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Synopsis

Understanding mechanisms of colorectal cancer chemoprevention using seAFOod polyp prevention trial outcomes and its Biobank: STOP-ADENOMA synopsis

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Abstract

Background: The 2 × 2 factorial seAFOod trial demonstrated that aspirin and omega-3 polyunsaturated fatty acid eicosapentaenoic acid reduce colorectal polyp number (a biomarker of colorectal cancer risk) during colonoscopy surveillance in the Bowel Cancer Screening Programme. The lack of a risk and/or therapeutic response biomarker limits a precision medicine approach to maximise efficacy of these chemoprevention agents. The seAFOod trial collected a comprehensive biobank of blood, urine and tissue samples.

Objective: To use the seAFOod Trial biobank and post-trial Bowel Cancer Screening Programme colonoscopy outcomes to (1) identify a biomarker(s) of colorectal polyp risk and therapeutic response, and (2) investigate the mechanism(s) of colorectal cancer prevention, of aspirin and eicosapentaenoic acid.

Design: Laboratory analysis of biobank samples and retrospective analysis of colonoscopy outcomes linked to clinical trial data.

Setting: Randomised, double-blind, placebo-controlled trial and the English Bowel Cancer Screening Programme colonoscopy surveillance programme.

Participants: seAFOod trial participants, who provided informed consent for use of trial samples and post-trial colonoscopy data.

Interventions: Aspirin 300 mg and/or eicosapentaenoic acid 2000 mg (or respective placebos) daily for 12 months during the seAFOod trial.

Main outcome measures: (1) Polyp outcomes from Bowel Cancer Screening Programme colonoscopy performed during and after the seAFOod trial; (2) plasma, red blood cell, urine and rectal mucosal polyunsaturated fatty acid and lipid mediator levels; and (3) genetic polymorphisms relevant to the pharmacology and metabolism of aspirin and eicosapentaenoic acid.

Results: The post-trial polyp detection rate (the number of individuals with ≥ 1 colorectal polyp) after randomisation to placebo was 71.1% compared with 80.1% for individuals, who had received aspirin (odds ratio 1.13, 95% confidence interval 1.02 to 1.24). Several genetic polymorphisms modified the polyp prevention efficacy of aspirin; for example, polyp risk reduction in aspirin users compared with non-aspirin users was restricted to rs4837960 (*PTGS1*) common homozygotes (incidence rate ratio 0.69, 95% confidence interval 0.53 to 0.90) and eicosapentaenoic acid [the presence of at least one *fatty acid desaturase 2* Indel (rs66698963) insertion allele identified eicosapentaenoic acid users with a reduction in colorectal polyp number (incidence rate ratio 0.50, 95% confidence interval 0.28 to 0.90). A high baseline urinary 11-dehydro-thromboxane B_2 level predicted increased polyp number (incidence rate ratio

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2.26, 95% confidence interval 1.11 to 4.58). A low (quartile 1) on-treatment urinary 11-dehydro-thromboxane B_2 level predicted reduced colorectal polyp number compared with placebo (incidence rate ratio 0.34, 95% confidence interval 0.12 to 0.93) for aspirin and eicosapentaenoic acid treatment compared with high on-treatment urinary 11-dehydro-thromboxane B_2 values (incidence rate ratio 0.61, 95% confidence interval 0.34 to 1.11).
Limitations: The seAFOod trial was relatively small with under-representation according to sex and ethnicity.
Conclusions: STudy Of Prevention by Aspirin anD EPA; kNowledge Of Mechanism of Action has taken a precision medicine approach to colorectal cancer chemoprevention and has generated novel findings that are applicable to the optimal use of aspirin and eicosapentaenoic acid in a targeted manner. Increased polyp number in trial participants that received aspirin suggests higher neoplastic risk after aspirin cessation. Genetic polymorphisms modify the polyp prevention efficacy of aspirin and EPA. The level of urinary 11-dehydro-thromboxane B_2 predicts polyp risk prior to treatment and polyp risk reduction during aspirin treatment.
Future work: Validation of risk and therapeutic response biomarkers for eicosapentaenoic acid (e.g. *fatty acid desaturase* Indel genotype) and aspirin (e.g. urinary 11-dehydro-thromboxane B_2) for colorectal cancer prevention (and other non-communicable diseases) is required in other human cohorts.
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Introduction

A comprehensive understanding of the natural history and molecular pathogenesis of colorectal cancer (CRC), combined with detailed knowledge of risk factors for colorectal carcinogenesis, that has been gained over the last five decades has, to date, not translated into effective prevention of this malignancy, which is the fourth most common cancer in the UK amounting to approximately 44,000 new cases per year.[1]

The preventability estimate for CRC is 54% based on known modifiable risk factors such as obesity and diet (e.g. red and processed meat intake).[1] Moreover, it is recognised that early diagnosis of CRC (the basis for occult blood test-based population screening in the UK) improves cancer outcomes,[2] and that removal of pre-malignant precursor lesions (polyps) by endoscopic polypectomy reduces CRC incidence and mortality.[3]

However, only 10% of CRCs are diagnosed through the UK Bowel Cancer Screening Programmes (BCSPs). Moreover, 'interval' CRCs occur despite careful endoscopic screening and surveillance, both within and outside the BCSP, with the post-colonoscopy CRC (PCCRC) rate (usually defined as a CRC diagnosis within 3 years) ranging between 2% and 8% of all colonoscopies at which a CRC is detected.[4] Lifestyle interventions (avoidance of obesity, physical exercise and dietary change) have yet to have any significant prevention impact.

A complementary prevention strategy is chemoprevention (the use of drugs or nutritional agents) used in a primary (general population) prevention context, or alternatively in a secondary prevention setting targeted at individuals at high risk of subsequent CRC after endoscopic polypectomy or surgical resection of colorectal neoplasia.[5,6]

Testing colorectal cancer chemopreventive efficacy

Colorectal polyp number is an established biomarker of subsequent CRC risk.[6] The colorectal polyp is also a clinically important lesion in its own right, leading to polypectomy (which is not without bleeding and/or perforation risk), as well as more frequent colonoscopic surveillance (thus increasing the overall burden on endoscopy resources).[6]

Therefore, the 'polyp prevention trial' has become the trial methodology of choice to test chemopreventive efficacy against 'sporadic' (non-familial) CRC using reduction in colorectal polyp recurrence (measured as the percentage of individuals with any polyp, or number of colorectal polyps per participant) during colonoscopic surveillance as a surrogate end point for CRC risk.[6]

There is increasing recognition that the molecular pathogenesis of the early stages of colorectal carcinogenesis is not uniform.[7,8] There are two main endoscopic and histological subtypes of polyp that have malignant potential (the dysplastic adenomatous polyp and the serrated polyp (previously known as serrated adenoma, but changed to 'polyp' based on the absence of genuine dysplastic change in the majority of serrated lesions), which map to distinct molecular profiles [chromosomal instability driven by loss of function of the *Adenomatous Polyposis Coli* gene, and microsatellite instability (MSI) usually accompanied by a CpG island hypermethylation phenotype, respectively].[7] Adenomatous polyps (termed adenomas) are distributed along the colo-rectum in a way that mirrors the relative frequency of CRCs throughout the colorectum [incidence in the left colon and rectum greater than the right colon

(defined as proximal to the splenic flexure)]. However, serrated polyps are over-represented in the right colon and are believed to contribute disproportionately to PCCRCs, which are frequently right-sided and display MSI.[7,8]

The seAFOod polyp prevention trial

A recent systematic review of randomised, placebo-controlled polyp prevention trials identified multiple agents that have been tested for CRC chemopreventive activity including non-steroidal anti-inflammatory drugs [NSAIDs; including aspirin, non-aspirin NSAIDs and selective cyclooxygenase-2 (COX-2) inhibitors], antioxidants (e.g. selenium), vitamin D and other nutrients (e.g. folic acid).[6]

The seAFOod polyp prevention trial was a multicentre, randomised, double-blind, placebo-controlled, 2 × 2 factorial trial of the omega-3 polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA) 2 g daily and aspirin 300 mg daily, nested in the English BCSP.[9] Proof-of-concept for CRC chemoprevention by EPA has been provided by a previous randomised trial in familial adenomatous polyposis (FAP) patients.[10] Chemopreventive activity of aspirin was supported by a meta-analysis of four previous polyp prevention trials, which highlighted uncertainty about the optimal dose and target population for aspirin chemoprevention.[11]

In the seAFOod trial, 709 individuals, who had been deemed 'high risk' on the basis of colorectal polyp number and size (≥ 5 polyps, or ≥ 3 polyps, if one or more are ≥ 10 mm in size), were randomised to receive the 12-month intervention before scheduled BCSP surveillance colonoscopy.[9] The seAFOod polyp prevention trial did not show any significant effect of EPA or aspirin on the primary end point of the percentage of individuals with any colorectal polyp [previously termed the adenoma detection rate but now more accurately called the polyp detection rate (PDR), to include both adenomatous and serrated polyps] at surveillance colonoscopy.[9] However, the trial demonstrated that both EPA and aspirin did have chemopreventive efficacy based on the reduction in colorectal polyp number, which was a secondary outcome measure.[9]

There was also evidence of selectivity for both EPA and aspirin based on polyp type (adenomatous or serrated polyp) and location within the colorectum (left vs. right colon).[9] Aspirin use was associated with a reduction in mean total colorectal polyp number per participant evidenced by an incidence rate ratio (IRR) of 0.78 [95% confidence interval (CI) 0.68 to 0.90], with preventive efficacy against adenomas [IRR 0.82 (0.71 to 0.94)], serrated [IRR 0.46 (0.25 to 0.87)] and right-sided [IRR 0.73 (0.61 to 0.88)] lesions, but not left-sided [IRR 0.85 (0.69 to 1.06)] colorectal polyps.[9] There was evidence of more modest chemopreventive efficacy of EPA on adenomas [IRR 0.86 (0.74 to 0.99)] and left-sided [IRR 0.75 (0.60 to 0.94)] colorectal polyps, but not on total colorectal polyp number [IRR 0.91 (0.79 to 1.05)], serrated [IRR 1.44 (0.79 to 2.60)] or right-sided [IRR 1.02 (0.85 to 1.22)] colorectal polyps.[9]

Total colorectal polyp number was reduced in the group that received EPA and aspirin together compared with the other groups that received either a single agent or placebos only.[9] However, the 2 × 2 factorial trial was not powered to perform a formal 'inside the table' analysis of the four treatment arms.

The seAFOod Trial concluded that both EPA and aspirin have CRC chemoprevention efficacy, based on reduction of colorectal polyp number.[9] The larger effect size of aspirin added to the weight of evidence for its use in combination with endoscopic screening and surveillance, which currently provides suboptimal protection, particularly against right-sided CRC.[12] The modest effect of EPA on left-sided colorectal adenomas mirrored its activity in FAP patients, who exclusively develop adenomatous polyps.[10]

The selectivity of EPA and aspirin for colorectal polyp prevention based on polyp type and location suggested that a precision medicine approach to CRC chemoprevention is necessary, which mirrors established best oncology practice in CRC treatment, which is now firmly based on molecular stratification of tumours.[13]

Mechanism(s) of the anticancer activity of omega-3 polyunsaturated fatty acids and aspirin

Despite decades of research into the mechanism(s) of the possible anticancer activity of the omega-3 PUFAs EPA and docosahexaenoic acid (DHA), as well as aspirin, the molecular basis of how these agents reduce CRC risk remains unclear.[12,14] The strongest line of evidence for both omega-3 PUFAs (particularly EPA) and aspirin is shared inhibition of COX-1 and COX-2.[12,14] COX-dependent synthesis of the lipid mediator (also termed eicosanoid or oxylipin) prostaglandin (PG) E_2 is believed to be critical for tumour growth and host antitumour immune evasion.[15] Moreover, inhibition of COX-1-dependent synthesis of thromboxane (TX) A_2 by aspirin underlies its antiplatelet activity and is likely to explain at least some of the anti-neoplastic activity of aspirin.[16] However, COX-independent mechanisms of action of both omega-3 PUFAs and aspirin have also been proposed, including production of other oxylipins [including specialised pro-resolving mediators (SPMs)], often based on in vitro cell studies without strong

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evidence of the relevance of findings from human studies.[17] In particular, there is a clear discrepancy between the consistent evidence of anti-CRC activity of EPA and DHA from multiple in vitro and pre-clinical studies and variable findings from epidemiological studies of fish (the primary source of dietary omega-3 PUFAs) intake and randomised trials of omega-3 PUFAs, suggesting that individual host factors may control efficacy of omega-3 PUFAs for non-communicable disease prevention in human populations.[18]

Another open question is whether the anti-neoplastic activity of omega-3 PUFAs and aspirin targets tumour (polyp) initiation at the very earliest stages of colorectal carcinogenesis and/or tumour (polyp) progression only after tumourigenesis.

Improved understanding of the mechanism(s) of action of omega-3 PUFAs and aspirin should lead to improved, precision use of these agents for CRC prevention based on use of risk and therapeutic response biomarkers to guide optimal targeting and timing of chemoprevention.

The seAFOod trial biobank and opportunity to investigate post-trial colonoscopy outcomes in the Bowel Cancer Screening Programme

A comprehensive −80 °C Biobank of blood, urine and rectal mucosa was established during the seAFOod polyp prevention trial.[19,20] One or more biological samples were received from 677 of 709 (95%) randomised seAFOod Trial participants. Seventy-three per cent (n = 519) of participants provided full sample sets of blood, urine and rectal mucosa from all three visits (baseline, 6 and 12 months). Overall, participant compliance with biological sample collection, defined as the proportion of sample sets expected (n = 2127) that were received with at least one sample aliquot, was 80% (blood), 78% (urine) and 74% (rectal mucosa). For primary analysis of the seAFOod trial, the only analysis that was carried out on Biobank samples was measurement of red blood cell (RBC) and rectal mucosal PUFA profiles,[9] which was prioritised for primary analysis of trial outcomes because of an unexpected change of capsule investigational medicinal product formulation during the trial intervention phase.[9,20]

The seAFOod polyp prevention trial was embedded in the English BCSP such that the BCSP screening colonoscopy prompted by a positive faecal occult blood test was used to determine eligibility for the trial and the 12-month 'high risk' BCSP surveillance colonoscopy was used to determine the primary and secondary polyp trial outcomes.[9,20] Following a 'high risk' BCSP surveillance colonoscopy, the English BCSP surveillance protocol required a further surveillance colonoscopy 3 years later, regardless of colorectal polyp findings, with subsequent surveillance colonoscopy dependent on the number and size of polyps detected.[21] Therefore, investigation of post-trial colorectal polyp outcomes is possible by linkage of trial data to surveillance data in the English BCSP.

Overall aims of the STOP-ADENOMA project

The overall aim of the STudy Of Prevention by Aspirin anD EPA; kNowledge Of Mechanism of Action (STOP-ADENOMA) project was to use the existing seAFOod Trial Biobank and collect post-trial colonoscopy outcomes in order to:

1. investigate the mechanism(s) of the CRC preventive activity of EPA and aspirin
2. identify potential biomarkers of colorectal polyp risk and prevention efficacy (therapeutic response) of EPA and aspirin.

Thereby promoting a biomarker-driven precision medicine approach to CRC chemoprevention by aspirin and EPA.

Integration of the seAFOod trial data and biobank directing the work-streams of the STOP-ADENOMA project

Close integration of the seAFOod trial data and laboratory measurements were required in the STOP-ADENOMA project (*Figure 1*).

Linkage of laboratory data derived from seAFOod trial biobank samples with the seAFOod trial database enabled several distinct hypothesis-driven, mechanistic questions to be addressed:

- Does chemoprevention by aspirin and EPA produce prolonged benefit (by inhibition of tumour initiation) or a 'rebound' increase in colorectal polyp incidence (by inhibition of tumour progression/growth)?
- Do polymorphisms in genes controlling PUFA synthesis or lipid mediator synthesis predict chemoprevention efficacy of aspirin and EPA?
- Do stable urinary metabolites of PGE_2 and/or TXA_2 (11-dehydro-TXB_2) predict colorectal polyp risk and/or chemoprevention efficacy of aspirin and EPA?
- Does individual EPA status at the start of chemoprevention and/or change in EPA level during treatment (regardless of the source of EPA) predict response?
- Does aspirin and EPA therapy lead to production of SPMs resolvin E and/or lipoxin A_4?

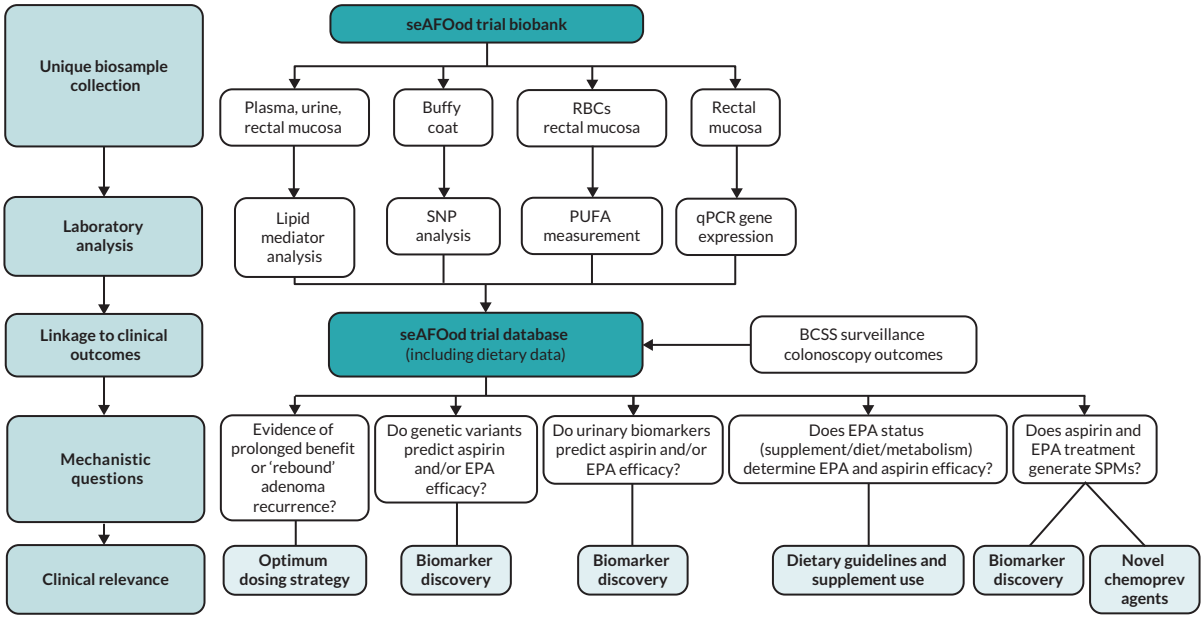

FIGURE 1 Linkage of seAFOod trial biobank analysis with seAFOod trial outcome data in order to address mechanistic questions and identify biomarkers for chemoprevention by aspirin and EPA. BCSS, Bowel Cancer Screening System; chemoprev, chemoprevention; qPCR, quantitative polymerase chain reaction; SNP, single nucleotide polymorphism.

The results and conclusions of the work addressing the above questions have been published as the following articles in peer-reviewed scientific journals:[22–28]

Discussion

Colorectal polyp outcomes in seAFOod trial participants during post-trial colonoscopy surveillance by the Bowel Cancer Screening Programme

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Investigation of colorectal polyp outcomes in seAFOod trial participants after trial participation was made possible by the prospective individual consent that each participant provided to allow access to his/her BCSP data during ongoing surveillance over a period of 6 years following their participation in the seAFOod trial.[20] The English BCSP (unlike its Scottish counterpart) includes a surveillance programme with the timing of colonoscopy examinations based on the colorectal polyp number and size detected at the last procedure. During the period covered by this post-trial investigation (November 2013–October 2021), individuals who had been deemed 'high risk' (≥ 5 polyps, or ≥ 3 polyps, if one or more are ≥ 10 mm in size) at the screening colonoscopy (the key inclusion criterion for seAFOod trial participation) were scheduled to undergo surveillance colonoscopy at 12 months (the trial exit colonoscopy for primary outcome analysis) and a minimum of 3 years later inside the BCSP.[21] Surveillance guidelines in the BCSP have now changed such that 'high risk' individuals are now scheduled to undergo a first surveillance colonoscopy 3 years after the screening procedure.[29]

The terms of the consent provided by seAFOod trial participants allowed access to BCSP records only and did not give approval to access other health data. A potential confounder of post-trial colonoscopy outcomes is ongoing use of either aspirin or EPA supplements. Therefore, we planned to link seAFOod trial and BCSP data to Hospital Episode Statistics data on myocardial infarction and cerebrovascular event incidence (as a proxy for regular use of low-dose aspirin). We also proposed to link seAFOod trial data to the National Cancer Registration and Analysis Service in order to perform an exploratory analysis of overall cancer incidence during the post-trial period given the data from the ASPirin in Reducing Events in the Elderly (ASPREE) Trial that aspirin use in an elderly population (≥ 70 years) may be associated with increased overall cancer incidence and mortality in the short term.[30] However, we did not receive NHS England (previously Public Health England) Office for Data Release approval for data linkage outside of the BCSP. Therefore, an extant

weakness of the post-trial outcomes work is absence of data on ongoing drug use. However, we have no reason to believe that aspirin and/or omega-3 PUFA supplement use has been widespread after seAFOod trial participation or that post-trial drug use would be unbalanced across treatment arms in the trial.[22]

The key finding of the study was that colorectal polyp risk was increased during post-trial follow-up in individuals who were randomised to aspirin use compared with placebo during the seAFOod trial.[22] The PDR after being randomised to placebo aspirin was 71.1%. The PDR was 80.1% for individuals, who had received aspirin [odds ratio (OR) 1.13 (95% CI 1.02 to 1.24); $p = 0.02$].[22] There was no difference in colorectal polyp outcomes between individuals that had been allocated EPA compared with its placebo [OR for PDR 1.00 (0.91 to 1.10); $p = 0.92$].[22]

We have highlighted that increased adenomatous polyp risk after chemoprevention treatment has been stopped has also been reported for selective COX-2 inhibitors.[22] We have argued that the effect size, a 10–15% increase in both outcome measures of colorectal polyp detection (the PDR and the mean number of colorectal polyps per participant/patient), has clinical significance based on the similarity of effect size for CRC risk reduction associated with aspirin in observational studies and endoscopic intervention studies.[12,22] Use of aspirin for primary and secondary prevention of vascular disease and cancer continues to be tempered by concern about bleeding risk, especially in the elderly, which has led to suggestions that aspirin dosing should have an age cut-off.[31] Our data imply that increased risk of colorectal neoplasia should be included in personalised risk–benefit considerations regarding stopping aspirin. Nevertheless, the findings from this study required corroboration from other studies, in which clinical outcomes are observed after aspirin is stopped, prior to formal inclusion of 'rebound' colorectal neoplastic risk into clinical decision-making and/or guidance regarding colonoscopy surveillance after aspirin prophylaxis is stopped.

Another key finding from this secondary analysis of colorectal polyp outcomes in the seAFOod trial is formal identification of an interaction between aspirin and EPA that was apparent in the primary trial publication data but was not analysed further as it was not a pre-specified analysis of the 2 × 2 factorial trial.[9] Potential mechanisms explaining a positive interaction between aspirin and EPA were addressed in other aspects of the STOP-ADENOMA project.[23] Formal investigation of a positive interaction between aspirin and omega-3 PUFAs is now required in an interventional clinical trial setting but can also be explored in observational cohorts that have linked data on aspirin use and fish intake/blood omega-3 PUFA levels.

Gene × drug interactions in colorectal polyp prevention

Work exploring the relationship between genetic variants and response to the seAFOod trial interventions, as well as baseline levels of PUFAs has been published in Davies *et al.*,[24] Sun *et al.*[25] and Sun *et al.*[26]

The first two papers describe analysis of the relationship of single nucleotide polymorphisms (SNPs) with seAFOod trial colorectal polyp outcomes and blood PUFA levels. The full list of SNPs that were studied using a custom-built Fluidigm microfluidic SNP genotyping assay (Fluidigm, San Francisco, CA, USA) are available in the supplementary material accompanying the paper by Davies *et al.*[24] In total, 78 SNPs in 18 genes were studied and these can be classified into several groups:

1. Genes controlling lipid mediator synthesis from omega-6 and omega-3 PUFAs [e.g. prostaglandin synthase (*PTGS*) and lipoxygenase (*ALOX*) isoforms], and their degradation [15-hydroxyprostaglandin dehydrogenase (*HPGD*)]
2. Genes previously implicated in modifying the anti-CRC response to NSAIDs, particularly aspirin (e.g. *TP53*)
3. Genes controlling PUFA synthesis [e.g. fatty acid desaturase 1 (FADS1) and *FADS2*]

We reported that SNPs in *PTGS1* and *PTGS2*, *ALOX5* and *ALOX12*, as well as *TP53*, were associated with differential polyp prevention efficacy of aspirin.[24] In each case, there is biological plausibility based on the known function of these genes in production of pro-inflammatory and pro-neoplastic oxylipins such as PGE_2 and LTB_4.[32] In particular, we demonstrated that total colorectal polyp risk reduction in aspirin users compared with non-aspirin users was restricted to rs4837960 common homozygotes (IRR 0.69, 95% CI 0.53 to 0.90) for *PTGS1*, which encodes the COX-1 enzyme that is believed to be the key target of aspirin.[24] However, the modification effect size for each gene variant was numerically small such that none of these SNPs are likely to have any clinical utility as a stand-alone biomarker of preventive activity of aspirin. Nevertheless, these SNPs in genes controlling lipid mediator synthesis could be incorporated in a future polygenic risk score (perhaps combined with other clinical factors known to influence aspirin efficacy and bleeding risk, e.g. age, body weight, hypertension) that would be used to target the use of aspirin for cancer prevention.[33]

The data on the relationship between *APOE* genotype and PUFA levels are an important addition to the literature on the interaction between *APOE* genotype and response to omega-3 PUFA supplementation, which, to date, has been restricted to studies of healthy volunteers and patients with neurodegenerative disease.[25] There is a small but statistically significant relationship between APOE haplotype and baseline PUFA levels such that *APOE2/2* individuals had lower levels, and *APOE4/4* participants had higher levels, of omega-3 PUFAs, including EPA, than wild-type *APOE3/3* counterparts.[25] After EPA supplementation in the seAFOod trial, omega-3 PUFA levels were not significantly different when stratified by *APOE* genotype, although *APOE4* carriers displayed lower plasma levels of the EPA-derived oxylipin metabolite 18-HEPE levels than individuals without an *APOE4* allele ($p = 0.002$).[25]

Several SNPs in FADS and elongation of very long-chain fatty acid (*ELOVL*) genes that control synthesis and levels of individual omega-3 and omega-6 PUFAs were included in the SNP characterisation by microfluidic genotype assay.[24] Characterisation of the *FADS2* insertion–deletion (Indel) polymorphism rs66698963 genotype in seAFOod trial DNA samples was performed separately by bench-top PCR by the Brenna laboratory, Austin, Texas under the terms of a Materials Transfer Agreement with University of Leeds.[26]

There is evidence that a 22 base-pair *FADS2* Indel in the first FADS2 intron is functional and controls neighbouring *FADS1* (delta-5 desaturase)-dependent omega-6 PUFA arachidonic acid (AA) synthesis from dihomo-γ-linolenic acid (DGLA) downstream of the major dietary omega-6 PUFA linoleic acid (LA).[34] Therefore, we tested the hypothesis that carriers of the insertion (I) allele who are 'high AA converters' will gain more benefit from an omega-3 PUFA intervention through presumed competition between AA and EPA.[34] We reported that I allele carriers display polyp prevention efficacy (using colorectal polyp number as the outcome measure) from EPA (IRR 0.50, 95% CI 0.28 to 0.90), although analysis of outcomes irrespective of *FADS2* Indel genotype did not demonstrate a reduction in colorectal polyp number in EPA users compared with the placebo group (IRR 0.92, 95% CI 0.74 to 1.16).[26] The polyp prevention effect size was similar to that observed for aspirin (IRR 0.67, 95% CI 0.51 to 0.90) in the same analysis.[26]

The FADS2 Indel displays a marked difference in allele frequency in different ethnic populations (probably explained by ancestral diet differences) with African American individuals having a much higher allele frequency than White races.[35] Consistent with this observation, the VITamin D and OmegA-3 TriaL (VITAL) reported that reduction in myocardial infarction and colorectal polyp incidence by a mixed omega-3 PUFA intervention was limited to African American participants.[36,37] A valid hypothesis is that the *FADS2* Indel genotype predicts omega-3 PUFA benefit for other disease outcomes that are driven by inflammation mediated by pro-inflammatory lipid signalling from lipid mediators downstream of AA such as cardiovascular events. There is now a need to validate our *FADS2* Indel findings in other randomised omega-3 PUFA intervention trials such as VITAL in order to strengthen the case for use of the FADS2 Indel (rs66698963) genotype as a therapeutic response biomarker for precision use of omega-3 supplementation and/or better use of dietary guidelines.

The SNP analysis in seAFOod participants included data on 2 *FADS1* SNPs (rs174546 and rs174556), 10 *FADS2* SNPs (rs77930731, rs174570, rs2072114, rs174589, rs2851682, rs174602, rs498793, rs174611, rs482548, rs76497692) and 3 *FADS3* SNPs (rs174450, rs1000778, rs174455).[24] Only *FADS1* SNP rs174546 was in weak linkage disequilibrium with rs66698963 ($R^2 = 0.23$ D'= 0.72 measured by *LDlink* in R). Comparison of *FADS2* Indel and *FADS1* rs174546 alleles in seAFOod trial participants with data on both polymorphisms showed good agreement between respective major alleles I and C (*Table 1*), which are both associated with increased *FADS1* expression and higher product-precursor PUFA ratios (AA/DGLA and AA/LA) in human blood fractions.[26,34,35,38]

TABLE 1 Agreement between genotypes for *FADS2* Indel rs66698963 (I/D) and *FADS1* SNP rs174546 (C/T) in seAFOod trial participants

		FADS1 SNP rs174546 genotype		
		C/C	C/T	T/T
FADS2 Indel rs66698963 genotype	D/D	80	135	71
	I/D	157	119	1
	I/I	61	1	0

Detailed genetic and functional analysis of the FADS gene cluster at the 'cancer hotspot' on chromosome 11q13 is required to fully understand the functional consequences of individual FADS polymorphisms, the relationship of FADS variants with cancer risk, and how omega-3 PUFAs affect FADS-dependent synthesis and cellular levels of AA.[39,40]

Urinary biomarkers of colorectal polyp risk and chemopreventive activity of aspirin and eicosapentaenoic acid

The stable urinary metabolites of PGE_2 (uPGE-M) and TXA_2 [urinary (u) 11-dehydro (d)-thromboxane (TX) B_2 (u11-d-TXB_2) are strong candidate biomarkers of colorectal polyp risk and prevention efficacy of EPA and aspirin based on the role of these eicosanoids in colorectal carcinogenesis and the putative mechanism(s) of action of EPA and aspirin as COX-1 and COX-2 inhibitors.[12,16,41]

A key overarching finding from the STOP-ADENOMA project was that intra- (over time) and interindividual variability in levels of uPGE-M and u11-d-TXB_2 are almost certainly too high to allow the use of these biomarkers to guide diagnosis and treatment efficacy of EPA and aspirin on an individual level in isolation from other biomarkers and clinical factors.[27] The seAFOod trial data are similar to those from another placebo-controlled trial of aspirin in colorectal polyp patients.[42]

However, the on-treatment reduction in uPGE-M and u11-d-TXB_2 levels confirmed the COX inhibitory activity of both EPA and aspirin in vivo with the effect size for EPA being much smaller than that for aspirin, especially for COX-1-dependent u11-d-TXB_2 synthesis.[27] One important finding of this urinary biomarker study was that an elevated u11-d-TXB_2 level predicted colorectal polyp recurrence at surveillance colonoscopy in an analysis restricted to individuals who were allocated to placebo in the seAFOod trial (IRR 2.26, 95% CI 1.11 to 4.58).[27] Prospective measurement of the u11-d-TXB_2 level in individuals undergoing colonoscopic follow-up in a surveillance programme will be required to understand further whether the u11-d-TXB_2 level could be a useful addition to a multifactor risk prediction model that could guide patient stratification for polyp surveillance. On the other hand, a low on-treatment u11-d-TXB_2 level (quartile 1) was associated with reduced polyp number at the end of the seAFOod trial intervention period *versus* placebo (IRR 0.34, 95% CI 0.12 to 0.93) for aspirin and EPA treatment compared with individuals on combination treatment that had a high on-treatment u11-d-TXB_2 value (IRR 0.61, 95% CI 0.34 to 1.11).[27] The use of u11-d-TXB_2 as a treatment response biomarker for polyp prevention by aspirin also requires prospective evaluation. This is a planned exploratory analysis of the ongoing COLO-PREVENT trial (ISRCTN13526628).

Another major learning point from the urinary biomarkers study (and also the plasma oxylipin study below) was the confounding effect of the seAFOod trial colonoscopy at the end of the intervention period on pro-inflammatory mediators.[23,27] Increased u11-d-TXB_2 levels in some seAFOod trial participants (even in individuals randomised to aspirin treatment) that provided a urine sample within a few days of colonoscopy suggest that the colonoscopy and/or bowel preparation beforehand may drive non-platelet-dependent TXA_2 synthesis during on-going aspirin treatment.[27] These findings tally with earlier observations of the inflammatory response to colonoscopy and the sensitivity of oxylipin levels to external stimuli.[43,44] Biospecimens should be collected prior to colonoscopy and the associated bowel preparation in subsequent translational and clinical studies of CRC chemoprevention agents.

Do eicosapentaenoic acid and aspirin treatment generate specialised pro-resolving mediators?

The primary finding that so-called SPMs EPA-derived resolvin (Rv) E1 and (aspirin-triggered) 15*R*-lipoxin (LX) A4 were not detectable above a sensitivity limit of 20 pg/ml in seAFOod trial plasma samples is an important addition to the contentious literature on the relevance of SPMs to human disease.[23,45,46] Of note, the trial cohort included individuals, who were allocated to aspirin and EPA, and for whom aspirin tablet compliance data and RBC EPA levels were available, to ensure as much as possible that

compliance was good. Even on-treatment plasma samples from these individuals did not have measurable RvE1 or 15*R*-LXA$_4$.[23] Since the STOP-ADENOMA publication on SPM detection, a report has cast doubt on the methodology used in several key clinical SPM studies.[47] Our findings add to the weight of evidence that endogenous RvE1 formation is unlikely to contribute to the anti-CRC activity of EPA, either in the presence or absence of aspirin.

The high variability in mono-hydroxy EPA metabolite 18-hydroxyeicosapentaenoate (HEPE) and AA-metabolite 15-hydroxyeicosatetraenoate levels between patients is in keeping with the wide range of values in the seAFOod trial cohort observed for urinary metabolites and RBC PUFA levels.[9,20,23,27] There was no evidence to suggest a role for the EPA metabolite 18-HEPE as a biomarker of EPA efficacy for colorectal polyp prevention.[23]

The relationship between dietary and supplemental omega-3 polyunsaturated fatty acid intake, blood and tissue omega-3 polyunsaturated fatty acid levels, and colorectal polyp recurrence in seAFOod polyp prevention trial participants

The fact that EPA was used as a pharmacological intervention in the seAFOod trial, although it is also obtained from dietary sources (mainly oily fish but also low-level synthesis from the precursor omega-3 PUFA alpha-linolenic acid found in nuts, seeds and derived oils[48]), prompted analysis of the relationship between dietary intake of omega-3 PUFAs, EPA capsule use and blood/tissue (rectal mucosa) omega-3 PUFA levels on an individual-participant level.[28]

Uniquely for a randomised omega-3 PUFA intervention trial, we estimated the total omega-3 PUFA intake per participant before and during the seAFOod trial as the sum of dietary intake (calculated from a food frequency questionnaire at baseline and at the end of the intervention period) *and* the EPA (or placebo) capsule intervention (adjusted for reported compliance).[28] We then performed a treatment-independent analysis of seAFOod trial outcomes according to the change in EPA level (measured as the percentage of total measured fatty acids) during the intervention phase of the seAFOod trial. The colorectal polyp risk reduction in individuals with a high ΔEPA value (regardless of treatment allocation to either active or placebo EPA in the trial) just missed statistical significance (IRR for the mean polyp number per participant for ΔEPA_{high} individuals 0.74, 95% CI 0.54 to 1.02) compared with ΔEPA_{low} individuals ($p = 0.06$).[28] Secondary treatment-independent investigation of trial outcomes regardless of the reason for the increase in omega-3 PUFA levels (which could be explained by the omega-3 PUFA intervention, alternative omega-3 PUFA supplement use, and/or dietary change) should be considered in subsequent omega-3 PUFA intervention trials.

Other important observations from the analysis of omega-3 PUFA levels from a large randomised, double-blind, placebo-controlled trial include confirmation that a pure EPA intervention does not lead to an overall increase in levels of the other main omega-3 PUFA DHA, and that 'placebo contamination' by over-the-counter own omega-3 PUFA supplement use was very rare with that possibility (based on an increase in RBC EPA and DHA levels in those allocated to placebo) occurring in < 5 participants.[28]

We also included RBC fatty acid analysis stratified according to the temperature and duration of blood sample storage at research sites in STOP-ADENOMA. During delivery of the seAFOod trial, it became increasingly recognised that storage of RBCs at temperatures above −80 °C is associated with loss of PUFAs over time, which is believed to be driven by oxidation in the presence of haem iron.[49–53] The reported rate of PUFA loss over time varies depending on several factors including RBC aliquot volume and presence of an antioxidant.[54] In the seAFOod trial, RBC samples were stored for a variable duration and at different temperatures. It was therefore important to consider a potential confounding effect of RBC sample storage and publish these data pertinent to interpretation of other STOP-ADENOMA research outputs.[9,20,23,26]

Given practical and financial constraints at seAFOod trial research sites, which limited > −80 °C storage capability and weekly temperature-controlled sample transfer, we adopted a pragmatic approach by collecting a large volume RBC aliquot (500 µl) in a sealed cryovial and coordinating sample transfer from several research sites to the central −80 °C trial biobank at the same time.[20] Using this approach, we observed a small amount of PUFA degradation in RBC samples that were stored temporarily at temperatures > −80 °C,[28] which we do not believe confounded the analysis of RBC omega-3 PUFA levels in the seAFOod trial and STOP-ADENOMA publications.[9,20,23,26] In the multivariate model that included

factors that could explain differences in the baseline RBC EPA + DHA level in seAFOod trial participants, the interaction term for storage duration and temperature > −80 °C was extremely small.[28] The rate of PUFA loss in seAFOod trial RBC samples appeared low compared with per cent reduction rates up to 26–52 weeks reported in other studies.[49–53] We postulate that the relatively large size of seAFOod trial RBC samples (thereby reducing the surface area to volume ratio) explains relatively low HUFA loss over time even at temperatures > −80 °C.[54]

Rectal mucosal gene expression studies

Original STOP-ADENOMA objectives included rectal mucosal gene expression analysis for *COX-2* (*PTGS2*), the gene that controls metabolism and inactivation of PGE_2 *HPGD* and the short-chain fatty acid receptor *FFAR2*/*GPR43* by quantitative real-time polymerase chain reaction (qPCR). As per seAFOod trial protocol, biopsies were taken from macroscopically normal rectal mucosa at the BCSP surveillance colonoscopy after 12 months of trial treatment.[19] Time and resource considerations limited total RNA extraction from frozen rectal mucosal samples (Monarch® Total RNA Miniprep Kit with DNase I treatment) to placebos-only and aspirin-only treatment groups. qPCR assays were developed for *PTGS2*, *HPGD*, *FFAR2* and *glyceraldehyde-6-phosphate dehydrogenase* (*GAPDH*) using a LunaScript® RT SuperMix Kit and a Luna® Universal qPCR Master Mix with SYBR-green kit (both New England Biolabs). *Table 2* lists the primers used for each transcript.[55–57]

Data were analysed as the ΔCt value for each gene compared with *GAPDH*. Relative gene expression was calculated as $2^{-\Delta Ct}$. This work has not been published elsewhere.

We investigated the relationship between mucosal *COX-2* expression and the corresponding uPGE-M level at 12 months in placebo users in order to test whether high mucosal *COX-2* expression was reflected by a correspondingly high uPGE-M level (supporting the use of uPGE-M as a biomarker of colorectal mucosal COX-2 activity). Rectal mucosal biopsies from $n = 101$ trial participants, who received placebos only, underwent RNA extraction resulting in sufficient RNA yield (≥ 24 ng/ml and $A_{260}/A_{280} \geq 1.5$) for qPCR in 69 cases, 47 of which had a corresponding uPGE-M value at the same (trial exit colonoscopy) time point. *Figure 2* shows that there was no significant difference in uPGE-M level according to mucosal *COX-2* expression.

Exploratory analysis of the relationship between mucosal *COX-2* expression and colorectal polyp number in trial participants, who did not receive an active intervention, did not suggest a link between *COX-2* expression in normal rectal mucosa and colorectal polyp risk for polyp number (Spearman's rank test $\rho = 0.088$, $p = 0.63$) or PDR (Mann–Whitney test, $p = 0.51$).

A previous report suggested that a high mucosal *HPGD* transcript level predicts lower colorectal polyp risk in aspirin users.[58] Therefore, we tested whether this relationship existed in seAFOod trial participants, who were allocated to aspirin only. In seAFOod trial participants, there was no statistically significant difference between individuals that had no colorectal polyps at exit colonoscopy after the 12-month intervention period {median [interquartile range] *HPGD* ΔCt 3.69 (2.74–4.29), using *GAPDH* as the reference gene; $n = 43$} compared with trial participants that had one or more colorectal polyps [3.33 (2.71–4.28); $n = 71$; $p = 0.65$ (Mann–Whitney *U* test)].

For *FFAR2* qPCR, only cDNA samples from participants who received aspirin were tested due to lack of resource. There were $n = 116$ samples that underwent PCR and provided a Ct value. Although melt curve analysis confirmed PCR product amplification, Ct values for *FFAR2* were universally above 30. This suggested a low abundance of *FFAR2* transcripts in rectal mucosa. With the proviso that interpretation of ΔCt values may be confounded by low *FFAR2* gene expression, there was no relationship observed between *FFAR2* transcript levels and colorectal polyp number in aspirin users (data not shown).

In summary, the qPCR analysis did not provide any new insights in addition to the investigation of uPGE-M levels. The small number of cases from only two of the trial

TABLE 2 Primer sequences for quantitative SYBR-green real-time PCR analysis

Gene	Protein	Forward primer sequence	Reverse primer sequence
PTGS2	COX-2	CCCTTGGGTGTCAAAGGTAA	GCCCTCGCTTATGATCTGTC
HPGD	15-PGDH	CTCTGTTCATCCAGTGCGAT	TCACTCCAGCATTATTGACCA
FFAR2	FFAR2/GPR43	TGCTACGAGAACTTCACCGAT	GGAGAGCATGATCCACACAAAAC
GAPDH	GAPDH	TCAACGACCACTTTGTCAAGC	CCAGGGGTCTTACTCCTTGG

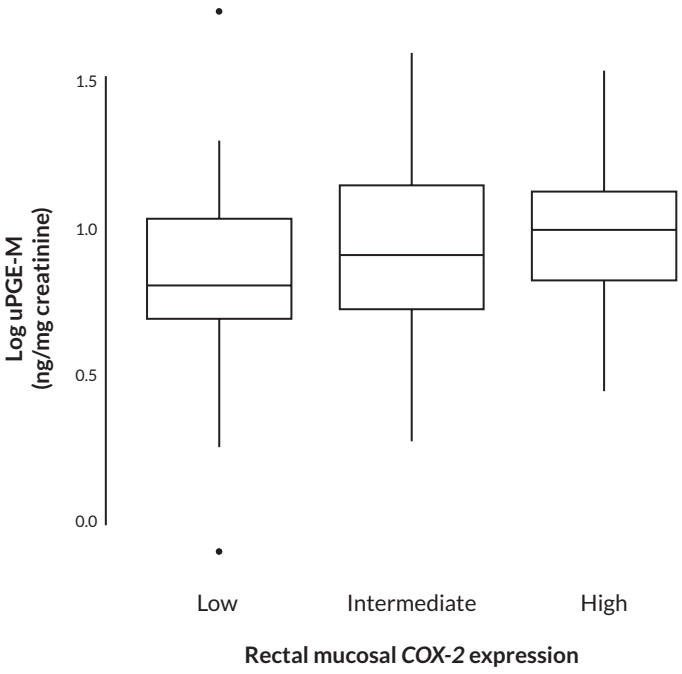

FIGURE 2 The relationship between rectal mucosal *COX-2* expression and uPGE-M level. Data are for 47 seAFOod trial participants who received placebos only before trial exit colonoscopy when rectal mucosa was biopsied and who had qPCR data for *COX-2* expression. Categories of mucosal *COX-2* expression were categorised as low (high *COX-2* Ct value > 35 and no PCR product peak on melt curve analysis) n = 22; medium (lower than the median $2^{-\Delta Ct}$ value) n = 14; and high (higher than the median $2^{-\Delta Ct}$ value) n = 11. Data are represented as the median value and interquartile range (box), with whiskers representing 1.5× the interquartile range and dots representing outlier data points. p = 0.45 for the comparison of the groups by the Kruskal–Wallis test.

treatment groups, as well as the relatively low expression level of *COX-2* and *FFAR2* in normal rectal mucosa made interpretation of the data challenging. Since the STOP-ADENOMA project was proposed, bulk RNA sequencing has become commonplace and affordable and is now the preferred method for gaining maximum insight from tissue RNA analysis rather than a limited number of gene targets addressing specific hypotheses.

Other collaborative work using seAFOod trial data

Medical Research Council Systematic Techniques for Assisting Recruitment to Trials study: a study within a trial

The seaFOod trial included a study within a trial (SWAT) in which potential participants were randomised to receive either standard written information about the trial prior to consent or a multimedia intervention (including video clips of patients discussing participation and the Chief Investigator explaining the rationale and conduct of the trial) accessed via a URL link or QR code, in addition to the standard written information about the trial. The seAFOod data are included in a random effects meta-analysis of the five studies that incorporated a SWAT of a multimedia information intervention.[59]

Of 728 individuals who were approached to discuss joining the seAFOod trial using either the multimedia intervention or standard written information in cluster randomised endoscopy units, 129 (18%) individuals were randomised to the seAFOod trial. There was no significant difference in recruitment between the group who received standard information [61/395 (15.4%)] compared with the multimedia intervention [68/333 (20.4%)], with an OR of 1.44 (95% CI 0.88 to 2.37).[59] Overall, multimedia alongside written information resulted in no significant difference in recruitment rate to trials included in the Medical Research Council (MRC) START project (pooled OR 0.96, 95% CI 0.79 to 1.17).[59]

Is recruitment to a colorectal polyp prevention trial associated with beneficial changes to diet related to factors associated with colorectal cancer risk?

A separate analysis of the seAFOod trial diet data, which addressed other dietary constituents such as red/processed meat intake as opposed to fish intake, was undertaken by Dr Elizabeth Williams, who supervised a PhD student at the University of Sheffield.[60]

Patient and public involvement

STOP-ADENOMA analyses were performed on biological samples collected during the completed seAFOod trial and on data obtained from the BCSP about colonoscopy outcomes during ongoing surveillance after trial participation according to the informed consent provided by all seAFOod trial participants. Therefore, there was no ongoing human subject involvement during STOP-ADENOMA.

A patient and public involvement (PPI) representative was a member of the Investigators group throughout the study. Key roles played by the PPI representative were writing and finalising submissions for approvals required by the Health Research Authority and Public Health England, as well as reviewing public-facing materials including description of STOP-ADENOMA findings on the Colorectal Cancer Screening Prevention Endoscopy and Early Diagnosis (COLO-SPEED) site.

We did not have identifiable data or contact details for seAFOod trial participants but we are aware that these individuals might want to know the results of the

STOP-ADENOMA research to which they contributed. In order to comply with the General Data Protection Regulation 2018 and Data Protection Act 2018, which came into force after individual informed consent had been provided for seAFOod trial participation, as well as for use of biological samples and linked clinical data in STOP-ADENOMA, we posted a Privacy Notice on the COLOSPEED website [STOP-ADENOMA_Privacy Notice.pdf (colospeed.uk)], which is a research platform that hosts research studies for prevention and early diagnosis of CRC [COLO-SPEED – Overview (colospeed.uk)] including STOP-ADENOMA. All scientific outputs from STOP-ADENOMA and its associated collaborations will be linked to the STOP-ADENOMA page with a Plain language summary written in conjunction with the STOP-ADENOMA PPI representative.

Equality, diversity and inclusion

A prominent factor which determined the seAFOod trial participant cohort, which was not predicted when the seAFOod trial protocol was developed in 2010, was that the English BCSP pathway (at the time based on guaiac faecal occult blood testing as a screening tool for colonoscopy) preferentially identified male individuals for screening colonoscopy.[61] Moreover, there was a significant male preponderance in individuals satisfying 'high risk' colorectal polyp criteria for subsequent annual colonoscopy surveillance under the previous BCSP guidelines.[62] Therefore, women were under-represented in the seAFOod trial cohort with 80% of participants being male.[9,20] The possibility of sex imbalance has been recognised in the ongoing COLO-PREVENT trial (ISRCTN13526628), which is recruiting patients who are deemed 'high risk' for colonoscopic surveillance in the English BCSP. However, the English BCSP surveillance pathway changed in 2020 such that initial population screening is based on the faecal immunochemical test and individuals deemed 'high risk' now require ≥ 5 polyps, or ≥ 2 polyps, if one or more are ≥ 10 mm in size, for scheduled 3-year surveillance colonoscopy.[29] It is possible that the change in screening methodology and criteria for BCSP surveillance might lead to more women undergoing screening colonoscopy and joining the BCSP surveillance pathway. Data from the English BCSP, as well as interim data on screening and recruitment to COLO-PREVENT, are awaited.

There was also a lack of ethnic and racial diversity in the seAFOod trial. Prospective data were not collected on race or ethnicity during recruitment between 2011 and 2016. However, anecdotally, the trial cohort was almost exclusively White British, which is consistent with the demographic profile of individuals undergoing screening colonoscopy in the English BCSP, in which uptake of occult blood testing and colonoscopy is reduced in areas with higher ethnic diversity.[63,64] The critical importance of racial diversity in CRC chemoprevention studies to maximise generalisability of research findings has been highlighted by the STOP-ADENOMA observation that the *FADS2* Indel (rs66698963) genotype, which predicts colorectal polyp prevention by EPA,[22] displays marked differences in allele frequency according to genetic ancestry.[35,65] It is predicted that the effect size for colorectal polyp prevention by EPA would be larger in a polyp prevention trial that includes a higher proportion of individuals of African or South Asian ancestry who display a higher *FADS2* Indel rs66698963 I allele frequency.[35]

Impact and learning

Design of subsequent polyp prevention trials

The COLO-PREVENT trial (ISRCTN13526628) is a randomised polyp prevention trial of aspirin alone *versus* aspirin plus metformin, as well as a placebo-controlled evaluation of the polyphenol resveratrol, in the patients undergoing colonoscopic surveillance in the BCSP. Many aspects of the design of this ongoing trial are based on the experience gained and protocol from the seAFOod trial.

The secondary analyses of the seAFOod trial for investigation of post-trial colonoscopy outcomes, as well as risk and therapeutic response biomarker discovery in STOP-ADENOMA, have all used colorectal polyp number, in addition to the PDR, as the read-out for neoplastic risk.[22,23,24,26,27,28] This has aided acceptance of colorectal polyp number per participant as the primary end point in future colorectal polyp prevention trials. COLO-PREVENT is using colorectal polyp number as the primary outcome measure.

Insight from the seAFOod trial biobank and STOP-ADENOMA project has already shaped the translational aspects (sample collection and storage) of COLO-PREVENT with increased emphasis on the need for −80 °C storage at research sites and addition of urine collection for measurement of u11-d-TXB_2 levels. End of intervention sampling in COLO-PREVENT occurs before bowel preparation for the 3-year colonoscopy in order to avoid any confounding effect of the procedure on biomarkers of inflammation and neoplastic risk.

The STOP-ADENOMA post-trial outcome data have prompted prospective approval for collection of post-trial colonoscopy outcomes in the BCSP after COLO-PREVENT participation.

Future work on the FADS2 Indel polymorphism as a therapeutic response biomarker

Funding is required to validate the use of *FADS2* Indel genotype for prediction of colorectal polyp prevention and cardiovascular risk reduction by omega-3 PUFAs with collaborators in Austin, TX and Rochester, NY. There are several historical randomised controlled trials with banked DNA samples that could be utilised including VITAL[36] and Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial,[66] prior to prospective evaluation of genotyping in other risk populations, for which omega-3 PUFA treatment might be considered.

Aspirin and DNA methylation

In collaboration with Dr Belinda Nedjai (Queen Mary University of London), a subset of seAFOod trial genomic DNA samples produced during STOP-ADENOMA [*n* = 15 each from placebo and aspirin only groups comparing baseline and end of treatment (12 months)] have undergone reduced representation bisulphite sequencing for detection and quantification of whole-genome DNA methylation. Several candidate differentially methylated positions have been identified prompting planned analysis of a larger sample set.

Whole genome sequencing

STOP-ADENOMA Investigators are currently exploring the possibility of using seAFOod trial genomic DNA samples for whole genome or exome sequencing subject to ethical approval and funding.

Implications for decision-makers

As an early phase translational 'discovery' study of mechanism(s) of action and related biomarkers of two interventions (the omega-3 PUFA EPA and aspirin) tested in a randomised clinical trial (seAFOod) that had a null primary end point,[9] the STOP-ADENOMA findings have no direct implications for practice or local service delivery.

However, the STOP-ADENOMA findings will inform research funding strategy and project prioritisation for cancer (and wider non-communicable chronic diseases) prevention by funding bodies, thereby promoting a precision approach to chemoprevention and nutritional prevention of cancer.

Research recommendations

The following priority areas for future translational and clinical research are proposed leading from STOP-ADENOMA findings:

- precision use of aspirin for cancer prevention and interception
- genetic factors underlying disease prevention by omega-3 PUFAs
- optimal use of colorectal polyp outcomes for cancer risk prediction.

Conclusions

The STOP-ADENOMA project has provided several new mechanistic and biomarker discovery insights from the seAFOod polyp prevention trial of aspirin and EPA using the trial biobank and linked BCSP colonoscopy data.

Investigation of candidate genetic variants that could modify the effect of aspirin and EPA has highlighted, in particular, a polymorphism in the *FADS2* gene that could be used to predict colorectal polyp prevention efficacy (and perhaps benefit for prevention of other diseases for which omega-3 PUFA treatment has been proposed) of EPA, when analysis of the whole trial population did not reveal any overall benefit from EPA use. Validation of this genetic marker in independent omega-3 PUFA intervention trial cohorts is necessary before prospective evaluation.

The importance of the antiplatelet activity of aspirin for anticancer activity is becoming increasingly recognised. Consistent with this, the stable urinary metabolite of platelet-derived TXA_2 u11-d-TXB_2 has shown promise as a biomarker of colorectal polyp prevention efficacy of aspirin and as a biomarker of colorectal polyp risk in untreated individuals. Further evaluation of the predictive ability of u11-d-TXB_2 is planned in ongoing trials such as COLO-PREVENT and Add-Aspirin.

The observation that colorectal polyp detection actually increased in the period following aspirin treatment during the seAFOod trial is consistent with suppression of polyp growth, rather than inhibition of tumour initiation, by aspirin. The finding highlights the need to study colorectal neoplastic risk carefully if a chemoprevention agent is stopped for clinical reasons.

Overall, STOP-ADENOMA has taken a precision medicine approach to CRC chemoprevention and has generated

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novel findings that are applicable to the optimal use of aspirin and EPA in a targeted manner.

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Data-sharing statement

The biomarker, as well as linked clinical and trial outcome, data sets used in STOP-ADENOMA contain identifiable data, which are stored in a secure virtual research environment at the University of Leeds. Data will be anonymised and exported upon request to the Principal Investigator (Professor Hull) and

the Study Sponsor (University of Leeds) as directed by a signed Data Access agreement. Data will be available from publication until July 2028. Any queries should be addressed to the corresponding author.

Ethics statement

The STOP-ADENOMA project gained approval from the London and Surrey Borders Research Ethics Committee (19/LO/1655). The study protocol is version 2.0 dated 21 April 2021. The study is registered (with the seAFOod trial) as ISRCTN05926847. Linkage of post-trial colonoscopy outcomes in the English BCSP with seAFOod trial data sets was approved by the NHS England Office for Data Release (ODR1920_199) and the BCSP Research Advisory Committee (BCSPRAC_285).[22]

Information governance statement

The University of Leeds is committed to handling all personal information in line with the UK Data Protection Act (2018) and the General Data Protection Regulation (EU GDPR) 2016/679.

Under the Data Protection legislation, the University of Leeds is the Data Controller, and you can find out more about how the University of Leeds handles personal data, including how to exercise your individual rights and the contact details for our Data Protection Officer here: https://dataprotection.leeds.ac.uk/research-participant-privacy-notice/.

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page at https://doi.org/10.3310/GJMH1530.

Primary conflicts of interest: Colin Rees has received grant funding from Medtronic and was an expert witness for Olympus and ARC Medical. Louise Brown was a member of the EME Strategy Advisory Committee (1 June 2018–1 June 2019), a member of the EME Funding Committee (1 July 2014–1 July 2020) and the EME Funding Committee Sub-Group Remit & Competitiveness Group (1 November 2017–31 October 2018). Mark Hull was a member of the EME Funding Committee (1 July 2014–1 July 2018) and the EME Funding Committee Sub-Group Remit & Competitiveness Group (1 November 2016–31 December 2017). None of the other authors declares any competing interest.

Copyright and credit statement

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Study registration

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Award publications

This synopsis provided an overview of the research award *Study Of Prevention by Aspirin anD EPA; kNowledge Of Mechanism of Action (STOP-ADENOMA)*. Other articles published as part of this thread are:

Downing A, Fenton H, Nickerson C, Loadman PM, Williams EA, Rees CJ, *et al*. Colorectal polyp outcomes after participation in the seAFOod polyp prevention trial: evidence of rebound elevated colorectal polyp risk after short-term aspirin use. *Aliment Pharmacol Ther* 2023;**58**:562–72. https://doi.org/10.1111/apt.17646

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For more information about this research please view the award page (https://www.fundingawards.nihr.ac.uk/award/NIHR128210).

Additional outputs

Other peer-reviewed research papers

Madurasinghe VW, Knapp P, Eldridge S, Collier D, Treweek S, Rick J, *et al*. Can we achieve better trial recruitment by presenting patient information through multimedia? Meta-analysis of 'studies within a trial' (SWATs). *BMC Med* 2023;**21**:425.

PhD thesis

El Mogassabi A. *Diet, Dietary Patterns and Colorectal Adenoma*. 2021. URL: https://etheses.whiterose.ac.uk/id/eprint/30211/ (accessed 28 August 2025).

Conference papers

Poster presentation: Do people change their diet after colorectal adenoma diagnosis? 13th European Nutrition Conference, FENS 2019, 15–18 October 2019, *Proc Nutr Soc* 2020;**79**:E462.

Poster presentation: *Plasma and Rectal Mucosal Oxylipin Levels during Aspirin and Eicosapentaenoic Acid Treatment in the seAFOod Polyp Prevention Trial*. International Society for the Study of Fatty Acids and Lipids 15th International Congress 2023, Nantes, France, 2–5 July 2023.

Poster presentation: FADS2 indel polymorphism rs66698963 predicts colorectal polyp prevention by the *n*-3 Fatty Acid EPA. *J Clin Lipidol* 2024:**18**:e535–6. National Lipid Association Meeting 2024, Las Vegas, NV, USA, 30 May–2 June 2024.

Seminars

Colorectal Polyp Prevention by Aspirin and the Omega-3 Fatty Acid EPA: Insights from the seAFOod Trial and Post-trial Follow-up. AsCaP Investigators Meeting, Paris, France, 15–16 May 2023.

Genetic Predictors of Colorectal Polyp Prevention Efficacy of the n-3 Fatty Acid EPA and Aspirin. UK Therapeutic Cancer Prevention Network Spring meeting 2023, 23 April 2023, online..

Genetic Determinants of the Colorectal Polyp Prevention Efficacy of n-3 Fatty Acids. NIHR Cancer and Nutrition Collaboration (Molecular Mechanisms sub-group), 11 November 2024, online.

Summarised in the International Collaboration on Nutrition in relation to Cancer (ICONIC) newsfeed May 2025.

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List of abbreviations

AA	arachidonic acid
BCSP	Bowel Cancer Screening Programme
COLO-SPEED	Colorectal Cancer Screening Prevention Endoscopy and Early Diagnosis
COX	cyclooxygenase
CRC	colorectal cancer
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FADS	fatty acid desaturase
FAP	familial adenomatous polyposis
GAPDH	glyceraldehyde-6-phosphate dehydrogenase
HEPE	hydroxyeicosapentaenoate
LA	linoleic acid
LX	lipoxin
MRC	Medical Research Council
MSI	microsatellite instability
NSAID	non-steroidal anti-inflammatory drug
PCCRC	post-colonoscopy colorectal cancer
PDR	polyp detection rate
PG	prostaglandin
PUFA	polyunsaturated fatty acid
qPCR	quantitative real-time polymerase chain reaction
RBC	red blood cell
Rv	resolvin
SNP	single nucleotide polymorphism
SPM	specialised pro-resolving mediator
STOP-ADENOMA	STudy Of Prevention by Aspirin anD EPA; kNowledge Of Mechanism of Action
SWAT	study within a trial
TX	thromboxane
u11-d-TXB_2	urinary (u) 11-dehydro (d)-thromboxane (TX) B_2
uPGE-M	urinary metabolites of PGE_2

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