PROTOCOL

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

Full trial title:	COLO-PREVENT – A phase 2/3 randomised platform trial assessing the efficacy of aspirin, aspirin plus metformin, or resveratrol, for colorectal polyp prevention in patients undergoing surveillance in the Bowel Cancer Screening Programme
EudraCT Number:	2022-000531-23
ISRCTN Number:	13526628
Sponsor:	University of Leicester (protocol number: 0834)
Funding Body:	Cancer Research U.K (CRUK/19/011)
IRAS ID:	1005142
CPMS ID:	53642
Ethics Committee:	East Midlands - Nottingham 2 (REC ref: 22/EM/0109)
Ethics Approval date:	14 June 2022
Version Number:	4.0
Date:	11 Dec 2024
Stage:	FINAL

Protocol Amendments:

Amend-ment No.	Protocol version no.	Date	Details of changes made
SA05	4.0	11 Dec 2024	• Exclusion criteria for the main trial: methotrexate use added • Exclusion criteria for the resveratrol signal-seeking sub-trial: capsule excipients (sorbitol, croscarmellose sodium, magnesium stearate and hydroxypropyl methylcellulose) added • Inclusion criteria for the resveratrol signal-seeking sub-trial: upper age limit increased to 74 years • Appendix I: updated to add Edoxaban as a not permitted medication for the main trial • Section 7.1.2: data on biological sex and ethnicity will be captured for patients screened for the trial. • Correction to typographical error on sub-trial assessments table 2 for telephone visit 2. This visit should take place at week 4 as detailed in trial assessments section 7.7. • Section vi has been updated to reflect the DMC's decision to meet annually. • Any other minor changes are administrative for the purposes of clarification
SA02	3.0	31 Aug 2023	• Change of Chief Investigator to Dr Ajay Verma on trial documentation • Inclusion criteria: GFR lowered to ≥35ml/min/1.73m^2 • Removal of ECG at baseline visit 1 • Annual testing for vitamin B12 required for participants on the metformin arm of the main trial • Clarification - research nurse led consent • Clarification - reimbursement of costs for delivery of IMP to a participant's home (reference to details in the mNCA and pharmacy manuals) • All other changes are administrative for the purposes of clarification or correction of errors
SA01	N/A	20 Jul 2023	• Change of Chief Investigator to Dr Ajay Verma
1.0	2.0	06 June 2022	Changes made in response to MHRA non-acceptance: • Clarification to wording in section 7 confirming safety blood results will be prioritised in order to determine eligibility. Investigators will make clear to the patient that if their blood results indicate they do not meet the requirements for the trial, they will not progress any further and their consent will be invalidated. Randomisation cannot take place until safety blood results have been confirmed.

Protocol Amendments:

Amend-ment No.	Protocol version no.	Date	Details of changes made
			• Blood serum pregnancy test added for women of childbearing potential (WOCBP) to visit 9 of the main trial and visit 5 of the resveratrol sub-trial • Sentence added to section 7.10 confirming trial treatment will be discontinued in the event a participant becomes pregnant during the study • Amendment to wording in section 8.10 to clarify true abstinence is only an acceptable method of contraception when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception. • The wording for permitted medications within section 9.9 has been consolidated into section 8.9 as the guidance is identical for both trials. • Sections 9.3 and 10.2 have been amended to update the reference safety information section number within the IB for resveratrol

Confidentiality Statement

All information contained within this protocol is regarded as, and must be kept confidential. No part of it may be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust, regulatory authorities and members of the Research Ethics Committee, by any Receiving Party to any Third Party, at any time, or in any form without the express written permission from the Chief Investigator and/or Sponsor.

This protocol has regard for the HRA guidance and order of content

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the trial publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

PROTOCOL: COLO-PREVENT: A platform for developing COLOrectal cancer PREVENTion therapies

VERSION: 4.0; 11/12/2024

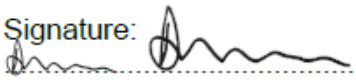
For and on behalf of the Trial Sponsor:

Signature:  Date: Feb 11, 2025

Name (please print): Dr Cat Taylor

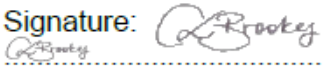
Position: Head of Research Governance

Chief Investigator:

Signature:  Date: Feb 11, 2025

Name: (please print): Ajay Verma

Statistician:

Signature:  Date: Feb 13, 2025

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II. LIST OF ABBREVIATIONS/GLOSSARY

Abbreviation	Explanation
ACF	Aberrant Crypt Foci
ADR	Adenoma Detection Rate
AE	Adverse Event
AMPK	Adenosine Monophosphate Protein Kinase
AR	Adverse Reaction
BCSP	Bowel Cancer Screening Programme
CI	Chief Investigator
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CRC	Colorectal cancer
CRF	Case Report Form
CRP	C-Reactive Protein
CTA	Clinical Trials Authorisation
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTU	Clinical Trials Unit
CTRG	Clinical Trials and Research Governance
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EOT	End of Treatment
EPIC	European Prospective Investigation into Cancer and Nutrition
FAP	Familial Adenomatous Polyposis
FBC	Full Blood Count (Haemoglobin [HB], White Cell Count [WCC], Platelets [Plt])
FFQ	Food Frequency Questionnaire
FIT	Faecal Immunochemical Test
GCP	Good Clinical Practice
GI	Gastrointestinal
GP	General Practitioner (Family Doctor)
HOMA index	Homeostasis Model Assessment-estimated insulin resistance index
HRA	Health Research Authority
HR	Heart Rate
IB	Investigator Brochure

Abbreviation	Explanation
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IGF-1	Insulin-like Growth Factor-1
IGFBP-1 or 3	Insulin-like Growth Factor Binding Protein-1 or 3
IMP	Investigational Medicinal Product
IRAS	Integrated Research Application System
MHRA	Medicines and Healthcare products Regulatory Agency
MPP	Total number of Polyps per Person
NAFLD	Non-Alcoholic Fatty Liver Disease
NHS	National Health Service
NQO1	NAD(P)H Quinone Dehydrogenase 1
NSAID	Non-Steroidal Anti-Inflammatory Drug
OTC	Over The Counter
PBMC	Peripheral Blood Mononuclear Cells
PDR	Polyp Detection Rate; proportion of individuals with one or more polyps at surveillance
PI	Principal Investigator
PIS	Participant/Patient Information Sheet
PPI	Patient & Public Involvement
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
R&D	Research and Development
RR	Relative Risk
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SSP	Specialist Screening Practitioner
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2DM	Type 2 Diabetes Mellitus
TSC	Trial Steering Committee
WOCBP	Women Of Child-Bearing Potential

III. TRIAL SUMMARY

Trial Title	COLO-PREVENT – A phase 2/3 randomised platform trial assessing the efficacy of aspirin, aspirin plus metformin, or resveratrol, for colorectal polyp prevention in patients undergoing surveillance in the national Bowel Cancer Screening Programme
Short title	COLO-PREVENT: A platform for developing COLOrectal cancer PREVENTion therapies
Trial acronym	COLO-PREVENT
Chief Investigator	Dr Ajay Verma
Lead Applicant	Professor Karen Brown
Joint Lead Applicant	Professor Mark Hull
Clinical Phase	Main trial: Phase III Signal-Seeking sub-trial: Phase II
Trial Design	Main trial: Interventional, preventative, multi-centre, open-label, parallel, randomised control trial. Signal seeking sub-trial: Interventional, preventative multi-centre, double-blinded, pharmacodynamic, pharmacokinetic, mechanistic, placebo, parallel, randomised controlled trial.
Trial Participants	Patients identified as high risk for colorectal cancer according to Bowel Cancer Screening Programme (BCSP) criteria. This includes: - patients with **'high risk'** findings after completion of a BCSP screening episode - patients with a **large non-pedunculated colorectal polyp**
Planned Sample Size	Main trial: 862 Sub-trial: 477 Total sample size for the two arms: 1339
Treatment duration	Main Trial: 3 years Sub-Trial: 1 year
Follow up duration	Participants are followed up until approximately 2 weeks after their exit surveillance colonoscopy
Planned Trial Period	97 months (from FPFV to LPLV)
Investigational Medicinal Product(s)	Aspirin Metformin Resveratrol
Formulation, Dose, Route of Administration	Aspirin - 75mg tablets (enteric coated) to be taken orally once daily Metformin – 500 mg tablets to be taken orally twice a day with breakfast and evening meal Resveratrol – participants in the sub-trial will be randomised to receive one of the following blinded treatments to be taken once daily: 5mg resveratrol (1X 5mg resveratrol and 3X placebo tablets) 1g resveratrol (4X 250mg resveratrol)

	Placebo (4X placebo tablets)
Eligibility criteria	**Inclusion criteria** *General inclusion criteria for both trials:* • Adequate renal function, defined as GFR ≥35ml/min/1.73m^2, at any time in the preceding 4 weeks • Willing and able to consent to participate in trial Participants must meet ONE of the following criteria: • Patients with high risk findings **(≥2 premalignant polyps including ≥1 advanced colorectal polyp; or ≥5 premalignant polyps)** at a completed screening episode according to BCSP criteria **OR** • Patients with a **large** (≥20mm) **non-pedunculated colorectal polyp** that is resected with **histological R0 *en bloc excision*** at a completed screening episode **OR** • Patients with a **large** (≥20mm) **non-pedunculated colorectal polyp** after **piecemeal excision.** These will only be eligible if the subsequent **2nd site check** is a **full clearance colonoscopy** *Inclusion criteria for the main trial but not the resveratrol Signal-Seeking trial:* • Aged 50-71 years *Additional inclusion criteria for the resveratrol Signal-Seeking trial only:* • Aged 50-74 years • Use of aspirin, including as an anti-platelet therapy, is permitted in the signal-seeking trial **Exclusion criteria** ***General exclusion criteria for both trials:*** • Malignant change in a polyp • Known clinical diagnosis or gene carrier of a hereditary CRC predisposition (FAP, hereditary nonpolyposis CRC) • Previous or newly diagnosed inflammatory bowel disease • Previous or planned colorectal resection • Known bleeding diathesis or concomitant non-aspirin anti-coagulant or anti-platelet agent • Abnormal liver function consisting of any of the following, at any time in the preceding 4 weeks: ○ Serum bilirubin ≥1.5 x ULN (except for participants with Gilbert's disease, for whom the upper limit of serum bilirubin is 51.3µmol/l or 3mg/dl)

	○ Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥2.5 x ULN • Inability to comply with trial procedures and use of therapies. • Pregnant or lactating women • Women of child-bearing potential unwilling to use appropriate methods of birth control (see protocol section 8.10) • Males with partners who are WOCBP and are unwilling to use effective methods of contraception • Serious medical illness interfering with trial participation including inability to have future colonoscopic surveillance. • Participants who have been administered an investigational medicinal product for another research trial in the last 30 days or ≤5 elimination half-lives ***<u>Exclusion criteria for the main trial but not the resveratrol Signal-Seeking trial:</u>*** • Methotrexate use • 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 • Current or previous treatment with metformin • Known history of peptic ulcer disease • Known history of lactic acidosis or predisposing conditions • 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 • Use of long-term systemic corticosteroids ***<u>Additional exclusion criteria for the resveratrol Signal-Seeking trial only</u>*** • Unable to abstain from ingestion of OTC supplements containing resveratrol for the trial duration • Known yeast allergy • Sensitivity or allergy to any of the capsule excipients (sorbitol, croscarmellose sodium, magnesium stearate and hydroxypropyl methylcellulose)	
	Objectives	**Outcome Measures**
Primary	1) To assess the benefits and harms of combining metformin and aspirin compared to aspirin	**Primary** Polyp number measured as MPP (Mean number of Polyps per Participant).

	alone for the prevention of colorectal polyps in patients with high risk findings, identified through the BCSP. 2) To assess whether Resveratrol prevents colorectal polyps in high risk individuals and identify the most active dose in a Signal-Seeking sub-trial.	**Secondary** • Polyp Detection Rate (PDR, proportion of individuals with one or more qualifying* pre-malignant polyp(s) at surveillance) • Advanced polyps (measured as MPP and PDR); these are defined as serrated polyp ≥10mm, serrated polyp with any dysplasia, adenoma ≥10mm, adenoma with high-grade dysplasia. • Polyp subtype based on histopathology (adenoma/serrated); also reported as MPP and PDR. • Location of polyps (right colon - any part of the colon proximal to the splenic flexure; left colon – the rectum and the colon distal to and including the splenic flexure). • Polyp size (maximum dimension in mm as described in the histopathology report [or endoscopic size if no histopathological size available) ** Please refer to definition in figure 2*
Secondary	1) To assess the safety and tolerability of aspirin and metformin compared to aspirin alone. 2) To assess the safety and tolerability of each resveratrol dose compared to placebo.	**Safety** Adverse events, including clinically significant bleeding episodes and GI tolerability. **Compliance** Assessment of compliance by counting residual numbers of tablets/capsules See protocol section 3.6 for list of exploratory outcomes.

IV. FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this trial)	**FINANCIAL AND NON FINANCIAL SUPPORT GIVEN**
Cancer Research UK	Financial support
ECMC (NIHR and Cancer Research UK)	Infrastructure support

V. ROLE OF TRIAL SPONSOR

The trial sponsor (University of Leicester) will be responsible for all aspects of the trial as per ICH-GCP and in conformance with the applicable regulatory requirements. The CI, delegated by the sponsor, is responsible for the proper conduct and management of the trial. The Leicester Clinical Trials Unit (LCTU), University of Leicester, will provide full research management services. A service level agreement will be put into place outlining the responsibilities of LCTU.

VI. ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITTES/GROUPS AND INDIVIDUALS

Trial Management Group (TMG)
The TMG will comprise of the trial Chief Investigator, Joint-Lead Applicants, Co-Investigators, Senior Trial Manager, Principal Statistician, and Trial Statistician. The TMG will oversee the operational aspects of the trial, which include the processes and procedures employed, and the day-to-day activities involved in trial conduct. The day-to-day management of the trial will be undertaken by the Trial Manager based in the Leicester Clinical Trials Unit. The TMG is responsible for all aspects of the trial (including protocol compliance, safety reporting, recruitment rate, budget management, etc.) and for ensuring appropriate action is taken to safeguard trial participants and the quality of the trial. Significant issues arising from management meetings will be referred to the Trial Steering Committee or Investigators, as appropriate.

Trial Steering Committee (TSC)
The TSC will comprise of an Independent Chairperson and two other independent members who may include clinicians and statisticians. The Chief Investigator, Lead Applicant and other members including Patient Representative(s) will also attend but do not having voting privileges. The TSC will provide oversight of the trial and monitor the progress of the trial taking into account reports and recommendations from the DMC, to ensure the trial is being conducted in accordance with the protocol, relevant regulations and the principles of GCP. The TSC are anticipated to meet approximately every 6 months; further details will be specified within the charter.

Data Monitoring Committee (DMC)
The DMC will consist of independent experts who will assess participant safety and conduct of the clinical trial. The DMC will adopt a DAMOCLES charter to define its terms of reference and operation and will make their recommendations to the TSC. The DMC will meet on a regular basis (anticipated at 12 monthly intervals) as specified in the DMC charter, prior to each TSC meeting to review the trial information and accruing data during the conduct of the trial.

VII. PROTOCOL CONTRIBUTORS

Dr Ajay Verma, Chief Investigator, Kettering General Hospital, University of Leicester (honorary contract)
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Professor Mark Hull, Joint Lead Applicant, University of Leeds
Professor Louise Brown, Co-Investigator, University College London
Professor Phil Quirke, Co-Investigator, University of Leeds
Professor Colin Rees, Co-Investigator, Newcastle University
Professor Ruth Langley, Co-Investigator, MRC/Clinical Trials Unit at UCL
Professor Matthew Rutter, Co-Investigator, University Hospital of North Tees
Cassey Brookes, Co-Investigator and Principal Statistician, Leicester Clinical Trials Unit
Dr Lynne Howells, Translational Research Manager, University of Leicester
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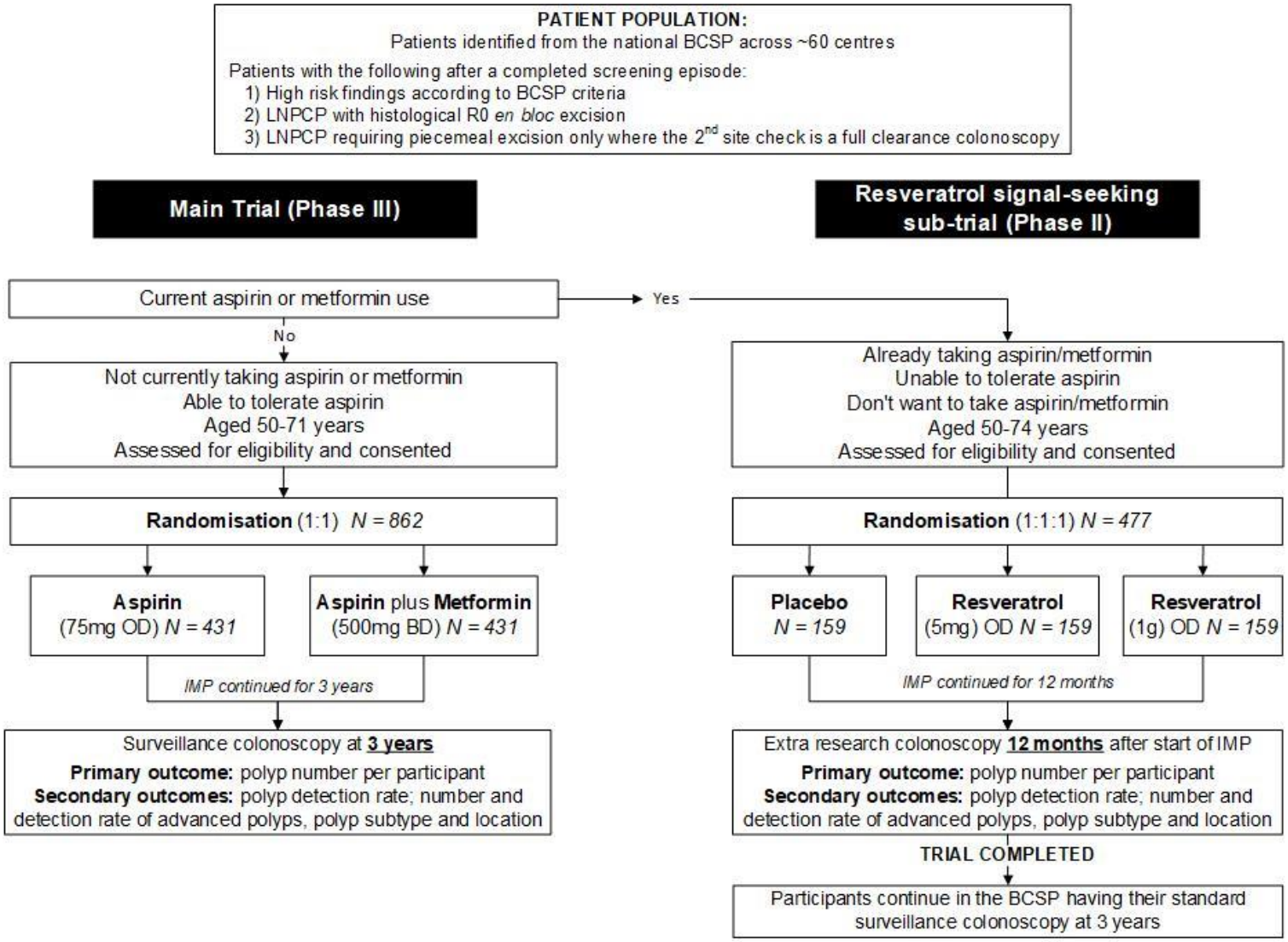
VIII. KEY WORDS

Cancer prevention, colorectal, aspirin, metformin, resveratrol, colonoscopy, adenoma, polyp, metabolic, microbiome, Bowel Cancer Screening Programme (BCSP)

FIGURE 1 - IX. TRIAL FLOW CHART



1. BACKGROUND

1.1 Trial setting

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 polyps; 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 phase 3 trial and integrated Signal-Seeking trial for evaluating the efficacy of cancer preventive therapies, that is embedded within the NHS Bowel Cancer Screening Programme. It will recruit patients deemed high risk for the development of metachronous colorectal cancer and/or polyps after a clearance colonoscopy.

The first intervention in COLO-PREVENT is aspirin, which is considered a standard of care comparator (7, 8) versus the combination of metformin plus aspirin, in an open-label phase 3 trial. The platform also incorporates a phase 2, randomised, placebo controlled, double blind signal-seeking sub-trial, which allows earlier phase testing of interventions for which there is not yet sufficient evidence to justify a large phase 3 trial. The dietary-derived agent, resveratrol will be tested in the phase 2 trial at two different doses versus placebo. Entry into this trial will be offered as an alternative to the main trial for those participants who cannot/do not wish to take aspirin or metformin, or who are already using these medications.

Colorectal polyps as surrogate markers in therapeutic prevention trials

Polyp prevention trials conducted within populations at high risk for developing CRC provide a model for testing the efficacy of preventive therapies within a relatively short period due to the use of polyp recurrence as the outcome, as opposed to CRC incidence. As precursors to most CRCs, polyps are the only validated surrogate biomarker of CRC risk and have been used as a primary colonoscopic outcome measure in multiple therapeutic prevention trials. Most notably, the reduction in adenoma recurrence observed in aspirin polyp prevention RCTs (9) has predicted the longer-term effect of aspirin on CRC incidence (8, 10).

COLO-PREVENT is embedded within the national Bowel Cancer Screening Programme and both builds on and extends the trial site infrastructure established by the seAFOod Polyp Prevention trial of eicosapentaenoic acid (EPA) and aspirin, which recruited over 700 patients from 53 sites across England (11, 12). Strict adherence to protocol-driven screening and surveillance care pathways, as well as careful colonoscopic and histological quality-assurance, contribute to the BCSP being an ideal setting for a polyp prevention RCT. Such trials involve recruiting patients identified as ‘high risk’ at index colonoscopy and randomising them to receive an intervention until their 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 trial size and duration (11). Adenoma recurrence post polypectomy may be due to both 'missed' and new lesions (11, 13). 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 (11, 13).

High risk patients in the BCSP

Following a recent review of the surveillance guidelines commissioned by the British Society of Gastroenterology (BSG), the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and Public Health England (PHE), changes have been made to the risk classification and timing of surveillance within the BCSP (Rutter). Previously, patients classed as high risk for CRC had their first surveillance colonoscopy at 12 months, but under the revised guidelines, this has been extended to 3 years. As part of the changes, the serrated and adenomatous polyp counts are now combined, such that patients are classed as having **high risk findings** if they have either

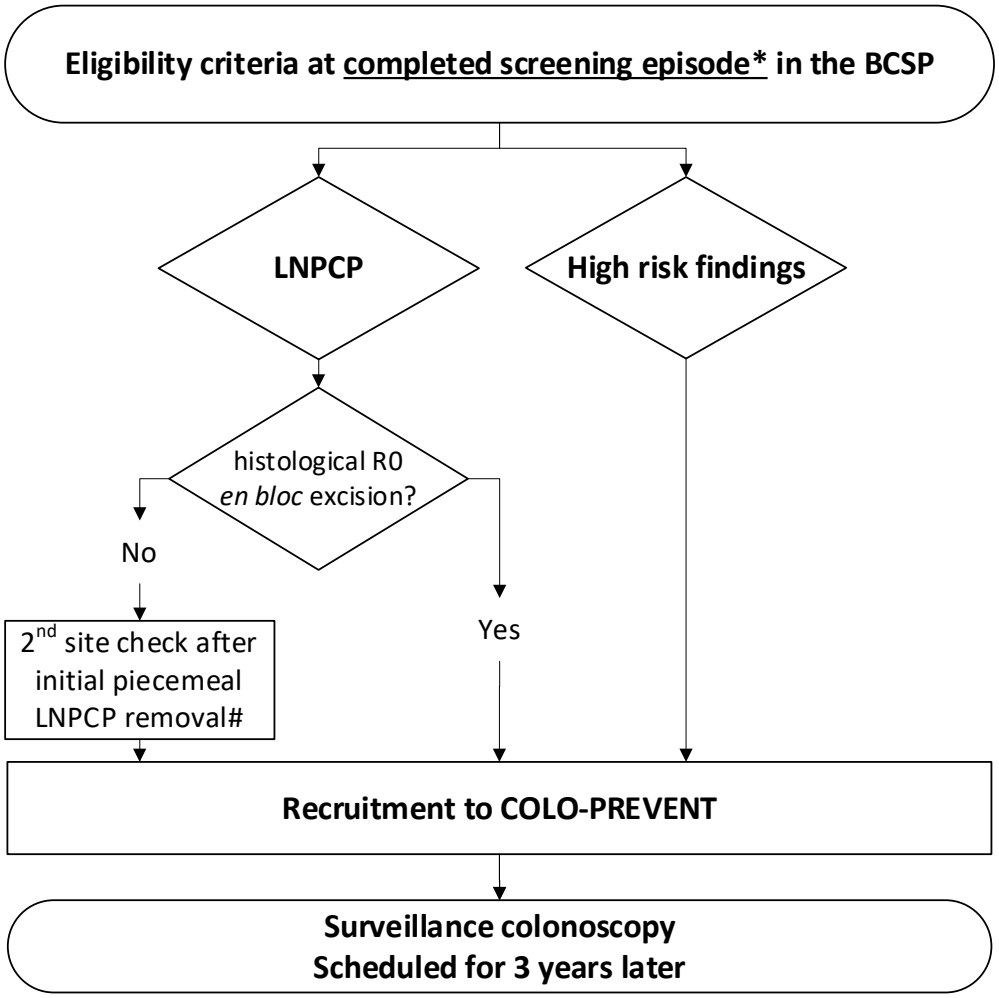
- ≥2 premalignant polyps including ≥1 advanced colorectal polyp; or
- ≥5 premalignant polyps

The term premalignant polyp includes both **adenomatous polyps** and **serrated polyps** (excluding diminutive [1-5mm] rectal hyperplastic polyps). **Serrated polyps** is an umbrella term for hyperplastic polyps, sessile serrated lesions, traditional serrated adenomas and mixed polyps. **Advanced colorectal polyps** are defined as serrated polyp ≥10mm, serrated polyp with any dysplasia, adenoma ≥10mm, adenoma with high-grade dysplasia (Figure 2).

Patients with high risk findings according to the above criteria are eligible for COLO-PREVENT **after completion of the index screening episode** (see Figure 2). A screening episode may include >1 diagnostic test, for example, patients requiring a site check at 2-6 months where histological completeness of excision cannot be determined for non-pedunculated polyps of 10–19 mm in size, or an adenoma containing high-grade dysplasia, or a serrated polyp containing any dysplasia.

Another group of patients classed as high risk for CRC and included in the BCSP surveillance algorithm (14), are those with **large** (≥20mm) **non-pedunculated colorectal polyp (LNPCP).** In cases where there is pathologically *en bloc* R0 endoscopic mucosal resection or endoscopic submucosal dissection of an LNPCP, patients will undergo post-polypectomy surveillance at 3 years. This group of patients would be eligible for inclusion in COLO-PREVENT after completion of the index colonoscopy episode. In other cases where there is piecemeal resection of an LNPCP, it is recommended that patients undergo a site check at 2-6 months and a further site check at 18 months after the original resection. These patients would become eligible for COLO-PREVENT only when the **second site check** is a **full clearance colonoscopy** and they would be recruited after this point (Figure 2).

The **primary outcome measure** in COLO-PREVENT is polyp number, quantified as mean polyp number per participant (MPP). Traditionally, polyp (or adenoma) detection rate (PDR or ADR; the percentage of individuals with one or more colorectal polyps) has been used as the primary outcome in prior polyp prevention trials, but recent advances in screening endoscopy and quality assurance in colonoscopy mean that PDRs are high and differences in PDR are difficult to detect. Consequently, polyp number is now considered to provide a better measure of efficacy in prevention trials (12, 15).



A screening programme **episode of care may include the screening colonoscopy and subsequent check procedures*
#Patients with LNPCP requiring piecemeal excision will only be eligible for recruitment if the second site check is a full clearance colonoscopy

High risk findings

- **≥2 premalignant polyps including ≥1 advanced colorectal polyp; or**
- **≥5 premalignant polyps**

Definitions

- ***Serrated polyps**: umbrella term for hyperplastic polyps, sessile serrated lesions, traditional serrated adenomas and mixed polyps*
- ***Premalignant polyps**: includes both **serrated polyps** (excluding diminutive [1-5mm] rectal hyperplastic polyps) and **adenomatous polyps***
- ***Advanced colorectal polyps**: serrated polyp ≥10mm, serrated polyp with any dysplasia, adenoma ≥10mm, adenoma with high-grade dysplasia*
- ***Large non-pedunculated colorectal polyp (LNPCP)**: Large (≥20mm) non-pedunculated colorectal polyp*

Figure 2 - Eligibility criteria for participants identified as high risk through the BCSP.

The algorithm also indicates the point at which patients become eligible for inclusion in COLO-PREVENT. Key definitions are included in the box.

1.2 Aspirin

1.2.1 Mechanisms

Aspirin has a long history of use as a preventive agent in cardiovascular disease primarily due to its ability to inhibit platelet activation and aggregation, but there is increasing evidence to support a role for aspirin in CRC prevention and treatment. The mechanisms by which aspirin exerts its biological activity are thought to be primarily due to its ability to interfere with the prostanoid biosynthetic pathway via irreversible inhibition of the cyclooxygenase (Cox) enzyme, which exists in 2 major isoforms: Cox-1 and Cox-2. Cox-1 and Cox-2 inactivation prevents conversion of arachidonic acid to the precursor prostaglandin PGH2 and subsequent production of thromboxane A2 and downstream prostaglandins (16-18), which are potent mediators of inflammation, cell migration, proliferation, apoptosis, angiogenesis, and the immune response (19), and have a profound influence on the carcinogenic process. Use of selective Cox-2 inhibitors to inhibit colorectal polyp formation in a clinical setting have provided credence for Cox-2 as a rational target for CRC prevention (20). The ability of a single low dose (100 mg) of aspirin to acetylate Cox-1 for up to 24 hours post dose in human colorectal mucosa with resultant decrease in PGE2 levels, suggests low dose aspirin may be sufficient for long term suppression of Cox-1 activity (21).

The direct effects of aspirin on platelet biology are also thought to play a pivotal role in its potential for CRC preventive efficacy. Aspirin-mediated attenuation of platelet reactivity and aggregation at the site of intestinal injury via Cox-1 inhibition prevents release of pro-carcinogenic inflammatory cytokines and growth factors. More recently, mouse models of intestinal tumourigenesis and subsequent clinical studies have revealed aspirin to have a direct antibiotic effect on the gut microbiota (22), affecting a number of gut microbes associated with increased CRC risk such as *Fusobacterium* and *Bacteroides* (23, 24).

1.2.2 Clinical evidence - efficacy

The Melbourne Colorectal Cancer Study was a large population-based epidemiological study which analysed numerous clinico-pathological features and their association with colorectal cancer incidence. This study was one of the first to recognise the potential benefit of aspirin in decreasing CRC risk (RR=0.57 (0.14-0.19), p=<0.001 (25). Twenty year follow up of 4 trials for prevention of primary and secondary vascular events evaluated the anti-cancer effectiveness of low dose (75-300 mg) aspirin administered over a median of 6 years. Here, the 20 year colon cancer incidence HR was 0.76 (01.60-0.96), p=0.02, and for mortality the HR was 0.65 (0.48-0.88), p=0.05. Increasing treatment duration (>5 years) was associated with decreased rectal cancer risk, but no enhanced benefit was observed with doses >75 mg/day (26). When comparing case-control studies versus randomised control trials, there was concordance in decreased risk of CRC death following regular aspirin use with ORs of 0.62 (0.58-0.67), p=<0.0001 and 0.58 (0.44-0.78), p=0.0002, respectively (27). In the Women's Health Study, women without a previous history of cancer took 100 mg aspirin every other day without any change in CRC incidence (28). However, at 10 year follow up of this cohort, the HR for CRC incidence was 0.80 (0.67-0.97), p=0.021 (29), similarly providing evidence for a latent benefit. Further evidence suggests that short-term effects of aspirin (3 years+) may also provide benefit in terms of cancer incidence and mortality (30).

Aspirin has also shown benefits in populations at high risk for CRC development. A meta-analysis of randomised trials evaluating aspirin for colorectal adenoma prevention across 4 trials and 2967 participants revealed a pooled RR of 0.83 (0.72-0.96) with absolute risk reduction of 6.7%. The aspirin doses in these studies ranged from 81-325 mg/day, with a more favourable risk reduction observed with lower dose aspirin (10). The Colorectal

Adenoma/Carcinoma Prevention Programme (CAPP) is a randomised placebo controlled trial assessing efficacy of aspirin for CRC prevention in Lynch syndrome. In CAPP2, participants received aspirin at a daily dose of 600 mg (+/- resistant starch), initially for 2 years with facility to continue for a further 2 years. Here, aspirin had negligible efficacy in preventing colorectal adenoma or CRC incidence following 2 years of dosing (31). However, follow up out to a mean of 55.7 months following completion of 2 years intervention gave an HR of 0.41 (0.19-0.86), p=0.02 for per protocol analysis (32), and at 10 year follow up, an HR of 0.56 (0.34-0.91), p=0.019 (33).

1.2.3 Clinical data – Safety

A key consideration when initiating long-term prevention strategies in healthy populations, is the safety of the drug under investigation. As aspirin has been used extensively in large populations over many years, there is much evidence upon which to base decisions on a risk versus benefit basis. A common and well-recognised complication of long-term aspirin use in the cardiovascular prevention setting has been increased risk of bleeding; primarily intracranial haemorrhage and acute gastrointestinal bleeding (34). It has previously been suggested that aspirin dose is related to the frequency and severity of bleeding-related adverse events, with key risk factors (such as older age, diabetes mellitus and renal dysfunction) requiring consideration prior to aspirin intervention (35). Across all outcomes, any aspirin dose increases bleeding risk, but low dose aspirin has the lowest bleed risk and does not increase fatal bleeds[ref]. In the previous seAFOod polyp prevention trial, which recruited high risk patients in the NHS BCSP aged between 55-73 years, there was no difference in the incidence of gastrointestinal bleeds in patients on aspirin (300mg daily) versus placebo, and there were no reports of haemorrhagic strokes (12).

1.2.4 – Clinical data – Choice of dose

The dose utilised in this trial will be 75 mg/day. Low doses of aspirin have shown efficacy in colorectal cancer prevention (36, 37), whilst at the same time minimising bleed risk (38, 39).

1.3 Metformin

1.3.1 – Mechanisms

Metformin is an oral biguanide that is prescribed for numerous conditions including type II diabetes (T2DM), gestational diabetes, and polycystic ovary syndrome (40). The primary actions of metformin are in the treatment of hyperglycaemia through its ability to suppress glucose synthesis in the liver and by improving insulin resistance in tissues, thus reducing circulating glucose levels. Diabetes is associated with increased risk for a number of cancers (41), with the hyperglycaemic state implicated in many pro-proliferative pathways. In addition to providing glucose control, metformin is able to independently modulate numerous pro-carcinogenic proliferative, inflammatory and metabolic pathways.

In CRC cell lines, metformin has been shown to induce apoptosis, cell cycle arrest and autophagy, inhibit proliferation and migration, and sensitises cells to chemotherapy (42). In the *APC*$^{Min/+}$ mouse model metformin reduced numbers of polyps (43) and decreased formation of azoxymethane-induced aberrant crypt foci (44). It is still unclear exactly how metformin may influence CRC prevention, but there are several key mechanisms which may contribute to its potential chemopreventive efficacy.

1.3.2. – Clinical data - Efficacy

Whilst metformin has been thought to primarily elicit its mode of action through effects on the liver, it has also been shown to have a high uptake in the small intestine which can directly affect localised glucose uptake within the gut (45). Metformin uptake in human colonic tissue generates levels approximately 150-fold higher than in plasma, representing concentrations that were consistent with anti-cancer efficacy observed in pre-clinical studies (46). There have been numerous large cohort studies of diabetic patients that have assessed the potential of metformin in decreasing incidence of, and improving outcomes for CRC. Recent meta-analyses consistently suggest a positive benefit of metformin in reducing risk for CRC incidence and mortality in T2DM patients.

A number of studies have also specifically examined polyp prevention. These are summarised in table 5, with the majority showing decreased risk for adenoma risk/recurrence following metformin treatment. 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 (47). 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/m2) (47) 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). 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.

1.3.3 – Clinical data - Safety

Whilst metformin is commonly prescribed across a number of conditions, there is still a risk of side effects, with 20-30% of patients having gastrointestinal (GI) symptoms, and up to 5% of patients discontinuing metformin due to their severity (48). Doses effective for the treatment of T2DM (1700 mg daily) have been used long-term within diabetes prevention trials in individuals with elevated glucose (49). This dose is also being investigated in several cancer studies involving non-diabetics, including the STAMPEDE trial, which aims to evaluate whether metformin improves survival of men with high risk localised or metastatic prostate Cancer (50). The MANSED trial administered 850 mg metformin BD to prostate cancer patients with no significant metformin-related side effects other than grade 2 diarrhoea (51). A sub-study from the 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 (52) 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.

1.3.4 – Rationale for combining aspirin with metformin

Ex vivo studies using CRC patient-derived spheroids suggest that the combination of aspirin with metformin may decrease viability and migratory capacity of tumour cells, but that not all patient-derived cultures were sensitive to metformin. In CRC cell lines, aspirin has a pronounced effect on AMPK activation and mTOR inhibition, acting via a similar mechanism to that observed for metformin (53).

In a recent population-based study, it was observed that patients with T2DM taking both metformin and aspirin had a higher five-year cancer-specific and relative survival for stage II and III CRC compared with diabetic patients not taking aspirin (54). Enhanced efficacy in delaying CRC incidence has also been observed, with the combination of aspirin and metformin (mean duration of 9.8 and 4.7 years use respectively) exhibiting an HR of 0.54 (0.39-0.74), p=<0.001, compared with aspirin alone (mean duration of use 7.5 years) with an HR of 0.83 (0.76-0.90), p=<0.001, and metformin alone (mean duration of use 4.4 years) with an HR of 0.92 (0.72-1.17), p=0.495 (55).

1.4 Resveratrol

Resveratrol (3,4',5-trihydroxystilbene) is a polyphenolic compound produced by plants in response to environmental stress. It is found in numerous foodstuffs, including grapes, peanuts and mulberries (56) and is widely available in health food stores in capsule format. In pre-clinical studies, it has been shown to exhibit numerous properties which may be beneficial to health, including anti-inflammatory, anticancer, lipid-lowering, hypoglycemic and vasodilatory effects (57). Whilst resveratrol has been used in many clinical trials across a variety of disease states, it currently does not have approval for medicinal use in any indication.

1.4.1 – Mechanisms

Early pre-clinical studies found resveratrol to act as an antioxidant, induce phase II drug-metabolizing enzymes and mediate anti-inflammatory effects through Cox-1 and Cox-2 inhibition (58, 59). Resveratrol is a pleiotropic molecule acting on numerous signalling pathways involved in the carcinogenic process, all of which may combine to contribute to its putative anti-cancer efficacy. In addition to Cox inhibition, resveratrol shares a number of mechanisms in common with aspirin and metformin, including regulation of the energy sensor AMPK. A recent systematic review exploring *in vitro* activity of metformin vs resveratrol in the management of diabetes-associated complications, suggested comparative efficacy in modulation of key metabolic targets including AMPK (60). This was further supported by a systematic review of resveratrol RCTs which found resveratrol to improve glucose, insulin and HbA1c levels in T2DM patients (61).

In $Apc^{Min/+}$ mice maintained on a high-fat diet, a very low resveratrol dose of 5 mg (equivalent to the amount contained in a large glass of red wine) suppressed intestinal adenoma development more potently than a 200-times higher pharmacological dose of 1 g (62). This phenomenon translated to human explant cultures, with maximal effects on AMPK signalling and autophagy at the lower concentrations.

Translation of pre-clinical biomarkers to the clinical setting has been undertaken in a number of studies. In colorectal cancer patients, resveratrol treatment (0.5-1.0g daily) resulted in decreased fasting insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) (63). A high serum IGF-1/IGFBP-3 ratio has been associated with increased CRC risk and increased angiogenesis in a number of meta-analyses (64, 65), contributing to pro-carcinogenic signalling and changes to glucose homeostasis.

Proliferative index, as measured by ki-67 expression in post- vs pre-dose tumour tissue, was significantly decreased with daily high dose resveratrol (0.5-1.0g), and a significant increase in apoptosis (via cleaved caspase-3) has been observed in malignant hepatic tissue from patients with colorectal metastases who took 5g resveratrol daily for two weeks (66, 67).

1.4.2 – Clinical data - Pharmacokinetics

Resveratrol is well absorbed from the GI tract following oral administration, but has poor systemic bioavailability due to its rapid metabolism (68). Plasma pharmacokinetics for resveratrol have already been well characterised in a number of healthy volunteer studies, eliciting a maximal plasma concentration approximately 1 hour after oral dosing, with conjugates still detectable for up to 24 hours (69-71). Maximum plasma concentrations of resveratrol sulfate and glucuronide conjugates were up to 20-fold higher than those observed for the parent compound. The sulfate conjugates are thought to contribute to efficacy, as they are readily taken up into CRC cells via specific membrane transporters, where they are converted back into resveratrol, so increasing intracellular resveratrol levels at concentrations sufficient to exert anti-proliferative and pro-autophagic effects (72, 73). In addition to assessing plasma pharmacokinetics, several studies have analysed the distribution of resveratrol and its metabolites in colorectal target tissues at doses ranging from 5mg to 1g. The average resveratrol colorectal tissue concentrations were approximately 70-times higher than the plasma C_{max}, with levels consistently higher in right- versus left-sided tissue (67). This may mean that resveratrol has potential for greater efficacy against tumours arising in the right side of the colon, which has similarly been observed for the adenoma preventive efficacy of aspirin (12).

1.4.3 – Clinical data – Efficacy

We have recently undertaken a systematic review (awaiting submission for publication) assessing all trials and studies performed using resveratrol in humans, which specify the resveratrol dose and formulation used. To date, 133 individual trials have been reported with a cumulative enrolment of nearly 5000 participants and a further 36 studies have described additional analysis of samples or data from these trials.

Relatively few studies have been conducted specifically within a cancer setting, but a number of recent systematic reviews and meta-analyses have assessed the potential efficacy of resveratrol in other clinical settings that may have relevance for cancer. Resveratrol appears to consistently downregulate inflammation in a number of disease states including T2DM, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). Furthermore, the meta-analyses reveal consistent decreases in plasma glucose and insulin, and an improvement to insulin sensitivity. Mechanistically, prolonged inflammatory responses and high circulating glucose play an important role in promotion of the carcinogenic process. In the hyperglycaemic state, free glucose acts as a potent signalling intermediary, promoting formation of advanced glycation end-products and reactive oxygen species, in addition to stimulating pro-inflammatory and pro-proliferative signalling pathways (74) such as NFκB (75)

1.4.4 – Clinical data - Safety, drug interactions and choice of dose

Of the reported 133 clinical trials involving resveratrol, just over half (54%) involved patients with an underlying disease or pathology, with the remainder enrolling healthy participants. The near even split between healthy individuals and patients with a diagnosed pathology reflects a focus on health maintenance and prevention as well as using resveratrol as a potential treatment. Furthermore, the good safety profile of resveratrol means it has been possible to conduct many of the studies addressing PK, metabolism and bioavailability in healthy people. Resveratrol has been investigated in adults of all ages including elderly populations over 70 years. In our recent systematic review, we found that out of the 90 RCTs undertaken with resveratrol, 38 studies report zero side effects, 30 studies do not make any mention of side effects, and the remaining 22 studies provide detail for side effects, which are shown in Table

6 (Appendix II). Of these 22 studies, 4 gave resveratrol in combination with standard of care drugs with known side effect profiles, but revealed no significant difference in side effects between groups. Of the remaining 18 studies, 11 of these administered resveratrol at or below our highest dose of 1 g/day, with 3 of these studies reporting that there were no significant differences in the number of participants experiencing side effects between the groups. Of the studies that do report side effects, the most common were dose-related and gastrointestinal in nature, comprising diarrhoea, constipation, nausea, abdominal cramps, vomiting, steatorrhoea, heartburn, reflux and bloating. Studies have shown that resveratrol is well tolerated at once-daily doses of ≤1g, and one of the longest published trials in elderly Alzheimer's patients has demonstrated an excellent safety profile at doses of up to 1g taken twice daily for one year (76).

Drug interactions and use of concomitant medication.

Based on pharmacokinetic studies, resveratrol may theoretically interfere with phase I metabolism of certain drugs due to its inhibitory effects on cytochrome P450s (77). In theory, drugs relying on first pass metabolism for activation/efficacy (such as some statins, anti-fungals and anti-histamines) may be rendered less effective during co-administration of resveratrol. Two *in vivo* studies have suggested that there is an increased bleed risk when resveratrol is combined with the anti-clotting agent warfarin (78, 79) and as a precaution, patients on warfarin are not be eligible for COLO-PREVENT. All concomitant medication must be carefully reviewed by the clinical team prior to trial enrolment to ensure that participant safety is maintained, and efficacy of concomitant medication is not compromised.

Justification of dose

Resveratrol will be administered at a dose of 1 g, or 5 mg daily. Pre-clinical *in vivo* data have revealed that both these doses have preventive efficacy in a mouse model of CRC. Mechanistic studies have demonstrated a non-linear dose response for the effects of resveratrol on AMPK signalling and the induction of autophagy and senescence in adenoma cells, supporting further investigation of both doses. In our studies these doses have been administered safely in daily dosing regimens to patients and healthy volunteers and have been shown to alter molecular biomarkers relevant to CRC in human tissue, suggesting they may both have efficacy in trials, potentially through different mechanisms (62). It is not currently possible to determine which of these doses might be more effective for CRC prevention in humans, therefore both doses will be compared against placebo in the COLO-PREVENT sub-trial.

Lay Scientific Summary

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 trials 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 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 trial we will test two doses against a placebo tablet in a way that neither patients nor medical staff know what treatment is being given (double blind). Patients already taking aspirin or metformin will be able to take part in the resveratrol sub-trial. The resveratrol sub-trial will be shorter and involve fewer

patients than the main aspirin and metformin trial 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 clinical trial in the future with the most effective dose of resveratrol.

Trial drugs will be given for 3 years in the main aspirin and metformin trial and 12 months in the resveratrol sub-trial, 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 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.

2. RATIONALE

2.1 Hypothesis

The Primary hypotheses of this trial are:

1) The combination of metformin and aspirin affords greater preventive efficacy in high risk polyp patients compared to aspirin alone

2) Resveratrol prevents colorectal polyps in high risk individuals

2.2 Need for a trial

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 polyps; the latter constitutes secondary prevention and may also serve to reduce the need for, or frequency of, surveillance colonoscopy.

Within the NHS Bowel Cancer Screening Programme, individuals classed as 'high risk' after an index colonoscopy have a high event rate over short periods of follow-up, with ~60% polyp recurrence within 12 months (11). Polyps detected post polypectomy may be due to both 'missed' and new lesions (11, 13). Evidence for *de novo* polyp growth comes from tandem colonoscopy studies in which there is as much as a 30% difference in polyp detection rate (PDR) at colonoscopy within three months of the index procedure compared with 12 months in high risk individuals (11, 13). Moreover, the occurrence of post-colonoscopy CRC, particularly right-sided tumours, is increasingly being recognised, even for those engaged in high quality

surveillance programmes (80-82). These facts highlight the opportunity for improvement by combining preventive therapies with endoscopic interventions in this population.

There is compelling evidence from epidemiology data and randomised clinical trials that aspirin can prevent colorectal cancer and polyps, and that this protection translates to reduced CRC associated mortality (9, 26, 83). However, aspirin does not work for everyone; therefore, there is a need 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 (32), implementation has been challenging due to limited awareness among potential prescribers and concerns over the safety of higher doses (84). In COLO-PREVENT we are adopting aspirin as standard of care in the main trial and we anticipate this action 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 clinical trial. 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 sub-trial provides a conduit for linking laboratory work with phase 3 assessment, in a unique package. We have selected resveratrol as the prime candidate for the sub-trial; 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 (62, 67, 85). Efficacy testing in a clinical setting is the next logical step.

2.3 Exploratory studies

Exploratory translational work 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.

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. To this end we will 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 IGFI, plus completion of EPIC Food Frequency Questionnaires (FFQs) and estimation of dietary fat content (86, 87).

2) We will examine the hypothesis that plasma drug concentrations and/or the metabolite profile correlate with efficacy for metformin and resveratrol. This will involve measurement of metformin blood concentrations and determining the plasma metabolite profile for resveratrol, including the proportion of human and bacteria-derived species since the pattern of resveratrol metabolites may influence, and therefore correlate with, 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 that will be 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. We will focus on markers of AMPK activation and/or mTOR inhibition in normal rectal tissue of patients that received aspirin, as well as the NF-kB pathway. Apoptosis will be quantified as a functional endpoint for the effects of aspirin and metformin on these pathways. Therapy-associated differences will be investigated for the three biomarkers in normal tissue of all designated patients by comparing between trial arms. Biomarker changes in adenomas will be determined for the proportion of patients presenting with a recurring polyp at 3 years by comparing results pre- and post-intervention and across groups.

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 (62, 67), and plasma MLX and CIDEB protein concentrations will be analysed across treatment groups and over time in the resveratrol signal-seeking sub-trial and associations with efficacy examined.

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 polyp recurrence. We will also conduct metabolomic and proteomic analysis of plasma and colorectal tissue to identify profiles associated with baseline microbiome composition or therapy-related changes to the microbiome. All research faecal samples will be obtained using faecal immunochemical test (FIT) kits and bacterial populations will be assessed by next generation sequencing of the 16S rRNA V4 region. 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 trial.

6) Assessment of total exposure to resveratrol:

Resveratrol: Resveratrol is widely available as a health food supplement, therefore adherence to the sub-trial requirements to abstain from ingestion of OTC supplements containing resveratrol for the trial duration will be explored in 20% of participants from randomly selected

sites that are willing and able to collect urine samples. Concentrations of urinary resveratrol will be quantified using established HPLC-UV assays.

Accounting for dietary resveratrol consumption in the sub-trial. To ensure that dietary resveratrol is not a confounding factor and examine whether individuals change their behaviour as a result of participating in the trial, we will compare resveratrol 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 can be used to estimate habitual resveratrol intake from a Food Frequency Questionnaire (88, 89).

2.4 Ethical considerations

The trial will be conducted in full conformance with the principles of the Declaration of Helsinki and to ICH Good Clinical Practice (GCP) guidelines. It will also comply with the Medicines for Human Use (Clinical Trials) Act 2004, subsequent amendments and University of Leicester Standard Operating Procedures (SOPs). All data will be stored securely and held in accordance with ICH GCP, the UK Policy Framework for Health and Social Care, the Data Protection Act and the EU General Data Protection Regulation (GDPR). Written informed consent will be gained prior to entry in the trial. Patient safety and well-being will be paramount and all patients will be treated with respect and dignity throughout the trial.

Participation in a randomised controlled trial means that the participant and clinician are not able to choose all aspects of treatment and a careful explanation of the different treatments and toxicities by arm will be given to participants. The protocol has been designed to reflect the standard care pathway for patients on the BCSP with the minimal number of additional hospital visits and tests (over and above routine care) possible. There will not be any routine reimbursement of costs incurred for patients within the grant however local centres may be able to cover travel and incidental expenses.

A placebo arm has been included in the resveratrol sub-trial to make the treatments seem as similar as possible from the participant's perspective. Importantly, even closer similarity between the trial arms is achieved by preventing investigators knowing which treatment the participant is receiving (double-blind).

For those who experience severe toxicity related to the study drugs and/or those who are unable to adhere to the protocol treatment schedule, active participation in the trial will end at this time. However, they will be asked if they wish to give consent to allow passive follow-up data collection using medical records.

Participants will be followed-up in the long-term through routinely collected healthcare databases provided by National Cancer Registration and Analysis Service (NCRAS) and other organisations. Participants will be asked to consent to this separately and can still participate in the trial if they do not give their consent to this aspect of the trial.

Blood sampling is required to answer trial objectives, and as per routine care some of these tests are required to be fasted. Usual clinical guidance will be followed and sites will be advised to book visits as early in the morning as possible. Section 7.1.1 details additional measures sites will take for patients to arrive fasted for visit 1 consent and baseline screening

assessments. Potential patients will be given the opportunity to come back for a further visit if they are not happy to undergo the consent process in a fasted state. Although rectal biopsies are taken as part of routine care during colonoscopies, this type of sampling poses a theoretical risk of perforation or serious bleeding, though this is rare. Only a very small amount of tissue is required for the research rectal biopsies and will be obtained by trained physicians. Participants will be asked to consent to this separately and can still participate in the trial if they do not give their consent to this aspect of the trial.

2.5 Assessment and management of risk

This trial is categorised as:

- Type B = somewhat higher than the risk of standard medical care

Use of low dose aspirin (75mg) will minimise the risk of adverse events; this is the lowest clinically used dose of aspirin in adults and is well tolerated.

To minimise the chances of GI toxicity, patients randomised to metformin will start at a dose of 500mg once daily and this will be increased after four weeks to the full dose of 500mg twice daily. Patients will be advised to take their tablets with or after food to help with treatment tolerance. If GI toxicities do occur this will be managed by dose reduction of metformin or a switch to a modified release (MR) preparation according to local policy. Following a drug safety update from the MHRA regarding monitoring patients at risk of reduced vitamin B12 levels, annual testing will be required by sites for participants randomised to the aspirin plus metformin arm only.

If participants experience GI symptoms in the resveratrol sub-trial trial, they will be advised to take their full dose with or after food; if symptoms do not improve, they can divide their dose such that two capsules are taken each morning and two in the evening. It will not be possible to manage these patients by reducing the dose without unblinding the trial because participants in the low 5mg resveratrol dose group take one 5mg capsule and three placebo capsules each day.

3. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objectives

1. To assess the benefits and harms of combining metformin and aspirin compared to aspirin alone for the prevention of colorectal polyps in patients with high risk finding identified through the BCSP.

2. To assess whether Resveratrol prevents colorectal polyps in high risk individuals and identify the most active dose in a Signal-Seeking sub-trial.

3.2 Secondary objectives

1) To assess the safety and tolerability of aspirin and metformin in combination compared to aspirin alone in the main trial.

2) To assess the safety and tolerability of each dose of resveratrol compared to placebo in the sub-trial.

3.3 Outcome measures/endpoints

Please refer to sections 1 and 2 for further details and rationale for selection of outcome measures/endpoints.

3.4 Primary endpoint/outcome

Polyp number measured by MPP (Mean number of Polyps per Participant)

3.5 Secondary endpoints/outcomes

Secondary outcome measures

- Polyp Detection Rate (PDR, proportion of individuals with one or more qualifying* pre-malignant polyp(s)at surveillance)
- Advanced polyps (measured as MPP and PDR); these are defined as serrated polyp ≥10mm, serrated polyp with any dysplasia, adenoma ≥10mm, adenoma with high-grade dysplasia
- Polyp subtype based on histopathology (adenoma/serrated); also reported as MPP and PDR
- Location of polyps (right colon - any part of the colon proximal to the splenic flexure; left colon – the rectum and the colon distal to and including the splenic flexure)
- Polyp size (maximum dimension in mm as described in the histopathology report or endoscopic size if no histopathological size available)

* *Please refer to definition in figure 2*

Safety

- Adverse events, including clinically significant bleeding episodes and GI tolerability

Compliance

- Assessment of compliance by counting residual numbers of tablets/capsules returned by each patient

3.6 Exploratory endpoints/outcomes

- Measurement of molecular (glucose, insulin (HOMA), HbA1c, triglycerides, cholesterol, IGFBP-3, free IGF-1) and physical (BMI, waist circumference) markers of metabolic status.
- Assessment of dietary patterns and fat intake using the EPIC FFQ.
- Plasma drug concentrations and metabolite profile for resveratrol and metformin.
- Urinary resveratrol/metabolite levels in 20% participants in the sub-trial from randomly selected sites that are willing and able to collect urine samples.
- In the main trial: Measurement of pharmacodynamic biomarkers common to both aspirin and metformin, including p65, pS6/S6 and cleaved PARP in tissue samples.
- In the resveratrol sub-trial: Analysis of tissue and plasma pharmacodynamic biomarkers, including NQO1, protein carbonyls, Ki67 proliferation index, MLX and CIDEB.
- Characterisation of the gut microbiome.

3.7 Table of endpoints/outcomes

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objectives 1) To assess the benefits and harms of combining metformin and aspirin compared to aspirin alone for the prevention of colorectal polyps in patients with high risk finding identified through the BCSP.	**Primary outcome** Polyp number measured by MPP (mean number of polyps per participant)	Exit surveillance colonoscopy 154 weeks for main trial 52 weeks for sub-trial
	Secondary outcomes Polyp Detection Rate (PDR, proportion of individuals with one or more qualifying* pre-malignant polyp(s) at surveillance)	Exit surveillance colonoscopy
2) To assess whether Resveratrol prevents colorectal polyps in high risk individuals and identify the most active dose.	Advanced polyps (measured as MPP and PDR) ; these are defined as serrated polyp ≥10mm, serrated polyp with any dysplasia, adenoma ≥10mm, adenoma with high-grade dysplasia * *Please refer to definition in figure 2*	Exit surveillance colonoscopy
	Polyp subtype based on histopathology (adenoma/serrated); also reported as MPP and PDR	Exit surveillance colonoscopy
	Location of polyps (right colon - any part of the colon proximal	Exit surveillance colonoscopy

	to the splenic flexure; left colon – the rectum and the colon distal to and including the splenic flexure)	
	Polyp size (maximum dimension in mm as described in the histopathology report or endoscopic size if no histopathological size available)	Exit surveillance colonoscopy
Secondary Objectives Safety	Adverse events, including clinically significant bleeding episodes and GI tolerability.	From first administration of IMP until the final visit at 156 weeks for the main trial and 52 weeks for the sub-trial
Compliance	Assessment of compliance (counting residual number of tablets/capsules).	Main trial: weeks 25, 52, 78, 104, 130 and 154 Sub-trial: weeks 25 and 52
Exploratory Objectives	Measurement of molecular (glucose, insulin (HOMA), HbA1c, triglycerides, cholesterol, IGFBP-3, free IGF-1) and physical (BMI, waist circumference) markers of metabolic status.	Main trial: weeks 0, 25, 52, 78, 104 and 154 Sub-trial: weeks 0, 25 and 52
	Assessment of dietary patterns and fat intake using the EPIC FFQ.	Main trial: weeks 0, 52, 104 and 154 Sub-trial: weeks 0 and 52
	Plasma drug concentrations and metabolite profile for resveratrol and metformin.	Main trial: weeks 0, 25, 52, 78, 104 and 154 Sub-trial: weeks 0, 25 and 52
	Urinary resveratrol/ metabolite levels exposure in 20% participants in the sub-trial from randomly selected sites that are willing and able to collect urine samples.	Weeks 0, 25 and 52
	In the main trial: Measurement of pharmacodynamic biomarkers common to both	Weeks 0, 25, 52, 78, 104 and 154

	aspirin and metformin, including p65, pS6/S6 and cleaved PARP in tissue samples.	
	In the resveratrol sub-trial: Analysis of tissue and plasma pharmacodynamic biomarkers, including NQO1, protein carbonyls, Ki67 proliferation index, MLX and CIDEB.	Weeks 0, 25 and 52
	Characterisation of the gut microbiome.	Main trial: weeks 0, 52 and 154 Sub-trial: weeks 0 and 52

4. TRIAL DESIGN

Both the main trial and the embedded resveratrol sub-trial are multi-centre, randomised, parallel, controlled interventional prevention trials. The main phase III trial will recruit 862 participants and the phase II signal-seeking sub-trial will recruit 477 participants; giving a combined total of 1339 participants.

For the open-label main trial, patients will be randomised to receive either aspirin (control group) or aspirin + metformin (intervention) daily for 3 years. A low dose of aspirin at 75mg once daily will be the backbone standard treatment in both arms, with those randomised to the interventional arm receiving metformin at a dose of 500mg twice a day. In the multi-arm, double-blinded, signal-seeking sub-trial, patients will be randomised to receive 5mg of resveratrol, or 1g of resveratrol, or placebo, once daily for 1 year.

The patient population will be adults aged 50-71 years for the main trial and 50-74 years for the sub-trial, who are identified as high risk for colorectal cancer at their **first complete screening episode** within the Bowel Cancer Screening Programme (BCSP). This includes individuals with 'high risk' findings according to the BCSP definitions (see Figure 2) or a large (≥20mm) non-pedunculated colorectal polyp (LNPCP). For patients with an LNPCP, these will be eligible for recruitment after their first complete screening episode if the resection is a histological R0 *en block* excision. Where there is piecemeal resection of an LNPCP, patients will be eligible **only** if their **2nd site check** is a **full clearance colonoscopy**; in these cases patients would become eligible after completion of this procedure (see Figure 2). For full inclusion/exclusion criteria see section 6.

We aim to assess the efficacy, safety and mechanisms of these potentially preventive treatments within this population. The primary endpoint is polyp number (MPP), secondary endpoints include polyp detection rate (PDR), advanced polyps, polyp subtype and location, adverse events and compliance. Research endpoints will focus on the association between metabolic status and treatment efficacy, assessment of pharmacodynamics biomarkers and role of the gut microbiome in predicting and mediating the effects of the three therapies.

5. TRIAL SETTING

This is a multi-centre trial. Recruitment will take place in approximately 60 NHS Bowel Cancer Screening Programme sites across England, Wales and Scotland. COLO-PREVENT will build on and extend the trial site infrastructure established by the SeAFOod trial (12).

6. PARTICIPANT ELIGIBILITY CRITERIA

6.1 Inclusion criteria

General inclusion criteria for both trials:

- Adequate renal function, defined as GFR ≥35ml/min/1.73m^2, at any time in the preceding 4 weeks
- Willing and able to consent to participate in trial

Participants must meet ONE of the following criteria:

- Patients with high risk findings **(≥2 premalignant polyps including ≥1 advanced colorectal polyp; or ≥5 premalignant polyps)** at a completed screening episode according to BCSP criteria **OR**
- Patients with a **large** (≥20mm) **non-pedunculated colorectal polyp** that is resected with **histological R0 *en bloc excision*** at a completed screening episode **OR**
- Patients with a **large** (≥20mm) **non-pedunculated colorectal polyp** after **piecemeal excision.** These will only be eligible if the **2nd site check** is a **full clearance colonoscopy**

Inclusion criteria for the main trial but not the resveratrol Signal-Seeking trial:

- Aged 50-71 years

Additional inclusion criteria for the resveratrol Signal-Seeking trial only:

- Aged 50-74 years
- Use of aspirin, including as an anti-platelet therapy, is permitted in the signal-seeking trial

6.2 Exclusion criteria

General exclusion criteria for both trials:

- Malignant change in a polyp
- Known clinical diagnosis or gene carrier of a hereditary CRC predisposition (FAP, hereditary nonpolyposis CRC)
- Previous or newly diagnosed inflammatory bowel disease
- Previous or planned colorectal resection
- Known bleeding diathesis or concomitant non-aspirin anti-coagulant or anti-platelet agent

- Abnormal liver functions consisting of any of the following, at any time in the preceding 4 weeks:
  - Serum bilirubin ≥1.5 x ULN (except for participants with Gilbert's disease, for whom the upper limit of serum bilirubin is 51.3µmol/l or 3mg/dl)
  - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥2.5 x ULN
- Inability to comply with trial procedures and use of therapies
- Pregnant or lactating women
- Women of child-bearing potential unwilling to use appropriate methods of birth control (see protocol section 8.10)
- Males with partners who are WOCBP and are unwilling to use effective methods of contraception
- Serious medical illness interfering with trial participation including inability to have future colonoscopic surveillance
- Participants who have been administered an investigational medicinal product for another research trial in the last 30 days or ≤5 elimination half-lives

Exclusion criteria for the main trial but not the resveratrol Signal-Seeking trial:

- Methotrexate use
- 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
- Current or previous treatment with metformin
- Known history of peptic ulcer disease
- Known history of lactic acidosis or predisposing conditions
- 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
- Use of long-term systemic corticosteroids

Additional exclusion criteria for the resveratrol Signal-Seeking trial only

- Unable to abstain from ingestion of OTC supplements containing resveratrol for the trial duration
- Known yeast allergy
- Sensitivity or allergy to any of the capsule excipients (sorbitol, croscarmellose sodium, magnesium stearate and hydroxypropyl methylcellulose)

6.3 Co-enrolment

Co-enrolment of COLO-PREVENT participants onto other interventional studies will be considered where there is no possible conflict with the objectives of this trial. A list of appropriate and agreed studies will be produced at a national level to guide co-enrolment. Co-enrolment will be discussed and confirmed with sites at the time of site set-up and monitored throughout the recruitment phase. In addition, the CI will review the protocols for other studies at sites and

will consider co-enrolment in conjunction with the Trial Management Committee where appropriate.

7. TRIAL PROCEDURES

See Tables 1 and 2 for detailed visit structure and trial procedures.

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Table 1: Trial Assessments for Main Trial (Aspirin or Aspirin + Metformin; 3 year intervention; open-label)

Procedure (Time (T) in weeks)	Pre-trial BCSP colonoscopy	Research visit Visit 1 T= 0 weeks	Telephone call Visit 2 T= 4 weeks	Telephone call Visit 3 T= 12 weeks	Research visit Visit 4 T= 25 weeks	Research visit Visit 5 T= 52 weeks	Every 6 months Research visits Visits 6, 7 & 8 6 monthly	Exit surveillance colonoscopy Visit 9 T= 154 weeks	Post-surveillance colonoscopy telephone visit Visit 10 T= 156 weeks
Colonoscopy (routine clinical procedure)	●							●	
Provide trial information (PIS)	●								
Colonoscopy results (routine care)	●								●
Eligibility assessment		●							
Informed consent		●							
Medical History and Demographics		●							
Fasting blood samples & processing -safety & research[b]		●			●	●	● (visits 6 & 7 only)	●	
Blood serum pregnancy test if applicable		●						●	
Food Frequency Questionnaire (FFQ)		●				●	● (visit 7 only)	●	
BP measurement		●							
Concomitant medication recording		●	●	●	●	●	●	●	●
Eligibility confirmation & Randomisation		●							
Trial drug dispensing		●[a]	**Increase metformin dose from 500mg OD to BD**		●	●	●		
Research faecal sample		●				●		● (prior to colonoscopy)	
Adverse Events		●[c]	●	●	●	●	●	●	●
Compliance (pill counting)					●	●	●	●	
Rectal biopsies								●	
Diagnostic FFPE blocks		●						●	
Last dose of trial drug								●	
End of trial participation									●

[a] Trial medication to start following collection of a baseline research faecal sample (obtained on a FIT kit). [b] Locally processed: fasting lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose; annual vit B12 testing for metformin arm participants. Research samples: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers. [c] AEs recorded from first dose. Visits 2 & 3 have a **±1 week** window, except for metformin arm participants where visit 2 has a window of **+1 week**. Visits 4-9 have a **±2 week** window. Visit 10 has a **+1 week** window.

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Table 2: Trial Assessments for Signal-Seeking Sub-Trial (Blinded allocation: resveratrol 5mg or 1g, or placebo; 1 year intervention)

Procedure (Time (T) in weeks)	Pre-trial BCSP colonoscopy	Research visit Visit 1 T= 0 weeks	Telephone call Visit 2 T= 4 weeks	Telephone call Visit 3 T= 12 weeks	Research visit Visit 4 T= 25 weeks	Surveillance (research) colonoscopy Visit 5 T= 52 weeks	Post-surveillance colonoscopy telephone call Visit 6 T= 54 weeks
Colonoscopy[a]	●					●	
Provide trial information (PIS)	●						
Colonoscopy results	●[f]						●
Eligibility assessment		●					
Informed consent		●					
Medical History and Demographics		●					
Fasting blood samples & processing -safety & research[c]		●			●	●	
Blood serum pregnancy test if applicable		●				●	
Food Frequency Questionnaire (FFQ)		●				●	
Concomitant medication recording		●	●	●	●	●	●
Eligibility confirmation & Randomisation		●					
Trial drug dispensing		●[b]			●		
Provision of urine sample[d]		●			●	●	
Research faecal sample		●				● (prior to colonoscopy)	
Adverse Events		●[e]	●	●	●	●	●
Compliance (pill counting)					●	●	
Rectal biopsies						●	
Diagnostic FFPE blocks		●				●	
Last dose of trial drug						●	
End of trial participation							●

[a] First colonoscopy is a routine clinical procedure, second colonoscopy is a research procedure. [b] Patient to commence their trial medication following collection of a baseline research faecal sample (obtained on a FIT kit). [c] Locally processed: FBC, LFT, U&E, eGFR, HbA1c, fasting lipid profile, glucose. Research samples: insulin, IGFBP-3, IGF-I, PK/PD, exploratory research biomarkers. [d] Urine will be collected from 20% of patients from randomly selected sites that are willing and able to collect urine. [e] Adverse events collected from first dose. [f] Where routine consultations are undertaken remotely following a screening colonoscopy, potential participants will need to be brought into hospital for visit 1.

Visits 2 & 3 have a **±1 week** window. Visits 4 and 5 have a **±2 week** window. Visit 6 has a **+1 week** window.

7.1 Recruitment

7.1.1 Participant identification

Potential participants will be identified at the point of their screening (index) colonoscopy as part of the Bowel Cancer Screening Programme (BCSP). Individuals confirmed as being 'high risk' according to current BCSP criteria will be considered for the trial at discharge from their completed index screening episode, where an episode may contain >1 diagnostic test. The exception is patients with **LNPCP** requiring site checks after **piecemeal resection** as these will only become eligible for COLO-PREVENT if their **2nd site check** is a **full clearance colonoscopy**. These individuals will be provided with a PIS at discharge from their 2nd site check, 12 months after the first complete screening episode.

'High risk' individuals will initially be approached by a member of their direct care team (e.g. BCSP Specialist Screening Practitioner (SSP), Endoscopist etc.). This clinical staff member will aim to have a brief discussion about the trial with the individual prior to discharge from their screening colonoscopy, and provide them with a Patient Infographic and Participant Information Sheet (PIS). This process will be clearly documented in the patient's medical notes, and details provided for the trial screening log (see further information in section 7.1.2).

On many occasions the direct care team will also be members of the research team. If the member of the direct care team has not been delegated the responsibility to undertake research tasks, they will ask the potential participant if they agree to be contacted by a member of the research team.

Individuals that have been provided with a PIS will be contacted by telephone to ask if they have had time to read the information and have any questions. This phone call will be made by a member of the direct care team, if permission to be approached by a member of the research team has not been obtained. If the potential participant is interested in participating in the trial they will be asked if they are happy to come into hospital fasted for collection of visit 1 blood samples. If subjects do take up the offer of arriving fasted, then they should be warned about the possible consequences (dizziness etc. and in the case of diabetics the potential for low/high blood sugars). This discussion will be recorded in the invitation to screening document (supplied in the Investigator Site File) and within the patient's hospital notes. **No samples or assessments will be taken until the patient has provided written consent for the trial.** Staff will endeavour to book the patient's visit 1 appointment as early in the morning as possible.

Adequate space and time for research activities will be provided at all clinics. Posters identifying the hospital as a COLO-PREVENT site, including contact details for the research team, will be displayed in the out-patients' clinic.

7.1.2 Screening

Patients interested in participating in the trial will be formally assessed for eligibility when they return for visit 1. During this visit, the trial will be explained in more detail and the patient will be provided the opportunity to ask questions.

Potential participants will be assessed for eligibility as per the inclusion and exclusion criteria. Once written informed consent has been provided, baseline assessments will be

undertaken, including safety bloods and blood pressure measurement. Safety bloods (serum pregnancy test for WOCBP [see section 8.10]; renal and liver function tests; blood glucose) will be reviewed by a delegated physician. These blood samples may be prioritised and fast tracked in order to determine eligibility if the patient intends to stay and wait for IMP dispensing. Alternatively, the participant may leave and return the next day for collection of IMP or the site may choose to arrange delivery of IMP to a participant's home (see pharmacy manual and schedule 3 of mNCA for details).

It will be made clear to the patient that if their blood results indicate they do not meet the requirements for the trial, they will not progress any further and their consent will be invalidated. **Eligibility will be confirmed by a delegated physician.** Randomisation will not be undertaken until the safety blood results have been confirmed. Participants will be randomised into the trial using a web-based randomisation system (access is provided by the LCTU).

Screening data will be captured in line with the CONSORT (Consolidated Standards of Reporting Trials) statement. The trial screening log, which forms part of the Investigator Site File (ISF), will be maintained by site research staff. Patients confirmed to be 'high risk' that have been screened for the trial, will be added to the trial screening log. Patients who are deemed not to be eligible or those that decline to take part in the trial will be screen failed; and the main reason for their non-enrolment will be documented. Screening data is likely to be captured electronically and non-identifiable pseudonymised data (patient initials, biological sex and ethnicity) will be submitted to the LCTU on a monthly basis.

7.1.3 Payment

This trial is funded by Cancer Research UK. The funding grant does not allow for reimbursement of patient travel expenses or their time taking part in this trial. Although travel expenses may be available for some patients through local centre arrangements.

7.2 Informed consent

Patients will have their routine consultation with their BCSP team typically within 14 days after their screening colonoscopy, allowing adequate time to consider the information and their participation in the trial.

The Investigator or their delegate, will inform the participant of all aspects pertaining to participation in the trial. The 'delegate' may be a research nurse or SSP, however agreement of the participating NHS Trust and evidence the staff member has been trained to obtain consent must be in place. Additionally, a delegated physician must confirm patient eligibility for nurse-led consent. The Investigator or their delegate, will answer any questions that the patient has concerning the trial.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available printed in other languages (apart from compliance with the Welsh Language Act). Participants who cannot read or write, require translators or have cognitive impairment would likely be from a minority within this patient population.

If the patient was provided the main trial PIS and they arrive at visit 1 stating they would prefer to participate in the resveratrol sub-trial (when this is open to recruitment), the investigator or their delegate will discuss the differences in information detailed in the sub-trial PIS. The patient will be informed that they may take longer to think about the study and either return for consenting on the same day or come back for a further visit. A further visit will also be arranged for patients not wishing to undergo the consent process whilst fasting, as failure to arrive in a fasting state would not preclude them from entering the study.

If the patient decides to participate in the trial, they will be asked to sign the informed consent form which will then be countersigned by the investigator or their delegate. The patient will retain one copy of the signed consent form. Another copy will be placed in the patient's medical records, whilst the original will be retained in the ISF. Further, the entire consent process will be fully documented within the patient's medical notes.

The process for obtaining informed consent will be in accordance with the REC guidance, and GCP and any other applicable regulatory requirements, which may be introduced.

The right of the patient to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Trial procedures, including baseline screening assessments, will not be undertaken until a fully signed informed consent form is in place.

If a participant should subsequently wish to withdraw their consent, it will be explained that we will retain information, data and samples collected up to this point; and their data will be used in the final analyses and may still be shared with our research collaborators. Trial participants may need to be re-consented following any amendments to the protocol which affect their safety or participation in the trial.

7.2.1 Additional consent for biological specimens and future research

Blood samples will be collected from participants at baseline, regular intervals during and at the end of each trial. These samples are mandatory to assess safety and for the analysis of exploratory biomarkers. Refer to tables in section 7.7 for more details on which tests will be performed locally.

Diagnostic formalin fixed paraffin embedded (FFPE) blocks of polyp tissue obtained during the initial screening colonoscopy and surveillance/research colonoscopy will be retrieved for analysis.

Faecal samples will be collected using a Faecal Immunochemical Test (FIT) kit, at baseline, and end of each trial. In addition, participants in the main trial will provide an interim faecal sample at 12 months. These faecal samples are a mandatory part of the trial.

Six biopsy samples of normal colorectal tissue will be obtained from each patient during the surveillance colonoscopy at 3 years in the main trial, or research colonoscopy in the resveratrol sub-trial at 12 months. These biopsies are optional.

For the sub-trial only, urine will be obtained from 20% of individuals randomly selected from sites that are willing and able to collect urine samples. Provision of urine is optional for participants.

Additionally, participants will be given the opportunity to consent to storage of their biological specimens for future ethically approved research in a Human Tissue Authority (HTA) licensed area of the Leicester Cancer Research Centre at the University of Leicester. This is optional and participants will be able to opt out of this without affecting their involvement in the trial. Samples and related data will be pseudonymised and may be transferred externally to collaborators (academic and industry partners elsewhere inside or outside the UK). All necessary agreements will be put into place before any transfer of data or samples. The right of the participant to refuse consent for the optional aspects of the trial, will be respected.

7.3 Randomisation

For the main trial, participants will be randomised in a 1:1 ratio to aspirin or aspirin + metformin, with minimisation by BCSP centre. The main trial is open-label, so the research team and participants will be aware of their treatment assignment. However, we will minimise potential bias at the surveillance colonoscopy, by asking participants and the research team not to reveal their treatment assignment to endoscopy staff.

For the resveratrol sub-trial, two doses of resveratrol (5mg and 1g) are compared against a placebo control group. Participants will be randomised equally in a 1:1:1 ratio to the three treatment arms and according to aspirin or metformin use, ensuring an even distribution across arms of these factors. The sub-trial will be double-blinded, so the research team and participants will not be aware of treatment assignment.

7.3.1 Method of implementing the randomisation/allocation sequence

The LCTU will supply a web-based randomisation system from a third party (Sealed Envelope Ltd.).

Once the participant has provided written informed consent to the trial and a physician has confirmed eligibility, randomisation will be performed. Site personnel (delegated and trained) will enter data pertaining to consent, eligibility, pseudonymised identification etc. onto the randomisation Case Report Form (CRF). These data are required to be input into the online randomisation system in order to allocate the participant to a treatment arm. The randomisation system will generate a unique trial ID and determine treatment consignment.

For the main trial, the research and pharmacy teams will be notified of which treatment the participant has been randomised to.

For the resveratrol sub-trial, the research and pharmacy teams will receive kit IDs for dispensing and will be blinded to the treatment allocation.

A copy of the confirmation email received following each randomisation will be printed out and filed in the Investigator Site File.

Access to both Sealed Envelope randomisation systems is restricted and will be provided to the trial statistician for safety and DMC reporting, site research staff, pharmacy staff, LCTU trial management staff and Sponsor.

7.4 Blinding

The main trial will be open-label and will not contain a placebo. Accordingly, steps were taken at the funding stage to investigate and minimise potential bias. A survey of Endoscopists conducted by Prof. Matt Rutter (co-investigator) 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. 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. To diminish potential bias at the surveillance colonoscopy, we will blind Endoscopy staff (outcome assessors) and will instruct the participants and research staff not to divulge what arm an individual patient has been randomised to. Furthermore, the DMC will regularly review the colonoscopy withdrawal times across treatment groups, which would flag up a potential change in Endoscopist behaviour. Lab staff will be blinded to the treatment allocation during all their analyses.

The resveratrol sub-trial will be blinded. Participants, investigators, and all involved in trial conduct, sample analysis, outcome assessors, sponsor, or with any other interest in this trial will remain blind to the randomised treatment assignments until after final analysis is complete. The exception to this is as follows;

- The trial statistician will have access to the web-based randomisation database of all randomised participants in order to prepare the unblinded DMC to make safety decisions and complete end of trial analyses. The trial statistician will receive the sequence and decode treatment allocations from the manufacturer for inclusion into the web-based randomisation system.

The capsules for the sub-trial will be manufactured to contain doses of 5mg and 250mg resveratrol, with matched placebo. Both of the active doses of resveratrol and the placebo will be filled into the same sized capsule and weight-matched. Each participant will receive the same number of capsules, regardless of which arm they are randomised to. All capsules will be matched to maintain blinding.

The sequence and decode of treatment allocations will remain concealed until after the creation of a locked analysis data set.

A DMC will review the trial data periodically to assess patient safety and will have access to unblinded data as outlined and agreed in the DMC Charter. Data presented at the DMC will be prepared by the trial statistician and take the format of an open blinded session followed by a closed unblinded session (as appropriate) to prevent unblinding of the CI, trial manager and the rest of the trial team.

7.5 Emergency Unblinding

Unblinding is only relevant for the resveratrol sub-trial.

Unblinding should generally only be considered in the event of a medical emergency (for example, an emergency operation is required or an overdose has been taken) where knowledge of the participant's treatment allocation would change clinical management. The scenario for this to happen for resveratrol is expected to be very unlikely. Wherever possible, unblinding should be avoided to protect the integrity of the COLO-PREVENT trial.

Unblinding can only be performed by the Investigator, an authorised delegate, the site pharmacy team or Sponsor via an access controlled system available through the web based Sealed Envelope System (https://www.sealedenvelope.com/access/).

If unblinding occurs, the Investigator must record the reason for unblinding, as well as the date and time of the event in the site file and medical notes. Corresponding information will be recorded in the CRF by the Investigator. It will also be documented at the end of the trial in any final trial report and/or statistical report. The site team will notify the Leicester Clinical Trials Unit and the Sponsor in writing as soon as possible following the code break, detailing the necessity of the code break. In the case of unblinding an individual participant for safety reasons, appropriate follow-up of safety related events is required until the event(s) has satisfactorily resolved. The written information will be disseminated to the Data Safety Monitoring Committee for review in accordance with the DMC Charter. SUSARs are required to be reported unblinded.

Unblinding of all participants will be undertaken by the trial statistician after the last participant has completed their final visit, and the database has been locked for the signal-seeking sub-trial.

7.6 Baseline data

Baseline measures will be completed following obtaining of written informed consent, and prior to first dosing (see section 8.6). Please see Trial Assessments (section 7.7) below for assessment details.

7.7 Trial assessments

The trial visit schedule and assessments are summarised in Tables 1 (main trial) and 2 (sub-trial). Trial assessments (e.g. FFQ) may be completed remotely by telephone consultation, where possible, and when necessary.

Randomisation occurs at visit 1 (T=0). Study drug will be started as close to randomisation as possible and following collection of the baseline faecal sample. Site staff will make telephone calls for visits 2 and 3 as close to the scheduled time points as possible, however a window of ±1 week is accepted. The exception is for participants randomised to the metformin arm on the main trial, where a window of +1 week will apply (in order to allow for the 4 week run-in period for dosing). All 6 monthly research visits and the exit surveillance colonoscopy (main trial: visits 4-8; resveratrol sub-trial: visit 4) visit have a window of ±2 weeks. For the final visit, which is in line with a routine consultation following the exit surveillance colonoscopy, a +1 week window applies to allow a sufficient period post last dose for collection of adverse events. Visit schedules are in relation to T=0 date.

Each participant's actual visit schedule should be determined taking into account practical considerations, the allowed visit window and ensuring the participant has sufficient drug.

Details of all trial visits are presented below.

MAIN TRIAL ASSESSMENTS	
H	**Pre-trial BCSP Colonoscopy** • Provide trial information (PIS) • Colonoscopy results provided to patient (routine care)

H	**Visit 1 (Week 0) – Research visit**
	The following procedures will be performed:
	• Eligibility assessment
	• Informed consent
	• Medical history
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Blood serum pregnancy test if applicable for WOCBP
	• Demographics
	• BP measurement
	• Concomitant medication recording
	• Eligibility confirmation & Randomisation
	• Faecal sample (using a FIT kit)
	• Food Frequency Questionnaire (FFQ)
	• Trial drug dispensed (first dose to be started after baseline faecal sample collected)
	• Retrieval of diagnostic FFPE blocks containing polyps
	• Adverse events (recorded from first dose, following visit)

☎	**Visit 2 (Week 4) – Telephone visit** The following procedures will be performed: • Adverse events • Concomitant medication recording • Increase metformin dose from 500mg OD to BD for patients on Arm B
☎	**Visit 3 (Week 12) – Telephone visit** The following procedures will be performed: • Adverse events • Concomitant medication recording
H	**Visit 4 (Week 25) – Research visit** The following procedures will be performed: • Adverse events • Concomitant medication recording

MAIN TRIAL ASSESSMENTS	

	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Compliance (pill counting)
	• Trial drug dispensed

H	**Visit 5 (Week 52) – Research visit**
	The following procedures will be performed:
	• Adverse events
	• Concomitant medication recording
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose; vitamin B12 test for metformin arm participants only.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Faecal sample (using a FIT kit)
	• Food Frequency Questionnaire
	• Compliance (pill counting)
	• Trial drug dispensed

H	**Visit 6, 7 and 8 (Six monthly) – Research visits**
	The following procedures will be performed:
	• Adverse events
	• Concomitant medication recording
	• Fasting blood samples & processing – safety and research **(visits 6 & 7 only)**
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose; **vitamin B12 test for metformin arm participants required at visit 7 only**
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Food Frequency Questionnaire **(visit 7 only)**
	• Compliance (pill counting)
	• Trial drug dispensed

H	**Visit 9 (week 154) – Exit surveillance colonoscopy**
	The following procedures will be performed:
	• Colonoscopy (routine clinical procedure)
	• Adverse events
	• Concomitant medication recording
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose; vitamin B12 test for metformin arm participants only.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers

MAIN TRIAL ASSESSMENTS	
	• Blood serum pregnancy test if applicable for WOCBP • Faecal sample **(prior to colonoscopy and using a FIT kit)** • Food Frequency Questionnaire • Compliance (pill counting) • Rectal biopsies (X6) of normal tissue • Retrieval of diagnostic FFPE blocks containing polyps, where available • Participant stops taking trial drug
☎	**Visit 10 (week 156) – Routine post-surveillance colonoscopy telephone consultation** • Colonoscopy results provided to participant by routine care team The following assessments will be performed: • Adverse events • Concomitant medication recording • End of trial participation

Guidance on procedures

Concomitant medication recording

A full history of baseline medications will be recorded at visit 1. At all subsequent visits the participant will only be asked about any new medications or any changes to concomitant medications.

Food Frequency Questionnaire (FFQ)

Research staff will go through the FFQ with the participant and explain how to complete this. The participant will complete and return the FFQ during the outpatient visit. Site research staff member to check the FFQ before the participant leaves to ensure the questionnaire has been completed fully and correctly.

Research faecal sample (collected on a FIT kit)

First FIT kit to be provided at visit 1 and sample obtained prior to commencement of trial drug(s). Participants will post faecal samples directly to the coordinating site in a pre-paid envelope.

Urine sample

For the resveratrol sub-trial only, participants will be asked to provide a urine sample at specified visits and will be provided with a 20 mL specimen bottle. Once the urine specimen has been received, record time of collection and place immediately on ice for processing.

Compliance

Participants will be asked to return their trial medication bottles at each visit even if they are empty. Residual pills will be counted for each bottle and recorded on the CRF. Returns will be taken to pharmacy for accountability.

RESVERATROL SUB-TRIAL ASSESSMENTS	
H	**Pre-trial BCSP Colonoscopy** • Screening colonoscopy • Provide trial information (PIS) • Colonoscopy results provided to patient (routine care)

H	**Visit 1 (Week 0) – Research visit**
	The following procedures will be performed:
	• Eligibility assessment
	• Informed consent
	• Medical history
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Blood serum pregnancy test if applicable for WOCBP
	• Demographics
	• Concomitant medication recording
	• Eligibility confirmation & Randomisation
	• Urine sample (only for 20% of randomly selected patients)
	• Faecal sample (using a FIT kit)
	• Food Frequency Questionnaire (FFQ)
	• Retrieval of diagnostic FFPE blocks containing polyps
	• Trial drug dispensed (first dose to be started after baseline faecal sample collected).
	• Adverse events (recorded from first dose, following visit)

☎	**Visit 2 (Week 4) – Telephone visit** The following procedures will be performed: • Adverse events • Concomitant medication recording
☎	**Visit 3 (Week 12) – Telephone visit** The following procedures will be performed: • Adverse events • Concomitant medication recording

H	**Visit 4 (Week 25) – Research visit**
	The following procedures will be performed:
	• Adverse events
	• Concomitant medication recording
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers

RESVERATROL SUB-TRIAL ASSESSMENTS	
	• Urine sample (only for 20% of randomly selected patients) • Compliance (pill counting) • Trial drug dispensed

🏥	**Visit 5 (week 52) – Surveillance (research) colonoscopy**
	The following procedures will be performed:
	• Research colonoscopy
	• Adverse events
	• Concomitant medication recording
	• Fasting blood samples & processing – safety and research
	  ○ Locally processed: Lipid profile, FBC, LFT, U&E, eGFR, HbA1c, glucose.
	  ○ Research samples processed centrally: IGFBP-3, IGF-I, insulin, PK/PD, exploratory research biomarkers
	• Blood serum pregnancy test if applicable for WOCBP
	• Provision of urine sample (only for 20% of randomly selected patients)
	• Faecal sample **(prior to colonoscopy and using a FIT kit)**
	• Food Frequency Questionnaire
	• Compliance (pill counting)
	• Rectal biopsies (X6) of normal tissue
	• Retrieval of diagnostic FFPE blocks containing polyps, where available
	• Participant stops taking trial drug

☎	**Visit 6 (week 54) – Post-surveillance colonoscopy telephone consultation** • Colonoscopy results provided to participant by routine care team The following assessments will be performed: • Adverse events • Concomitant medication recording • End of trial participation

7.8 Long term follow-up assessments

There is no active follow-up stage of the trial after surveillance colonoscopy. Participants will be followed-up for up to 10 years after the trial has ended through routinely-collected healthcare databases including the Bowel Cancer Screening System, the National Cancer Registration and Analysis Service (NCRAS), and other organisations. Specifically this will include access to anonymised BCSP data on colonoscopic outcomes. This is optional and will only be conducted if the participant provides consent.

7.9 Qualitative assessments

All participants will be requested to complete a validated Food Frequency Questionnaire (FFQ) at specified times: at baseline (visit 0) and end of each trial, as well as 52 and 104 weeks in the main trial. The purpose of the FFQ is two-fold; it will enable us to perform exploratory analysis to investigate whether dietary patterns and particularly fat intake, influence the effectiveness of aspirin, metformin and resveratrol. Secondly, in the sub-trial

the information will be used to identify any changes in dietary intake of resveratrol during trial involvement.

7.10 Withdrawal criteria

Every effort should be made by the research team to keep the participants in the trial. However, participants have the right to withdraw their consent to take part in the trial at any time without having to give a reason and this will not affect their future care. Withdrawal of consent details and reasons (if known) should be recorded on the CRF and in the medical notes.

In the unlikely event that a trial participant becomes pregnant during the study, trial treatment will be discontinued. Please refer to section 10.5 for guidance on pregnancy reporting.

The investigator may discontinue treatment of a participant at any time if they are not compliant with the trial protocol or to protect the participant's safety and well-being after consultation with the CI. The IMP may also be discontinued in the case of an adverse event which the investigator considers sufficient to jeopardise the safety of the participant. Participants who discontinue treatment, for whatever reason, will be encouraged to remain in the trial for collection of important outcome and safety data. These treatment ceased participants will be asked to continue attending visits for collection of data and samples. Participants not wishing to attend all subsequent visits, will be asked for their permission to collect data and samples at their exit surveillance colonoscopy. Additionally, some trial assessments (e.g. Food Frequency Questionnaire) may be collected over the telephone if the participant agrees to be contacted. As a minimum, important outcome and safety data will be collected remotely from the participant's medical record. Those declining this will be withdrawn from the trial (thus withdrawing their consent).

Withdrawals from the trial should be discussed with the CI or their deputy and the Trial Manager. If a participant has withdrawn/discontinued from the trial for any reason, then his/her screening and trial ID number(s) cannot be issued to another participant.

Loss to follow-up will be minimised by diligent liaison with the participant, their medical team and if required the general practitioner. If a participant fails to attend for a trial visit, the research team should contact the participant and re-schedule the missed visit. If the participant cannot be contacted or misses their next appointment, the research team should make every effort to contact the participant again by phone and letter. If the participant still cannot be contacted, they will be approached at their exit colonoscopy visit to request their permission for collection of data and samples. Alternatively they may agree to remote collection of important outcome and safety data from their medical records. If the participant fails to attend their exit colonoscopy visit they will be recorded as 'lost to follow-up'.

7.11 Storage and analysis of clinical samples

Precise details of sampling requirements are provided in the COLO-PREVENT Laboratory Manual provided in the ISF. Sampling strategies will consist of samples required for Safety

(which will be analysed at participating sites as per standard local NHS protocols) and Research (which will be held by sites until they can be sent to the coordinating site at the University of Leicester).

In brief, the research samples we will collect are blood (plasma and serum), fresh tissue, FFPE diagnostic samples where available, and faecal samples from all 60 sites over the full duration of recruitment and follow-up (97 months) (see also sections 7.2.1 and 7.7). Urine samples will be collected for 20% of participants on the resveratrol sub-trial only. For the 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.

The Lead Applicant and Chief Investigator will have overall responsibility for custodianship and use of all clinical samples collected in COLO-PREVENT for the duration of the trial. Frozen blood products/tissue/urine samples will initially be stored at the individual sites in specified temperature-monitored freezers then shipped in batches to Leicester where they will be stored in designated and secure facilities within the Cancer Prevention Group laboratories (CPG) at the Leicester Cancer Research Centre (LCRC), University of Leicester. The laboratories operate under standards of Good Clinical Laboratory Practice (GCLP) and GCP and all samples will be stored, processed and analysed according to SOPs. Frozen plasma, urine, tissue and faecal samples will be stored in temperature monitored (cloud-based system from Haier Biomedical) -80ºC freezers. After retrieval from each participating site, diagnostic FFPE specimens will be stored within dedicated ambient temperature-monitored areas within the CPG-LCRC laboratories. Patients will send research faecal samples (via FIT collection kits) directly to the University of Leicester research team in a pre-paid envelope. Faecal samples will then be frozen, batched and sent for further processing and microbiome analysis at the University of Leeds, with the Leeds Microbiome Laboratory subsequently acting as custodian for these samples.

Samples may be shared with other researchers for work directly related to the objectives of COLO-PREVENT. These may be universities, NHS organisations or companies involved in health and care research inside and outside of the UK and Europe. Samples will not be transferred to other researchers until a Material Transfer Agreement (MTA) is in place.

The trial consent form will ask whether patients are willing for their samples to be stored and used in future ethically approved research projects that are not related to the current COLO-PREVENT objectives. These samples may be disposed of in certain conditions, such as withdrawal of patient consent for long-term storage and analysis.

7.12 Pandemic guidance

In the event of a pandemic (e.g. COVID-19) or local/national lockdowns which may affect COLO-PREVENT participants, the site research team will check ahead of scheduled trial visits that participants are able to attend in line with local NHS Trust policy. Government guidelines with regards to testing and self-isolation should also be followed if a participant experiences symptoms, tests positive and/or must self-isolate. Trial visits will be rescheduled following completion of the government required self-isolation period/a negative test.

Trial participants will need to attend hospital for collection of safety bloods as a minimum. The local research team will ensure participants spend the least amount of time in hospital as possible in order to reduce the risk of transmission in participants and staff. Solutions such as posting trial drug to participants will be considered by site. Data may be collected by telephone consultation (e.g. FFQ, adverse events, concomitant medications etc.) where possible and when necessary.

Collection of samples and data will be consistent with clinical care without compromising the scientific validity of the trial, ensuring safety assessments and key endpoints of the trial are met.

A pandemic risk assessment will be undertaken by the trial management team, CI and sponsor who will provide further guidance to research sites.

7.13 End of Trial

This trial will end when the specified number of participants have been recruited, all participants have completed their last follow up visit, data validation has taken place and the database is locked and statistical analysis complete.

Data reported in the final report to REC and MHRA within 12 months of the End of Trial will include the primary and secondary outcomes (see sections 3.4 and 3.5).

The trial will be stopped prematurely if:

1. Mandated by the Ethics Committee or the Sponsor
2. Mandated by the Medicines and Healthcare products Regulatory Agency (MHRA)
3. Following recommendations from the Data Safety Monitoring Committee (DMC) or otherwise the Trial Steering Committee (TSC) determine the trial should stop
4. Funding for the trial ceases

8. MAIN TRIAL THERAPIES

8.1 Name and description of investigational medicinal products

The **main trial** is open-label, and participants will be randomised to receive either **aspirin**, or the combination of **metformin** plus aspirin for 3 years.

Both aspirin and metformin will be prescribed off-label and are therefore both defined as Investigational Medicinal Products (IMPs) for the main trial. Aspirin 75 mg Gastro-Resistant tablets will be taken orally once daily. Metformin 500 mg tablets will be taken orally once daily for the first 4 weeks and increased to twice daily for the remainder of the trial.

Drug Name	Dosage	Description
Metformin	500 mg (BD)	Any brand/manufacturer, with a marketing authorisation in the UK, can be dispensed but film-coated tablets are recommended. *Description from generic SmPC:* White coloured, round tablet containing metformin hydrochloride 500mg.
Aspirin	75 mg (OD)	Any brand/manufacturer of gastro-resistant aspirin tablets, with a marketing authorisation in the UK, may be dispensed depending on the individual NHS Trust. *Description from generic SmPC:* Gastro-Resistant, white, film-coated, round tablet containing 75 mg aspirin.

8.2 Regulatory status of the drug

Aspirin and metformin are commonly used generic drugs. Neither drug has a marketing authorisation to be used as a CRC preventive therapy and will be prescribed off-patent in the main trial.

Aspirin is currently licensed in the UK for the secondary prevention of thrombotic cerebrovascular or cardiovascular disease and following by-pass surgery.

Metformin is currently licensed in the UK for the treatment of type 2 diabetes mellitus, particularly in overweight patients, when dietary management and exercise alone does not result in adequate glycaemic control.

8.3 Product Characteristics

The Summary of Product Characteristics (SmPC) for aspirin 75mg Gastro-Resistant tablets and metformin 500mg will be used to obtain information on how to use the medicine. Section 4.8 of the latest trial approved version of the SMPCs will be used to assess expectedness of adverse events (known as the reference safety information). The trial approved SmPCs will be provided in the Investigator and Pharmacy Site Files.

8.4 Drug storage and supply

Aspirin 75mg Gastro-Resistant tablets and Metformin 500 mg tablets will be sourced by the research site pharmacy; the purchase of both drugs has been designated an NHS treatment cost. Any suitable brand/manufacturer, with a marketing authorisation in the UK, may be used.

As these are generic drugs these will likely be dispensed from general pharmacy stock. Storage conditions will be in compliance with the SmPC. Aspirin requires storage at room temperature below 25^{o}C. Metformin does not require any special storage conditions. Site pharmacy teams will not dispense aspirin that has undergone a temperature excursion above 25^{o}C.

The local site investigator will delegate responsibility to the Clinical Trials Pharmacist to ensure drug accountability, including reconciliation of trial drug and maintenance of trial drug records, throughout the course of the trial in accordance with UK regulatory requirements. Trial drug may be dispensed only by specifically authorised personnel. Responsibility for certain tasks related to the management of the trial drug can be delegated to the site pharmacy clinical trials staff and other members of the site team.

Trial drug allocated to a participant must not be used for any other purpose than the present trial. Prescriptions should be made available for review as source data.

Dispensing will be recorded on the appropriate trial specific accountability forms. Trial drug that has been dispensed to a participant must not be re-dispensed or re-issued to a different participant.

Unused drug, partially used drug and empty packaging will be returned to research staff at the 6 monthly visits (see table 1), the exit surveillance colonoscopy or post-colonoscopy visit. Unused drug, partially used drug and empty packaging will be taken to the local pharmacy to be recorded and final accountability performed before local destruction is approved by the Sponsor.

Further details are included in the Pharmacy Manual.

8.5 Labelling of Investigational Medicinal Product

For both aspirin and metformin, site pharmacies will add regulatory approved labels to the secondary (outer carton) packaging according to the requirements of the COLO-PREVENT trial and Annex 13 of EU Guidelines to Good Manufacturing Practice.

8.6 Dosing schedules

The main trial is open-label; the participants, research team and pharmacy team will be aware of the treatment assignment.

Eligible participants will be randomised at visit 1 to receive Aspirin 75 mg Gastro-Resistant tablets once daily OR Aspirin 75 mg Gastro-Resistant tablets once daily AND Metformin hydrochloride 500 mg film-coated tablets twice daily.

All participants will be given a dose instruction card describing when to take the medicine, how to take the medicine and the dose.

There will be no weight-based dosing for this trial. All trial drugs are self-administered.

One 75 mg tablet of aspirin will be taken daily and swallowed whole with plenty of liquid (e.g. a glass of water) preferably after a meal. The participant will take their aspirin dose at a similar time each day ensuring sufficient intervals between dosing. A low dose of aspirin has been selected for the trial reducing the likelihood of toxicity. The maximum daily aspirin dose for this trial is 75 mg, and no dose interruptions are anticipated in the absence of specific adverse events. See section 8.7.

Metformin 500mg will be started once daily for 4 weeks. One tablet will be taken orally with breakfast. For the purposes of this clinical trial we are allowing a 4 week run-in to maximise gastrointestinal tolerability. The research team will contact the participant by telephone, 4 weeks after randomisation to check for any adverse effects. If the participant is tolerating the 500 mg dose of metformin, they will be advised to increase their dose twice daily with one tablet to be taken with breakfast and one tablet to be taken with their evening meal. If tolerated, the maximum daily dose of metformin will be 1 g for the remainder of the trial. See section 8.7 for guidance on definition of tolerability and dose modification in the presence of specific adverse events.

The trial pharmacy team will dispense sufficient IMP to last for the full 6 month interval between out-patient visits, see schedule below.

Dispensing schedule	
Visit #	Time point
Visit 1	Baseline (week 0)
Visit 4	Week 25
Visit 5	Week 52
Visit 6	Week 78
Visit 7	Week 104
Visit 8	Week 130
Visit 9	No dispensing. Stop dosing on day of exit surveillance colonoscopy - anticipated at week 154

The participant will stop taking trial drug on the day of their exit surveillance colonoscopy and the site research team/pharmacy team will ensure the participant is dispensed an adequate supply. If a participant misses a dose on one day, they should resume taking their normal daily dose the next day. If the participant vomits straight after dosing they will be advised not to take an additional dose to make up for it.

8.7 Dosage modifications

Aspirin

Aspirin dose cannot be modified, only stopped.

Patients will be made aware that aspirin should be used with caution before surgery, including tooth extraction. Temporary discontinuation of treatment may be necessary.

Participants that experience any aspirin-related severe toxicity (defined as ≥grade 3 Common Terminology Criteria for Adverse Events (CTCAE v5)) or significant gastrointestinal bleeding (grade 3 and 4), active gastrointestinal ulceration, new cases or worsening tinnitus (grade 2 or above), intracranial bleeding or hypersensitivity to aspirin should permanently discontinue aspirin immediately.

Metformin

Run-in
Undesirable effects of metformin are most frequent during initiation of therapy and usually resolve spontaneously. To prevent them, it is recommended that metformin is taken during or after meals. Metformin does not cause hypo-glycaemia in non-diabetic patients and participants will be reassured about this prior to dosing. Participants will have a run-in period of 4 weeks at the once daily 500 mg metformin dose to allow time for gastrointestinal tolerance. If more than 80% of the prescribed dose is taken during this time, the research team will instruct the participant to increase their dose to twice daily 500 mg metformin tablets.

If the patient does not tolerate the 500 mg metformin then it is possible for the patient to have another 4 week run-in period using a MR formulation 500 mg dose. If this is deemed tolerable then patients will increase their dose to twice daily 500 mg MR release metformin tablets. If the participant is unable to tolerate the lowest dose of metformin then they can continue on the trial with aspirin alone.

Main trial
It is advised that metformin treatment will be paused for approximately 72 hours around the time of contrast-enhanced CT scans.

Metformin must be discontinued at the time of surgery under general, spinal or epidural anesthesia. Therapy may be restarted no earlier than 48 hours following surgery or resumption of oral nutrition and provided that renal function has been re-evaluated and found to be stable.

Metformin must be permanently discontinued in patients where the GFR < 30mls/min. Patients who develop acute and unstable heart failure, while on the study should discontinue metformin.

For patients who wish to stop metformin due to adverse events then they should be offered a trial of twice daily 500 mg MR formulation to improve tolerability. If adverse events persist then the metformin dose can be reduced to 500 mg once per day. No further dose modifications are permitted and therefore if adverse events continue the metformin should be permanently discontinued.

If a participant misses >20% of doses for aspirin in combination with metformin or aspirin alone, then it is at the discretion of site investigators as to whether it is appropriate to restart trial treatment.

8.8 Known drug reactions and interaction with other therapies

The most common (>1/100 to <1/10) side effects of aspirin are increased bleeding tendencies and dyspepsia.

The most common (>1/100 to <1/10) side effects of metformin are taste disturbance and gastrointestinal disorders such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite.

For the full list of undesirable effects please refer to sections 4.8 of the regulatory approved Summary of Product Characteristics (SMPCs) for aspirin and metformin provided in the Investigator and Pharmacy Site Files (ISF and PSF).

8.9 Concomitant medication

Permitted Medication
Participants should be encouraged to take any regular medication throughout the trial unless they are not permitted (see below). All non-contraindicated medications that the responsible clinician feels are appropriate, for example, PPI, paracetamol and remedies for cough and colds, are allowed in the trial. If a patient develops diabetes while on the study they can remain on the study providing they continue to take metformin as part of their hypoglycaemic regimen.

The potential for interaction of trial treatments with all medicines should be checked against the information provided by the BNF (www.bnf.org) or local equivalent. Information on concomitant medication that has potential anti-cancer activity will be collected at each follow-up visit and call.

Not-permitted medication
ASPIRIN
Medications not permitted include anti-coagulant and anti-platelet medication, aspirin (including over the counter preparations) and others including methotrexate. See appendix I for a list. Regular NSAID use is not permitted. NSAIDs should be avoided wherever possible but short-term intermittent/occasional NSAID use is allowed (maximum of 2 consecutive weeks of regular use). Long-term systemic corticosteroids are not permitted but short-term intermittent use is allowed (maximum of two consecutive weeks). See appendix I for further details. Participants should be counselled about over the counter aspirin and NSAID use prior to consent and this should be reinforced at follow-up visits.

METFORMIN
Patients should be advised to avoid acute alcohol intoxication as this is associated with an increased risk of lactic acidosis.

Medication to be used with caution
ASPIRIN
Aspirin has the potential to interact with the following medications: Selective Serotonin Reuptake Inhibitors (SSRIs), Iloprost, kaolin, metoclopramide, phenytoin, probenecid, spironolactone, sulfinpyraxone, thiopental, valproate, venlafaxine and zafirlukast. See the BNF for further details and a full list of interactions.

METFORMIN
Intravascular administration of iodinated contrast media may lead to renal failure, resulting in metformin accumulation and an increased risk of lactic acidosis.

Some medicinal products can adversely affect renal function which may increase the risk of lactic acidosis, e.g. selective cyclo-oxygenase (COX) II inhibitors, ACE inhibitors, angiotensin II receptor antagonists and diuretics, especially loop diuretics.

Metformin is a substrate of both organic cation transporters OCT1 and OCT2. Precautions are advised when considering co-administration of metformin with inhibitors of OCT1 (such

as verapamil), inducers of OCT1 (such as rifampicin), inhibitors of OCT2 (such as cimetidine, dolutegravir, ranolazine, trimethoprime, vandetanib, isavuconazole); inhibitors of both OCT1 and OCT2 (such as crizotinib, olaparib).

MANAGEMENT WHERE A MEDICATION THAT IS NOT PERMITTED BECOMES INDICATED DURING THE TRIAL

We would not expect patients who require a medication that is not permitted in the trial (see appendix I) to be consented to COLO-PREVENT because this should have been identified during the eligibility screening. However there may be participants who develop a medical condition during the trial where a medication that is not permitted becomes clinically indicated. Examples include but are not limited to: anti-coagulation for the treatment of pulmonary embolism, deep vein thrombosis or atrial fibrillation and anti-platelet therapy for the management of cardiovascular events. The participant would need to cease trial drug in these circumstances but should be encouraged to remain in the trial for collection of important outcome and safety data.

8.10 Trial restrictions

Eligibility criteria have been formulated so that individuals with an increased risk of serious toxicity from either aspirin or metformin are excluded. Details of any concomitant illness (any illness present at the start of the trial) should be recorded at trial entry. If a change influences the participant's eligibility to continue in the trial, the investigator must be informed.

There are no special dietary requirements or restrictions for COLO-PREVENT as this could adversely affect recruitment, compliance and retention. Blood glucose is amongst the measures being monitored at all out-patient visits for safety.

Regular use of aspirin may adversely affect a pregnancy and/or foetal development. Regular aspirin or metformin use whilst breast feeding can also cause complications in the neonate/infant and should be avoided.

Therefore, participants joining COLO-PREVENT should not be pregnant or lactating at trial entry and should be advised against becoming pregnant during the trial treatment period.

Male participants and their female partners of child bearing potential must use medically acceptable forms of contraception during the trial.

A woman is defined as being of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal, unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

Acceptable birth control methods include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide
- cap, diaphragm or sponge with spermicide

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner (provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success)
- True abstinence: When this is in line with the preferred and usual lifestyle of the participant (defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception).

8.11 Assessment and compliance with treatment

Residual numbers of tablets will be recorded to assess adherence in the CRF, this will need to be completed by a member of the research team before returning to pharmacy.

Adherence

A high rate of adherence to treatment is required and Investigators are responsible for ensuring that participants understand this. Prior to consent, Investigators and any other trial staff involved in the consent process should ensure that participants fully understand the trial treatment and the importance of adherence, and this should be re-iterated at face-to-face (or telephone) follow-up visits at the specified time points and adherence should be positively encouraged. Patient-reported adherence should be recorded on follow-up CRFs.

Compliance with trial drug will be assessed by pill counting (returns of unused medication). Returns of residual pills will be recorded in accountability logs retained in pharmacy and the electronic Case Report Form (eCRF).

- If treatment ceased for 6 or less days no action taken.
- If ≥7 days record on the CRF.
- If ≥14 days consider restarting with once daily dosing and escalating to twice daily dosing.
- If treatment is paused and >20% of doses are missed for any reason, it is at the discretion of site investigators as to whether it is appropriate to restart trial treatment.
- All treatment pauses must be recorded in the CRF.

9. SUB-TRIAL THERAPIES

9.1 Name and description of investigational medicinal products

The Signal-Seeking **Sub-Trial is anticipated to start in early 2023**. Once all required data is obtained from the manufacturer, a major Substantial Amendment (SA) will be submitted. The resveratrol sub-trial will not commence until regulatory approval is in place for the SA.

The Investigational Medicinal Product (IMP) for the sub-trial is **resveratrol**.

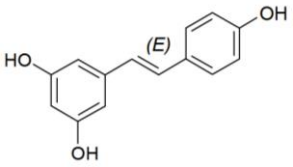
Veri-te™ *trans*-Resveratrol (CAS number: 501-36-0) is the Active Pharmaceutical Ingredient (API) being used to manufacture capsule strengths of 5 mg and 250 mg

Chemical product name:

(E)-5-(4-hydroxystyryl)benzene-1,3-diol;

5-[(1E)-2-(4-Hydroxyphenyl)ethenyl]-1,3-benzenediol

Molecular formula:



***trans* (*E*) resveratrol**

The Veri-te™ *trans*-Resveratrol is produced yeast (Saccharomyces cerevisiae) fermentation (using renewable feedstock/source as the carbon source, followed by separation and purification). Resveratrol API is manufactured in Italy, in a facility operating under cGMP and HACCP standards. Veri-te™ is a trademark of Evolva SA (headquarter address: Duggingerstrasse 23, 4153 Reinach, Switzerland).

The sub-trial is double-blinded, and eligible participants will be randomised to receive 5 mg resveratrol or 1 g resveratrol (4X 250 mg) or placebo to be taken once daily for 1 year.

Drug Name	Dosage	Description
Resveratrol	5 mg (OD)	Hydroxypropyl methylcellulose (HPMC) capsules containing 5 mg *trans*-Resveratrol; excipient blend TBC
Resveratrol	250 mg (OD)	Hydroxypropyl methylcellulose (HPMC) capsules containing 250 mg *trans*-Resveratrol; excipient blend TBC

The resveratrol capsules and matching placebo are being manufactured by Catalent Pharma. Final labelling and release of blinded IMP/placebo packs will be carried out by Catalent Pharma. All sites involved in the manufacturing process have appropriate licenses in place.

Resveratrol 5 mg, resveratrol 250 mg and placebo will be filled into identical, same sized capsules and weight-matched. Both of the active doses of resveratrol and the placebo will be packaged in an identical manner. A unique pack ID will be used to identify each pack and its contents.

9.2 Regulatory status of the drug

There are no marketing authorisations for resveratrol in the UK or indeed worldwide.

Resveratrol is a naturally occurring phytoalexin, an antifungal and antibacterial chemical produced by plants in response to stress, and is found in a variety of food and drinks including red wine, grapes, and peanuts. Resveratrol is widely sold as a single supplement or constituent of a supplement in health food shops in the UK, and resveratrol-containing nutraceutical products are available in capsule, tablet, film, and chewing gum formats.

9.3 Product Characteristics

The Investigator Brochure (IB) for resveratrol will be used to obtain information on how to use the medicine and to assess expectedness of events (section 8.0 of the IB is known as the reference safety information). The IB will be provided in the Investigator and Pharmacy Site Files.

9.4 Drug storage and supply

Catalent Pharma will store bulk IMP supply and distribute blinded trial drug to participating centres. The resveratrol/placebo will be provided to sites free of charge.

Participating sites will be allocated an initial supply of resveratrol/placebo at site activation. Stock levels will be monitored by the clinical trials pharmacy team and the trial management team at LCTU. When trial drug supplies reach a pre-determined level re-supply will be arranged.

Storage conditions will be in compliance with the Investigators Brochure. At site the resveratrol/placebo will be stored between 15-25°C. Storage of trial drug at site will be in a secure location, in a temperature controlled environment, with a temperature log maintained for each working day.

Any temperature excursions must be reported to the Leicester CTU and Sponsor as soon as they are identified.

The local site investigator will delegate responsibility to the Clinical Trials Pharmacist to ensure drug accountability, including reconciliation of trial drug and maintenance of trial drug records, throughout the course of the trial in accordance with UK regulatory requirements. Trial drug may be dispensed only by specifically authorised personnel. Responsibility for certain tasks related to the management of the trial drug can be delegated to the site pharmacy clinical trials staff and other members of the site team.

Trial drug allocated to a participant must not be used for any other purpose than the present trial. Prescriptions should be made available for review as source data.

Dispensing will be recorded on the appropriate trial specific accountability forms. Trial drug that has been dispensed to a participant must not be re-dispensed or re-issued to a different participant.

Unused drug, partially used drug and empty packaging will be returned to research staff at the 6 monthly visits (see table 1), the surveillance colonoscopy or post-colonoscopy visit. Unused drug, partially used drug and empty packaging will be taken to the local pharmacy to be recorded and final accountability performed before local destruction is approved by the Sponsor.

9.5 Preparation and labelling of Investigational Medicinal Product

Final labelling and QP release of blinded trial drug packs will be carried out by Catalent Pharma, in line with Annex 13 of the EU Guidelines for Good Manufacturing Practice.

Catalent Pharma will arrange for delivery of the labelled resveratrol/placebo to the recruiting site at regular intervals according to trial requirements. Allocation of trial medication (resveratrol 5mg or resveratrol 1 g or placebo) will be managed by Sealed Envelope (web based randomisation system). The participant, research team and pharmacy team will all be blinded to the treatment allocation.

9.6 Dosing schedules

The sub-trial is double-blinded; the participants, research team and pharmacy team will not be aware of the treatment assignment.

Eligible participants will be randomised at visit 1 to receive one of the following BLINDED allocations:

- **5 mg resveratrol intervention arm**
- **1 g resveratrol intervention arm**
- **Placebo arm**

The capsules will be packaged into bottles. Each patient kit will contain 4 bottles such that patients take one capsule from each bottle daily. The 3 types of blinded kit will comprise of the following:

- **5mg resveratrol intervention arm:** 1 bottle of 5mg resveratrol capsules + 3 bottles of placebo
- **1g resveratrol intervention arm:** 4 bottles of 250mg resveratrol capsules
- **Placebo arm:** 4 bottles of placebo capsules

There will be no weight based dosing for this trial. All trial drugs are self-administered.

The web based randomisation system will generate a unique kit ID which will be added to a trial specific prescription, along with the participant's trial number and details.

All participants will be given a dose instruction card describing when and how to take the medicine.

The research team will be conducting telephone consultations at visits 2 (week 4) and 3 (week 12) and will ask the participant about any symptoms or new medical problems since the last contact. Participants will be reminded to take their trial drug as directed.

Dispensing will be recorded on the appropriate trial specific accountability forms. Trial drug that has been dispensed and prepared for administration to a participant must not be re-dispensed or re-issued to a different participant.

Further details are included in the Pharmacy Manual.

The trial pharmacy team will dispense sufficient trial drug for 6 months' supply at visit 1 (week 0) and visit 4 (week 25).

The participant will stop taking trial drug on the day of their research surveillance colonoscopy (visit 5; approximately week 52).

If a participant misses a dose on one day, they should resume taking their normal daily dose the next day. If the participant vomits straight after dosing they will be advised not to take an additional dose to make up for it. If the investigator feels that the reason for inadequate adherence is temporary, the participant will be advised to resume taking their trial drug. The participant will stop taking trial drug on the day of their exit surveillance colonoscopy.

9.7 Dosage modifications

Dose reduction for the sub-trial is not permitted. If participants experience GI symptoms, they will be advised to take their full dose with or after food. If symptoms persist, participants can divide their dose such that two capsules are taken each morning and two in the evening. In this situation the participant must take the capsules from the same 2 bottles each morning and evening to allow for the dose blinding. In order to assist with this, each bottle within a treatment pack will be easily distinguished (e.g. numbers 1-4/different colours/letters A-D) from one another.

9.8 Known drug reactions and interaction with other therapies

The most common side effects of resveratrol are gastrointestinal in nature, comprising diarrhoea, constipation, nausea, abdominal cramps, vomiting, steatorrhoea, heartburn, reflux and bloating.

For the full list of undesirable effects are detailed in the regulatory approved Investigators Brochure (IB) for resveratrol provided in the Investigator and Pharmacy Site Files (ISF).

9.9 Concomitant medication

There is evidence from two *in vivo* studies in preclinical models suggesting that taking resveratrol in combination with the **anti-clotting agent warfarin** increases the risk of bleeding, therefore patients taking warfarin would be ineligible for COLO-PREVENT.

Permitted medications
The guidance for permitted medications is as per the main trial; see section 8.9.

Not-permitted medication
Medications not permitted include warfarin

Medications to be used with caution
Based on pharmacokinetic studies, resveratrol may theoretically interfere with phase I metabolism of certain drugs due to its inhibitory effects on cytochrome P450. In theory, drugs relying on first pass metabolism for activation/efficacy (such as some statins, anti-fungals and anti-histamines) may be rendered less effective during co-administration of resveratrol. All concomitant medication should be carefully reviewed by the clinical team prior to trial enrolment to ensure that participant safety is maintained, and efficacy of concomitant medication is not compromised.

If a contraindicated medication is required for treatment of a clinical event while the participant is on study they should be withdrawn from trial treatment and permission requested to continue to collect data.

9.10 Trial restrictions

Eligibility criteria have been formulated so that individuals with an increased risk of serious toxicity are excluded. Details of any concomitant illness (any illness present at the start of the trial) should be recorded at trial entry. If a change influences the participant's eligibility to continue in the trial, the investigator must be informed.

Trial participants must abstain from ingestion of OTC supplements containing resveratrol for the trial duration.

There are no special dietary requirements or restrictions for the sub-trial. Following consultation with PPI representatives, it is not believed to be practical to attempt to restrict dietary consumption of foods/drinks containing resveratrol as this could adversely affect recruitment, compliance and retention. The sub-trial is designed to be pragmatic and applicable to a real world setting.

Male participants and their female partners of child bearing potential must use medically acceptable forms of contraception during the trial. See section 8.10 on definitions of WOCBA and adequate contraception; definition on pregnancy reporting process is described in section 10.5.

9.11 Assessment and compliance with treatment

<u>Adherence</u>
A high rate of adherence to treatment is required and Investigators are responsible for ensuring that participants understand this. Prior to registration, Investigators and any other trial staff involved in the consent process should ensure that participants fully understand the trial treatment and the importance of adherence, and this should be re-iterated at face-

to-face (or telephone) follow-up visits at the specified time points and adherence should be positively encouraged. Patient-reported adherence should be recorded on follow-up CRFs.

Compliance with trial drug will be assessed by pill counting (returns of unused medication). Returns of residual pills will be recorded in accountability logs retained in pharmacy and the electronic Case Report Form (eCRF).

- If treatment ceased for 6 or less days no action taken.
- If ≥7 days record on the CRF.
- If treatment is paused and >20% of doses are missed for any reason, it is at the discretion of site investigators as to whether it is appropriate to restart trial treatment.
- All treatment pauses must be recorded in the CRF.

9.12 Name and description of each Non-Investigational Medicinal Product (NIMP)

The two doses of resveratrol will be compared to placebo in the sub-trial. The placebo will be manufactured to match the 5mg and 250 mg resveratrol capsules, and will contain no active ingredients.

10. ADVERSE EVENT MANAGEMENT / PHARMACOVIGILANCE

10.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. It is important to note that this is entirely separate to the known side effects listed in the SmPC. It is specifically a temporal relationship between taking the drug, the half-life, and the time of the event or any valid alternative etiology that would explain the event.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that:

	• results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the reference safety information: • in the case of a product with a marketing authorisation, this could be in the summary of product characteristics (SmPC) for that product, so long as it is being used within its licence. If it is being used off label an assessment of the SmPCs suitability will need to be undertaken. • in the case of any other investigational medicinal product, in the Investigator's Brochure (IB) relating to the trial in question

10.1.1 Adverse Events (AE)

Main trial

Adverse events will be collected from first dose up to visit 10 (week 156). All bleeding and GI adverse event grades (Common Terminology Criteria for Adverse Events, CTCAE v5), including grades 1-2, will be recorded on the AE eCRF. Any AE classified as related to the study IMP or procedures will be recorded and followed up on. For all other types of events only grade 3 and above will be collected and recorded in the AE eCRF.

Participants that experience any of the following should permanently discontinue aspirin immediately:

- aspirin-related severe toxicity (defined as ≥grade 3 CTCAE v5)
- significant gastrointestinal bleeding (grade 3 and 4)
- active gastrointestinal ulceration
- new cases or worsening tinnitus (grade 2 or above)
- intracranial bleeding
- hypersensitivity to aspirin.

Sub-trial
All Adverse Events will be collected from first dose up to visit 6 (54 weeks), and recorded in the AE section of the eCRF.

10.1.2 Exemptions to reporting

The following adverse events, in the context of this trial, do not require expedited reporting on Serious Adverse Event (SAE) forms, unless they result in death.

- Elective medical or surgical procedures or social admissions
- Pre-existing disease or a condition present that was diagnosed before trial treatment that does not worsen

10.2 Recording and reporting SAEs, SARs AND SUSARs

All SAEs, except those defined as exempt above, and SUSARs occurring from first administration of trial drug up to the routine visit post-surveillance colonoscopy (visit 10 for the main trial; and visit 6 for the resveratrol sub-trial) or 14 days post cessation of trial treatment where this occurs earlier than protocolised, are subject to expedited reporting. This should be conducted in accordance with the Sponsor SOP for reporting SAEs and on the COLO-PREVENT SAE Report Form. Completed forms should be emailed to the Sponsor (rgosponsor@leicester.ac.uk) and Leicester CTU (coloprevent@leicester.ac.uk) immediately and within **24 hours** of the research staff becoming aware of the event. All SAEs will be reviewed and tracked by the Sponsor (or their delegate) until resolution. Monthly line listings of all SAEs are reviewed by the Director of Research and Development on a monthly basis. Please note: the 14 day safety monitoring period following last dose is greater than 5X half-lives of all trial drugs.

For each **SAEs / SUSARs** the following information will be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator
- whether the event would be considered expected or unexpected.

Any change of condition or other follow up information should be emailed to Sponsor and Leicester CTU as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

The causality and expectedness of the SAE should be assessed by the Investigator(s) or another delegated medic on the SAE form.

For participants on the main trial, section 4.8 of the SmPC for aspirin and metformin will be used to assess expectedness of events (known as the reference safety information).

For participants on the resveratrol sub-trial, section 8.0 of the IB for resveratrol will be used to assess expectedness of events (known as the reference safety information).

Any SAE assigned by the Investigator or delegate as being related to the allocated treatment and unexpected according to the approved reference safety information will be classified as a "SUSAR" and is subject to expedited reporting to the Competent Authority (the MHRA) and the REC. The Sponsor or delegate will liaise with the Principal Investigator and Chief Investigator to ensure that the relevant eSUSAR report is completed and submitted to the Competent Authority (the MHRA) within the required timeframes. The Sponsor will then submit the eSUSAR report and safety report to the REC. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. Follow up reports must be provided in a timely manner and as per the request of the Sponsor or delegate.

10.3 Responsibilities

Principal Investigator (PI) (or delegate):

1. Checking for AEs and ARs at every visit following first administration of IMP
2. Using medical judgement in assigning seriousness, causality and expectedness using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs and SARs (including SUSARs) are recorded and reported to the Sponsor and Leicester CTU immediately and within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
4. Ensuring that AEs and ARs are recorded and reported to the Leicester CTU in line with the requirements of the protocol.

Chief Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning seriousness, causality and expectedness of SAEs where it has not been possible to obtain local medical assessment.
3. Immediate review of all SUSARs.
4. Review of specific SAEs and SARs in accordance with the trial risk assessment and protocol.
5. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Sponsor (or delegate):

1. Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol.
2. Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit.
3. Reporting safety information to the independent oversight committees identified for the trial (Data Monitoring Committee (DMC) and / or Trial Steering Committee (TSC)).
4. Expedited reporting of SUSARs to the Competent Authority (MHRA in UK) and REC within required timelines.
5. Notifying Investigators of SUSARs that occur within the trial.
6. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
7. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the MHRA and REC.

Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data and liaising with the DMC regarding safety issues.

Data Safety & Monitoring Committee (DMC):

In accordance with the Trial Terms of Reference for the DMC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

10.4 Notification of deaths

Deaths will be reported to Leicester CTU and the Sponsor, where applicable in accordance with the SAE requirements described previously, and within 24 hours of the investigator becoming aware. A death notification CRF will be completed for all participants that die following randomisation up to the final visit.

10.5 Pregnancy reporting

Guidelines around pregnancy are detailed in section 8.10.

All pregnancies within the trial (either the trial participant or the participant's partner) should be reported to the Chief Investigator, the LCTU and the Sponsor using the relevant Pregnancy Reporting Form within 24 hours of site awareness.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE.

The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child in accordance with Sponsor and local reporting

requirements and timelines. The relevant pregnancy PIS will be provided to the participant or partner and consent to follow up the pregnancy will be obtained.

Any SAE occurring in association with a pregnancy, brought to the investigator's attention after the participant has completed the trial, and considered by the investigator as possibly related to the trial treatment, must be promptly reported to sponsor in accordance with Sponsor and local reporting requirements and timelines.

10.6 Overdose

The Sponsor and Leicester CTU should be informed about any overdoses of trial drug and the appropriate adverse event reporting should be followed.

Aspirin overdose
Should an aspirin overdose be suspected, the trial drug should be withheld and local guidance and pathways should be followed.

Metformin overdose
Participants should be urgently assessed in the event of an overdose and hospital admission considered. The management of metformin overdoses should be as per standard clinical care by the local team. The most effective way to remove lactate and metformin is haemodialysis.

Resveratrol/placebo
The investigator should use clinical judgement in treating the symptoms of a suspected overdose.

10.7 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.

Please refer to the following website for details on clinical trials safety reporting: http://www.mhra.gov.uk/Howweregulate/Medicines/Licensingofmedicines/Clinicaltrials/Safetyreporting-SUSARSandASRs/index.htm

10.8 The type and duration of the follow-up of participants after adverse reactions

Following an adverse reaction (AR) the participants will be followed-up for an appropriate length of time as dictated by the nature of AR and their clinical needs. ARs will continue to be recorded and reported for up to 14 days after the last dose of IMP has been administered. Any SUSAR will need to be reported to the Sponsor and Leicester CTU irrespective of how long after IMP administration the reaction has occurred until resolved.

10.9 Development safety update reports

Within 60 days following the anniversary of the authorisation date for the trial, a Development Safety Update Reports (DSURs) will be sent by the Chief Investigator to the MHRA and the Main Research Ethics Committee. A copy of the report will also be sent to the trial Sponsor. The LCTU will prepare the DSUR report on behalf of the CI and submit to the Sponsor who retain responsibility for submitting to the Competent Authority (MHRA) within the specified time frame.

11. STATISTICS AND DATA ANALYSIS

11.1 Sample size calculation

For all sample size calculations we have assumed a negative binomial distribution (12, 90), with over-dispersion of 2.0 and used equation from Cundill & Alexander (91). Replication of the sample size found was done using the NCSS PASS 16.1 software. We have designed COLO-PREVENT with recent 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 has changed such that it is estimated the new high risk group is composed of individuals that previously fell under this category, plus a proportion (~50%) of the patients previously classed as intermediate risk who used to have their first surveillance colonoscopy at 3 years. A consequence of these changes is that real data do not yet exist on MPP 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 MPP as compared to aspirin alone. Data from the aspirin arms of the SeAFOod trial (12) and 1st surveillance in the BCSP (2006 -2016, 1 year MPP for high risk patients and 3 year MPP for intermediate risk patients) have been provided (M Rutter, personal communication). Assuming a MPP at 3 years in the untreated population of 1.2 polyps, this leads to a control MPP of 0.94 in the standard of care arm, after accounting for a 22% reduction in total MPP due to aspirin use, based on the SeAFOod data (12). This trial is powered to detect a reduction of 30% (0.66 vs 0.94) in the MPP 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 minimisation for BCSP Centre. We have predicted the dropout rate based on the 10% value reported for the one-year seAFOod trial (12) and have increased this figure to 15% due to the longer intervention period in COLOPREVENT; 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 trial 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 MPP at 1 year from the aspirin groups in seAFOod and the untreated population from the BCSP (2006 -2016) data, and estimate the MPP in the control arm for this trial to be 1.2. As this is a signal-seeking trial, we will aim for a 1-sided 10% significance level and will not adjust for multiple testing due to the two active treatment arms. We aim for 80% power to ensure a high probability of detecting a Signal-Seeking reduction in MPP for this group of 30%, which will lead to a MPP of 0.84. We are aiming to recruit 477 patients (159 per arm) which includes a 10% drop out at 1 year. Therefore 477 individuals will be randomised equally (1:1:1 ratio) to the three treatment arms with minimisation 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 Signal Seeking sub-trial. Note that the overall predicted MPP value for the new HR group in the BCSP could be underestimated as we are using the one-year MPP for the previous high risk patients for three year MPP predictions, since data do not currently exist for recurrence at three years in this population. Furthermore, when factoring in the contribution from the previous intermediate-risk patients we are using the average three-year MPP 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 fully implemented, 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 MPP value compares with our predicted value and make any adjustments to our sample sizes; the findings will be made available to the DMC.

The effect on clinical outcomes in the long term is important and all participants will be consented for additional follow-up (10 years) and clinical events will be ascertained through the BCSS, NCRAS, and other appropriate datasets.

11.2 Planned recruitment rate

We plan to exploit the legacy of the seAFOod trial utilising the infrastructure set up within the BCSP. We have been able to review the recruitment data from the trial to robustly confirm the feasibility of recruitment into COLO-PREVENT. Moreover, we have planned for an increased number of trial sites and with the efficient trial design can recruit patients into the COLOPREVENT sub-trial 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. 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 the COLO-PREVENT sub-trial. 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.

As explained in Section 11.1 above, 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. As 'real world' data become available for this new high risk group, we will conduct a formal review (at ~48 months) of adenoma recurrence (MPP) for control patients within the BCSP and compare to our predicted values. The findings from this review will be made available to the DMC.

11.3 Statistical analysis plan

Statistical Analysis: All statistical analyses will be carried out according to a detailed Statistical Analysis Plan (SAP), to be agreed by trial statisticians, the CI, and the TSC. The analysis will primarily follow an intention-to-treat (ITT) approach to the analysis i.e. participants will be analysed in the groups to which they were randomised and not treatment received. Additional analyses to evaluate the extent to which following the protocol is important will be pre-specified in the SAP e.g. per protocol and complier-average causal effects analyses.

11.3.1 Summary of baseline data and flow of patients

The flow of the patients in the trial will be described according to CONSORT guidelines for RCTs (92). The baseline characteristics will be summarised by randomised group without formal statistical comparison.

11.3.2 Primary outcome analysis

The primary outcome will be analysed using a generalised linear model to estimate incident rate ratios between randomised groups, assuming a negative binomial distribution, MPP 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.

11.3.3 Secondary outcome analysis

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 polyp detection rate, polyp subtype and location. The relative recurrence of 'advanced' polyp 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 polyps 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.

11.3.4 Biomarker analysis

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 (MPP) of the interventions, which may have potential as pharmacodynamic biomarkers that can be monitored over time as surrogates for efficacy.

A separate SAP will detail the analysis to be performed. All the proposed biomarker work is exploratory and therefore no sample size estimates have been given. 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 polyp number per person (MPP).

The prognostic and predictive ability of each of the biomarkers will be explored univariately on the primary outcome measure (MPP), 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 MPP 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 MPP 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.

11.4 Subgroup analyses

There are currently no planned subgroup analysis.

11.5 Adjusted analysis

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.

11.6 Interim analysis and criteria for the premature termination of the trial

There is no planned interim analysis for the main trial. The result of the main trial will be analysed once the last patient has completed the 3 year post-surveillance colonoscopy (last visit).

For the Signal-Seeking sub-trial we will review the polyp rate (MPP) in the placebo control arm 12 months after the first 100 patients have been recruited to this arm, to assess whether the control polyp rate is in line with our assumption of 1.2, which was used to calculate the sample size. Should the placebo control MPP value be higher than estimated the sample size will be re-calculated to see if the trial could complete recruitment earlier than planned. However should the current sample size estimation be an overestimate for the control arm polyp rate, then options will be explored for expanding the sample size or introducing a stop/go decision based upon futility at that stage.

Ultimately, the final results from sub-trial will be used to make a 'stop/go' decision for each dose of resveratrol and the plan would be to then transfer the most effective dose to the main trial within the COLO-PREVENT platform, subject to securing additional future funding.

11.7 Procedure(s) to account for missing or spurious data

The impact of missing data will be explored through the use of multiple imputation.

12. DATA MANAGEMENT

LCTU will be responsible for Data Management for the trial and will undertake data validation, database queries/reviews in line with their SOPs.

12.1 Data collection tools and source document identification

Source Data is defined as the first place data is recorded, this will include:

- Medical Records
- Paper CRFs
- Laboratory Reports
- Printouts from equipment
- Participant reported outcome questionnaires

Data collection tools will comprise of:

- Macro Database (transcribed from CRFs) and direct source data entry
- Participant reported outcome questionnaires

The trial researchers will seek consent from participants to re-contact them about taking part in future ethically approved research. Participants will also be asked for permission to collect long term data up to 10 years after the end of this trial, from routinely-collected healthcare databases including the Bowel Cancer Screening System, the National Cancer Registration and Analysis Service (NCRAS), and other organisations. This is outlined in the PIS and consent form and participants will be able to opt out without affecting their involvement in the trial.

12.2 Data handling and record keeping

Records of trial participant data will be made on trial specific electronic CRFs. Trained member(s) of the site research team will enter data directly into a commercially available web based Clinical Data Management System (CDMS) provided by the LCTU (MACRO). On-entry validation checks will be applied where required and data entered will be checked for completeness, accuracy and timeliness by the site research team/trial manager/trial coordinator/data manager, with queries managed using the data clarification functionality within the CDMS system.

A copy of the patient consent form and information sheet will be placed in the hospital notes of all participants and original copies in the Investigator Site File. A sticker will be placed on the cover of the notes (or inside cover) detailing the trial title, contact details of the PI and the fact that the notes should not be destroyed for 25 years from the end of the trial. All trial visits and AEs/SAEs will be recorded in the hospital notes. Where electronic or hybrid medical notes are used it is expected that electronic flags, scanned documents and annotation are included in the medical notes.

During the trial any paper CRFs and source data documentation will be stored in a secure area accessible to trial site and sponsor staff. Each enrolled participant will be allocated a unique trial ID so that the CRFs and electronic database remains pseudonymised.

According to the ICH guidelines for Good Clinical Practice, the monitoring team must check the CRF entries against the source documents, except for the pre-identified source data directly recorded in the CRF. The informed consent form will include a statement by which the patient allows the Sponsor and LCTU's duly authorised personnel, the Ethics Committee, and the regulatory authorities to have direct access to original medical records which support the data on the CRFs (e.g., participant's medical file, appointment books, original laboratory records, etc.). These personnel must maintain the confidentiality of all personal identity or personal medical information (according to confidentiality and personal data protection rules).

A Data Management Plan will be created with specific details on data handling and record keeping.

12.3 Access to Data

All trial documentation containing identifiable patient data will be managed in accordance with ICH GCP, the UK Policy Framework for Health and Social Care, the Data Protection Act and the EU General Data Protection Regulation (GDPR).

Information will only be obtained from the participant if necessary for the trial. The site research team will use the participant's name, NHS number and contact details, to contact them about participating in the trial and to make sure that relevant information about the trial is recorded, to ensure patient care and for quality assurance purposes. Individuals from the Sponsor, the LCTU, and regulatory organisations may look at participants' medical and research records to check the accuracy of the research trial but the identifiable data will not leave the site. The only people in the Sponsor organisation and LCTU who will have access to identifiable information will be those requiring it for trial purposes or for

audit of the data collection process. The trial statistician only involved in analysis will not have access to identifiable information.

The trial team will keep participant contact information for 10 years after the trial has finished where participants have consented to being contacted about future research. Consent forms and details of record linkage (i.e., trial ID numbers/pseudonyms) will be archived for 25 years as part of the research data so that in the event of the data being challenged, this will allow for verification of the quality of the data.

Explicit (optional) consent will be sought to store identifiable contact details in order to invite participants to take part in future research. Participant name and contact details will be stored on a separate secure database by the University of Leicester (and its clinical trials unit, the Leicester Clinical Trials Unit) for up to 10 years after the end of this trial.

For the purposes of this clinical trial, the Data Controller will be the Sponsor.

12.4 Archiving

Personal identifiable data generated by the trial will be retained for 10 years following the notification of the end of the trial before being destroyed in a confidential manner.

Following completion of the trial data analysis, data and essential trial records, including the final trial report, will be archived in a secure location, for 25 years after the completion of the trial, in accordance with EU regulations. No trial-related records, including hospital medical notes, will be destroyed unless or until the Sponsor gives authorisation to do so.

13. MONITORING, AUDIT AND INSPECTION

The University of Leicester, as Sponsor, operates a risk-based monitoring and audit programme, to which this trial will be subject. The LCTU operates a risk-based Quality Management System which will apply to this trial with Quality Checks and Quality Assurance Audits performed as required.

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

As part of the quality management process, the trial will be subject to a risk assessment and a monitoring plan will be developed by the Sponsor in accordance with the level of risk identified to participant safety, integrity of the trial and trial data validity. All trial monitoring will be conducted in accordance with the monitoring plan and will be undertaken by the trial Sponsor. All monitoring will be performed by staff who are ICH GCP trained and are competent in monitoring to all applicable regulatory guidelines. A documented monitoring log and audit trail will be maintained throughout the lifetime of the trial. The trial may also be subject to audit by the Sponsor delegate.

The trial manager will also undertake quality checks and assurance audits to ensure compliance with protocol, ICH GCP, and regulatory requirements.

All source data, trial documents, and participant notes will be made available for monitoring, audits and inspections by the Sponsor (or their delegate), NHS Host Organisation, and the regulatory authorities.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Research Ethics Committee (REC) review & reports

Before the start of the trial, approval will be sought from a REC for the trial protocol, informed consent forms and other relevant documents e.g. advertisements. Any substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion for the trial.

All correspondence with the REC will be retained in the Trial Master File and an annual progress report (APR) will be submitted to the REC by or on behalf of the CI within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The CI will be responsible for informing the REC of the end of trial. After completion of the trial, CI will submit a final report with the results to the REC.

14.2 Peer review

This trial is funded by Cancer Research UK and has been subject to full peer review as part of the standard grant review process under the remit of the Clinical Committee. Prior to submission of the application to Cancer Research UK, the trial concept was presented to several national groups, including the NCRI Colorectal Screening Prevention and Early Diagnosis Advisory Group, the UK Therapeutic Cancer Prevention Network, the NCRI Colorectal Cancer CSG and was also discussed at the CRUK Prevention Trials Unit Bowel Cancer Workshop (Barts, QMUL). All groups were supportive and provided constructive feedback that was used to refine the trial design.

14.3 Public and Patient Involvement (PPI)

Patient and public representatives have contributed to the design of the research (including patient facing documentation) and will be involved in the management of the research and dissemination of findings. We have three PPI members fully engaged and contributing to the project who have been involved in COLO-PREVENT since the very first meeting when the concept was proposed several years ago. 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, the PPI group 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.

14.4 Regulatory compliance

The trial will not commence until approval has been received from the REC/MHRA Combined Review process. The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments, and be conducted in accordance with the UK Policy Framework for Health and Social Care Research (2017). The Sponsor responsible for checking research governance arrangements will be the University of Leicester and Sponsor Green Light will be issued for each participating NHS Trust prior to any trial related activity taking place.

14.5 Protocol compliance

The LCTU trial team will monitor and review protocol compliance, deviations from the protocol will be captured both within the source data and the LCTU CDMS (MACRO). Where deviations frequently reoccur this may meet the criteria for a Serious Breach of GCP and will be reported in line with Section 14.6 below. The research team will not be required to report protocol deviations in instances where a participant is unable to produce a sample. Protocol deviations are not required for trial assessments omitted during a pandemic, or where the visit window might be impacted as a result.

14.6 Notification of Serious Breaches to GCP and/or the protocol

Any serious breach (a breach which is likely to effect to a significant degree the safety or physical or mental integrity of the participants of the trial; or the scientific value of the trial) will be reported to Sponsor immediately and within 24 hours of discovery. Sponsor will be responsible for the onward notification to the MHRA within 7 days.

14.7 Data protection and patient confidentiality

All investigators and trial site staff will comply with the requirements of relevant legislation with regards to the collection, storage, processing and disclosure of personal information.

The personal information that is collected will be kept secure and maintained by:

- The creation of a unique trial ID number, depersonalised data where the participant's identifying information is replaced by an unrelated sequence of characters;
- Secure maintenance of the data, in both electronic and paper forms and the linking code in separate locations;
- Limiting access to the minimum number of individuals necessary for quality control, audit, and analysis;
- Paper based pseudonymised trial records will be stored in locked filing cabinets within a locked office. Electronic records will be stored on secure NHS servers at site;
- Where a participant has provided consent for their contact details to be held on a central database (in order to invite them to participate in future research) this information will be held securely on University of Leicester server systems;
- The trial database(s) will be password protected and only researchers collecting data will be provided access to enter data for their site. All data collected during the trial will be stored pseudonymously;
- At site participants' contact details should be held separate to the trial visit data and used to arrange data collection visits by the research team or direct care team.

Participants will be followed-up in the long-term through routinely collected healthcare databases provided by the Bowel Cancer Screening System (BCSS), National Cancer Registration and Analysis Service (NCRAS) and other organisations. Participants will be asked to consent to this separately and can still participate in the trial if they do not give their consent to this aspect of the trial.

Any data transmitted will be done securely in approved University of Leicester methods (i.e. encrypted file transfer, internal email system) in accordance with LCTU SOPs.

14.8 Financial and other competing interests for the Chief Investigator, PIs at each site and committee members for the overall trial management

All real and perceived conflicts will be recorded and reported to the TSC. Members of the TSC and DMCs will be required to sign a declaration of conflicts of interest forms which will be retained in the TMF.

14.9 Indemnity

Sponsorship and insurance for the trial design and management will be provided by the University of Leicester.

If a participant is harmed due to negligence, this would be covered by the local NHS Trust(s) indemnity arrangements for all participants in clinical trials. If a trial participant wishes to make a complaint about any aspects of the way they have been treated or

approached during the research project, the standard National Health Service complaint system will be available to them, the contact details for which are in the participant information sheet.

14.10 Amendments

Amendments will be submitted by or on behalf of the CI after approval by the Sponsor and will be implemented following all required ethical, competent authority and Sponsor approvals.

14.11 Post-trial care

After trial completion participants will continue in the BSCP and will be discharged back to the care of their GP. They will not be followed up by the site research team, apart from in the case of an adverse reaction to IMP or an unresolved SAE.

Incidental findings from routine assessments (e.g. abnormally elevated LFTs) will be referred to the participant's GP or suitable clinician for follow up. Incidental findings from non-routine/exploratory assessments, particularly those analysed after the completion of the trial, will not be referred for investigation.

14.12 Access to the final trial dataset

CI and her appointed deputies will have access to the analysed trial dataset following execution of the SAP and completion of the End of Trial Report.

15. DISSEMINATION POLICY

15.1 Dissemination policy

The results of the trial will be reported first to trial collaborators. The main report will be drafted by the Leicester CTU, and the final version will be agreed by the Trial Steering Committee before submission for publication, on behalf of the collaboration.

The success of the trial depends on the collaboration of doctors, nurses and researchers from across the UK. Equal credit will be given to those who have wholeheartedly collaborated in the trial.

The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).

All publications and presentations relating to the trial will be authorised by the Trial Management Group. Authorship will be determined according to the internationally agreed criteria for authorship (www.icmje.org).

A lay summary of the results will be written by the PPI representatives and made available on: www.coloprevent.co.uk. Here, trial participants will also be directed towards the full scientific results of the study.

15.2 Authorship eligibility guidelines and any intended use of professional writers

Authorship will be determined in line with the International Committee of Medical Journal Editors.

16. APPENDICES

16.1 Appendix I – Medicines not permitted during trial treatment in the Main Trial

The following lists are not exhaustive and clinical judgement should be exercised. For further information please refer to the British National Formulary or local equivalent.

Anti-coagulants:	Warfarin Acenocoumarol Phenindione Dabigatran Unfractionated Heparin Low molecular weight heparin[1] Rivaroxaban Apixaban Argatroban Edoxaban	Anti-platelets:	Clopidogrel Dipyridamole Prasugrel Ticagrelor Abciximab Tirofiban Eptifibatide Epoprostenol Fondaparinaux
LONG-TERM NSAIDS:[2]	Ibuprofen Naproxen Diclofenac Acelofenac Fenoprofen Flurbiprofen Ketoprofen Dexketoprofen Tiaprofenic acid Etodolac Indomethacin Meloxicam Tenoxicam Nabumetone Phenylbutazone Ketorolac Piroxican Sulindac Tolfenamic acid Celecoxib Etoricoxib	Aspirin: (including over the counter)	Nu-seals Anadin Beechams powders Alka-seltzer Disprin Codis 500
		Others:	Methotrexate Long-term corticosteroids[3] (e.g. dexamethasone, prednisolone, hydrocortisone)

1. Low molecular weight heparin at a prophylactic dose for inpatient thromboembolism is permitted.

2. Non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided wherever possible but short term intermittent NSAID use is allowed. NSAIDs should not be co-administered with the trial treatment for more than 2 consecutive weeks). Paracetamol can be considered as an alternative analgesic and is permitted within the trial.

3. Short term intermittent systemic corticosteroids are permitted (and are likely to prescribed alongside chemotherapy) however longer term use (longer than 2 continuous weeks) is not permitted.

16.2 Appendix II - Background supporting evidence

Table 3 - Summary of updated analyses for effects of aspirin on CRC.

CI= confidence interval; HR = hazard ratio; OR = odds ratio; PLCO=prostate, lung, colorectal cancer screening trial; RR = relative risk.

Author, date, PMID	**Article type**	**Nº of studies; Cohort name; Nº cases;**	**HR, RR or OR (95% CI)**	**Aspirin dose**	**Dose- or follow up duration**
Bosetti, 2020 PMID: 32272209	Meta-analysis	45; N/A; 256,019 participants	RR for CRC=0.73 (0.69-0.78); p=<0.001	75-100 mg/day gives 10% reduction 325 mg/day gives 35% reduction	5 years; 20% risk reduction 10 years; 30% risk reduction
Bowman, 2018 PMID: 29653635	RCT	1; ASCEND; 15,480 participants	No change to CRC incidence	100 mg/day	7.4 years follow up
Burn, 2020 PMID: 32534647	RCT	1; CAPP2; 957 participants, 98 incident cases	HR for CRC=0.56 (0.34-0.91), p=0.019	600 mg/day	25 months dose duration. >7 year follow up
Chudy-Onwugaje, 2021 PMID: 33974712	Cohort analysis	1; PLCO; 154,952 participants, distal adenoma=1221 Recurrent adenoma=862 CRC incidence =2826	OR for recurrent adenoma=0.56 (0.36-0.87), p=0.006 HR for CRC incidence=0.88 (0.81-0.96), p=<0.0001	Compared >30 pills/month vs <4/month	13 year follow up
Elwood, 2021 PMID: 34567243	Systematic review and meta-analysis of observational studies	24; N/A; case numbers not stated	OR for CRC mortality (1 study)=0.78 (0.66-0.93)	Not stated	Not stated

Author, date, PMID	Article type	Nº of studies; Cohort name; Nº cases;	HR, RR or OR (95% CI)	Aspirin dose	Dose- or follow up duration
			HR for CRC mortality=0.72 (0.63-0.82)		
Figueiredo, 2021 PMID: 33528005	Cohort analysis	1; Cancer Prevention Study II Nutrition Cohort; 2686 incident cases	HR for CRC mortality (pre-diagnosis use) = 0.69 (0.52-0.92) HR for CRC mortality (post diagnosis use) = 0.60 (0.36-0.98) OR for distant metastasis (pre diagnosis use) = 0.73 (0.53-0.99)	>15 pills /month;	Not stated
Ghaddaf, 2021 PMID: 33682036	Meta-analysis of RCTs	11; N/A; 66,979 participants	RR for colorectal adenoma at 3 yrs=0.84, p=<0.05 RR for advanced lesion at 5 yrs=0.68, p=<0.05	Not stated	10 months – 10 years dose duration
Hull, 2018 PMID: 30466866	RCT	1; seAFood; 709 participants,	RR for all colorectal adenomas=0.78 (0.68-0.90)	300 mg/day	348 day dose duration
Hurwitz, 2021 PMID: 33104910	Pooled cohort analysis	2; NIH-AARP diet and health study (1995-2011); PLCO (1993-2009); 6902 incident cases	HR for CRC incidence= 0.85 (0.80-0.89)	Daily aspirin use	16 years dose duration

Author, date, PMID	Article type	Nº of studies; Cohort name; Nº cases;	HR, RR or OR (95% CI)	Aspirin dose	Dose- or follow up duration
Loomans-Kropp, 2019 PMID: 31800071	Cohort analysis (older adults 65+)	1; PLCO (1993-2008); 146,152 participants, 814 CRC deaths	HR for CRC mortality=0.62 (0.46-0.85), p=0.003	Aspirin use >1 times/week	Consistent use from baseline through follow up (13 years)
Ma, 2021 PMID: 33594505	Meta-analysis of RCTs	16; N/A	RR for CRC incidence=0.97 (0.84-1.12), P=0.66 RR for recurrence of adenoma=0.83 (0.72-0.95), P=0.006 RR for CRC mortality=0.79 (0.64-0.97), P=0.02	50-500 mg/day	RR for mortality with aspirin dose duration of <7 years = 0.69 (0.50-0.94)
McNeil, 2021 PMID: 32778876	RCT	1; ASPREE; 19,114 (>70 years old)	HR for all cancer incidence = 1.04 (0.95-1.14) HR for incident cancer that had metastasised = 1.19 (1.00-1.43)	100 mg/day	Median of 4.7 year follow up
Tsoi, 2018 PMID: 29665624	Cohort study	1: Hong Kong cohort; 612,509 (65%> 65 years old)	HR for CRC mortality =0.59 (0.56-0.52)	Median dose = 80 mg/day for at least 6 months	14 years

Table 4 - Shows some of the key latest updates for aspirin in terms of bleeding risk.

ICH = intracranial haemorrhage; GIB = gastrointestinal bleeding; H_2B = H2 blocker; HR = hazard ratio; PPI = proton pump inhibitor; RCT = randomised control trial; RR = relative risk

Author, date, PMID	**Study type**	**Nº in study**	**Dose**	**HR, OR, RR (95% CI)**	**Other observations**	**Conclusions**
Abdelaziz, 2019 PMID: 31196447	Systematic review of 15 RCTs	65,502	75-500 mg/day (12 studies 100 mg or less)	RR for major bleeding =1.5 (1.33-1.69) RR for ICH =1.32 (1.12-1.55) RR for GIB =1.52 (1.34-1.74)	No difference in fatal bleed rates between aspirin and control	Aspirin increases non-fatal bleeding rates
Bowman, 2018 PMID: 29653635	RCT (ASCEND)	15,480	100 mg/day	Rate ratio for serious bleeding events of 1.29 (1.09- 1.52), p=0.003	No change to GI cancer risk	Aspirin prevented serious vascular events in people with diabetes
Bouget, 2020 PMID: 32764775	Cohort study	69,911	Low dose =<100 mg/day High dose =100-325 mg/day	HR for ICH high vs low dose =1.80 (1.10-2.95)	Major bleeding also twice as likely with dual anti-platelet therapy	Low incidence of major bleeding and mortality. Low dose aspirin was safest option
Gaziano, 2018 PMID: 30158069	RCT (ARRIVE)	12,546	100 mg/day	HR for GIB =2·11 (1·36–3·28), p=0·0007	GIB were mostly mild	The overall incidence of treatment-related adverse events was low (16·75%) vs (13·54%) in the placebo group

Author, date, PMID	Study type	Nº in study	Dose	HR, OR, RR (95% CI)	Other observations	Conclusions
Haykal, 2019 PMID: 31098750	Systematic review of 16 RCTs	104,018	75-1200 mg/day	RR for any bleed = 1.63 (1.31-3.03), p=<0.01 RR for major bleed = 1.41 (1.26-1.57), p=<0.01 RR for GIB = 1.85 (1.38-2.48), p=<0.01	No reduction in cancer-related incidence or mortality	Increased risk of bleeding compared to placebo
Jones, 2021 PMID: 33999548	RCT (ADAPTABLE)	15,076	81 mg/day (low dose) 325 mg/day (high dose)	HR for hospitalisation due to major bleeding = 1.18 (0.79-1.77)	Patients on high dose had high incidence of dose switching and fewer days on assigned dose	No significant difference in cardiovascular or major bleeding events between dose levels
Trolsen, 2020 PMID: 32719046	Cohort study	1,648,762	75-150 mg/day (defined as low dose)	RR for GIB = 2.79 (2.4-3,24)	Increase in endoscopies due to increased GIB	Decrease in CRC risk possibly due to increased pre-malignant polyp bleeds resulting in increased endoscopy uptake and polyp removal
Tsoi, 2018 PMID: 29665624	Cohort study	612,509	Median 80 mg/day	RR for GIB-related mortality = 1.09 (1.00-1.19)	PPI and H_2B use decreased OR for GIB-related mortality from 1.74 (1.34-2.26) to 0.80 (0.70-0.91)	Long term aspirin use decreases CRC incidence and mortality, with bleed risks mitigated by

Author, date, PMID	Study type	Nº in study	Dose	HR, OR, RR (95% CI)	Other observations	Conclusions
						use of anti-acid agents
Veronese, 2020 PMID: 32488906	Umbrella review of systematic reviews	67 meta-analyses;780,553 participants (in RCT group)	Not stated	From observational studies RR for GIB=2.28 (1.97-2.64) From RCTs RR for GIB=1.47 (1.26-1.72) and for ICH = 1.34 (1.8-1.53)	Low dose aspirin has lower comparative efficacy compared to other medications	Low dose aspirin showed strong evidence for lower risk of bleeding compared to other active medication

Table 5 - Studies showing effects of metformin use on colorectal adenoma incidence.

CI= confidence interval; FAP = familial adenomatous polyposis; HR = hazard ratio; OR = odds ratio; RR = relative risk; *T2DM = type 2 diabetes mellitus.*

Author, date, PMID	Article type	Nº of studies; Cohort name; Nº cases;	HR, RR or OR (95% CI)	Observations	Metformin dose	Dose- or follow up duration
Han, 2017 PMID: 28210856	Retrospective cohort analysis of T2DM patients	1 study; NA; 423	OR for colorectal adenoma recurrence = 0.434 (0.260-0.723), p=0.001	Metformin use decreases risk of adenoma recurrence	Not stated	Not stated
Higurashi, 2016 PMID: 26947328	RCT in non-diabetics undergoing polypectomy	1 study; not stated; 151	RR for total polyp recurrence = 0.67 (0.47-0.97) RR for adenoma recurrence =	Low dose was safe and well tolerated in non-diabetic patients	Placebo vs 250 mg/day	1 year

Author, date, PMID	Article type	Nº of studies; Cohort name; Nº cases;	HR, RR or OR (95% CI)	Observations	Metformin dose	Dose- or follow up duration
			0.60 (0.39-0.92)			
Hou, 2017 PMID: 27903961	Meta-analyses of T2DM patients	7 studies; NA; 7,178	OR for colorectal adenoma risk = 0.73 (0.58-0.90)	OR for advanced adenoma risk = 0.52 (0.38-0.72)	Not stated	Not stated
Jung, 2017 PMID: 28449338	Systematic review and meta-analysis	10 studies; NA; 8,726	OR for adenoma risk = 0.76 (0.63-0.92)	Reduced risk of colorectal adenoma in high risk populations	2 studies described doses of 990 mg and 250 mg	Duration not associated with risk reduction
Liu, 2017 PMID: 27926481	Systematic review and meta-analysis in T2DM patients	20 studies; NA; not stated	OR for adenoma risk = 0.80 (0.71-0.90), p=0.0002	Decreased risk for CRC also observed	Not stated	Not stated
Mansourian, 2018 PMID: 29383018	Traditional and Bayesian meta-analysis	11 studies; NA; not stated	OR for advanced adenoma = 0.51, p=<0.001 OR for total adenoma = 0.86, p=0.274 OR for adenoma recurrence = 0.89, p=0.137	Metformin had no protective effect on total and recurrent adenoma. Insulin increased risk of total adenoma	Not stated	Not stated
Marks, 2015 PMID: 26377195	Retrospective cohort study in T2DM patients	1 study; Northern	HR for adenoma recurrence in	Metformin decreased polyp recurrence risk	Total dose quartiles:	1 – 10 years

Author, date, PMID	Article type	Nº of studies; Cohort name; Nº cases;	HR, RR or OR (95% CI)	Observations	Metformin dose	Dose- or follow up duration
	with adenoma diagnosis	California; 2,412	Q1= 0.90 (0.72-1.12) HR for adenoma recurrence in Q2= 0.89 (0.70-1.12) HR for adenoma recurrence in Q3= 0.80 (0.63-1.01) HR for adenoma recurrence in Q4= 0.50 (0.42-0.60)	after polypectomy	Q1=50-399 mg Q2 =400-799 mg Q3 =800-1499 mg Q4 =>1500 mg	
Park, 2021 PMID: 33509804	RCT in FAP patients	1 study; PREVENTION RELEVANCE; 34	Not stated	No significant differences in colorectal and duodenal polyp number; significant decrease in mTOR expression in polyps	Placebo vs 500 mg/day vs 1500 mg/day	7 month dosing

Table 6 - Side effects for resveratrol vs placebo across all RCTs.

AE – adverse event; GI – gastrointestinal; PCOS – polycystic ovary syndrome; RSV – resveratrol; SAE – serious adverse event; T2DM – type 2 diabetes mellitus;

Author/date, PMID	**Participants**	**Dose/schedule**	**Nº of participants**	**Nº AEs**	**AE description**
Almeida, 2009 PMID: 19194969	Healthy volunteers	25, 50, 100,150 mg or placebo 6 times a day for 48h	8 on each dose	15 for RSV (9 possibly related); 3 for placebo	Mild AEs. Headache, myalgia, somnolence, epididymitis, dizziness and occipital headache.
Anton, 2014 PMID: 24866496	Healthy, overweight, older adults	150 mg, 500 mg or placebo twice daily for 90 days	14 on 300 mg, 13 on 1 g daily, 12 on placebo	17 at 300 mg/day; 12 at 1000 mg/day; 10 for placebo	Diarrhoea (5); Constipation (3); Muscle cramps/pain (4); Fatigue (4); Memory loss (4); Allergies/URI (3); Difficulty swallowing (2); Rash (2); Headache (3); Others1 (7)
Arzola-Paniagua, 2016 PMID: 27221771	Obese adults	100 mg RSV 3 times per day; orlistat alone; RSV + orlistat; placebo	40 on RSV alone; 40 on orlistat alone, 41 on the combination; 40 on placebo	Number of patients withdrawing due to AEs: 3 in orlistat + RSV group, 6 in RSV group, 2 in orlistat group, 4 in placebo group.	No differences in AEs between groups. No SAEs. Abdominal pain; Constipation; Diarrhoea; Nausea; Steatorrhoea
Banaszewska, 2016 PMID: 27754722	Individuals with PCOS	1500 mg daily for 3 months	17 on RSV; 17 on placebo	2 AEs for RSV.	Transient numbness of hands in 2 patients.

Author/date, PMID	Participants	Dose/schedule	Nº of participants	Nº AEs	AE description
Ding, 2017 PMID: 28993436	Women with pregnancy-induced pre-eclampsia	All patients received nifedipine plus either 50 mg RSV or placebo every 15 min until blood pressure was ≤150/100 mmHg (maximum of 250 mg resveratrol)	174 on RSV; 175 placebo group	23 in RSV group; 28 in placebo group.	No significant differences for maternal or neonatal AEs. RSV + nifedipine Nausea; Vomiting; Maternal tachycardia; Mild headache; Dizziness; Chest pain
Goh, 2014 PMID: 23918588	Male patients with T2DM	RSV or placebo at 500 mg starting dose. Dose increased by 500 mg/day every 3 days to a maximum dose of 3 g per day. Duration was 12 weeks	5 on RSV; 5 on placebo	4 AEs overall.	Proportion of patients with AEs did not significantly differ between groups. Mild elevation of ALT; Diarrhoea; Mild hypoglycemia which resolved spontaneously
Harper, 2021 PMID: 33068691	Older adults	500 mg RSV once or twice daily, or placebo + exercise regimen. Duration of 12 weeks	20 on 1000 mg RSV 20 on 500 mg RSV 20 on placebo	19 related or possibly related to RSV across the two dose groups	AE frequency and type were similar between all groups. Gastrointestinal issues; Musculoskeletal; Dizziness
Heeboll, 2016 PMID: 26784973	Patients with transaminasemia and suspected NAFLD	RSV or placebo 500 mg three times a day for 6 months	15 on RSV 13 on placebo	33 AEs with RSV 14 with placebo.	Number of patients reporting AEs similar between resveratrol (9/15) and

Author/date, PMID	Participants	Dose/schedule	Nº of participants	Nº AEs	AE description
					placebo group (6/13). Constipation; Diarrhea; Abdominal pain; Nausea; Heartburn; Flatulence; Less appetite; More appetite; less fatigue; more fatique; Miscellaneous; Bicytopen fever (SAE); Dizziness; Hot flushes;
Hendouei, 2019 PMID: 31714621	Children with autism spectrum disorder	RSV or placebo (250 mg) twice daily for 10 weeks. Both groups also had risperidone twice daily	35 on RSV 35 on placebo.	There were 48 AEs with RSV + resperidone	AEs similar between groups. Constipation; Abdominal pain; Restlessness; Headache; Drowsiness; Fatigue; Dry mouth; Increased appetite; Decreased appetite; Nausea; Diarrhoea
Howells, 2011 PMID: 21680702	Patients with colorectal liver metastases	5 g SRT501 microparticle resveratrol powder or placebo taken once daily for ~14 days	6 on RSV 3 on placebo	17 with RSV; 3 with placebo	Primarily GI and mild in grade. Anal pruritus; Diarrhoea; Nausea; Chills; Lethargy;

Author/date, PMID	Participants	Dose/schedule	Nº of participants	Nº AEs	AE description
					Peripheral neuropathy; Rash; Skin irritation; Flushing
Kantartzis, 2018 PMID: 29484808	Overweight and insulin resistant subjects	150 mg RSV or placebo once daily for 12 weeks	54 on resveratrol; 54 on placebo	73 with RSV; 43 with placebo	No safety issues with RSV. Numbers of participants having at least 1 AE were: 19/54 for placebo and 24/54 for RSV.
Lin, 2016 PMID: 26727506	Patients undergoing peritoneal dialysis	150 mg RSV, 450 mg RSV or placebo once daily for 12 weeks	24 on each dose of RSV; 24 on placebo	4 patients discontinued RSV due to AEs.	Diarrhea; Constipation; Muscle cramps/pain; Fatigue; Headache; Memory loss
Lokken, 2021 PMID: 33934389	Patients with mitochondrial myopathy	500 mg RSV or placebo twice daily for 8 weeks	10 (crossover)	Number not stated	AEs observed for both RSV and placebo were mild gastrointestinal symptoms including diarrhoea.
McDermott, 2017 PMID: 28403379	Older people with peripheral arterial disease	125 mg RSV, 500 mg RSV or placebo once daily for 6 months	21 on 125 mg, 23 on 500 mg, 22 on placebo	14 in 125 mg group; 27 in 500 mg group; 13 in placebo group.	Diarrhoea; Abdominal pain; Pruritic exanthem
Ornstrup, 2014 PMID: 25322274	Men with metabolic syndrome	75 mg RSV, 500 mg RSV, or placebo twice daily for 16 weeks	23 on 75 mg RSV; 25 on 500 mg RSV; 26 on placebo	7 in 500 mg group; 3 in 75 mg group; 4 in placebo group.	Gastrointestinal complaints were mild, and primarily in relation to increased frequency

Author/date, PMID	Participants	Dose/schedule	Nº of participants	Nº AEs	AE description
					and/or softer stool
Pollack, 2017 PMID: 28329397	Adults without T2DM	1.5 g RSV twice daily. Dose was reduced to 1 g twice daily due to GI side effects.	30 participants, crossover	3 AEs, 1 SAE	SAE of GI symptoms at 3 g/day. Subsequent dose de-escalation to 2g/day with no further side effects.
Poulsen, 2013 PMID: 23193181	Obese healthy male volunteers	500 mg RSV or placebo three times daily for 4 weeks	13 on resveratrol; 12 on placebo	4 in RSV and 4 in placebo.	RSV AEs were: flatulence, reflux, rash.
Rafeiy-Torghabeh, 2020 PMID: 32449130	Children with attention-deficit/hyperactivity disorder	250 mg RSV or placebo twice daily combined with methylphenidate for 8 weeks.	33 on RSVl; 30 on placebo.	38 (RSV + methylphenidate); 47 (placebo + methylphenidate).	There was no significant difference in side effects between groups. Headache; Insomnia; Drowsiness; Fatigue; Dry mouth; Decreased appetite; Nausea; Vomiting; Diarrhoea; Abdominal pain
Samaei, 2020 PMID: 33372679	Patients With Stable Schizophrenia	200 mg RSV or placebo for 8 weeks	26 on RSV; 26 on placebo	43 AEs across both groups.	All AEs were tolerable with mild to moderate severity, with no significant difference in frequency between

Author/date, PMID	Participants	Dose/schedule	Nº of participants	Nº AEs	AE description
					groups. AEs were: Headache, Constipation, Diarrhoea, Fatigue, Nausea, Increased appetite, Abdominal pain, Nervousness.
Sattarinezhad ,2019 PMID: 29983230	Patients with T2DM and newly confirmed albinuria	250 mg RSV or placebo twice daily for 90 days	32 on RSV; 32 on placebo	2AEs across both arms	Mild dyspepsia
ThaungZaw, 2021 PMID: 32900519	Postmenopausal women	75 mg RSV or placebo twice daily for 12 months, crossover.	125 for both RSV and placebo.	13 AEs for placebo and 6 for RSV. AEs were not necessarily related to resveratrol	Possibly related AEs: Itching, Menses, Exacerbation of gastric reflux
Turner, 2015 PMID: 26362286	Patients with mild to moderate dementia due to Alzheimers Disease	500 mg RSV or placebo once daily with a dose escalation by 500 mg increments every 13 weeks, ending with 1000 mg twice daily for 52 weeks	64 on RSV; 55 on placebo	355 on RSV; 302 on placebo. 19 SAEs on RSV; 17 SAEs on placebo.	No significant difference in AEs or SAEs between groups. Most common AEs were nausea and diarrhoea (42% of individuals on RSV vs 33% on placebo).

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