done as a phase IV trial in primary care or within endoscopy units.

If either dose of resveratrol was shown to reduce polyp recurrence in the Signal-Seeking trial, this would justify translation to the main trial for full assessment of efficacy over three years. We could envisage testing resveratrol versus aspirin alone and the combination, if the mechanistic results or location/sub-type specificity outcomes from COLO-PREVENT suggested combining the two might be advantageous. The aim of such a trial would be to ascertain whether resveratrol can serve as an alternative to the standard of care for those individuals who cannot tolerate aspirin and whether it provides additional benefit when taken with aspirin.

1.12 Provide details of any input to this study from the relevant NCRI Clinical Studies Groups (CSGs), NCRI Advisory Groups, CTRad or CT-PAG.

We strongly recommend approaching the relevant group(s) for their comments on the proposal before submission.
Please note that the groups typically meet twice a year so you must approach them in good time. A letter of support will usually only be provided if your study has been discussed formally at a meeting of the group, or the relevant sub-group. To engage with the Clinical Studies Groups, please contact. To engage with CTRad, please contact. To engage with CT-PAG, please contact cmpath@ncri.org.uk.

The COLO-PREVENT platform has been presented at a meeting of the NCRI Colorectal Screening Prevention and Early Diagnosis Advisory Group, chaired by Professor Colin Rees. The trial concept received strong support and was judged deliverable through the BCSP (see letter). It was also discussed at a meeting of the UK Therapeutic Cancer Prevention Network (K Brown and R Langley are Co-chairs) where there was very strong support for using aspirin as a standard of care comparator and much enthusiasm for inclusion of the combination arm (see letter). COLO-PREVENT has also been presented at the CRUK Prevention Trials Unit Bowel Cancer Workshop (Barts, QMUL) and NCRI Colorectal Cancer CSG Trials Meeting in March.

Additionally, COLO-PREVENT was well received when it was presented to the NCRI Colorectal CSG. The only criticism was that they wanted us to include sites from Scotland and Wales. We have initially focussed on the English BCSP and the 53 sites that were involved in seAFOod, due to the existing infrastructure and experience of staff at these locations, but we would aim to include sites from Wales within the additional 7 sites needed for COLO-PREVENT. Scotland is currently less amenable to inclusion because surveillance colonoscopies take place outside of the Scottish Bowel Screening Programme (SBoSP). Furthermore, the SBoSP has less QA control and the colonoscopists

do not require additional accreditation to participate in the programme as they do in the English BCSP. A recent comparison of the adenoma detection rate, the most widely used metric for assessing colonoscopy quality, has shown significantly lower rates in the SBoSP compared to the English BCSP[105], therefore, inclusion of Scottish sites could impact the results of COLO-PREVENT. We will monitor the situation and if anything changes that makes it feasible to include sites in Scotland we will explore this possibility.

1.13 Provide up to 10 key words to describe the study.

Cancer prevention, colorectal, aspirin, metformin, resveratrol, colonoscopy, adenoma, polyp, metabolic, microbiome

2. STUDY DELIVERY

2.1 Describe how the specific expertise of the study team will facilitate successful delivery of the proposed study.

Please include information about how your knowledge and experience, and that of the team you have assembled, will help you to deliver the study. You may want to include evidence of successful study delivery of similar studies by you or members of your team. You must include a statistician in the study team. Where biomarker work is included in your application, it is strongly recommended that the team includes members with both clinical and translational experience, and it is expected that a statistician with translational expertise will be included in the study team. If you intend to collect samples as part of this study, then a pathologist must also be included. The statistician and, if relevant, the pathologist must also be named as supporting roles on eGMS.

To deliver COLO-PREVENT we have assembled an exceptional multidisciplinary team with patient involvement at the heart, consisting of experts in colorectal cancer prevention, large scale nation-wide therapeutic prevention trials and interventions within endoscopy units (including seAFOod, Add Aspirin and BADENOMA), and design of complex adaptive platforms (e.g FOCUS4).

Prof Karen Brown (Lead) is a translational scientist with a focus on therapeutic prevention and international expert on resveratrol and its use in the treatment and prevention of cancer. She has led several early phase trials to establish its safety, clinical pharmacokinetics, pharmacodynamics and distribution in target tissues. **Prof Anne Thomas** (Joint Lead and Chief Investigator) has been Chief Investigator on numerous Clinical Studies in the UK, spanning phase I-III, and is Director of the Hope Clinical Trials Unit in Leicester, which conducts a portfolio of approximately 100 studies annually and has ~5000 patient attendances per year.

Prof Mark Hull (Joint Lead, University of Leeds) will provide support in the design and delivery of COLO-PREVENT, using his invaluable experience gained as CI for the seAFOod Polyp Prevention Trial in the Bowel Cancer Screening Programme. He brings considerable knowledge of large randomised therapeutic prevention trials, including an understanding of regulatory approvals and pharmacy considerations specific to studies with re-purposed drugs and nutritional agents.

Lead and Co-Investigators occupy pivotal roles in UK endoscopy practice. The involvement of **Prof Matt Rutter** (Co-I) in particular, as the Bowel Cancer Screening Programme (BCSP) Research Lead and Chair of the National Endoscopy Database, will provide a direct link to the BCSP and access to key BCSP data to ensure we will be at the forefront of changes occurring in screening and prevention practice and will be well equipped to respond in a timely manner. **Prof Colin Rees** (Co-I, Newcastle University) will provide expertise in delivering collaborative multidisciplinary endoscopy and colorectal cancer research, and advice on service delivery. He also leads **COLO-SPEED North** which amounts to significant funding for regional research infrastructure in Endoscopy units. This framework will be used to support delivery of COLO-PREVENT across multiple sites in the North of England. This model will be extended throughout the UK and support national trial delivery and should ultimately increase capacity for recruitment to the COLO-PREVENT platform.

Statistical input will be provided by a local team at LCTU, with oversight of the design and statistical analysis by **Dr Louise Brown** (Co-I, MRC CTU at UCL), who has considerable expertise in complex adaptive platform trials. **Seid Mohammed** (Co-I, LCTU) will provide statistical input into study design and be responsible for managing and processing data, conducting the statistical analysis and preparing the report. **Cassandra Brookes** (Co-I, LCTU Principal Statistician) will be the statistical biomarker lead and responsible for trial statistics oversight locally.

Prof Ruth Langley (Co-I, MRC CTU at UCL) will contribute expertise in the design and conduct of complex clinical trials and knowledge of aspirin from her role as lead of the Add-Aspirin study, a long-term large-scale trial of aspirin for the prevention of cancer recurrence.

The patient and public perspective will be provided by **Dr Barry Sandywell** (Co-I, University of York), who is also on the BCSP Research Advisory Committee and has experience of contributing to projects directly relevant to our target patient population and the BCSP environment. Further PPI input will come from **Jacqui Gath** (Collaborator, Yorkshire and Humber CRN Consumer Research Panel) and **Mairead MacKenzie** (Collaborator, Independent Cancer Patients' Voice) who have both been involved in the development of COLO-PREVENT. **Dr Kyle Montague**, (Collaborator, Open Lab, Newcastle University) will provide expertise in human-computer interactions, accessibility and health technology and will support the PPI group in the development of digital and interactive tools for disseminating news about the trial.

Prof Phil Quirke (Co-investigator, University of Leeds) will provide expert histopathology support and advice on the collection/storage of faecal samples as well as overseeing the microbiome analysis conducted in his laboratory. He will also provide a direct link to the **CRUK OPTIMISTICC** (Opportunity to Investigate the Microbiomes Impact in the Science and Treatment of Colorectal Cancer) **Grand Challenge team** (see letter of support).

Support with metabolic phenotyping and dietary analysis will be provided by colleagues in the Diabetes Research Centre, University of Leicester, namely our collaborators **Prof Tom Yates**, **Dr Emma Baldry** (Senior Clinical Research Dietitian) and **Prof Kamlesh Khunti**, an internationally renowned expert on diabetes and primary care, who can also advise on the use of metformin. We will also benefit from the support of Dr Augustin Scalbert (**International Agency for Research on Cancer, Lyon**) who will advise on the use of Phenol-Explorer to estimate resveratrol dietary intake.

We will integrate with the existing **ASCaP CRUK Catalyst** award led by Prof. Jack Cuzick (see letter of support) and can use our sample set to validate aspirin-associated risk/benefit biomarkers emanating from ASCaP as well as taking advantage of new biomarker assays developed by the catalyst team. We will also collaborate with **Dr Farhat Din** (University of Edinburgh) who has an ongoing preclinical programme investigating the combined effects of aspirin and metformin for CRC prevention. She will identify new mechanistic targets and biomarkers that can be interrogated in our patient samples.

2.2 Provide a work plan outlining key milestones for study set up and delivery.

Use of a Gantt chart or other visual aid as an appendix is required. If applicable, please include HRA submission, first site open, first patient recruited, all sites open, recruitment completed, follow up completed. Please also highlight any aspects which may present challenges in trial set up, and details of how these will be overcome (e.g. radiotherapy or surgical quality assurance, contracts with industry partners, international participation). Where biomarker work is included in your application, please also include milestones for this aspect. Note that the earliest your grant can start is 2 months after the Committee meeting so please take this into account when setting out your work plan.

Milestone	Time post grant award letter	Predicted date
Grant start date	0	01/06/2020
Protocols & approvals	9 months	28/02/2021
First site open	10 months	31/03/2021
First patient first visit	11 months	30/04/2021
All sites open	35 months	30/04/2023
Recruitment completed for main trial	72 months	31/05/2026
Recruitment completed for SS trial	86 months	31/07/2027
Follow-up for SS trial completed	98 months	31/07/2028
Follow-up for main trial completed	108 months	31/05/2029
Close out and report SS trial	110 months	31/07/2029
Close out and report main trial	120 months	31/05/2030

Also see the accompanying Gantt chart that includes timelines associated with biomarker work.

2.3 Provide details of the recruitment plan and a graph of projected recruitment for the duration of the proposed study.

Please include how many patients will be recruited, how many centres will be involved, the duration of the recruitment period, evidence that recruitment is feasible (e.g. a pilot or feasibility study) and whether international collaboration has been considered. Please also comment on whether there are likely to be any issues or challenges with recruitment (e.g. patient acceptance or competing studies) and describe how these will be overcome. If your application is successful, our expectation is that the first patient will be recruited within a year of the Committee approving your study and the Year 2 Grant Award Letter may be delayed if this milestone has not been reached.

Recruitment plan: We plan to exploit the legacy of the seAFOod study utilising the infrastructure set up within the BCSP[16, 17]. We have been able to review the recruitment data from the study to robustly confirm the feasibility of recruitment into COLO-PREVENT. Moreover, we have planned for an increased number of study sites and with the efficient trial design can recruit patients into COLO-PREVENT–SS who are already taking/intolerant to aspirin or metformin. Excluding the 12-month run-in period, seAFOod recruited on average 16 patients per month (range 7-26) from 53 centres, which equates to 18% of the high-risk patients screened for enrolment (Appendix 8). A further 20% of this high-risk group were excluded due to existing aspirin/NSAID use or aspirin intolerance; these patients would all be eligible for COLO-PREVENT-SS. Therefore, with the wider eligibility, we realistically expect to recruit 30% of the high-risk patients, and by increasing the number of sites to 60, we anticipate being able to recruit an average of 30 individuals per month once all the sites are open and recruiting to both trials (Figure 8).

As explained in Section 1.6, it is likely that our prediction of adenoma recurrence at 3 years for the new high-risk group of patients under the updated BCSP criteria is an underestimate of the true value; this would mean our trial is overpowered (see Appendix 4). As 'real world' data become available for this new high-risk group, we will conduct a formal review (at ~48 months) of adenoma

recurrence (MAP) for control patients within the BCSP and compare to our predicted values. The findings from this review will be made available to the IDMC.

A significant feature of COLO-PREVENT is the stringent QA and unified practices for patients within the BCSP; as other screening programmes (including international) reach this level, we will seek to involve them to facilitate recruitment, as appropriate. In recent years ~8000 patients have been recruited to trials led by applicants Rees and Hull (DISCARD 2, ADENOMA, BADENOMA, seAFOod)[17, 106-108]. The BADENOMA trial successfully recruited 3,222 patients from 16 sites in just 9 months (9 months ahead of target)[106]. These studies represent faster recruitment than any other endoscopy trials worldwide (clinical trials.gov). The co-investigators occupy the pivotal roles in UK endoscopy practice, ensuring that research will be delivered. In summary, 1458 patients will be recruited from 60 centres to COLO-PREVENT and COLO-PREVENT-SS.

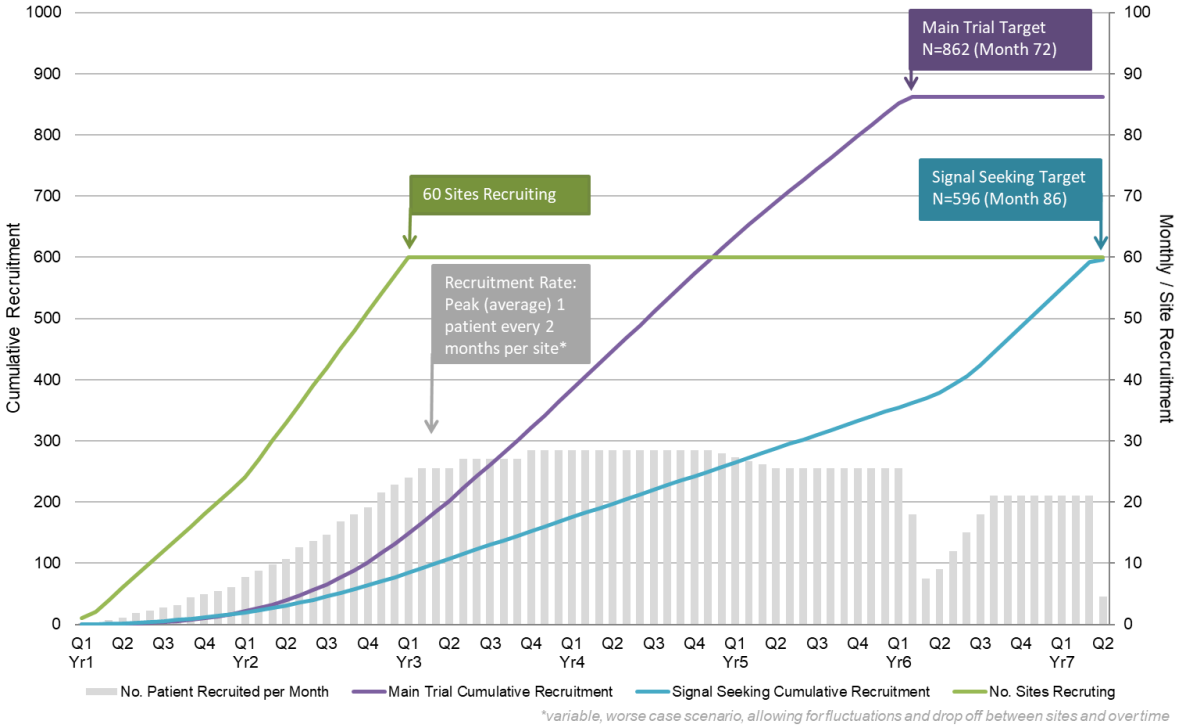

Figure 8. COLO-PREVENT predicted recruitment rate for both trials. The start of year 1 corresponds to month 11 in the timeline shown in Section 2.2 above.

Delivery: The trial will be run by the Leicester Clinical Trials Unit (UKCRC 43), with expert knowledge and experience in the design, conduct and analysis of randomised clinical trials and a track record in supporting complex intervention RCTs. It will take 10 months to set up and open the first site and a further 25 months to open all 60 sites. Recruitment to the main trial will be complete after an additional 37 months, and 51 months for the SS study. The SS trial will then require 12 months follow-up, whilst the main trial will continue for 2 more years to follow everyone up under the 3-year surveillance ruling. Participants in the Signal-Seeking trial will require an additional research colonoscopy at 12 months. Results from COLO-PREVENT-SS will be reported at the end of year 9, with the main trial reporting and closing down at 10 years. **Calculations and timelines are based on the assumption that recruitment will be solely through the BCSP** to ensure strict QA and unified practices for all participants. There are no planned competing studies in this space.

Future proofing the trial

Changes to the BCSP: As long-term infrastructure, it is vital that the COLO-PREVENT platform is adaptable, can deal with any future changes to the BCSP and will recruit the high-risk patients,

however they are defined. If we are to be successful, it is also essential that the trial protocol is pragmatic, aligns with standard practice, and does not introduce significant additional workload into the screening programme. We have therefore taken the decision to incorporate impending changes to the BCSP into the design of COLO-PREVENT now. We are uniquely positioned to assimilate these changes because we have assembled an expert team, including key figures in UK endoscopy practice who are leading the current BCSP reviews and have kept us informed of likely alterations during the development of COLO-PREVENT.

Changes will be across the following areas: **1**) **Substitution of FIT instead of gFOBT.** This could potentially alter the risk profile of patients undergoing initial colonoscopy, however, the aim is to set the haemoglobin threshold so that similar numbers of colonoscopies will be performed and we do not anticipate the roll-out of FIT to adversely affect the numbers of people eligible for COLO-PREVENT, in fact it is more likely to increase numbers. **2**) **Changes to the surveillance guidelines**; Matt Rutter (co-investigator) has led a review for BSG/ACP/PHE which recommends increasing the length of the surveillance interval for high-risk patients, such that the first surveillance colonoscopy will be extended from 1 to 3 years, which is in-line with European Society of Gastrointestinal Endoscopy Guidelines. Since we want to embed COLO-PREVENT within standard practice, this means a 3 year intervention in each arm of the main trial (the smaller Signal-Seeking trial will remain as 1 year to provide a quicker readout of efficacy). We consider this change to be a major positive as it would enable us to detect the combined effect on both the slower growing polyps, as well as those that occur within 12 months, to gain a measure of long-term preventive potential. **3**) **The risk classification** has also been reviewed and although still subject to final agreement, it is likely that the new high-risk group will be identified by the following criteria: adenomas ≥20mm or high-grade dysplasia, or serrated lesions ≥10mm or any dysplasia, or ≥4 polyps. This means that it will include individuals currently classed as high-risk, plus a proportion of those that fall into the upper end of the existing intermediate-risk group. Consequently, the overall colorectal neoplasia risk profile of the new high-risk surveillance group will be altered slightly; we have accounted for this in our statistical model by predicting the group will be composed of 69% high-risk and 31% intermediate-risk patients, and have factored in the associated different MAP values. This is based on the assumption that the new high-risk group encompasses all current high-risk patients, plus half of the intermediate-risk patients and has been calculated using the number of patients falling under these classifications from the BCSP data (2006 -2016). As indicated in Section 1.6, in using the most accurate and appropriate data currently available, we are most likely to be underestimating the MAP, such that the trial is underpowered.

Blinding: After careful consideration and discussion with our PPI members, we have opted not to include a metformin placebo in the main COLO-PREVENT trial and to conduct it non-blinded. This is primarily driven by the significant logistical and financial considerations associated with the formulation, packaging and labelling of placebos for academic trials of off-patent drugs, which would likely delay the trial and certainly increase the cost significantly (by ~£1M for supply/distribution of metformin and matched placebo). Looking to the future, a requirement for placebos would make it difficult on a practical level to add on new arms, as well as considerably less efficient. Consequently, we believe the advantages of performing the trial non-blinded outweigh the disadvantages, and have devised strategies to minimise risks. The BCSP is a quality assured system in which the ADR of each endoscopist is closely monitored and withdrawal times for each procedure are recorded. A survey of endoscopists conducted by Prof. Matt Rutter suggests bias at surveillance colonoscopy is unlikely to be an issue as the majority won't have any preconceptions around the benefits of each intervention, whilst the close monitoring, high standards and strict protocols in the BCSP means it is unlikely that endoscopists will modify their behaviour because a patient is in a particular arm of the trial. However, to diminish potential bias at the surveillance colonoscopy, we will blind Endoscopy staff and will instruct the participants and research staff not to divulge what arm an individual patient has been randomised to.

Since all patients in the main trial are receiving aspirin, self-medication with this drug is unlikely to be a significant issue. Metformin is a prescription-only-medicine and whilst use of OTC resveratrol containing supplements is a possibility, in the seAFOod trial there was little indication of self-medication for EPA, which is also freely available, based on red blood cell EPA levels in the placebo arms[17, 89]. **However, to mitigate against possible bias and compliance issues, we will operate a rolling testing programme of blood samples for our designated compliance markers (in addition to 'pill counting') and the IDMC will regularly review these data alongside the colonoscopy withdrawal times across treatment groups, which would flag up a potential change in Endoscopist behaviour.**

2.4 Provide details of all the centres involved in the study.

Please provide a signed letter confirming participation from the 5 -10 centres anticipated to be the highest recruiters in the uploads section on eGMS. It is strongly recommended that you ask PIs at the recruiting centres to engage with their local R&D office to confirm they will be able to participate in the study and provide details of the numbers of patients they expect to be able to recruit.

PI at centre	Centre name	Letter of support attached (Y/N)	Estimated no. of eligible patients per year	Expected annual recruitment	Expected total recruitment
Paul Dunckley	Cheltenham & Gloucester	Y	66	20	111
Keith Dear	Chesterfield	Y	36	11	60
Richard Robinson	Glenfield	Y	40	12	67
Deven Vani	Mid Yorkshire	Y	23	7	39
Alistair King	West Herts	Y	35	11	59
Richard Baker	Leeds	Y	25	8	42
James Turvill	York	Y	21	6	35
Ajay Verma	Kettering	Y	20	6	34
Colin Rees	Gateshead	Y	27	8	45
Alexandra Kent	Lewisham	Y	26	8	44
The above locations correspond to the top 10 recruiting sites in the seAFOod trial					
				Total recruitment:	**534**

2.5 Where patient samples are to be collected as part of this proposal, describe the sample acquisition plan.

Please include how many centres will be involved, the duration of the sample acquisition period, and evidence that acquisition is feasible (e.g. a pilot or feasibility study). Please also comment on whether there are likely to be any issues or challenges with sample acquisition (e.g. patient acceptance) and describe how these will be overcome. A graph or table of projected acquisition for the duration of the proposed sample collection should be included here or uploaded as an appendix.

We will collect blood (whole, plasma and serum), fresh tissue and FFPE diagnostic blocks, faecal samples and urine from all 60 sites over the full duration of recruitment and follow-up (97 months) (see Fig. 9 and Appendix 5 & 6). Our sample requirements replicate the blood and tissue samples that were successfully collected in the seAFOod trial. The seAFOod biobank contains 7323 clinical samples (16,258 aliquots). Samples were collected from 95% of participants, with 80% collected and stored per protocol. Furthermore, 73% or participants provided a full sample set. For the additional faecal samples required in COLO-PREVENT, the patients have already opted to participate in the BCSP, therefore they will be familiar with the sampling process. We are therefore confident

that we can collect and store all the samples needed for COLO-PREVENT and we do not anticipate any problems with patient acceptability.

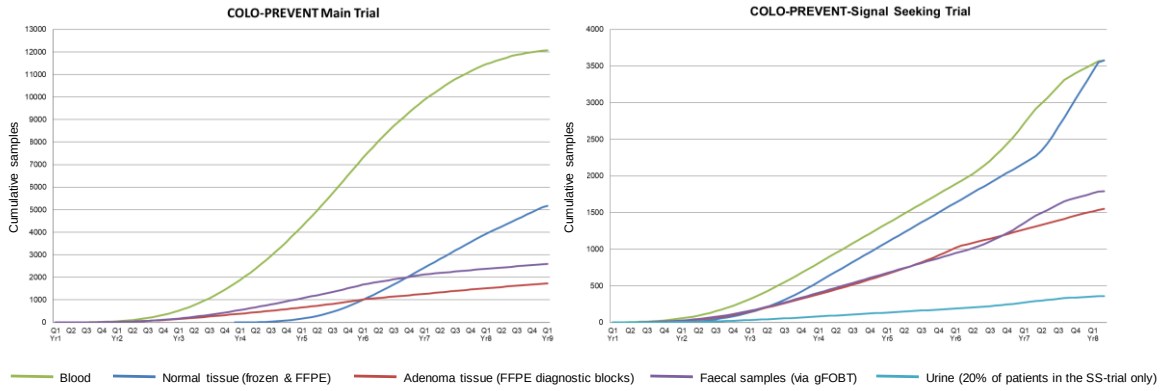

Figure 9. Predicted sample collection for the main trial and Signal-Seeking trial. The start of year 1 corresponds to month 11 in the timeline shown in Section 2.2 above. See also Appendix 9 for a larger version of this figure.

2.6 Where patient samples are to be collected as part of this proposal, provide details of the custodianship and day-to-day management of the sample collection and how you plan to support the long-term maintenance of samples collected.

The Lead and Chief Investigator will have overall responsibility for custodianship and use of all clinical samples collected in COLO-PREVENT for the duration of the study. Frozen plasma/tissue/urine samples will initially be stored at the individual sites in specified freezers then shipped in batches to Leicester where they will be stored in designated and secure facilities within the Leicester Experimental Cancer Medicine Centre (ECMC) Laboratories of the Cancer Prevention Group (CPG, Leicester Cancer Research Centre, Leicester Royal Infirmary). The laboratories operate under standards of Good Clinical Laboratory Practice (GCLP) and GCP and all samples will be collected, stored, processed and analysed according to SOPs. Frozen plasma, urine and tissue samples will be stored in temperature monitored -80^{o}C freezers. After retrieval from each centre, diagnostic FFPE specimens will be stored within dedicated areas within the CPG-ECMC laboratories. All samples will be barcoded, logged and tracked within a LIMS system. Patients will send faecal samples (as gFOBT cards) directly to the University of Leeds for batch processing and microbiome analysis and this laboratory will act as custodian for these samples. Samples may be shared with other researchers for work directly related to the objectives of COLO-PREVENT, or for additional translational studies approved by the trial management group. Approvals will be obtained to share samples and a Material Transfer Agreement (MTA) put in place. Samples will be kept for a maximum of 10 years and any surplus material may be used for future translational research. Samples may be disposed of in certain conditions, such as withdrawal of patient consent for storage and analysis, or use of the whole specimen.

2.7 Where patient samples are to be collected as part of this proposal, describe the tissue discoverability and access arrangements.

Please include details of the approximate timeframe within which it is expected that the collection will become openly accessible, details of the access policy, and a description of what resources are available for the processing of sample requests and distribution of samples. Note that it is expected that for any award involving sample collection, information regarding this aspect of the project is made publicly available on the UKCRC Tissue Directory within the first year of commencing the collection. We expect that samples are made available to facilitate high quality translational research, and you should confirm this will be the case. Whilst the questions you are aiming to address in your proposal can take priority, there should be clear mechanisms in place to facilitate access to the samples and associated clinical data by others. It is also suggested that you establish an Access Committee with independent representation and that you have a system for logging and responding to approaches.

Once samples have been used for analysis of the designated exploratory endpoints we will welcome requests for any surplus material from other researchers. In particular, we expect to exchange samples and data with the ASCaP Catalyst (led by Jack Cuzick) to maximise the combined output from these projects, as well as other members of the UKTCPN. Applications for use of the samples or access to data can be made to the trial management group who will evaluate the scientific quality in a timely manner and release samples to successful applicants under an MTA.

3. PATIENT AND PUBLIC INVOLVEMENT (PPI)

3.1 Provide a lay summary of the proposed study.

Please focus on the purpose of the study, the way(s) in which patients will be asked to participate and the main potential benefits and risks for patients.

One way of preventing bowel cancer is to take drugs or dietary supplements (this is called therapeutic prevention). Most bowel cancers develop over many years from a polyp. A polyp is a growth on the bowel wall; also known as an adenoma. Several clinical studies have shown that aspirin use reduces the risk of developing polyps and the drug metformin, which is used in patients to treat diabetes, can also reduce the number of polyps. We want to test whether combining aspirin with metformin is able to prevent more bowel polyps forming than aspirin alone. We will test this in 'high-risk' patients taking part in the National Bowel Cancer Screening Programme (BCSP), who have already had several polyps removed at the bowel camera test (colonoscopy). We are also keen to understand whether the dietary agent resveratrol which is found in red grapes, reduces the number of polyps formed. For this part of the study we will test two doses against a 'dummy' tablet in a way that both patients and medical staff do not know what treatment is being given. Patients already taking aspirin or metformin will be able to take part in the resveratrol sub-study. The resveratrol sub-study will be shorter and involve fewer patients than the main aspirin and metformin study because we are just seeking an indication that resveratrol might protect against polyp recurrence; if the results are positive then they would provide justification to conduct a longer-term study in the future with the most effective dose of resveratrol.

Trial drugs will be given for 3 years in the aspirin and metformin study and 12 months in the resveratrol sub-study, until patients have another planned BCSP colonoscopy, at which time the number and size of polyps will be measured. We will collect blood, faeces, urine and tiny samples of bowel tissue (biopsies) during the trial so that we can learn more about how the therapies work, as well as develop 'biomarker' tests to predict who will or won't respond to each therapy. We are particularly interested in examining the effects of the therapies on gut bacteria, which will be analysed using faecal samples. The expected benefit is that the therapies will reduce the number of polyps returning and therefore potentially the risk of developing a bowel cancer. A major advantage of metformin, aspirin and resveratrol is that they are safe, have few side-effects and are already widely used by people with diabetes, heart and/or stroke disease or as a dietary supplement.

3.2 Describe how PPI has contributed to the research question and rationale, study design, plans for continuing PPI in the governance, management and delivery of the study, and how PPI will contribute to dissemination plans.

For example, you may have engaged with patient groups, sought input from consumer representatives on the relevant NCRI CSG(s), or appointed a patient representative to your trial management group.

Patient and public representatives have been involved in COLO-PREVENT since the very first meeting when the concept was proposed ~4 years ago. We have three PPI members fully engaged and contributing to the project. They have participated in discussions on all aspects, ranging from technical issues relating to drug dosage/pharmaceutical formulations to ethical issues about patient experience and maintaining involvement. They have informed many features of the design, for example, they suggested we should not request a change in patient behaviour by stipulating

participants exclude resveratrol containing foods/drink from their diet in the signal-seeking trial, and they supported the use of a lower metformin dose in this healthy population to reduce the chances of gastrointestinal side effects. Furthermore, given that the trial requires long-term interventions which are not blinded, they have highlighted the need to fully engage patients, creating an ethos of participation, with an emphasis on active-and-continuous collaboration to maximise compliance. To achieve this environment the three PPI representatives led by Barry Sandywell (Co-investigator) with Mairead MacKenzie and Jacqui Gath (collaborators) will assemble a wider PPI panel of study-naïve people to provide continuous input to the trial and assist in devising a communication and dissemination strategy. There is no doubt that during the study, digital platforms will evolve, therefore Kyle Montague (Open Lab), will provide expertise in the use of digital and interactive tools that empower individuals to feel part of the research with relevant news updates.

3.3 Describe how patients participating in the study and/or their families will be able to find or obtain the results.

We propose to use a variety of strategies that we have successfully delivered in other studies to disseminate results of the trial, both during and at trial end. These include YouTube videos and digital abstracts in lay language to accompany publications and newsletters. Through Leicester ECMC, patients have enjoyed meeting for 'result-sharing' evenings; these also provide opportunities for focus group meetings to develop any emerging patient related themes. To develop this model nationally, we propose setting up 'result sharing' through virtual meetings using webinars. All of the digital platforms will be accessible through the COLO-PREVENT Website.

4. JUSTIFICATION OF SUPPORT REQUESTED

4.1 Provide details of the roles of the staff to be employed on the grant, including clear justification for each post.

You should include information about the purpose of each post and an explanation of any changes in %FTE in different years.

Leicester Clinical Trials Unit (LCTU): The trial has been costed for full support by LCTU, which includes trial management, statistics, database provision, data management and randomisation.

- ***Grade 7 Research Statistician (96mths, 30% FTE)*** responsible for SAP preparation, input into protocol and CRF development, data validation and randomisation checks, preparation of reports to DSMC, TSC and Trial Management Group meetings, day to day statistical support. In the last 24 months, the statistical input increases to ***66% FTE*** reflecting the cleaning and final analysis of the two trials and generation of the final report.
- ***Grade 5 Database Developer (18mths 100% FTE)*** to assist the senior trial manager with the design of the CRF and then to build, test and validate the two trial MACRO databases, support access to the MACRO system by the 60 participating sites. Thereafter ***(90mths 20% FTE)*** to provide continued support for the duration of the trial i.e. generation of data query reports, undertaking database amendments and data extractions as required by the trial statistician. In the final ***12 months this reduces to (0.15 FTE)*** reflecting the decrease in activity.
- ***Grade 6 Trial Database Development Manager (120mnths 10% FTE)*** to provide oversight and quality management of the Database Developer, ensuring project milestones are achieved.
- ***Grade 7 Senior Trial Manager (120mths 100% FTE)*** planning and delivery of the trial entailing: CRF and protocol development and finalisation, obtaining the necessary regulatory approvals (HRA MHRA), set up of 60 participating sites to recruitment, providing study-specific training to participating sites, monitoring and reporting of study progress to funder and oversight committees, act as the main contact point for the trial, work closely with the Chief Investigator and core trial team, troubleshooting, close down of sites and archiving of the trial.
- ***Grade 5 Trial Co-ordinator (42mths 100% FTE)*** to support the senior trial manager in the first 3 ½ years in the set-up of participating sites.

- ***Grade 4 Trial Assistant (66mnths 100% FTE)*** providing trial administration to the core trial team, servicing meetings, compiling monthly screening and recruitment data, trial master file maintenance and being the first point of contact for participating site queries. The support required will decrease to ***(42mths 60% FTE)*** once the participating sites are open to recruitment.
- ***Grade 8 Heads of Trial Management and Quality Management (120mnths, 5% FTE)*** providing trial oversight and quality management for the duration of the trial.

Leicester Laboratory staff

- ***Senior translational scientist (50% FTE*)** with extensive experience in the analysis of resveratrol/metabolites in clinical samples as well as developing and validating new analytical assays, ELISA, IHC and immunofluorescence protocols. She will be responsible for establishing and validating assays then conducting or supervising all the biomarker work in Leicester.
- ***Laboratory technician (100%, years 2-10)*** responsible for ensuring quality compliance for all aspects of laboratory work including validation reports and supporting biomarker analysis. During the period of maximum recruitment and sample collection, we request a ***second technician (50%, years 4-8)*** to help with the increased workload.
- ***Administrative support (40% year 1, 100% years 2-10)*** will set up the database system for incoming samples, and subsequently be responsible for receiving, logging and storing all clinical samples at central site. Further ***admin support*** will be required ***(50%, years 4-8)*** to help with increased workload.

Staff in Leeds for microbiome analysis

- ***A Technician (60%, years 1-9)*** will be required for receiving and logging all the faecal samples, extracting DNA for analysis and preparing samples for sequencing.
- ***Experienced Bioinformatician (25%, years 4-9)*** will be responsible for analysis of the sequencing data, interpreting and presenting the microbiome results.

4.2 Provide details of the purpose of each line of running expenses requested, including clear justification for each aspect.

You should include information about the purpose of each line of running expenses requested. Please note that running expenses should be clearly set out in the costs section of eGMS with a line for each different expense type. If you set out your running expenses in large broad categories (for example having a running expense line for 'Research Part a Costs') your application will be returned.

Leicester Clinical Trial Unit (LCTU)

Consumables: Funding has been requested to cover the cost of Trial Master Files and 60 Investigator Site files and trial related documents over the duration of the study. A sum for trial postage has also been included and costs associated with trial promotion including the production of a trial-specific website and for dissemination of the research findings.

Travel costs: have been requested to cover four investigator meetings and for the trial management team to visit sites with the expectation that the majority of site initiations will take place via teleconference.

Other Costs: Randomisation is provided by Sealed Envelope and we request funding for the set-up cost for a code list (double blinded study), amendments to randomisation and archiving. We have also specified a sum for set-up and testing of the system by LCTU. Licensing fees have been requested for the duration of the study for the MACRO database and the LCTU Q-Pulse quality management system. An amount has been included to cover MHRA fees and any subsequent amendments. Finally, an archiving cost has been requested to cover archiving of the trial master file for 25 years.

Laboratory costs:

Laboratory running expenses incorporate biomarker/safety analyses conducted through the NHS and central Leicester Labs. NHS analyses are critical to ensure patient safety throughout the trial,

and allow retrieval of material that will set the baseline for future research analyses. These running expenses include: retrieval of FFPE blocks; fresh tissue processing (block and save); lipid panels; glucose/insulin panels; liver/kidney function; urea and electrolytes; research colonoscopies for the resveratrol Signal-Seeking arm. General plasticware consumables are costed for all sites and are an essential component of the trials pack. FOBT samples will enable pre- and post-trial microbiome analyses to be undertaken.

Biomarker analyses are tailored specifically to research questions or will be analysed as part of exploratory endpoints likely to give new insights into mechanisms of action following intervention. Established analyses critical to ascertain trial compliance include levels of resveratrol and metformin, with 11-dehydro thromboxane B2 used as a measure of response to aspirin. Tissue/plasma biomarkers for the resveratrol Signal-Seeking trial include NQO1 and protein carbonyls, MLX and CIDEB, which will be assessed as a panel of pharmacodynamic biomarkers in addition to giving further insight into potential mechanisms of action of resveratrol. Immunohistochemistry and state-of-the-art fluorescence microscopy will be used to determine phenotypic endpoint markers of efficacy (Ki67/cleaved PARP) in addition to determining localisation and levels of proposed aspirin pharmacodynamic biomarkers such as NFkB. Microbiome analyses and associated bioinformatics will be outsourced to Leeds and funding is requested for DNA extraction, sequencing and PCR. All samples will be posted (where appropriate) or couriered back to the central labs on a 6-monthly basis from all sites where samples have accrued, ensuring that sample stability is suitably maintained.

NHS/Research (listed as other) costs will incorporate provision of the aspirin and metformin, plus resveratrol and placebo, which encompasses encapsulation, packaging, QP release and distribution. Pharmacy costs over the 10 years incorporate set-up, sponsor-related activity, amendment fees, additional support costs, placebo dispensing and an advisory services retainer. University of Leicester Sponsor costs will include both remote and on-site monitoring and audit programmes for the duration of the study, ensuring compliance and trial integrity.

PPI events are essential to engage with prospective patient cohorts, maintain engagement and feed back the findings and critical healthcare messages. Video clips accessed via social media platforms will facilitate engagement with a much wider audience and reduce overall engagement costs using more traditional face-to-face events.

4.3 Provide details of the purpose for each line of equipment costs requested, including clear justification for each aspect.

We request equipment costs at the **central Leicester lab** for sample storage, which covers one -80°C freezer, cryogenic gloves, racking systems and boxes for racking, and a real time alarm probe that can be added on to our current monitoring system, thus decreasing costs considerably.

Site-specific equipment: We will benefit from the equipment infrastructure provided by the SeAFOod trial at each original site, but anticipate a proportion of the 60 centres will need replacements, therefore, we have budgeted for 12 sets of essential equipment, each consisting of a benchtop refrigerated centrifuge, freezer, temperature monitor plus software and infrared transmitter, plus sample storage racks and cryogenic gloves.

Computers: We request funding for eighteen PCs in total – three for staff at the central Leicester lab, 3 for CTU staff, and 1 each to be situated at 12 BCSP sites (as described above) where extra equipment is required.

5. STUDY GOVERNANCE

5.1 Provide details of the membership of the Trial Steering Committee (TSC).

It is Cancer Research UK's policy that a TSC should be set up for all randomised trials. The membership should include an independent chairman, other independent members, chief investigators and ideally a PPI representative. Please use the table provided to give the names and affiliations of any members who have been appointed. If your application is successful, we'll ask you for full details of the TSC when you submit your first scientific milestone report.

Name	Affiliation	Role on TSC *(e.g. Independent Chairman, Independent Member, Chief Investigator, Member of Study Team)*
Prof Andrea DeCensi	Galliera Hospital, Genova, Italy & Wolfson Institute of Preventive Medicine, QMUL	Independent Chairperson
Prof Karen Brown	Leicester Cancer Research Centre, University of Leicester	Joint Lead
Prof Anne Thomas	Leicester Cancer Research Centre, University of Leicester	Chief Investigator and Joint Lead
Prof Mark Hull	St James's University Hospital, University of Leeds	Joint Lead, Member of Study Team
Dr Louise Brown	MRC CTU at UCL	Member of Study Team
Prof Ruth Langley	MRC CTU at UCL	Member of Study Team
Mr Seid Mohammed	University of Leicester CTU	Member of Study Team
Prof Colin Rees	Newcastle University	Member of Study Team
Dr Barry Sandywell	Member of BCSP Research Advisory Committee	PPI Member of Study Team
Prof Kamlesh Khunti	Leicester Diabetes Centre, University of Leicester	Member of Study Team
TBC	TBC	Independent Member
TBC	TBC	Independent Member

5.2 Provide details of the membership of the Independent Data Monitoring Committee (IDMC).

It is Cancer Research UK's policy that for all trials where the investigators are blinded to the data, an IDMC should be set up. Please use the table provided to give the names and affiliations of any members who have been appointed. If your application is successful, we'll ask you for full details of the IDMC when you submit your first scientific milestone report.

Name	Affiliation	Role on IDMC
Prof Gareth Griffiths	Southampton CTU, University of Southampton	Independent Chairman and statistician
Mangesh Thorat	Barts CTU, Queen Mary University of London	Independent Clinician
TBC	TBC	Independent Clinician
Seid Mohammed	University of Leicester CTU	Study Statistician

5.3 Please provide details of the EU Directive Sponsor, where applicable.

If the proposed study involves a medicinal product, please provide details of the sponsor as required under the EU Clinical Trials Directive.

University of Leicester

5.4 Please provide details of the UK Research Governance Sponsor.

If the proposed study does not involve a medicinal product, please provide details of the UK Research Governance sponsor.

University of Leicester

6. REFERENCES

6.1 Provide full details of any references

Please provide full details of any references, including authors, publication year, title and journal name, volume, page numbers. We won't accept shortened references. Number your references in the order in which they appear in the text, and list them in the Vancouver style (as outlined by the US National Library of Medicine). ***References will not be included in the page count.***

1. CRUK. 2019; Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer.
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COLO-PREVENT (Phase III)

Total sample size in two trials = 1,458

INCLUSION CRITERIA

Patients identified from English BCSP across 60 centres

Polyps removed
Patient classified as **high-risk** for surveillance colonscopy
(according to new criteria)

Current aspirin or metformin use?
N= 4400

Predict
30% uptake

Not currently taking aspirin or metformin and able to tolerate aspirin

(Expect ~75%)

Screen ~ 3200

Seeking a 30%↓
80% power

Consent/randomisation (1:1)
N= 862

Aspirin
300mg or 75mg OD based on BW
N=431

Aspirin
plus
Metformin
(500mg BD)
N=431

Colonoscopy at **3 years** (per new BCSP guidelines) for primary outcome – mean adenoma number per person (MAP)

Assume 15% drop out
&
3% crossover in each arm

COLO-PREVENT-SS (Phase II)

Signal-seeking sub-trial

Predict
50% uptake

Already taking aspirin/metformin.
Unable to tolerate aspirin.
Don't want to take aspirin/metformin

(Expect ~25%)

Screen ~ 1200

Seeking a 35%↓
80% power

Consent/randomisation stratified by aspirin/metformin use (1:1:1)
N= 596

Placebo
N=199

Resveratrol
5mg OD
N=199

Resveratrol
1g OD
N=199

Colonoscopy at **1 year** to use as interim analysis of MAP for stop/go decision for each dose

Assume 10% drop out

Transfer most effective dose to main trial
(subject to additional future funding)

Logos

[TO BE PRINTED ON HEADED PAPER]
PARTICIPANT INFORMATION LEAFLET

COLO-PREVENT: A platform for developing COLOrectal cancer PREVENTion strategies

MAIN STUDY

Invitation

You are invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve for you. Please take time to read the following information carefully, and discuss it with others if you wish.

Please ask your Bowel Cancer Screening Programme (BCSP) Screening Practitioner or the Research Nurse if there is anything that is not clear, or if you would like more information from a Doctor working on the research study. Take time to decide whether or not you wish to take part.

1. What is the purpose of the Study?

The purpose of the study is to determine whether treatment with aspirin, or aspirin combined with metformin is better at preventing bowel polyps (small growths on the bowel lining) from re-growing. Aspirin has been used widely as a pain-killer and for symptoms of fever for over a century. Metformin is used regularly in patients with diabetes to help control their blood sugar.

At the moment, the standard way to identify bowel polyps is by a colonoscopy, which is a camera test of the large bowel. If polyps are seen with the camera test, they are removed at the same time. Removing bowel polyps reduces the risk of bowel cancer in the future, but it does not prevent all cases of bowel cancer. That is why we want to find a more effective way to reduce bowel polyps, which in turn will reduce bowel cancer risk. One option is to treat patients with drugs or food supplements (called 'therapeutic prevention') after they have had their polyps removed. We also hope that therapeutic prevention will reduce the number of colonoscopies each patient needs, and may even remove the need for repeat colonoscopy in some patients.

We already know that aspirin can reduce the number and size of bowel polyps in patients with a rare inherited condition. These patients have many polyps, and they are quite similar to the more common polyps (called 'sporadic') that most people have removed when they have their colonoscopy. We think that taking daily aspirin may help to prevent these sporadic bowel polyps from growing and so also reduce bowel cancer risk, although we are still not sure what the best dose is and who would benefit most from taking it. There are also studies showing that metformin can reduce the number of bowel polyps, and we want to know if more polyps can be prevented if it is used together with aspirin.

In this study, we want to test whether, for people who have already had bowel polyps removed, re-growth of polyps is reduced most by aspirin alone, or by metformin and aspirin given together.

2. Why have I been chosen?

You have been chosen because you have had a colonoscopy in the NHS Bowel Cancer Screening Programme (BCSP) to remove bowel polyps and you need another colonoscopy in approximately 36 months' time.

3. Do I have to take part?

You can choose whether or not to take part in this study. Before you decide please ask the doctor or research nurse if anything is not clear or if you need more information. If you do decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. Once you decide to take part you are still free to withdraw at any time without giving a reason. Doing so will not affect the standard of care you receive. It is possible that you might be suitable to receive a different form of experimental therapy and we are happy to discuss this with you if you would like to know more. It is also possible that in the future there may be other treatment options; again, we can discuss these with you if, and when, they become available.

4. What are the benefits of taking part in the study?

There is no guarantee that you will personally benefit from taking part. The study will contribute to the medical knowledge about preventing the formation of bowel polyps.

5. What will happen to me if I take part?

We will discuss the study with you during your routine follow-up appointment after your colonoscopy. If you are happy to proceed you will be asked to sign a consent form. Once you are in the study, you will be asked to take study medication every day until the day before your surveillance colonoscopy in approximately 36 months' time. You will see a Screening Practitioner or a Research Nurse four times during the study and she/he will also contact you three or four times by phone.

This study is a 'randomised study' which means that a computer randomly allocates you to one of the treatment arms. You have an equal chance of being allocated to each arm.

The treatments in the study are aspirin with and without metformin tablets.

The treatments in the two groups are:
Arm 1 – aspirin tablets once a day
Arm 2 – aspirin tablet and metformin tablets

If you agree to take part in the study, in Arm 1 you will take one aspirin tablet once a day; either 300mg or 75mg depending on your body weight. In Arm 2 you will take one tablet of aspirin once a day and 500mg of metformin twice a day. You will be asked to take the tablets with food. You will be given an initial six months' supply of study medication.

The study has been designed to fit into your normal Bowel Cancer Screening care. We also will ask you to attend extra clinic visits every six months so we can find out how you are getting on with the study treatment and give you a further six months' supply of the study medication. The Screening Practitioner or Research Nurse will also contact you by telephone on another three or four occasions during your treatment to ask how you are getting on.

Your participation in the study will end when you have seen the Screening Practitioner after your surveillance colonoscopy. You will then continue in the Bowel Cancer Screening Programme as normal. If you need them, you may have further colonoscopy tests while in the Programme. We would like your permission to look at the results of any future colonoscopy tests you may have, to see if any polyps have returned. We would access the additional results up to 6 years after your participation in the study. You do not have to agree to the long-term follow-up to be able to participate in the study. We will specifically ask your permission for this on the consent form.

We would like to take several samples while you are in the study. We wish to perform a blood test before treatment starts, at the extra 6 month clinic visit and at the second routine colonoscopy after your treatment has ended. We will also ask you to do another Faecal Occult Blood Test (FOBT), which is the same test that you did as part of the Bowel Cancer Screening Programme before being invited for your camera test. The amount of blood we take each time is approximately 2 dessert spoons. We will also ask for a urine specimen at these times. We will take 4 biopsies (small pieces of tissue) from the lining of the bowel at your surveillance colonoscopy.

Furthermore, we would like your permission to obtain and analyse the polyp tissue that has been removed from your bowel during your first colonoscopy and any polyp tissue that may be removed at the second colonoscopy. At the end of the study, this tissue will be returned to the hospital in which you had your colonoscopies.

If we lose contact with you during the study, for whatever reason, we will try a maximum of three times to contact you.

The flow chart below summarises what the study involves from the first screening colonoscopy to the end of the study after the second colonoscopy.

COLO-PREVENT Study patient care pathway

Time Study activity

BCSP colonoscopy – You receive this Patient Information Leaflet

Visit 1 Week 0 BCSP follow-up clinic –
You can discuss the study and ask any questions
If you decide to take part, you will be asked sign a consent form
You will be asked to give a blood sample and a urine sample, and to complete a food questionnaire
You will have blood tests taken
You will be given six months' supply of your study medication

Visit 2 Week 2 Telephone follow-up call – To enquire about your progress

Some patients may be asked to come back for another bowel camera test between week 2 and 12. This is not part of the Study but is routine BCSP care for some patients.

Visit 3 Week 12 Telephone follow up call – To enquire about your progress

Visit 4 Week 25 Out-patient visit –
You will be asked about how you are
You will be asked to give a blood sample
You will be given another six months of your Study medication

Visit 5 Week 37 Telephone follow up call – To enquire about your progress

Visit 6 Week 52 Out-patient visit –
You will be asked about how you are
You will be asked to give a blood sample
You will be given another six months of your Study medication

Visit 7-9 Every 6 months Out-patient visit –
You will be asked about how you are
You will be asked to give a blood sample
You will be given another six months of your Study medication

Visit 10 Week 154 (36 months) Surveillance BCSP colonoscopy
You will have your planned colonoscopy in the BCSP. This will be the same as the routine procedure in the BCSP except that 4 biopsies will be taken from the lining of the bowel.
A blood sample will also be collected before or after the colonoscopy
Some patients may have their surveillance BCSP colonoscopy later than week 50 due to the extra bowel camera test that they had between weeks 2 and 12

Visit 11 Week 156 BCSP follow-up clinic – This is the appointment when you will receive the results from your latest colonoscopy and when follow-up arrangements will be discussed.
You will be asked to complete another food questionnaire

This appointment will be 2 weeks after your surveillance colonoscopy and so for some patients this will be later than week 52 This is the end of the Study

6. During your time on the study

You will need to sign and date the consent form before you can participate in the study. While in the study you will have to visit the hospital for any scheduled appointments. You will also need to take your study medication as directed by the Screening Practitioner or Research Nurse. If you experience any problems while taking the study medication then contact the Screening Practitioner or Research Nurse on the number provided at the end of this leaflet.

While you are taking the study medication we ask that you avoid taking any other medication that contains aspirin. Examples of medication you can buy in a pharmacy or shop that contain aspirin are Askit Powders, Beechams Powders, Disprin and Anadin. If you need pain relief, you can take medication that doesn't contain aspirin, such as paracetamol. If you need to take a prescription non-steroidal anti-inflammatory drug (NSAID) such as ibuprofen more than three times a week you will not be allowed to join the Study. If you are taking an NSAID tablet or capsule less frequently, we ask you to try an alternative medication such as paracetamol. Please note that ibuprofen is also available 'over-the-counter' from Pharmacies or shops (an example is Nurofen). If you are unsure whether a preparation contains ibuprofen please check with the Pharmacist or the Shop Assistant.

7. How long will I be involved in the study?

As described in Section 5 we will ask you to attend appointments for at least 3 years. There are some circumstances when we might consider it in your best interests to withdraw you from the study, for example if we feel that the treatment is doing you harm, if your condition worsens or if new information becomes available. If so, we will explain the reasons to you and arrange for your care to continue.

8. What is the alternative to entering the study?

The alternative to entering the study would be to not take any of the study medications but have your planned colonoscopy in the Bowel Cancer Screening Programme at 36 months after the last colonoscopy, as is the usual practice for patients like you who had polyps removed at the first colonoscopy. A decision not to take part will not affect the quality of care you receive in the BCSP in any way.

9. What are the possible disadvantages and risks of taking part?

Disadvantages for participants include the small number of extra visits and tests that will need to be made.

If you have private medical insurance, you should check with your insurance company before agreeing to take part to ensure that your medical insurance is not affected.

Taking the blood sample may cause some pain where the vein is punctured and some bruising but this is usually short lived.

The taking of biopsies during your colonoscopy is a routine procedure and very safe. Although there is a theoretical risk of perforation or serious bleeding this is very rare as the biopsies we take are very small. You usually cannot feel the actual biopsy though you may be aware of a tugging sensation as it is taken.

Male participants with female partners of childbearing potential must be abstinent from sexual intercourse or practice a reliable form of contraception, such as condom or barrier with spermicidal jelly, oral contraceptive, hormonal implant, IUCD for the duration of the Study.

10. What are the potential side-effects of study medication?

Both metformin and aspirin have an excellent safety record and are well tolerated, although there are some recognised side-effects.

Aspirin has been used for many years and has an excellent safety record. A low dose of aspirin is being used in this study in order to minimise any stomach upset or other gastrointestinal problems. Some minor bleeding (e.g. after having blood taken) and bruising may be experienced by some people. Serious bleeding from the stomach and upper bowel can occur but is rare. It is thought to occur once or twice every time one thousand patients take treatment for one year. There is an even smaller risk of stroke in people using aspirin.

Metformin has been used in patients without diabetes in a number of studies. Although it is used to reduce blood sugar levels in patients with diabetes, it does not do this in patients with normal sugar levels. Metformin can cause some gastrointestinal side effects and so in the study we start at a very low dose and build this up to a dose that we know from other studies, the majority of patients manage without problems. Finally we know that metformin can cause problems with the kidneys if they are not monitored properly, and will therefore measure your kidney function while you are on study and advise if you need to alter the dose of metformin.

Combining metformin with aspirin has not been tested before in a randomised study like this. Therefore, although we do not think there will be a problem, we will provide you with very clear advice as to when you should stop the drug before any CT scan, surgical procedure or repeat bowel camera test that you are due to undergo. We will provide you with an information card for you to show any doctor who you see when on study.

Other prescribed treatments can be taken as usual and there are no other lifestyle or dietary restrictions required. We will ask you about the other medication you take and advise about any precautions. For example we would not want you to take regular non steroidal anti-inflammatory drugs while taking the study medication and we would need to monitor your kidney function closely if you were admitted to hospital with an infection requiring a specific antibiotic.

If you experience any problems or are told that you need surgery you need to tell us at one of the visits or when you are contacted by telephone. You can also contact us yourself at any time on the contact number provided later.

11. What additional samples would you like me to donate?

Blood samples

We would like to understand how these drugs work to prevent polyps. We would like to take up to 15ml of extra blood (about 2 dessert spoonfuls) in addition to the 35ml of blood being taken for the study. This sample can be taken at the same time as any routine blood tests you need.

Tissue samples from previous operations

We would like to look at tissue that has been taken in the past when you had previous operations or biopsies. These samples will be stored in the pathology laboratory of the hospital you had your previous surgery or biopsy but we would like your permission to request these samples for our research.

Additional optional research biopsies

We would like to take extra samples of tissue from you at colonscopy to try to understand why polyps come back and how treatment can affect this.

Stool samples

We may ask you to provide a stool specimen, in the same way you did the original FOBT test as part of the bowel cancer screening programme.

12. What will happen if I don't want to carry on with the study?

If you change your mind you can withdraw from the study *at any time*. This will not affect your relationship with the doctors and nurses or your subsequent care in any way.

If you withdraw from the study, we will keep the information about you that we have already obtained. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. To safeguard your rights, we will use the minimum personally identifiable information possible. If you consent to the study but subsequently become unable to give consent, then you will be withdrawn from the study and no further samples or information will be collected from your records. Previously obtained samples and data will still be used in the study.

13. What if new information becomes available?

During the course of a study, new information about the study treatment sometimes becomes available, for example a previously unknown potential side effect. If this happens, we will tell you and ask you if you wish to stay in the study. If you decide to leave the study, your standard care will continue. If you stay in the study you may be asked to sign a new consent form.

14. What will happen to any samples I give?

With your consent, any samples that remain once the trial tests have been done may be retained for future research. Samples will be coded and kept anonymous, which means that the laboratory researchers who are carrying out any tests cannot identify you. Coded samples may also be linked to study data from which all identifying information has also been removed. All information that is collected will be kept strictly confidential. Nothing that could reveal your identity will be disclosed. In addition, future work may be undertaken by us and other research groups to understand how we can treat polyps better in the future. In all cases, before any groups are given access to your donation, they will have to submit an application to an NHS research ethics committee to ensure

the quality and integrity of the proposed research. Your tissue will not be used for non-medical or non-scientific purposes.

15. Are there expenses payments?

We are unable to reimburse you for your travel expenses and your time taking part in this study.

16. What will happen when the research ends?

The research study will end once all the participants needed for the study have been followed-up following their surveillance colonoscopy at 36 months.

17. Will any genetic testing be done?

DNA will be obtained from one of the blood samples and this DNA will be used to test whether a person's genetic make-up can predict who will respond well to therapeutic prevention and who might be resistant to it. We will only test genes relevant to the way that aspirin and metformin work (for example, the cyclooxygenase gene) and we will not do any other genetic testing. Therefore, there will be no insurance implications involved in taking part. The results will not be linked back to you.

18. Who is organising and funding the research?

This research is being organised by the University of Leicester. This study is being carried out by a network of doctors across the UK. The study is co-ordinated by the Leicester Clinical Trials Unit and is sponsored by the University of Leicester. The study is funded by Cancer Research UK. Your doctor is not receiving any payment for including you in the study.

19. What if there is a problem?

You will be monitored closely and offered whatever help is available to cope with any side effects. Some participants need to short stay in hospital to treat side effects, which on rare occasions can be serious.

If something goes wrong and you are harmed by taking part in this study, there are no special compensation arrangements beyond your rights as an NHS patient. If you are harmed due to someone's negligence, then you may have grounds for a legal action against the [insert name of Trust] but you may have to pay the legal costs for undertaking this.

If you have a concern about any aspect of the study, you should speak to your research doctors or nurses who will do their best to answer your questions. If you remain unhappy and wish to complain formally, the normal National Health Service complaints mechanism will be available to you. Details can be obtained from the Patient Advice and Liaison Service (PALS) at your hospital.

PALS Contact Details:
Tel: XXXX XXXXXX between the hours of 10.00am and 4.00pm
Email: pals@XXXXXX.nhs.uk
Write to: Patient Advice and Liaison Service, {Name of Hospital, Address of Hospital]

20. Will my taking part in this study be kept confidential?

The University of Leicester is the sponsor for this study based in the United Kingdom.

We will be using information from you and/or your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The Leicester Clinical Trials Unit will keep identifiable information about you for 20 years after the study has finished.

All information about you obtained from this study will be kept confidential and stored securely in line with the General Data Protection Regulation established in 2018.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by visiting:
https://www2.le.ac.uk/offices/ias
https://www2.le.ac.uk/offices/ias/information/public/public
https://www2.le.ac.uk/offices/ias/dp/subject-access-request

Information from your medical records for this research study will be collected in accordance with our instructions. We will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study.
You will be given a unique study reference number, which will be used to collect and record all study-related information to preserve your confidentiality. All study data will be kept securely with restricted access. Information recorded in your medical records may need to be seen by authorised members of the research study team (including clinical and non-clinical staff from your hospital), individuals who audit the study and data collection process, and staff from the Leicester Clinical Trials Unit who are managing this study and with CRUK the study's main funder. The people who analyse the information, who are not designated members of the research team, will not be able to identify you and will not be able to find out your name, NHS number or contact details.
Your study data may also be sent to countries outside of the UK. Please be aware that the laws in such countries may not provide the same level of data protection as in the UK but any transferred data will be coded and will not contain any personal information.

Contacting your GP
It is standard practice for your GP to be told if you are taking part in research. With your consent, your GP will be informed that you are taking part in the COLO-PREVENT study.

21. What will happen to the results of the research study?
The results will be analysed by scientists and doctors who specialise in developing cancer prevention treatments. Any positive results from this study will provide a basis for helping to develop future treatment schedules for people with polyps. The results may be published in scientific or medical journals. Participants will of course remain anonymous. Please tell your Study Doctor if you wish to see a copy of the published report.

22. Who has reviewed and checked the study?
All research in the NHS is reviewed by an independent group of people called a Research Ethics Committee, who work to protect your safety, rights and wellbeing. The

study has been reviewed and approved by the Research Ethics Committee. In addition, the Research Ethics Committee will observe the progress and results of this study. The suitability of [insert the name of the local hospital] to carry out this trial has been assessed by the University of Leicester Research and Development department, which is responsible for the study. Your hospital has also reviewed and approved the study and the study was reviewed by independent experts during its development.

23. Whom do I contact if I have questions or problems?

If, at any time, you have any questions about the study or a research-related injury, you should contact your hospital team.

[please insert local contact details]

Emergency contact no: XXXXXXXXXX

Patient Advice and Liaison Service (PALS): XXXXXXXXXX

For [insert name of Trust]
Dr XXXXXXXXXXX Tel: XXXXXXXXXXX
Research Nurse Team Tel: XXXXXXXXXXX Fax: XXXXXXXXXXX

If you feel unwell after your treatment:

If you are concerned about any problems please ring the hospital hotline.
The hotline is open 24 hours a day. Do not delay your call.
[insert name of Trust] Tel: XXXXXXXXXX

24. Where can I get more information?

If you wish to discuss this study and your rights as a research subject with an independent person then please contact the person below, who is not directly involved in the study, but will be happy to discuss any issues you have
Contact Person [insert name of Trust]

Sources of General Information about clinical studies

CancerHelp UK provides general information for participants about cancer and its treatment on their website www.cancerhelp.org.uk

Cancer Research UK has cancer information nurses who provide a confidential service.
Tel: 020 7061 8355 or email: cancer.info@cancer.org.uk

Thank you for taking the time to read this information.

SPONSOR/TRUST HEADER

CONSENT FORM

COLO-PREVENT: A platform for developing COLOrectal Cancer PREVENTion strategies

SITE REFERENCE NUMBER ☐

PATIENT IDENTIFICATION NUMBER ☐

Please initial all boxes

1. Patient Information Sheet: I have read and understood the Patient Information Sheet (version xx, dated xxx). I have had the opportunity to consider this information and to ask any questions. ☐

2. Samples: I consent to the donation of tissue, urine, fecal and blood samples whilst enrolled on this study. ☐

3. Samples: I consent to storage of my samples by the research team, and for their use in future research. Yes ☐ No ☐

4. Samples: I consent to my samples being sent to other research sites, including outside of the UK. I understand that my samples will be fully anonymised and no personal information will be released to these sites. Yes ☐ No ☐

5. Genetic Analysis: I consent to genetic analyses being done on my samples, to determine whether genetic makeup has any influence on disease or treatment. I understand that this will be purely for research purposes only, and that I will not receive any information on these analyses. Yes ☐ No ☐

6. Personal Information: I understand that relevant sections of my medical notes and study data may be looked at by responsible members of the research team, the Sponsor, NHS Trust, regulatory authorities or external monitors, where it is relevant to my taking part in the research. I give permission for these individuals to access my records. I understand that information about me will be stored securely and kept confidential. ☐

7. Freedom to Withdraw. I understand that I am free to withdraw my consent at any time, without giving any reason and without it affecting my medical care. I understand that my samples would then be destroyed. I also understand that any data from research already performed cannot be withdrawn. ☐

8. Use of future samples. It is understood that if I need another operation in the future, then a blood sample and surplus tissue from the operation would be of use for research. I give consent for my future samples to be used as part of this research. Yes ☐ No ☐

9. Use of past samples. I understand that UHL NHS-Trust will have stored diagnostic samples from previous operations/procedures I have had. I give consent for my past samples to be used as part of this research. Yes ☐ No ☐

10. Follow-up of medical records on the Hospital database. I consent to researchers looking at my medical records from the past and also in the future. Yes ☐ No ☐

11. I agree to my GP being informed of my participation in this study. ☐

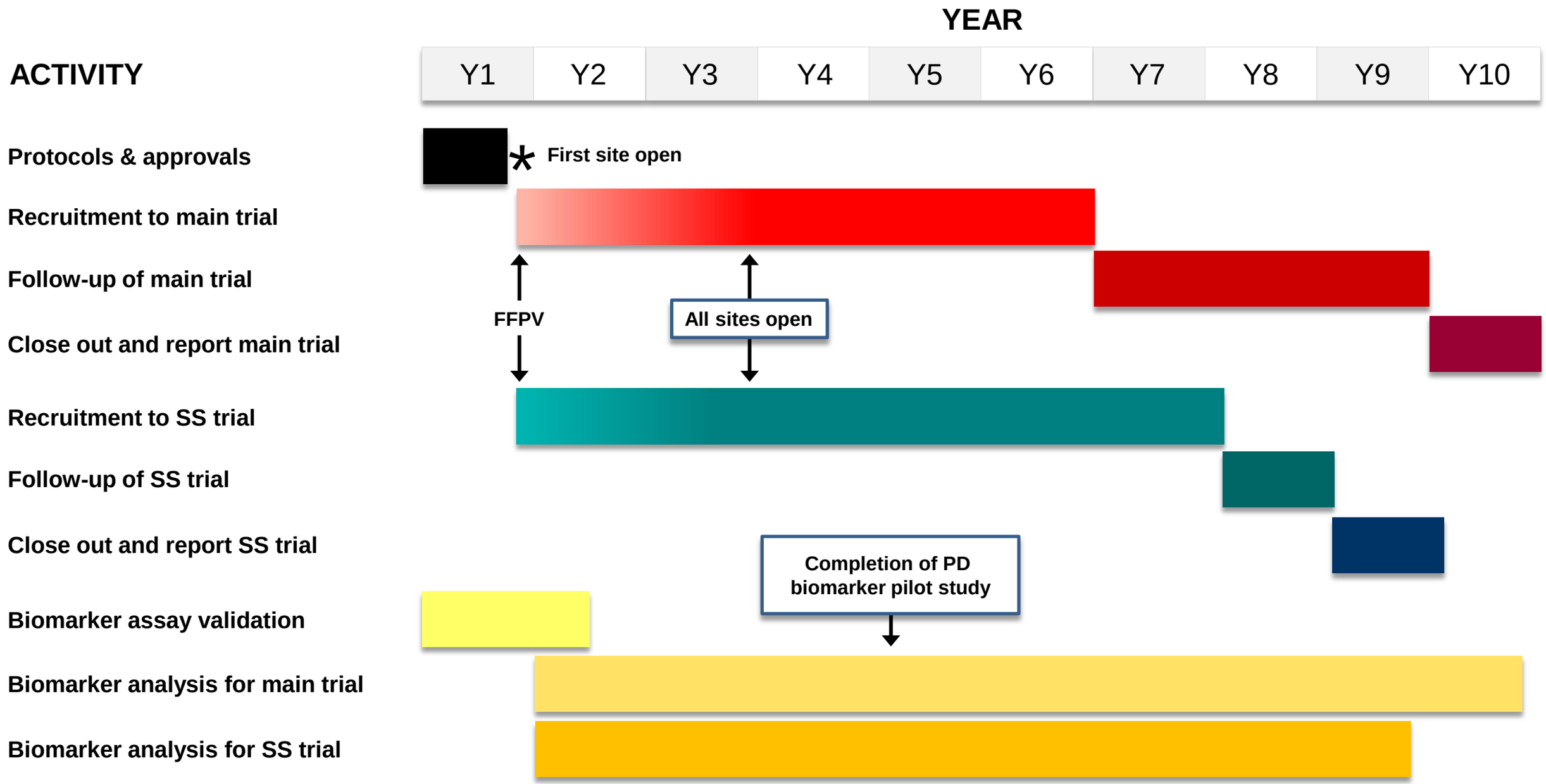
12. I agree to participate in this Research ☐

Person taking consent (Print):_____ Signature:_____ **Date:_____**

I have explained to the donor the reasons for collecting, storing and using samples for research. I am satisfied that the donor signing this form understands the content and purpose of the consent form.

Donor (Print):_____ Signature:_____ **Date:_____**

Gantt chart for the main COLO-PREVENT trial and COLO-PREVENT-SS



SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Quality Policy**
Number ID:	**QM CS001 v01**
Review Date:	Every 2 years
Aim:	To provide a general Quality Policy for the Department of Cancer Studies for work conducted as part of, or in support of, commercial studies, clinical trials or studies utilising human samples

History:

Version	Author	Date	Changes
1	L Howells	08/08/16	

Cross references:

Reviewed By: **L Primrose, C Cowley**

Training Requirement (please tick appropriate box)	
No Action	x
Familiarly with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised By 1)	**Departmental Manager**	9/11/16
Authorised By 2)	**Head of Department** C.A. Primrose	14/11/16

1. PURPOSE

It is the policy of the Department of Cancer Studies to assure that analytical studies undertaken by this facility as part of, or in support of, commercial studies, a clinical trial or study using human samples, are conducted in compliance with the relevant government and facility policies and guidelines such as The Medicines for Human Use (Clinical Trials) Regulations 2004 and the Human Tissue Act 2004. This includes commonly accepted principles of good clinical laboratory practice.

2. SCOPE

Individual groups may use this SOP template with additional appropriate group-specific information incorporated.

2.1. Projects undertaken within the Department of Cancer Studies as part of, or in support of, commercial studies or clinical trials/studies fall under the remit of this Quality Policy.

3. RESPONSIBILITIES

3.1. The Head of the Department is responsible for supporting implementation and continued adherence to the Quality Policy.

3.2. Departmental personnel are responsible for its implementation.

4. PROCEDURE

4.1. The policy is a statement by Departmental Management of the organisation's overall philosophy on Quality and describes its commitment to a Quality Management system which **assures the integrity of data** produced by the Department in activities associated with commercial studies or clinical trials/studies.

4.2. The policy provides a framework for establishing assurance:

4.2.1. That relevant groups under the Departmental umbrella meet regulatory requirements.

4.2.2. That either findings which form part of a commercial or clinical study are reported in formal reports which are presented to the responsible management or as specified in the trial protocol.

4.2.3. That applicable good practices are applied to all scientific work and their advancement is encouraged and fostered through open communication which involves personnel staff at all levels within the Department.

4.2.4. That record creators and contributors remain identifiable with respect to any given scientific record.

4.2.5. That the experimental results which form part of a commercial or clinical study can be traced back to original records of data and the procedures, materials and equipment used to obtain that data.

4.2.6. That the procedures, materials and equipment can be verified as fit for purpose.

4.2.7. That the records reliably present objective facts, are easily identified and are available for review when required.

4.2.8. That the persons responsible for record creation and records handling or management are adequately skilled in the appropriate principles and procedures through training and/or experience.

4.2.9. That general principles of quality assurance shall be implemented using a combination of formal training, controls, and cross checking as well as informal review, including facilitation of peer review.

4.2.10. That errors or other deficiencies in data can be identified and properly corrected.

4.2.11. That records are securely stored for assigned time periods and retrievable when required.

4.2.12. That there is a commitment to the health, safety and welfare of all personnel and visitors to the laboratory.

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Working towards GCLP**
Number ID:	**QA CSMM038**
Review Date:	Every 2 years
Aim:	To provide the basic structures that need to be in place prior to undertaking any laboratory work that relates to human tissues

History:

Version	Author	Date	Changes
1	L Howells L Howells	15/07/16	

Cross references:

Reviewed By: **L Primrose** LPrimrose 10/08/2016.

Training Requirement (please tick appropriate box)	
Relevant laboratories should be able to demonstrate that they are working towards these criteria	X

Authorised By 1)	**L Howells**	L Howells 10/8/16
Authorised By 2)	**J Strupish**	NOT AVAILABLE JmH 10/8/16

Implemented on 15/08/16

1. PURPOSE

1.1. Each laboratory that works with human tissues or is expected to deliver an analytical service, should have the following management structures in place.

1.2. It is the responsibility of the PI for the study to ensure that the laboratory facilities are fit for purpose, and that their staff have access to the necessary training to conduct the study appropriately.

2. SCOPE

This SOP sets out the management structure within the Chemoprevention section in the Department of Cancer Studies. It is envisioned that this will also provide a useful template for the wider Department, should other groups require advice on working to GCLP.

3. PROCESS

There are several aspects to be considered and consist of:

- General laboratory
- Equipment
- People
- SOPs
- COSHH

If GCLP is to be implemented within a fully functioning laboratory, it is advisable to introduce this as a phased process to SMART objectives.

General Laboratory phase 1:

- Unlabelled/expired materials disposed of or archived away from current.
- All solutions labelled using template label.
- Expiry/opening dates should be clear on all chemicals
- All chemicals to be stored under correct conditions

General Laboratory phase 2:

- Fridges and freezers tidied
- Contents all correctly labelled
- Designated fridge/freezer for clinical sample storage
- Fridge/freezer monitoring to be in place with appropriate log system

Equipment:

- List of all equipment and serial numbers
- Label all equipment not currently in use
- Ensure that service contracts are in place for appropriate equipment
- Designation of responsible person
- Log books – calibration, QA, usage

People:

- Induction for all new staff
- Training folders to be made for each employee containing:
- Job description
- Signed and dated CV
- Training certificates (eg, ICH-GCP, IGT, HTA)
- Signed training logs
- DESIGNATE QA PERSON FOR EACH GROUP
- Designate a document controller

SOPs:

- Required for all equipment, laboratory procedures, QA processes (particularly record keeping)
- Users of SOPs will be logged by document controller
- Controlled document recall procedure in place

COSHH:

- Ensure that COSHH forms exist for ALL non-archived chemicals/reagents
- Copies to be in area accessible to all
- COSHH attached to all orders

Please note. GCLP does not represent an accredited ISO standard. It is designed to try and ensure that a quality standard is maintained, and can be applied across laboratories within a University environment.

SOP

- Check that this SOP is the current version, cross check with database.

Title: **Receipt and storage of clinical trial test material**

Number ID: **QA CSMM009**

Review Date: 18/03/10

Aim: To describe how to log and store incoming clinical samples

History:

Version	Author	Date	Changes
1	L. Howells L. Howells	13/03/08	

Cross references:

Sample log form 001	Sample aliquot use form 002	Sample receipt form 003

Reviewed By: **F Teichert** F. Teichert 19/03/08

Training Requirement (please tick appropriate box)	
No Action	
Familiarly with changes	X
Documented Instruction	
Documented instruction and assessment	

Authorised By: **K Brown** Karen Brown 30/3/08

J Strupish J Strupish

PROCEDURE

1. Introduction

1.1. This Standard Operating Procedure documents the procedure to follow for the receipt of test materials for pharmacokinetic and pharmacodynamic evaluation as part of clinical trials. The objective of this procedure is to ensure that sample condition is assessed on receipt, labelling is adequate and controlled and that the sample is appropriately stored to ensure demonstrable integrity until analysis is commenced or sample disposal.

2. SCOPE

2.1. This procedure covers any patient sample that will be analysed or stored as part of a clinical trial in the Department of CSMM – chemoprevention.

3. RESPONSIBILITIES

3.1. It is the responsibility of the Unit Head of the Analytical Department (Karen Brown/Andy Gescher) to ensure that sample logging is performed by staff of adequate experience and training.

3.2. The senior analyst responsible for the specific analytical process or trial must ensure that details of sample handling, labelling, processing and storage are made available, in writing, to the individual responsible for logging in the sample.

3.3. Staff logging in samples are responsible for following this procedure and ensuring the safe and documented receipt of sample, labelling and placement in appropriate storage.

3.4. Any problems with logging in of samples, due to their condition or associated documentation should be reported immediately to the Unit Head.

4. RELATED DOCUMENTS

4.1. Sample Receipt Logbook.

5. PROCEDURE

5.1. Definition of Test material.

5.1.1. A test material is a material supplied by a Sponsor or a Clinical Centre (on behalf of a Sponsor) for analysis or storage as part of a clinical trial.

5.2. Pre-shipment Arrangements

5.2.1. Whenever possible sample shipment from the clinical trials unit or Sponsor should be prearranged. The recipient should ensure that he/she or a suitably trained colleague is available at the expected time of arrival and that the sender has received the full destination address including the laboratory and/or receiver name for inclusion on the package labelling.

5.3. Sample Receipt

The key objectives are to assess sample integrity, assign a unique internal reference code and ensure appropriate storage.

5.3.1. Unopened package - to assess and record the condition of the package in which the sample arrives i.e. damaged/undamaged, labelling adequate.

5.3.2. Opened package – cool packs present/absent/warm/cold/ presence/absence of dry ice. Sample containers intact/damaged/leaking.

5.3.3. To confirm the completeness of accompanying information (datasheet etc).

5.3.4. To confirm unequivocally the identity of every vial/primary container.

5.3.5. To confirm identity of samples received against the supplied data sheet.

5.3.6. To issue an internal sample identity code.

5.3.7. All information to be recorded in the sample receipt logbook.

5.4. The Sample Logbook

5.4.1. The sample logbook is a study specific record *(can be a general or multi-study logbook instead)* of the samples received, condition of samples on arrival, sample identification, accompanying information and details of any known missing samples or labelling queries.

5.4.2. The sample logbook is the only source where the internal identification code is linked with the other sample information. This information defines how the results from analysed samples are linked to the patient information and sample timing.

5.4.3. The sample logbook is kept as a hard cover, bound notebook and stored in a secure location.

5.4.4. An example of the layout of the sample logbook is given in sample receipt log form 003.

5.5. Assignment of the Internal Identification Code
Each trial and each patient sample within the trial receives a unique internal identification code. The internal sample code should not be taken to signify any sequence or other sample information. Any sample information must only be derived from the information in the sample logbook.

The study code will take the following form:-

5.5.1. Study identifier
This will be the clinical trial protocol number (EUDRACT).

5.5.2. Patient number
Capital letter P plus a two digit number (P01, P02, P03...). The patient numbers are assigned in sequence of arrival of sample. Check to see if this is the first time a sample has been received from this patient. If a sample has been received from this patient previously, use the existing patient number. If a new patient generate a new patient number.

5.5.3. Sample sequence number
Capital letter S plus a two digit number (S01, S02, S03...) is assigned to each sample. For ease of handling/checking the code will be assigned according to the sequence in which the samples were taken. An additional (small) letter differentiates duplicate samples (two vials with identical contents) e.g. S03b or different sample types. Details should normally be given in specific trial appendices, in the absence of an appendix ensure full descriptor is recorded in the sample logbook.

5.5.4. If more than one type of sample is received from the sample patient the internal code can be extended to reflect this. The code should retain the alphanumeric elements specified in this SOP. Additional information should be recorded in a practical mnemonic format following on from the standard code. It is mandatory that an individually coded sample can be linked unequivocally to the full description of the sample in the sample log book.

5.5.5. Missing samples
Missing samples should not be given a number in the sequence but identified by 'Sample Missing' in the corresponding internal identification code column in the logbook. The sender should immediately be notified of a missing sample.

5.5.6. Missing patient information, unclear labels
A preliminary sample number should be assigned as above. In the sample logbook a detailed record should be made of any available as well as missing or unclear information. If the sample identity can be established unequivocally (e.g. label fallen off on one vial only, label in bag) the reasoning should be given in the sample logbook. The sample may then be taken forward for storage / analysis.

5.5.7. Documents supplied by the sender
These should be filed with the trial records held by the analytical group.

5.5.8. Sample storage
On completion of logging of the sample it is transferred to an appropriate storage area such as a freezer. Storage conditions will be as defined in study specific documentation such as the clinical trial protocol. Samples for each specific trial will be held in a container labelled with a minimum of the Cancer Research-UK trial number, alternatively a shelf dedicated to the trial may be used. Storage location will be recorded in the sample logbook. Samples from different trials should be physically separated to avoid potential mix-ups and cross contamination. All sample storage areas should be monitored on a regular basis

to ensure the desired storage conditions are maintained – temperature records must be kept.

5.5.9. Sample use.
Samples should be logged into a particular storage area, e.g. designated freezer, and each time the sample is removed or frozen/thawed, this should be recorded in the sample aliquot use form (002)

5.5.10. Deviations from procedure
Any deviations from this procedure should be recorded in the sample logbook or the storage area log, as appropriate.

SOP

- Check that this SOP is the current version, cross check with database.

Title: **Extraction of plasma samples for HPLC or LC-MS analysis of resveratrol and its metabolites**

Number ID: **HPLC CSMM004**

Review Date: Every 2 years

Aim: To provide a method for users analysing resveratrol and its metabolites in plasma

History:

Version	Author	Date	Changes
1	H Cai	11/06/19	

Cross reference:

HPLC use		

Reviewed By: **L Howells** L. Howells 11/6/19

Training Requirement (please tick appropriate box)	
No Action	
Familiarity with changes	x
Documented Instruction	
Documented instruction and assessment	

Authorised by 1) **K Brown** Karen Brown 11/6/19.

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation	COSHH
Concentrated (5M) HCl		
Methanol	HPLC grade (Fisher)	
Water	Nanopure diamond dispenser	
Propan-2-ol	HPLC grade (Fisher)	
Ammonium Acetate	HPLC grade (Fisher)	

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

1) Plasma containing resveratrol/metabolites will be stored at –80°C before extraction.
2) As far as logistically possible, all extraction will be carried out swiftly and in the dark.
3) The samples are thawed at room temperature or in the hand whilst kept in the dark and mixed well.
4) 250 µl of the plasma is pipetted into a clean 1.5 mL eppendorf tube and acidified with 4.38 µl of concentrated HCl.
5) 250 µl of room temperature methanol is added to the acidified plasma.
6) **The remaining sample is re-frozen at –80°C as quickly as possible.**
7) The sample is then vortex mixed for 1 minute – the plasma should turn cloudy as the protein precipitates out.
8) Place the sample in a –20°C freezer for 5-10 minutes.
9) Spin the samples at 13,000 g in a microcentrifuge for 15 minutes.
10) Pipette off the supernatant into a fresh labelled eppendorf.
11) Dry down the supernatant at room temperature in the dark under a stream of nitrogen. This can take up to 45 minutes.
12) Once dry – reconstitute in 200 µl of 50:50 MeOH:H_2O and mix well.
13) Re-spin the samples at 13,000 g in a microcentrifuge (at 4°C) for 15 minutes.
14) Pipette the supernatent into appropriate HPLC analysis vials, and if not injected immediately – keep in the dark (preferably chilled to 4°C) in the autosampler or fridge.

HPLC method details for sample analysis can be found in the relevant SOP.

DISPOSAL

After running, HPLC vials should be disposed of in glass bins.

SOP

- Check that this SOP is the current version, cross check with database.

Title: **Extraction of urine samples for HPLC or LC-MS analysis of resveratrol and its metabolites**

Number ID: **HPLC CSMM005**

Review Date: Every 2 years

Aim: To provide a method for users analysing resveratrol and its metabolites in urine

History:

Version	Author	Date	Changes
1	H Cai	11/06/19	

Cross reference:

HPLC use		

Reviewed By: **L Howells** L. Howells 11/6/19

Training Requirement (please tick appropriate box)	
No Action	
Familiarity with changes	x
Documented Instruction	
Documented instruction and assessment	

Authorised by 1) **K Brown** Karen Brown 11/6/12

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation	COSHH
Concentrated (5M) HCl		
Methanol	HPLC grade (Fisher)	
Water	Nanopure diamond dispenser	
Propan-2-ol	HPLC grade (Fisher)	
Ammonium Acetate	HPLC grade (Fisher)	

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

1) Urine containing resveratrol/metabolites will be stored at –80 C before extraction.
2) As far as logistically possible, all extraction will be carried out swiftly and in the dark.
3) The samples are thawed at room temperature or in the hand whilst kept in the dark and mixed well.
4) 250 µl of the urine is pipetted into a clean 1.5 mL eppendorf tube and acidified with 1.33 µl of concentrated HCl.
5) 500 µl of room temperature methanol is added to the acidified urine.
6) **The remaining sample is re-frozen at –80 C as quickly as possible.**
7) The sample is then vortex mixed for 1 minute and placed in a –20 C freezer for 5-10 minutes to allow any protein to precipitate out.
9) Spin the samples at 13,000 g in a microcentrifuge for 15 minutes.
10) Pipette the supernatent into appropriate HPLC analysis vials, and if not injected immediately – keep in the dark (preferably chilled to 4 C) in the autosampler or fridge.

DISPOSAL

After running, HPLC vials should be disposed of in glass bins.

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Collection of urine samples for HPLC or LCMS analysis of resveratrol and its metabolites**
Number ID:	**SAM CSMM003**
Review Date:	Every 2 years
Aim:	To ensure correct sample requirements for resveratrol PK analysis in urine

History:

Version	Author	Date	Changes
1	H Cai	11/06/19	

Cross references:

Reviewed By: **L Howells** L. Howells 11/6/19

Training Requirement (please tick appropriate box)	
No Action	
Familiarly with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised by 1) **K Brown** Karen Brown 11/6/19.

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation	COSHH

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

Aliquot urine as soon as possible after collection.

5ml aliquots of each sample should be added to a 15ml polypropylene tube and placed on dry ice in the dark.

Store samples in a -80 freezer until analysis.

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Collection of serum samples**
Number ID:	**SAM CSMM010**
Review Date:	Every 2 years
Aim:	To provide a collection method for serum samples

History:

Version	Author	Date	Changes
1	L Howells	10/06/19	

Cross references:

Reviewed By: **L Howells** L Howells

Training Requirement (please tick appropriate box)	
No Action	x
Familiarly with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised By 1) **K Brown**

Authorised By 2) K Brown 10/6/19.

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

1. Collect the blood sample in 6.0ml (Serum Z) tubes
2. Leave the serum sample to clot at room temperature for 20-30 minutes (no longer than 1hour).
3. Centrifuge the blood sample at 2600xg for 20mins at room temperature. This will separate the blood into an upper serum layer and a lower red blood cell (RBC) layer.
4. Without disturbing the RBC layer, transfer all serum to a sterile container. Divide serum sample into aliquots of approx 1mL volume in fresh Eppendorf (or similar) screw top cryovial tubes, and label appropriately.
5. Store samples at -20ºC until transport.

DISPOSAL

Dispose of as per appropriate COSHH instructions

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Validation of new antibodies for IHC**
Number ID:	**IHC CSMM010**
Review Date:	Every 2 years
Aim:	To provide a procedure for the validation and use of new antibodies for IHC methods

History:

Version	Author	Date	Changes
1	L Howells L-Howells	03/10/12	

Cross references:

IHC CSMM003,004,005,007		

Reviewed By: **P Greaves**

Training Requirement (please tick appropriate box)	
No Action	x
Familiarity with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised By 1)	**L Howells** L-Howells	10/1/14.
Authorised By 2)	**J Strupish** JS	21/01/14

Implemented on 03/1/14

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

Validation of new antibodies for immunohistochemistry (IHC)

THIS PROTOCOL IS NOT DESIGNED FOR USE IN DIAGNSOTIC EVALUATION THAT WILL HAVE DIRECT IMPACT ON PATIENT HEALTHCARE PATHWAYS.

Pre- analytical factors to consider.

1) Is the antibody commercially available and validated for use in IHC?
2) If the antibody has been synthesised in-house, has its specificity been checked using other platforms such as western blotting?
3) What fixatives are being used for the tissues?
4) How long are the standard fixation procedures prior to automated processing?
5) Are standard reagents/kits being used throughout, and is this to the manufacturer's recommended instructions.

Commercially available antibodies that are used for diagnostics have been optimised and validated according to a specific platform. If lab use deviates in any way from that used by the manufacturers then the antibody will have to be re-validated for use on the laboratory platform in question.

Fixation

Validation of antibodies for IHC used in lab506 RKCSB requires the following:

Tissue is fixed in 10% phosphate buffered formalin for a minimum of 8 hours (tissue penetration by formaldehyde is approx. 1mm/h). Tissues fixed for greater than 8 hours are generally more amenable to standard amounts of antigen retrieval.

Fixation (pre-processing) does not exceed 24 hours. Over fixation can result in high background.

Tissues are as uniform in size as possible to ensure comparable fixation.

There are a variety of factors that may affect antibody optimisation, including:

Antigen retrieval, heating device used, primary antibody incubation time, detection system, chromogen.

Antigen retrieval

There are a variety of antigen retrieval protocols (IHC CSMM003), including heat, pH, and enzyme digestion. However, a minimum amount of energy must be applied to a sample to restore its antigenicity and the greater the standardisation of the fixation process, the less the method of applying this energy matters. Too little energy and the antigenic sites will not be fully revealed, too much energy and the sample integrity may compromised (eg, sections likely to float off slides).

In lab 506 CSMM, antigen retrieval is standardly heat retrieval via an 800W microwave. Standard retrieval time for samples that have been fixed according to conditions above are 20 minutes at full power. Standard antigen retrieval buffers consist of sodium citrate (10mM, pH 6.0) or Tris/EDTA (pH 9.0).

Antibody Optimisation

Antibodies that are in diagnostic use will come with manufacturer recommendations which should be followed in the first instance. Where specific antibody clones are being used, the user must be sure that they are appropriate for the antigenic binding site in the specific tissue of interest. Non-diagnostic antibodies will require a full optimisation protocol from the start.

Tissues

Antibody optimisation should use tissues serially cut from the same block to ensure as much uniformity as possible. If the antibody has not yet been tested in the specific tissue of interest, then it should be tested using this tissue in conjunction with a tissue which is known to express the appropriate antigenic site.

Detection systems

Not all detection systems will produce the same signal strength. For example, some antibodies will produce weak signals with a one-step system, so it is worth evaluating a two-step system such as streptavidin/biotin as well.

Validation procedure

This procedure is based upon the fixation criteria above being met. Tissues are processed according to standard pathology protocols on level 3. Standard antigen retrieval in lab 506 is via an 800W microwave for 20 mins at full power. SOPs associated with this protocol include:

IHC CSMM003 – antigen retrieval methods for paraffin wax sections

IHC CSMM004 – immunohistochemistry using the novolink polymer detection system

IHC CSMM005 – H/E staining on paraffin wax sections

IHC CSMM007 – recipe for antigen retrieval buffers

If antibodies are already validated to diagnostic standards by the manufacturer, then the initial protocol should follow the manufacturer's specific recommendations. If this fails to produce the expected results, then the validation procedures below should be followed.

1. Sections to be analysed need to include a known positive and a serial section for a corresponding negative. (The negative should consist of IgG from the host species of the primary antibody, at the same concentration as the primary antibody.) If the positive differs from the tissue type to be analysed, then positive and negative slides for the specific tissue are to be included. This should come from more than one source due to potential variability in antigen expression between patients. Ultimately, high, medium and low expressing samples should be used. It is useful if this data can be obtained pre-validation using alternate techniques such as western blot/flow cytometry.
2. Antigen retrieval (AR) should initially be undertaken using the standard AR technique above. One set of slides should undergo AR in high pH buffer and a corresponding set in low pH buffer (see IHC CSMM007). All of the following validation steps must be carried out for both AR methods. Note – not all antibodies require an antigen retrieval step.
3. Antibody dilutions should follow the recommendation by the manufacturer. If, for example, the dilution given is 1:100, then a higher and lower dilution should be included (eg, 1:50 and 1:200). If no guidance is given, then a more comprehensive set of antibody dilutions is required (range 1:20 to 1:1000).
4. Antibody incubation times. Standard incubation times are for 2 hours at room temperature or overnight at 4°C. This can be altered if necessary following the results from antibody dilution optimisation. *Different batches of antibody should be retested under the conditions decided upon for the original antibody in a high, medium and low expressing sample, in order to ensure uniformity of staining patterns and signal intensity between batches.*

5. Detection systems. If there is no recommended detection system in the manufacturer's instructions, or the detection systems available in the laboratory differ from the recommendations, then 2 differing platforms should be used. This will typically consist of a one-step and a two-step system (see SOPs IHC CSMM004 and xxx).
6. Data interpretation. Scoring of slides should consist of 2 separate components: the extent of immunoreactivity (eg, percent of positively stained cells in target area), and the intensity of immunoreactivity. Scoring should ideally be undertaken by 2 pathologists according to standard institutional scoring protocols.
7. Validation reports. Within the report, there should be:
    - Details of SOPs used
    - Antibody details and conditions of assay
    - Localisation, intensity and extent of immunoreactivity
    - Tissue types and block numbers used for validation

Once the standard assay conditions have been decided upon, the assay should be repeated a further 3 times using tissue derived from serial sections in order to assess consistency of performance.

DISPOSAL

Dispose of as per appropriate COSHH instructions

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Plate reader verification**
Number ID:	**E CSMM011**
Review Date:	Every 2 years
Aim:	To ensure accuracy of plate readers used in association with clinical trial samples

History:

Version	Author	Date	Changes
1	L. Howells	13/03/08	
1	L Howells L. Howells	22/01/10	none

Cross reference:

Reviewed By: **F Teichert** F. Teichert 22-01-10

Training Requirement (please tick appropriate box)	
No Action	
Familiarly with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised By:

K Brown K Brown 28/1/10.

J Strupish 29/01/10

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation	COSHH
Azorubin S (amaranth A)	Sigma	
Sodium azide	Sigma	

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

1.1. Uniformity of Reading

1.1.1. Prepare a stock dye solution:-

1.1.2. *2g* Azorubin *S.*
Dissolve in 100ml Phosphate Buffered Saline (PBS), pH 7.2.
Add 0.1g sodium azide.
Mix until dissolved . Filter through Whatman 113v filter paper and check that no undissolved stain is left on the filter paper.
Store in a glass bottle, labelled as "Azorubin S Stock Solution with an expiry date 6 months from date of preparation.
Store at room temperature.
Prepare working strength dye solution by diluting the stock dye solution 1:500 with PBS, pH 7.2 to achieve working strength dye solution.

1.1.3. Using a multichannel pipette dispense 100ul into each well of a microtitre plate.

1.1.4. Read the plate x5 in quick succession at 540nm.

Calculate the mean value and coefficient of variation.

1.1.5. If the coefficient of variation is >5% prepare a fresh plate and repeat analysis to obtain a further ten plate readings. If the CV is >5%, withdraw the plate reader from service and advise the Laboratory Manager.

1.1.6. Print out the data from a spreadsheet, add details of instrument, date and sign.

1.1.7. Retain all test records in the instrument maintenance and calibration file.

1.1.8. Carry out this check on a monthly basis.

SOP

- Check that this SOP is the current version, cross check with database.

Title:	**Validation of VEGF assay**
Number ID:	**ELISA CSMM004**
Review Date:	Every 2 years
Aim:	To assess storage conditions required for human serum VEGF analysis

History:

Version	Author	Date	Changes
1	L Howells	20/03/08	
1	L Howells L-Howells	26/01/10	none

Cross reference:

VEGF assay SOP	Collection and storage of clinical samples	Use of fluostar plate reader
Validation of fluostar plate reader		

Reviewed By: **S Sale** 26.1.10

Training Requirement (please tick appropriate box)	
No Action	x
Familiarly with changes	
Documented Instruction	
Documented instruction and assessment	

Authorised By:

K Brown Karen Brown 28/1/10

J Strupish 28/01/10

Implemented 15/04/08

MATERIALS AND REAGENTS

Material	Grade, Source, Preparation
Human VEGF ELISA kit (Biosource)	
Microtiter plate reader (450nm)	BMG Fluostar
Human serum	Sigma
Human serum (sigma – H4522)	
Dry ice/ethanol	

HEALTH AND SAFETY

- Read appropriate COSHH forms for all of the chemicals used in the method.

PROCEDURE

Obtain fresh blood from volunteers into an untreated tube. Allow blood to clot. Remove serum following centrifugation at 3300xg at 4 degrees C for 10 minutes.

Aliquot serum into 1ml aliquots and store in a -80 freezer until required (avoid freeze thaw cycles).

Analyse serum samples using the Biosource VEGF kit.

- Day 1 post freezing: Defrost 1x1ml sample on ice and split into 4 aliquots. Keep aliquot (a) on ice. Freeze aliquot (b) x 1 in a dry ice/ethanol bath and re-thaw on ice. Freeze – thaw aliquot (c) x2, and aliquot (d) x3.
- Perform the assay as per the analysis SOP. Analyse each sample in triplicate.
- Day 90 post freezing: Repeat as for point 1, using the same kit if possible.

STANDARDISED SAMPLES

Use human serum from sigma and spike with samples used to make up the standard curve.

Analyse triplicate serum samples spiked with 0, 100pg/ml, 200pg/ml and 750pg/ml recombinant VEGF.

Repeat this (freshly made up) when analysing the 90 day post freezing samples.

Calculate VEGF levels in each sample from the standard curve. Compare freeze-thaw cycles, and also day 1 post-freezing vs day 90 post-freezing for stability. Assess reproducibility of standard spiked sample at day 1 vs day 90.

For stability demonstration, samples should fall within 30% of values obtained for day 1 post freezing sample (a).

UNIVERSITY OF LEEDS

Instructions for collecting the stool (poo) sample

If after reading these instructions you have any questions, please email caroline.young4@nhs.net You will receive a response the same day.

1. If you have recently had a colonoscopy (camera test of the bowel) please wait at least **14 days** before you collect the sample.

2. Remember to complete a **food diary** for the **day before** you collect the sample.

3. Collecting the stool sample will take approximately **5-10 minutes**. You should collect the stool sample from the privacy of your bathroom/toilet at home. When you're ready to collect the sample, write the **date & time** on the front of the card provided (ignore the back of the card, which is marked with a cross).

4. Take the card, stick (provided) and bag labelled '1' (provided) with you to the toilet. Open the 3 flaps on the front of the card to reveal 6 squares.

5. Go to the toilet and catch the stool **before** it goes into the toilet bowl. You can do this using folded toilet paper, a plastic glove over your hand or a disposable container (e.g. a clean plastic disposable cup or a clean plastic fruit carton).

6. Use the stick to smear a **thin** sample of the stool onto all **6 squares** of the card.

7. Close the 3 flaps, tucking them under the semicircle flaps at the bottom of the card.

8. Place the card back into the bag labelled '1'. Seal the bag.

9. Flush the remaining stool down the toilet. Wrap the stick in toilet paper and dispose of it in an outside bin (do **not** flush the stick down the toilet.) Wash your hands.

10. Place the bag labelled '1' inside the bag labelled '2'. (Please note, the bag labelled '2' also contains a square of white absorbent material – please do not remove this). Seal the bag labelled '2'.

Thank you for collecting the sample. For information on how to return it, please follow the **'Return of study pack'** instructions (page X).

Laboratory checklist for receiving samples

ONLY EVER DEVELOP ONE CARD AT A TIME TO AVOID MIXING UP STUDY DOCUMENTS

	Take the sample to the extraction room. Wear gloves and a green lab coat. Open the hood, clean it with ethanol and distel.	Tick
1	Record the **number** and **letter** code on the edge of the box: _____	
2	Remove the bag from the box. Open the outer bag to remove the inner bag containing the card. Place the card onto a paper towel in the hood.	
3	Record the following details from the card: ABC code _____ *Does this match the number from Q2?_____ (if not, email CY)* Date on card _____ Time on card _____	
4	Turn the card over and peel off the strip from the back. Apply **3 drops of developer solution** to the 6 brown areas (*bottle of developer solution is marked 'ABC' and is stored in the top drawer to the LHS of the hood*). Leave for **10 minutes**. Remove gloves.	
5	Write the **number and letter code from the edge of the box** on relevant pages (marked with - - - in the footer) of the following documents: • Consent form x2 • Questionnaire • Food diaries Place all documents inside a plastic wallet.	
6	Once the 10 minutes is over, place the card back inside the bag that it came in. Store this in the plastic box to the LHS of the hood.	
8	Waste: • The card box can be disposed of in cardboard waste. • The outer bag with absorbent sheet can be disposed of in the yellow-lined bin in the extraction lab. • The strip from the back of the card should be disposed of in the sharps bin inside the hood (gently close the lid). Clean the hood with distel and ethanol and close the hood.	
9	Date sample was received _____ Date sample was developed _____ Sample developed by (name) _____	
10	Temporarily store plastic wallet containing the study documents in locked drawer and email CY to let her know. Only one plastic wallet should be used per sample.	

Any questions, please text/call CY on 07578675774 or email caroline.young4@nhs.net Thank you!

Extraction Checklist (24gFOBT cards)

- This protocol is a modified version of the protocol: *Taylor, M., Wood, H.M., Halloran, S.P. and Quirke, P. Examining the potential use and long-term stability of guaiac faecal occult blood test cards for microbial DNA 16S rRNA sequencing. J Clin Pathol. 201*6.
- It uses the QIAamp DNA Stool Mini Kit (Qiagen) Cat No./ID: 51504 and Pathogen Lysis Tubes S (Qiagen) Cat No./ID: 19091
- Extraction is undertaken within a biohazard fume-hood in batches of twenty-four.

Collect and UV:

- 24 x pathogen lysis tubes
- 72 x 2ml eppendorfs
- 48 x 1.5ml eppendorfs
- 20 ml Buffer ASL QIAamp DNA Stool Mini Kit *(see preparation step)*
- 5 ml Ammonium acetate (10M)
- 19 ml Isopropanol
- 39 ml 70% ethanol
- 5 ml 100% ethanol
- 5 ml TE buffer (9ml molecular grade water: 1ml TE)
- 2.4 ml molecular grade water

Collect (do not UV):

- 24 x QIAmp DNA Stool Mini Kit spin columns
- 72 x 2ml QIAmp DNA Stool Mini Kit collection tubes

Preparation:

- Set shaker incubator 23C, 850rpm
- Set water bath at 70C: Warm buffer ASL for 10 minutes *(as precipitates out of solution)* - decant into falcons – UV. Keep buffer ASL warm until ready to use *(to prevent it precipitating).*
- Collect: paper towels, 48 scalpel blades, samples, 24x2ml eppendorfs (UV'd)

	Action	Additional action/explanation
1	Dissect 3 alternate squares into a 2ml Eppendorf using sterile scalpel blades.	*Cut by lifting the card off the work surface so that you don't scratch it, and quarter by resting the square on the back of the card.*
2	Add Buffer ASL (QIAamp DNA Stool Mini Kit): **800µl.**	*Push the solid down with pipette tip. Dissolves faeces from the card.*
3	Shaker incubator at **23°C at 850rpm for 1 hour**.	*Clean hood, change gloves. Prep for tomorrow.*
4	Briefly spin in centrifuge.	
5	Increase shaker incubator to 95°C.	
6	Transfer liquid into pathogen lysis tubes.	*Bacterial cell lysis.*
7	Vortex shaker **1800rpm-2200rpm for 10 minutes**.	
8	Shaker incubator **95°C, 850rpm for 15 minutes**.	Meanwhile, add **173µl of 10M Ammonium Acetate** to 2ml eppendorfs.
9	Centrifuge at **14000rpm for 1 minute.**	*Get ice.*
10	Decrease shaker incubator to 70°C.	
11	Add supernatant to the eppendorfs containing ammonium acetate.	*Precipitates impurities.*
12	Vortex, leave on ice for **5 minutes**.	Meanwhile, add an equal volume of **Isopropanol (approx. 725ul)** to clean 2ml eppendorfs.
13	Centrifuge **14000rpm for 5 minutes.**	*NB the pellet is debris.*
14	Add **supernatant** to eppendorfs containing isopropanol.	*DNA precipitation.*
15	Vortex, and leave on ice for **30 minutes**.	*Have lunch!*
16	Centrifuge **14000rpm for 10 minutes**.	
17	Discard supernatant, **do not disturb the pellet.**	
18	**Carefully** add **1ml of 70% Ethanol**, centrifuge for 5 minutes at 14000rpm.	
19	Discard supernatant.	
20	Carefully add **500ul 70% ETOH**. Centrifuge 14000rpm for 3 minutes.	
21	Discard supernatant.	
22	Leave the eppendorfs open to air dry. Ensure that all ethanol has evaporated (approx. 10 minutes).	
23	Re-suspend pellet by adding **200ul of 1x TE buffer**: leave with lids closed for 10mins.	*Re-suspend the DNA pellet.* Meanwhile, to clean 1.5ml eppendorfs, add: **200µl of Buffer AL (**QIAamp

		DNA Stool Mini Kit**).**
24	After 10mins vortex the eppendorfs containing the DNA and centrifuge briefly.	
25	Add the re-suspended DNA to the eppendorfs containing Buffer AL (QIAamp DNA Stool Mini Kit).	
26	Add **15µl** of Proteinase K (QIAamp DNA Stool Mini Kit).	
27	Vortex; leave on the shaker incubator at **70°C** and **650rpm** for 10 minutes.	*Meanwhile, label 1.5ml eppendorfs and get 72 collection tubes and 24 QIAamp spin colums.*
28	Centrifuge briefly.	*Turn off the shaker incubator.*
29	Add **200µl of 100%** Ethanol; vortex; centrifuge briefly.	
30	Transfer everything to a QIAamp Mini Spin Column (QIAamp DNA Stool Mini Kit).	
31	Centrifuge at 14000rpm for 1 minute; transfer column to a clean 2ml collection tube, and discard the flow through.	
32	Add 500µl of Buffer **AW1 (**QIAamp DNA Stool Mini Kit**).**	
33	Centrifuge at 14000rpm for 1 minute. Transfer columns to clean 2ml collection tubes and discard the flow through.	
34	Add 500µl of Buffer **AW2 (**QIAamp DNA Stool Mini Kit**).**	
35	Centrifuge at 14000rpm for **3 minutes**. Transfer columns to clean 2ml collection tubes and discard the flow through.	
36	Centrifuge at 14000rpm for 1 minute to dry the membranes.	
37	Transfer columns to clean labelled 1.5ml eppendorfs.	
38	Add 100µl of Molecular Grade water to each column; leave at room temperature for 5 minutes.	
39	Centrifuge at 14000rpm for 1 minute. Discard columns.	
40	Measure DNA concentration (Nanodrop).	

Amplification protocol

This protocol is adapted from the Earth Microbiome Project 16S protocol (http://www.earthmicrobiome.org/protocols-and-standards/16s/)

Stock primer dilutions

Details of how to order stock plates of primers are found here:
http://www.earthmicrobiome.org/protocols-and-standards/16s/

Details of how to re-suspend primers are found here:
http://www.earthmicrobiome.org/protocols-and-standards/primer-ordering-and-resuspension/

- Stock primers are at 100µM.
- Make **10µM** aliquots of the Forward and Reverse primers.
- Do **not** dilute: read1_seq, read2_seq, index_seq.
- Store at -20C.

DNA sample dilutions

1. Collect DNA from cold room; allow to come to room temperature (30minutes).
2. Calculate the volume of water needed to dilute 3µl DNA sample to a concentration of 20ng/µl. If the DNA sample concentration is <20ng/µl then do not add any water.
3. UV:
   - molecular water
   - 96 well plate
   - poppers
4. Cover 96 well plate with poppers.
5. Vortex and centrifuge DNA samples.
6. Add the required volume of molecular water to the first row of the 96 well plate.
7. Add 3µl DNA sample to each well. Re-cover row with poppers.
8. Complete for remaining rows.
9. Label plate. Freeze at -20C.

Amplification

Reagents:

PCR Hot Start PCR Master Mix (2x) from ThermoFisher (which contains PCR-grade water) Cat no 13000012 or 13000013 or 13000014
EMP primers
E.coli DNA (Sigma-Aldrich Cat no D4889)
human C2020 DNA (Sigma-Aldrich Cat no 941208132)

1. Allow the following to come to room temperature (30minutes):
   - Master Mix (PCR Hot Start PCR Master Mix (2x) from ThermoFisher)
   - Microbial-free water (PCR Hot Start PCR Master Mix (2x) from ThermoFisher)
   - DNA samples
   - Forward and Reverse EMP primers
   - Positive control (*E.coli* DNA)
   - Negative control (human C2020 DNA)

2. Vortex and centrifuge reagents. Place in cool box.

3. UV:
    - Plate
    - 1 x Eppendorf

4. Make the following mastermix in an Eppendorf (make enough for the required number of samples +1):

Reagent	**Volume**
PCR grade water	13 µl
PCR master mix(2x)	10 µl
Reverse primer (10 µM)	0.5 µl

5. Vortex and centrifuge. Add 23.5 µl to the relevant wells.
6. Add 0.5 µl relevant Forward Primer to the relevant wells.
7. Vortex and centrifuge DNA.
8. Add 1µl DNA to relevant wells using the multipipette.
9. Add 1 µl controls to relevant wells:

    - positive control = *E.coli* DNA
    - negative control = PCR grade water
    - negative control = human C2020 DNA

10. Seal plate. Scrape around the edges and in between each row and column.
11. Vortex plate, centrifuge.
12. Place plate in thermocycler.
13. Select the following programme:

Temperature	**Time**	**Repeat**
94C	3 minutes	
94C	45 seconds	X 35
50C	60 seconds	X 35
72C	90 seconds	X 35
72C	10 minutes	
4C	Infinite hold	
Reaction volume = 25 µl		

14. Once amplification is complete check that:

    - wells contain an equal volume
    - there is no condensation
    - liquid has not spread to wells which you would expect to be empty

15. Scrape around the plate and take to the post-PCR room.

Suitable freezing point.

Post-amplification gel electrophoresis

Reagents:

Agarose
Ethidium bromide

1. Weigh 1.5g agarose into a conical flask.
2. Add100ml TBE.
3. Microwave until agarose has dissolved, be careful the liquid does not bubble over.
4. Cool the conical flask under running water.
5. Add 5µl ethidium bromide, swirl until dissolved.
6. Pour liquid slowly into tray with combs and leave for 30 minutes to set. Once set, remove combs and place tray with gel into tank filled with TBE.
7. Put 1.5µl of orange loading dye/well into a new plate.
8. Vortex and centrifuge plate of PCR products.
9. Add 5µl PCR product to each well of the plate containing orange loading dye and seal.
10. Briefly centrifuge the plate.
11. Load 5µl 50bp blue ladder dye into the first well of the gel and 5µl sample into subsequent wells.
12. Turn on the BioRad pack:100V, 25min.
13. View gel using a GelDoc.
14. The expected band size is 300-350bp.

Post-amplification quantification

Reagents:

1. Quant-iT 'dsDNA Assay Kit, broad range' (Invitrogen, Thermo Fisher Scientific, USA) Cat no Q33130. This requires special plates (Costar, Life Sciences, USA) and a special machine (Fluoroskan Ascent, Thermo Fisher Scientific, USA).
   NB. The EMP protocol uses an alternative kit.

2. Create the following mastermix:

Reagent	Volume per sample (µl)
Quant-iT dsDNA BR reagent	1
Quant-it Buffer	197

3. Add 198µl master-mix to the wells of a 96 well plate (Costar, Life Sciences, USA).
4. Add 2µl PCR product.
5. Add 2µl of the Quant-iT 'dsDNA Assay Kit, broad range' standard to the wells of the final column.
6. Read fluorescence using a microplate fluorometer (Fluoroskan Ascent, Thermo Fisher Scientific, USA).
7. Calculate the concentration.

Post amplification pooling and cleanup

Reagents:

MinElute PCR Purification kit (Qiagen, Germany) Cat no 28004 or 28006. *NB the EMP protocol uses a different kit.*
If the MinElute PCR Purification kit is new, make up Buffer PE by adding 100% ethanol according to the instructions.

1. Combine **240ng** of each sample into a single sterile Eppendorf (max 5µg/Eppendorf according to the MinElute PCR Purification kit i.e. max 20 samples/eppendorf).
2. UV clean eppendorfs (you will need 2 for each pool).
3. Take (5x volume of each pool) of Buffer PB (MinElute PCR Purification kit) and put the Buffer PB in a clean eppendorf.
4. Vortex and centrifuge pool.
5. Transfer pool to eppendorf containing Buffer PB and mix with pipette.
6. Transfer the mix to a min-elute column (MinElute PCR Purification kit).
7. Centrifuge at 13,000rpm for 1 minute.
8. Discard the flow through into glass container. Dab the top of the collection tube on a tissue. Re-insert the column.
9. Add 750µl of Buffer PE (MinElute PCR Purification kit) to the column. Close the lid carefully.
10. Centrifuge at 13,000rpm for 1 minute.
11. Discard the flow through. Dab the top of the collection tube on a tissue. Re-insert the column.
12. Centrifuge the column empty at 13,000rpm for 1 minute to dry the membrane.
13. Transfer the column to a pre-labelled 1.5ml eppendorf.
14. Add 10µl Buffer EB (MinElute PCR Purification kit) to the center of the membrane **without touching the membrane** and leave for 1 minute.
15. Centrifuge 13,000rpm for 1 minute.
16. Repeat steps 14. and 15 (so that the final eluted volume is 20µl).

Sequencing

- Due to the low eluted volume, you may wish to take 1µl of cleaned sample and dilute this for any subsequent quantification steps.
- Measure the concentration of the cleaned pool using either:
    - Nanodrop: A260/A280 ratio should be between 1.8-2.0 (as per EMP protocol but not recommended)
    - Fluoremetric measurement
    - qPCR
    - Tape station: ensure there is no adaptor peak

- Send the pool for sequencing. Also send read1_seq, read2_seq, index_seq.

- For running libraries on the MiSeq and HiSeq, please make sure you read the supplementary methods of Caporaso et al. (2012). You will need to make your sample more complex by adding PhiX to your run.

Sample Size Stata Code

```
*******************************************************************************************
* Project Name: COLOPREVENT                                                               *
* Document: Sample Size Calculation                                                       *
* Date: May 2019                                                                          *
* Software: Stata Version 15.1                                                            *
*******************************************************************************************
```

**** 1, TRIAL-1 Main polyp reduction trial**

```
** Assuming the MAP rate of 0.94 for the Aspirin arm after subtracting the effect of Aspirin based on the Seafood
*result... let's call it crate

* 30% reduction in MAP rate to be clinically meaningful and estimate a MAP rate of 0.66 in the treatment arm .....
let's call it arate

* Z alpha and Z beta function for 80% power i.e z_alpha = 1.96 and z_beta = 0.84

clear

input alpha_twosided crate arate z_alpha z_beta

0.05 0.94 0.66 1.96 0.84

end

list
*******************************************************************************************
*Equation 8 from Cundill and Alexander for calculating a sample size assuming a negative binomial distribution
*******************************************************************************************
* Assuming the over dispersion is equal 2 from the BSCP data (2006 - 2016) for the new high risk group

* Q1 and Q0 = 0.5 for equal allocation ratio

* k1 and k0 are estimated using the following equation from Cundill & Alexander:

* Dispersion = Var(μ)/ μ = 1 + (μ/k),  Where μ = the MAP rate in each arm. Thus, for this example where
*dispersion=2, k=μ.

** Using Over-dispersion of 2.0

gen dispersion=2

*Therefore in our case the dispersion parameter for the active and control arm k1 and k0 will be

gen k0= crate/(dispersion - 1)

gen k1= arate/(dispersion - 1)

*** Equal allocation ratio

gen q0=0.5

gen q1=0.5

*** generating the denominator and numerator for equation 8

gen top_eqn_8= ((1.96 + 0.84)^2)*(((1/q1)*((1/arate)+(1/k1))) + ((1/q0)*((1/crate) + (1/k0))))

gen bot_eqn_8=(ln(crate)-ln(arate))*(ln(crate)-ln(arate))
```

```
* Calculating the sample size for 80% power assuming negative binomial and using the above denominator and
*numerator for equation 8

gen n_80_power_eqn_8= top_eqn_8/bot_eqn_8

*** 80% power including 15% drop out

gen n_power_80_a_15_drop=n_80_power_eqn_8/0.85

**** Number per arm

foreach var of varlist  n_80_power_eqn_8 n_power_80_a_15_drop  {

gen `var'_arm=.

replace `var'_arm= `var'/2

}

*

order alpha_twosided crate arate n_80_power_eqn_8 n_80_power_eqn_8_arm  ///

n_power_80_a_15_drop n_power_80_a_15_drop_arm

** There will be some crossovers for example if patients in the Aspirin alone arm diagnosed with diabetic they may
*start taking metformin some patients from Aspirin + metformin may found metformin very toxic (4% from the
*STAMPED paper) so they may stop taking metformin which means they will be in Aspirin alone arm

****the proportion withdrawing or non-compliant from Aspirin alone

gen Q_a=0.03

*** The proportion withdrawing or non-compliant from Aspirin plus metformin

gen Q_a_m=0.03

** Assuming 3% crossover in each arm the inflation factor will b

gen inflation_factor= 1/(1 - Q_a - Q_a_m)^2

rename (n_80_power_eqn_8 n_80_power_eqn_8_arm n_power_80_a_15_drop ///

n_power_80_a_15_drop_arm) (n_80_power n_80_power_arm n_power_80_a_15_drop ///

n_power_80_a_15_drop_arm)

foreach var of varlist n_power_80_a_15_drop n_power_80_a_15_drop_arm {

gen `var'_infl= `var'*inflation_factor

}

*

** Keeping important variables only

keep alpha_twosided crate arate n_80_power n_80_power_arm n_power_80_a_15_drop ///

n_power_80_a_15_drop_arm n_power_80_a_15_drop_infl n_power_80_a_15_drop_arm_infl

format n_80_power-n_power_80_a_15_drop_arm_infl %9.0fc
```

******* End of Do file for the main trial (Trial-1) **********

**** 2, TRIAL-2 Resveratrol signal-seeking trial**

* Assuming the MAP rate of 1.2 for the placebo arm ... let's call it crate. 35% reduction in MAP rate to be clinically *meaningful and estimate a MAP rate of 0.78 in the two Resveratrol arms ..... let's call it arate

** Z alpha and Z beta function for 80% power i.e z_alpha = 1.96 and z_beta = 0.84

clear

input alpha_twosided crate arate z_alpha z_beta

0.05 1.2 0.78 1.96 0.84

end

** Using Over-dispersion of 2

gen dispersion=2

** Therefore in our case the dispersion parameter for the active and control arm k1 and k0 will be

gen k0= crate/(dispersion - 1)

gen k1= arate/(dispersion - 1)

*** Equal allocation ratio

gen q0=0.5

gen q1=0.5

*** generating the denominator and numerator for equation 8

gen top_eqn_8= ((1.96 + 0.84)^2)*(((1/q1)*((1/arate)+(1/k1))) + ((1/q0)*((1/crate) + (1/k0))))

gen bot_eqn_8=(ln(crate)-ln(arate))*(ln(crate)-ln(arate))

*** Calculating the sample size for 80% power assuming negative binomial and using the above denominator and *numerator for equation 8

gen n_80_power_eqn_8= top_eqn_8/bot_eqn_8

*** 80% power including 10% drop out

gen n_power_80_a_10_drop=n_80_power_eqn_8/0.9

**** Number per arm (Three arms)

foreach var of varlist n_80_power_eqn_8 n_power_80_a_10_drop {

gen `var'_arm=.

replace `var'_arm= `var'/2

}

*** Total sample size need for the three arms

gen total_ss_3arm=n_power_80_a_10_drop_arm*3

** Keeping important variables only

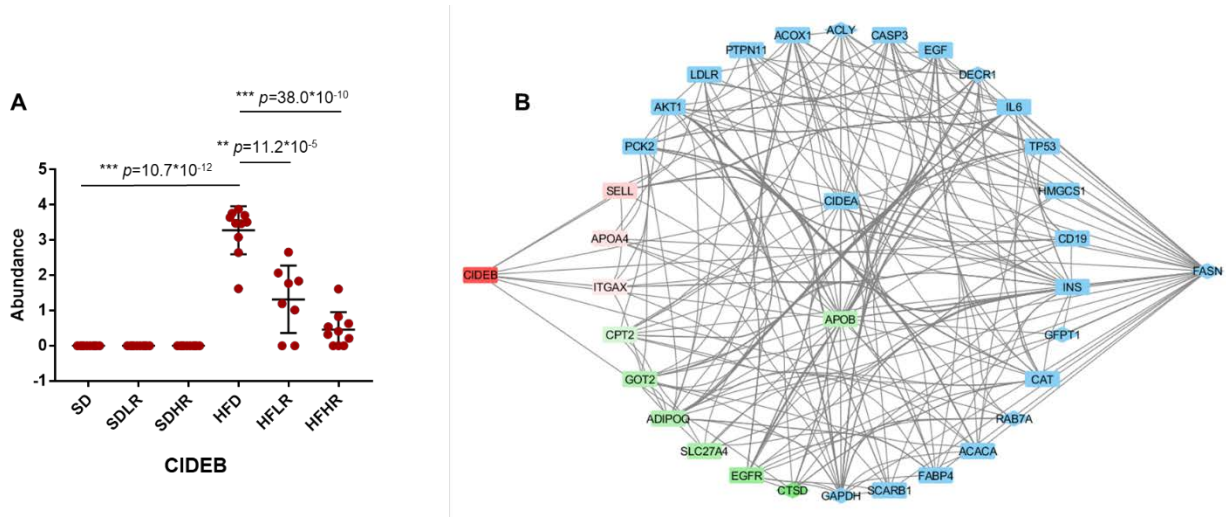
keep alpha_twosided crate arate n_80_power_eqn_8 n_80_power_eqn_8_arm ///

n_power_80_a_10_drop n_power_80_a_10_drop_arm total_ss_3arm

format n_80_power_eqn_8-total_ss_3arm %9.0fc

********** End of Do File for the Signal Seeking Trial (Trial 2) **********

Appendix 1



Proteomic analysis of mouse plasma samples reveals CIDEB as a candidate pharmacodynamic biomarker of resveratrol efficacy *in vivo*. Cell death activator b (CIDEB) was identified as a potential biomarker that correlates with resveratrol efficacy, using a dual proteomics approach, in which the plasma proteome of a *Apc*^{Min} mice from our previous published study[7] was analysed together with the chemical proteome of resveratrol from resveratrol pull-down studies using CRC cell lines. **A.** CIDEB expression is markedly increased in the plasma of *Apc*^{Min} mice maintained on a high-fat diet (HFD) compared to animals on the standard diet (SD). Dietary supplementation with resveratrol at 0.7ppm (HFLR) and 143ppm (HFHR) begins to significantly restore CIDEB to baseline levels. **B.** CIDEB is associated with fatty acid synthase (FASN) via cell death activator a (CIDEA) and apolipoprotein B (APOB). Network analysis combining significant plasma proteome changes and direct resveratrol interactors (as observed from chemical proteomics studies) highlights potential mechanistic insights. Networks generated from plasma proteomics data using Ingenuity Pathway Analysis (Qiagen) were further combined with chemical proteomic data and analysed using Cytoscape (STRING) to highlight protein-protein interactions within networks.

A. SD – Standard diet; SDLR – Standard diet + 7ppm resveratrol; SDHR – Standard diet + 143ppm resveratrol; HFD – High-fat diet; HFLR – High-fat diet + 7ppm resveratrol; HFHR – High-fat diet + 143ppm resveratrol. Abundance shown as arcsine transformed data with mean and SD. *p*-values calculated by unpaired t test.
B. Upregulated by high-fat diet – red; downregulated by high-fat diet – green; IPA network members – blue; resveratrol interactors – diamonds.

Appendix 2: Visit table for COLO-PREVENT: 3 year intervention - both arms

	Pre-trial BCSP colonoscopy T=-2 weeks	Post-screening colonoscopy out-patient visit **Visit 1** T=0 weeks	Telephone call **Visit 2** T=4 weeks	Telephone call **Visit 3** T=12 weeks	Out-patient visit **Visit 4** T=25 weeks	Telephone call **Visit 5** T=37 weeks	Out-patient visit **Visit 6** T=52 weeks	**Every 6 months** Out-patient visit **Visits 7-9**	Exit surveillance colonoscopy **Visit 10** T=154 weeks	Routine Visit Post-surveillance colonoscopy **Visit 11** T=156 weeks
Provide trial information (PIL)	X									
Check eligibility		X								
Informed consent		X								
Medical history/demographic data		X								
BP measurement and ECG		X								
Medication log		X	X	X	X	X	X	X	X	
Colonoscopy	X								X	
Colonoscopy results		X								X
Randomisation		X								
Trial drug dispensing		X[a]	Increase metformin dose from 500mg OD to BD		X		X	X		
Fasting blood sample & processing -safety & research[b]		X			X		X	X	X	
FOBT samples		X					X		X (prior to colonoscopy)	

FFQuestionnaire		X					X	X (visit 8)	X	
AE recording			X	X	X	X	X	X	X	X
Compliance			X	X	X	X	X	X	X	
Rectal biopsies									X	
Retrieval of diagnostic FFPE blocks		X							X	

[a]Patient to commence their trial medication the day after they have collected a baseline FOBT sample.

[b]To include Fasting lipid profile, FBC, LFT, U&E, eGFR, HbA1c, insulin, IGFBP-3, IGF-I, glucose

Appendix 3: Visit table for COLO-PREVENT-SS: 1 year intervention - All 3 arms

	Pre-trial BCSP colonoscopy T=-2 weeks	Post-screening colonoscopy out-patient visit **Visit 1** T=0 weeks	Telephone call **Visit 2** T=2 weeks	Telephone call **Visit 3** T=12 weeks	Out-patient visit **Visit 4** T=25 weeks	Telephone call **Visit 5** T=37 weeks	Exit surveillance colonoscopy **Visit 6** T=50 weeks	Routine Visit Post-surveillance colonoscopy **Visit 7** T=52 weeks
Provide trial information (PIL)	X							
Check eligibility		X						
Informed consent		X						
Medical history/demographic data		X						
Medication log		X	X	X	X	X	X	
Colonoscopy[a]	X						X	
Colonoscopy results		X						X
Randomisation		X						
Trial drug dispensing		X[b]			X			
Fasting blood sample & processing - safety & research[c]		X			X		X	
Provision of urine sample[d]		X			X			X
FOBT samples		X			X		X (prior to colonoscopy)	
FFQuestionnaire		X					X	
AE recording			X	X	X	X	X	X

Compliance			X	X	X	X	X	
Rectal biopsies							X	
Retrieval of diagnostic FFPE blocks		X					X	

[a]First colonoscopy is standard, second is research

[b]Patient to commence their trial medication the day after they have collected a baseline FOBT sample

[c]To include FBC, LFT, U&E, eGFR, HbA1c, fasting lipid profile, insulin, IGFBP-3, IGF-I, glucose

[d]Urine will be collected from 20% of randomly selected patients for compliance testing

Appendix 4

These Tables illustrate the effect of higher than predicted mean adenoma numbers per person (MAP) on the sample size for each trial.

COLO-PREVENT: Main polyp reduction trial with aspirin and metformin

Sample sizes assuming a negative binomial model with over-dispersion of 2.0; Alpha = 0.05 (two sided)

Power (%)	MAP rate in the untreated population	Control Rate$_0$*	% reduction in MAP	Active Rate$_1$	N per arm	Inflate for 15% drop-out N per arm	Total sample for two-arm trial	Inflated sample sizes assuming 3% crossover in each arm (i.e inflation factor of 1.13)	
								N per arm	Total for two arm
80	1.5	1.17	30	0.82	257	303	606	343	685
80	1.4	1.09	30	0.76	269	317	634	359	718
80	1.3	1.01	30	0.71	303	356	712	403	806
80	**1.2**	**0.94**	**30**	**0.66**	**323**	**380**	**806**	**431**	**862**

*includes 22% reduction in total MAP from Aspirin use based on the seafood trial; NB: The row grey out is the current sample size used for this application.

COLO-PREVENT-SS: Resveratrol Signal-Seeking trial

Sample sizes assuming a negative binomial model with over-dispersion of 2.0; Alpha = 0.05 (two sided)

Power (%)	Control Rate$_0$*	% reduction in MAP	Active Rate$_1$	N per arm	Inflate for 10% drop-out	Total sample for three-arms trial
80	1.5	35	0.98	146	162	486
80	1.4	35	0.91	153	170	510
80	1.3	35	0.85	169	188	564
80	**1.2**	**35**	**0.78**	**179**	**199**	**596**

*Assuming 50:50 split of patients unable to tolerate aspirin versus already taking aspirin; NB: The row grey out is the current sample size used for this application.

Appendix 5: COLO-PREVENT main trial: Details of sample collection and biomarker analysis

Sample type	Timing and frequency of collection	Total per patient and Trial total	Biomarkers measured	Reason for analysis	Evidence samples are fit for purpose	Assay method	Level of validation
Blood sampling requirements							
Blood - whole blood and plasma	Baseline, every 6 months and end of study	Blood collection on 7 occasions from all patients. **Total = 6034 samples**	Fasting glucose, insulin, HbA1c, IGF1, IGFBP-3, triglycerides and cholesterol	Assessment of metabolic status and associations with efficacy	Collected as for standard clinical care	Standard NHS laboratory methods	ISO accredited labs at each trial site or centralised analysis (for IGF1, IGFBP-3)
Blood – plasma and serum.	Baseline, every 6 months and end of study	Plasma and serum collection on 7 occasions from all patients **for all further biomarker work described below** **Total = 6034 samples**	See below	See below	See below	See below	See below

Appendix 5: COLO-PREVENT main trial: Details of sample collection and biomarker analysis

All numbers below this point relate to numbers and type of analyses to be undertaken on the above samples and NOT to further blood sampling requirements							
		Analysis of samples from 20% patients in COLO-PREVENT on 3 occasions ***519 samples***	Serum thromboxane B2	Aspirin compliance	The assay method has been applied by a colleague at the University of Leicester for the analysis of plasma samples in cardiovascular clinical trials involving aspirin[1].	ELISA based assay where the difference between the level of thromboxane B2 in serum and plasma ($[TxB2]_{S-P}$) is measured to provide a direct indicator of the effect of aspirin on platelets[1].	Full in house validation is needed to GCLP standards. We will do this in accordance with the FDA guidelines for Bioanalytical method validation.
		All patients in COLO-PREVENT metformin + aspirin arm on 2 occasions (midpoint and trial end) ***1212 samples***	Metformin plasma concentrations	Compliance and any association between plasma levels and efficacy.	We will set up and validate an LC-MS/MS assay that has previously been used for determining metformin plasma and tissue concentrations in published clinical trials[2-4].	LC-MS/MS assay.	We will validate to GCLP standards, according to FDA guidelines for Bioanalytical method validation.

Appendix 5: COLO-PREVENT main trial: Details of sample collection and biomarker analysis

Tissue sample requirements							
From all patients: biopsies of normal mucosa at surveillance colonoscopy	6 biopsies per patient at surveillance. 3 for immediate freezing and 3 for FFPE	6 biopsies per patient, taken on a single occasion. Total: ***2586 FFPE samples 2586 frozen samples***	Analysis of PD markers in normal tissue for 20% of randomly selected patients: NF-kB expression & localisation, apoptosis (cleaved PARP), pS6/S6 expression/localisation.	**Pilot study** to investigate whether the combination leads to additive effects on mechanistically relevant biomarkers and whether there is an association with preventive efficacy	High quality biopsy samples of normal tissue were previously obtained in the seAFOod trial using comparable protocols for collection and storage.	Immunofluoresence of FFPE samples using a Vectra Polaris multi-spectral imaging system. This will enable simultaneous imaging & measurement of multiple overlapping biomarkers within a single tissue section	These assays will be developed and validated to GCLP standards in our laboratory. Cleaved PARP is already established.
Where available - retrieved diagnostic FFPE blocks of adenoma tissue	From the screening and surveillance colonoscopies	Two per patient, all patients, where available. ***Maximum of 1724 samples***	Analysis of PD markers in adenoma tissue in the same 20% of randomly selected patients as above: NF-kB expression & localisation, apoptosis (cleaved parp), pS6/S6 expression/localisation).	**Pilot study** to investigate whether the combination leads to additive effects on mechanistically relevant biomarkers and whether there is an association with preventive efficacy	Diagnostic blocks from pathology within the BCSP will be of high quality.	Immunofluoresence of FFPE samples using a Vectra Polaris multi-spectral imaging system. This will enable simultaneous imaging & measurement of multiple overlapping biomarkers within a single tissue section	These assays will be developed and validated to GCLP standards in our laboratory.
Faecal samples							
Faecal samples	Baseline, 12 months and end of study.	Samples will be provided by all patients	Microbiome profiling	Investigate whether aspirin and metformin	Phil Quirke (Co-investigator) has validated methods	Next generation sequencing of the 16S rRNA V4	Validated protocols already exist.

Appendix 5: COLO-PREVENT main trial: Details of sample collection and biomarker analysis

collected using a gFOBT card		on 3 occasions. **Total: 2586**		alter the gut microbiome and whether the effects might contribute to efficacy	for the collection, storage and transport of faecal samples collected using a gFOBT card from BCSP patients. The resulting samples are compatible with high quality sequencing results for microbiome analysis.	region using our standard robust methodology	

References cited in Appendix 5

1. Good RI, McGarrity A, Sheehan R, James TE, Miller H, Stephens J, et al. Variation in thromboxane B2 concentrations in serum and plasma in patients taking regular aspirin before and after clopidogrel therapy. Platelets. 2015;26(1):17-24.
2. Paleari L, Burhenne J, Weiss J, Foersch S, Roth W, Parodi A, et al. High Accumulation of Metformin in Colonic Tissue of Subjects With Diabetes or the Metabolic Syndrome. Gastroenterology. 2018;154(5):1543-5.
3. DeCensi A, Puntoni M, Gandini S, Guerrieri-Gonzaga A, Johansson HA, Cazzaniga M, et al. Differential effects of metformin on breast cancer proliferation according to markers of insulin resistance and tumor subtype in a randomized presurgical trial. Breast Cancer Research and Treatment. 2014;148(1):81-90.
4. Grun B, Kiessling MK, Burhenne J, Riedel KD, Weiss J, Rauch G, et al. Trimethoprim-metformin interaction and its genetic modulation by OCT2 and MATE1 transporters. Br J Clin Pharmacol. 2013;76(5):787-96.

Appendix 6: COLO-PREVENT-SS resveratrol trial: Details of sample collection and biomarker analysis

Sample type	Timing and frequency of collection	Total per patient and Trial total	Biomarkers measured	Reason for analysis	Evidence samples are fit for purpose	Assay method	Level of validation
Blood sampling requirements							
Blood – whole blood and plasma	Baseline, 6 months and end of study	Plasma and serum collection on 3 occasions from all patients. **Total = 1788 samples**	Fasting glucose, insulin, HbA1c, IGF1, IGFBP-3, triglycerides and cholesterol	Assessment of metabolic status and associations with efficacy	Collected as for standard clinical care	Standard NHS laboratory methods	ISO accredited labs at each trial site or centralised analysis (for IGF1, IGFBP-3)
Blood – plasma and serum.	Baseline, 6 months and end of study	Plasma, and serum collection on 3 occasions from all patients **for all further biomarker work described below** **Total = 1788 samples**	See below	See below	See below	See below	See below

Appendix 6: COLO-PREVENT-SS resveratrol trial: Details of sample collection and biomarker analysis

All numbers below this point relate to numbers and type of analyses to be undertaken on the above samples and NOT to further blood sampling requirements							
		All patients on 3 occasions ***1788 samples***	Plasma resveratrol and metabolite levels	Explore associations between, human/bacterial metabolite profile and concentrations with efficacy. Evidence of an interaction with metformin.	We will follow our established SOPs for collection, processing and analysis that have been used for resveratrol/metabolite determination in our previous trials[1 2].	Our in-house validated HPLC-UV assay.	Assay is validated to GCLP standards, according to FDA guidelines for Bioanalytical method validation.
		All patients on 3 occasions ***1788 samples***	Plasma MLX, and CIDEB protein concentrations	Assessment of candidate resveratrol PD biomarkers that correlate with efficacy in preclinical models and are altered in plasma samples of patients in our previous trials. Will also provide mechanistic insight.	We will follow our established SOPs for collection and processing of plasma samples as these methods were suitable for the analysis of MLX1 and CIDEB proteins in plasma from colorectal and prostate cancer patients in our resveratrol trials[3].	Commercial ELISA kits (My Biosource; Biovendor)	MLX ELISA assay is currently being validated to GCLP standards. CIDEB ELISA kit will need to be validated to GCLP standards according to FDA guidelines.

Appendix 6: COLO-PREVENT-SS resveratrol trial: Details of sample collection and biomarker analysis

Tissue sample requirements							
From all patients: biopsies of normal mucosa at surveillance colonoscopy	6 biopsies per patient at surveillance. 3 for immediate freezing and 3 for FFPE	6 biopsies per patient, taken on a single occasion. **Total = 1788 FFPE samples 1788 frozen samples**	Analysis of resveratrol PD markers: protein carbonyls, NQO1 protein expression, Ki67 index	Assessment of whether candidate resveratrol PD biomarkers identified from previous work correlate with efficacy.	Samples will be collected according to our SOPs followed in previous resveratrol trials where we have detected changes in these PD markers[3 4].	ELISA (NQO1, My Biosource Biovendor), IHC (Ki67) OxiSelect™ protein carbonyl spectrophotometric assay (Cell Biolabs Inc.),	Ki67 analysis - ISO accredited lab University Hospitals of Leicester. ELISA/spectrophotometric assays will be validated to GCLP standards in our laboratory.
Where available - retrieved diagnostic FFPE blocks of adenoma tissue	From the screening and surveillance colonoscopies	Two per patient, all patients, where available. Total: **Total = 1192 samples**	Analysis of resveratrol PD markers: Ki67 index	Assessment of whether candidate resveratrol PD biomarkers identified from previous work correlate with efficacy.	Diagnostic blocks from pathology within the BCSP will be of high quality and Ki67 is a routinely measure marker.	Standard NHS laboratory methods	ISO accredited lab, University Hospitals of Leicester
Urine samples							
Urine	Baseline, 6 months and end of study	Three samples collected from 20% of patients. **Total = 358 samples**	Concentrations of parent resveratrol and major metabolites.	Assessment of compliance.	We will follow our established SOPs for collection, processing and analysis that have been used for resveratrol/metabolite determination in urine in our previous trials[5].	Our in-house validated HPLC-UV assay.	Assay is validated to GCLP standards, according to FDA guidelines for Bioanalytical method validation.

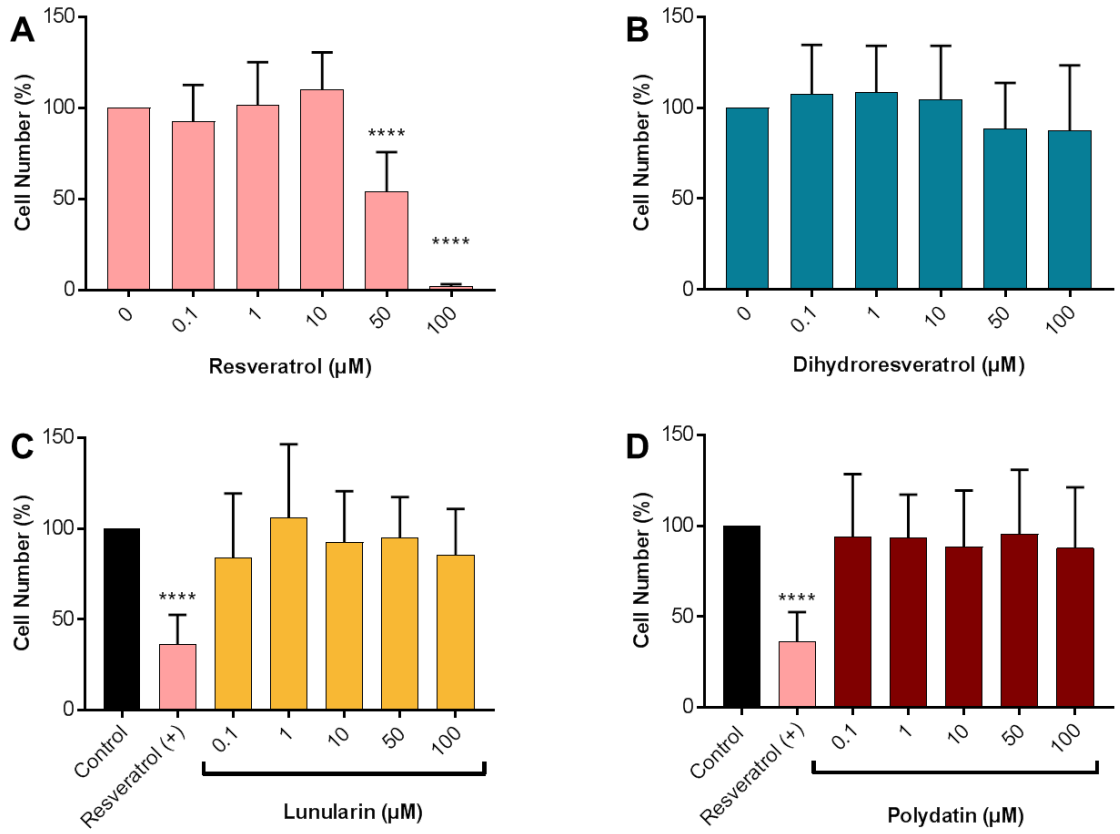
Appendix 6: COLO-PREVENT-SS resveratrol trial: Details of sample collection and biomarker analysis

Faecal samples							
Faecal samples collected using a gFOBT card	Baseline, 6 months and end of study.	Samples will be provided by all patients on 3 occasions. **Total = 1788**	Microbiome profiling	Investigate whether resveratrol alters the gut microbiome and whether the effects might contribute to efficacy.	Phil Quirke (Co-investigator) has validated methods for the collection, storage and transport of faecal samples collected using a gFOBT card from BCSP patients. The resulting samples are compatible with high quality sequencing results for microbiome analysis.	Next generation sequencing of the 16S rRNA V4 region using our standard robust methodology	Validated protocols already exist.

References cited in Appendix 6

1. Patel KR, Andreadi C, Britton RG, Horner-Glister E, Karmokar A, Sale S, et al. Sulfate Metabolites Provide an Intracellular Pool for Resveratrol Generation and Induce Autophagy with Senescence. Science Translational Medicine. 2013;5(205).
2. Brown VA, Patel KR, Viskaduraki M, Crowell JA, Perloff M, Booth TD, et al. Repeat Dose Study of the Cancer Chemopreventive Agent Resveratrol in Healthy Volunteers: Safety, Pharmacokinetics, and Effect on the Insulin-like Growth Factor Axis. Cancer Research. 2010;70(22):9003-11.
3. Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, et al. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Science Translational Medicine. 2015;7(298).
4. Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, et al. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res. 2010;70(19):7392-9.
5. Boocock DJ, Patel KR, Faust GES, Normolle DP, Marczylo TH, Crowell JA, et al. Quantitation of trans-resveratrol and detection of its metabolites in human plasma and urine by high performance liquid chromatography. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences. 2007;848(2):182-7.

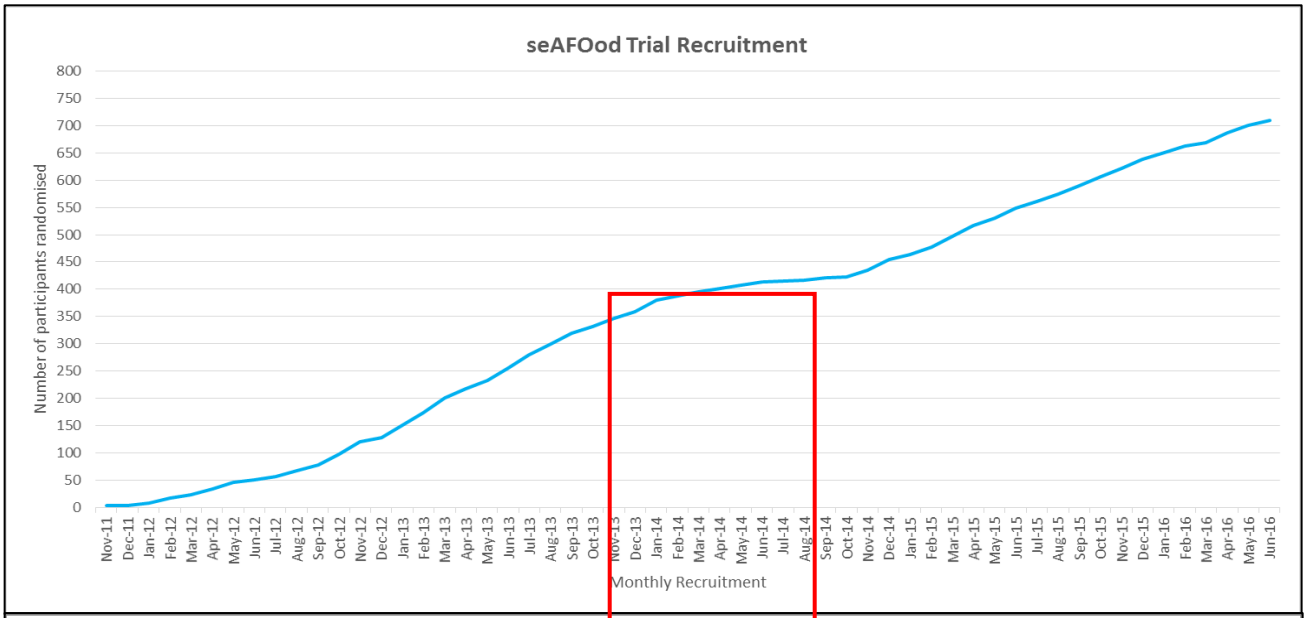
Appendix 7

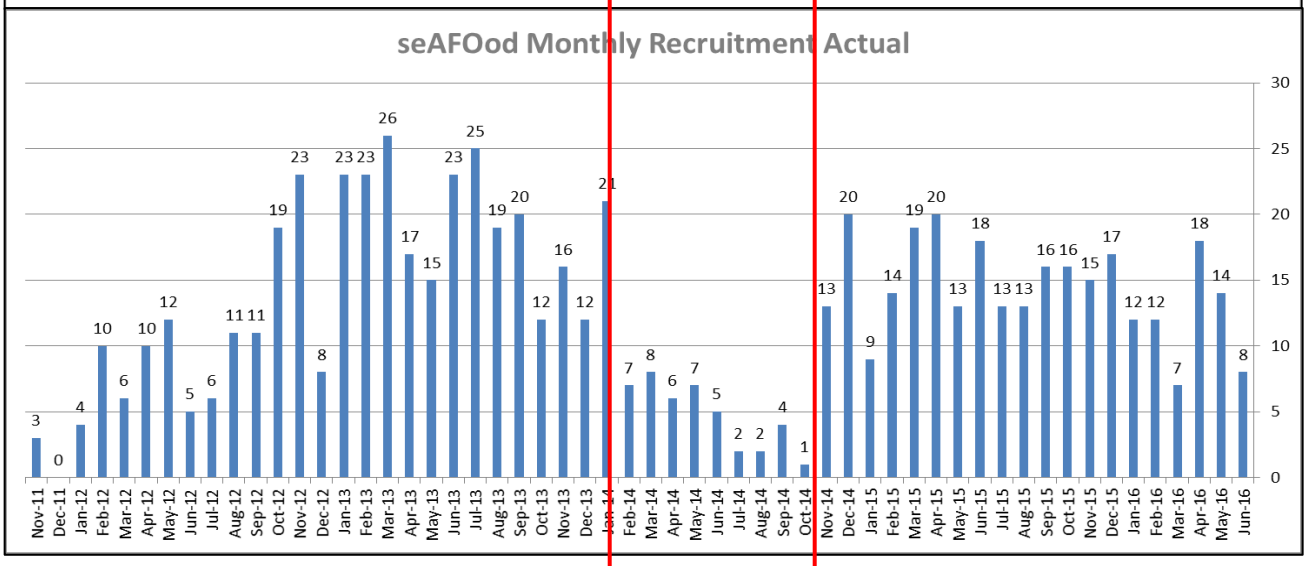


In contrast to the parent compound, the bacterial metabolites of resveratrol have no effect on the proliferation of HT-29 colorectal cancer cells. Incubations were conducted for 72h with fresh resveratrol/metabolite added in daily and cells were counted at 72h. The bacterial metabolites tested were **B**) dihydroresveratrol, **C**) lunularin and **D**) polydatin, up to a concentration of 100μM. Cell number is expressed as a percentage relative to the solvent control (DMSO) and resveratrol was included as a positive control in the lunularin and polydatin incubations. Experiments were conducted in triplicate wells on three independent occasions and error bars represent the mean+SEM. Significant differences are indicated by ****p<0.0005.

seAFOod
eicosapentaenoic acid (EPA)/aspirin
Polyp Prevention Trial

Appendix 8

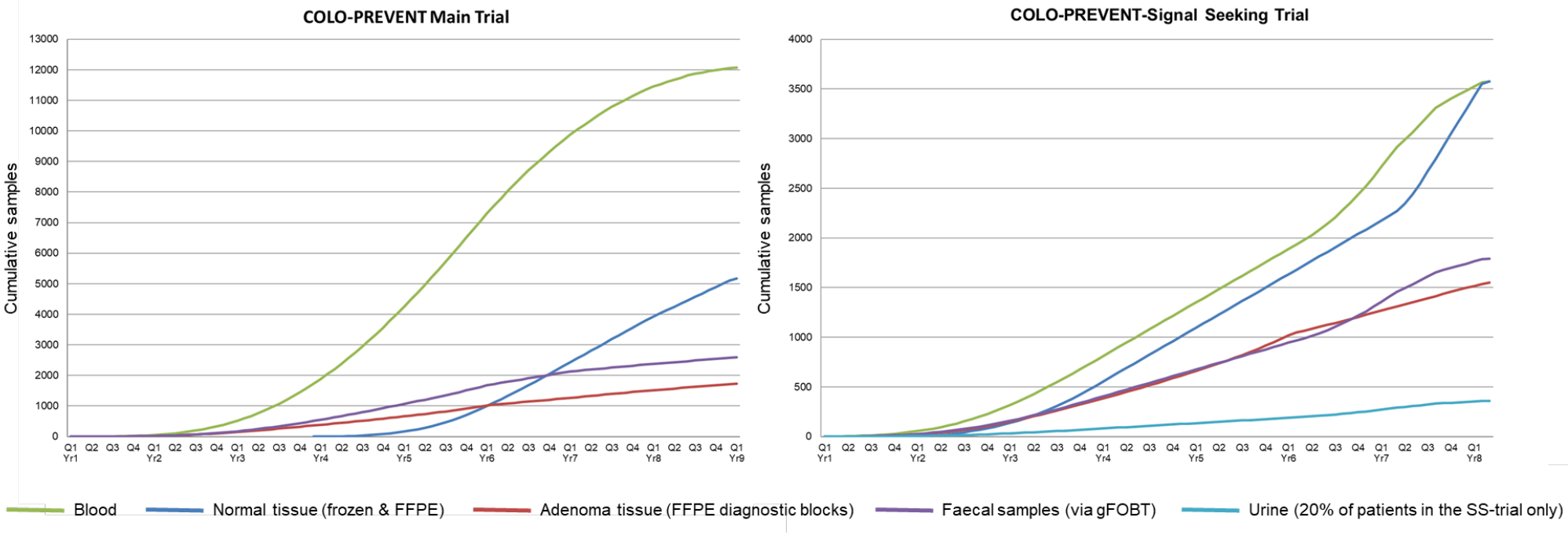




Trial 're-boot'

Recruitment in the seAFOod Polyp Prevention trial of eicosapentaenoic acid and aspirin. Midway through the trial, the eicosapentaenoic acid IMP was discontinued and an alternative manufacture had to be found. This led to a large drop in recruitment, as stocks of the first IMP dwindled, until the supply was reinstated. This period is indicated by the red box. Excluding a 12-month 'run-in' period and the time when first IMP supply was limiting, there was a mean of 16 randomisations per month (range 7-26), which equates to ~18% of all high-risk cases.

Appendix 9:



Copy of Figure 9 from the main proposal: Predicted sample collection for the main trial and Signal-Seeking trial. The start of year 1 corresponds to month 11 in the timeline shown in Section 2.2of the proposal.