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**NHS**
*National Institute for Health Research*

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# Abstract

## Eicosapentaenoic acid and/or aspirin for preventing colorectal adenomas during colonoscopic surveillance in the NHS Bowel Cancer Screening Programme: the seAFOod RCT

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**Background:** The omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) and aspirin both have proof of concept for colorectal cancer (CRC) chemoprevention, aligned with an excellent safety profile.

**Objectives:** The objectives were to determine whether or not EPA prevents colorectal adenomas, either alone or in combination with aspirin, and to assess the safety/tolerability of EPA, in the free fatty acid (FFA) form or as the triglyceride (TG), and aspirin.

**Design:** This was a randomised, blinded, placebo-controlled, 2 × 2 factorial trial.

**Setting:** The NHS Bowel Cancer Screening Programme (BCSP).

**Participants:** Patients (aged 55–73 years) identified as 'high risk' (i.e. those who have five or more colorectal adenomas of < 10 mm in size or three or more colorectal adenomas if one is ≥ 10 mm in size) at screening colonoscopy.

**Interventions:** The interventions were capsules containing 2000 mg of 99% EPA–FFA or 2780 mg of 90% EPA–TG (equivalent to 2000 mg of FFA) taken daily, or identical placebo capsules; and 300 mg of aspirin taken daily, or an identical placebo, enteric-coated tablet. Both were taken for ≈1 year until surveillance colonoscopy. All participants and staff were unaware of treatment allocation.

**Main outcome measures:** The primary outcome was the number of participants with one or more colorectal adenomas [adenoma detection rate (ADRa)] at surveillance colonoscopy. Outcomes were analysed for all participants with observable follow-up data by an 'at-the-margins' approach, adjusted for

BCSP site and by the need for repeat baseline endoscopy. Secondary outcome measures – these included the number of colorectal adenomas per patient [mean adenomas per patient (MAP)], 'advanced' ADRa and colorectal adenoma location (right/left) and type (conventional/serrated).

**Results:** Between November 2011 and June 2016, 709 participants were randomised, with 707 providing data (80% male, mean age 65 years). The four treatment groups (EPA + aspirin, $n = 177$; EPA, $n = 179$; aspirin, $n = 177$; placebo, $n = 176$) were well matched for baseline characteristics. Tissue EPA levels and tolerability were similar for FFA and TG users. There was no evidence of any difference in ADRa between EPA users (62%) and non-users (61%) [risk difference –0.9%, 95% confidence interval (CI) –8.8% to 6.9%] or for aspirin users (61%) versus non-users (62%) (risk difference –0.6%, 95% CI –8.5% to 7.2%). There was no evidence of an interaction between EPA and aspirin for ADRa. There was no evidence of any effect on advanced ADRa of either EPA (risk difference –0.6%, 95% CI –4.4% to 3.1%) or aspirin (risk difference –0.3%, 95% CI –4.1% to 3.5%). Aspirin use was associated with a reduction in MAP [incidence rate ratio (IRR) 0.78, 95% CI 0.68 to 0.90), with preventative efficacy against conventional (IRR 0.82, 95% CI 0.71 to 0.94), serrated (IRR 0.46, 95% CI 0.25 to 0.87) and right-sided (IRR 0.73, 95% CI 0.61 to 0.88) lesions, but not left-sided (IRR 0.85, 95% CI 0.69 to 1.06) adenomas. There was evidence of chemopreventive efficacy of EPA on conventional (IRR 0.86, 95% CI 0.74 to 0.99) and left-sided (IRR 0.75, 95% CI 0.60 to 0.94) adenomas, but not on total MAP (IRR 0.91, 95% CI 0.79 to 1.05) or serrated (IRR 1.44, 95% CI 0.79 to 2.60) or right-sided (IRR 1.02, 95% CI 0.85 to 1.22) adenomas. EPA and aspirin treatment were well tolerated, with excess mild/moderate gastrointestinal (GI) adverse events (AEs) in the EPA alone group. There were six GI bleeding AEs.

**Conclusion:** EPA and aspirin treatment were not associated with a reduction in ADRa. However, both agents displayed evidence of chemopreventive efficacy, based on adenoma number reduction, which was specific to adenoma type and location, and is compatible with known anti-CRC activity of aspirin.

**Limitations:** Limitations of the trial included the failure to recruit to the target sample size of 853, and an unexpected switch of EPA formulation mid-trial.

**Future work:** A future objective should be to understand the mechanism(s) of action of EPA and aspirin using the trial biobank. Established trial infrastructure will enable future trials in the BCSP.

**Trial registration:** Current Controlled Trials ISRCTN05926847.

**Funding:** This project was funded by the Efficacy and Mechanism Evaluation (EME) programme, a MRC and NIHR partnership.

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# List of abbreviations

| | | | |
|---|---|---|---|
| AA | arachidonic acid | FFQ | Food Frequency Questionnaire |
| ADR | adverse drug reaction | FIT | faecal immunochemical test |
| ADRa | adenoma detection rate | FOBt | faecal occult blood test |
| AE | adverse event | FPFV | first participant, first visit |
| AF | atrial fibrillation | FS | flexible sigmoidoscopy |
| ALA | alpha-linolenic acid | GI | gastrointestinal |
| APC | adenomatous polyposis coli | HR | hazard ratio |
| BCSP | NHS Bowel Cancer Screening Programme | ID | identification |
| CACE | complier-average causal effect | IMP | investigational medicinal product |
| CI | confidence interval | IRR | incidence rate ratio |
| CIMP | CpG island methylator phenotype | ITT | intention to treat |
| CIN | chromosomal instability | LA | linoleic acid |
| COX | cyclooxygenase | LPLV | last participant, last visit |
| CRC | colorectal cancer | MAP | mean adenomas per participant |
| CRN | Clinical Research Network | MDT | multidisciplinary team |
| CTA | Clinical Trials Authorisation | MHRA | Medicines and Healthcare products Regulatory Agency |
| CTIMP | Clinical Trial of an Investigational Medicinal Product | MMR | mismatch repair |
| CTPL | Clinical Trials Pharmacology Laboratory | MRC | Medical Research Council |
| | | MSI | microsatellite instability |
| CVD | cardiovascular disease | NCRI | National Cancer Research Institute |
| DHA | docosahexaenoic acid | NCTU | Nottingham Clinical Trials Unit |
| DMC | Data Monitoring Committee | NIHR | National Institute for Health Research |
| DNA | deoxyribonucleic acid | | |
| EE | ethyl ester | NSAID | non-steroidal anti-inflammatory drug |
| EME | Efficacy and Mechanism Evaluation | OTC | over the counter |
| EPA | eicosapentaenoic acid | PCCRC | post-colonoscopy colorectal cancer |
| EPIC | European Prospective Investigation of Cancer | PG | prostaglandin |
| | | PGDH | prostaglandin dehydrogenase |
| FAP | familial adenomatous polyposis | PI | principal investigator |
| FFA | free fatty acid | PIL | patient information leaflet |
| FFAR | free fatty acid receptor | PPI | patient and public involvement |
| FFPE | formalin fixed paraffin embedded | PUFA | polyunsaturated fatty acid |

| | | | |
|---|---|---|---|
| QA | quality assurance | SAP | statistical analysis plan |
| RBC | red blood cell | SCFA | short-chain fatty acid |
| RCT | randomised controlled trial | seAFOod | Systematic Evaluation of Aspirin and Fish Oil |
| R&D | research and development | | |
| REC | Research Ethics Committee | SSPr | specialist screening practitioner |
| R&I | research and innovation | TG | triglyceride |
| RN | research nurse | TMG | Trial Management Group |
| RSI | Reference Safety Information | TSC | Trial Steering Committee |
| Rv | resolvin | WHO | World Health Organization |
| SAE | serious adverse event | w/w | weight per weight |

# Plain English summary

Bowel cancer kills > 15,000 people every year in England and Wales. Most bowel cancers develop from a polyp, also known as an adenoma, which is a fleshy growth on the bowel wall. Polyps are found and removed at colonoscopy, which is a large-bowel camera test, but colonoscopy does not prevent further polyps. Use of drugs or dietary supplements (called chemoprevention) may be able to reduce polyp growth and the possibility of developing bowel cancer.

The Systematic Evaluation of Aspirin and Fish Oil (seAFOod) trial tested the effects of naturally occurring omega-3 eicosapentaenoic acid (EPA) (a dose roughly equivalent to two oily fish portions every day) and aspirin on bowel polyp growth. Patients took EPA on its own, aspirin on its own, EPA and aspirin together or placebo (dummy) medication.

The trial recruited 709 participants who had three or more adenomas found and removed at a NHS Bowel Cancer Screening Programme colonoscopy and needed a check-up colonoscopy 1 year later. Allocation to one of the four treatment options before the check-up was by chance and 'blinded', so that all participants and trial staff did not know what treatment was taken.

The results showed that there was no reduction in the number of patients who had at least one adenoma at check-up (≈60%) in either EPA or aspirin users. However, EPA and aspirin were found to reduce the number of certain types of adenoma in different parts of the bowel by 10–20%. Both EPA treatment and aspirin treatment were safe for patients, with no increased bleeding risk, but EPA caused 10% more symptoms of mild stomach upset, including diarrhoea.

It is concluded that both EPA and aspirin have chemoprevention benefits, which are limited to certain bowel polyp types. The results also suggest that aspirin (possibly with EPA) could be used to help prevent bowel cancers that occur despite colonoscopy.

# Scientific summary

## Background

Chemoprevention (the use of drugs or nutritional agents) is one strategy for the prevention of colorectal cancer (CRC), the development of which occurs predominantly via a benign colorectal lesion termed an adenoma (also known as a polyp). The molecular pathogenesis of the early stages of colorectal carcinogenesis is complex, and is reflected by two main histological types of precursor lesion [(1) conventional (i.e. tubular, tubulo-villous, villous) adenoma and (2) serrated adenoma (now termed polyp, recognising that no dysplasia is present in most serrated lesions)], which map onto different molecular characteristics, such as chromosomal instability or deoxyribonucleic acid (DNA) hypermethylation, and are both believed to progress to CRC.

The omega-3 (ω-3) polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA) and aspirin are candidate CRC chemoprevention agents: both have proof of concept in humans, aligned with an excellent safety and toxicity profile. Therefore, a randomised, Phase III, polyp-prevention trial was performed to investigate the chemoprevention efficacy of both agents in individuals at risk of 'sporadic' colorectal adenoma recurrence within a colonoscopy screening and surveillance programme.

Different 'nutraceutical' formulations of EPA exist, including EPA in the free fatty acid (FFA) form, as a triglyceride (TG) conjugate, or as ethyl ester. All three forms of EPA have anti-CRC activity in pre-clinical studies. A direct comparison between EPA bioavailability and tolerability of different formulations in a randomised trial has not been reported previously.

## Objectives

### Primary objective
The primary objective was to determine whether or not EPA prevents colorectal adenomas, either alone or in combination with aspirin.

The following primary hypotheses were tested:

- 2 g of EPA-FFA or 2780 g of EPA-TG (equivalent to a 2-g FFA dose) daily is more effective than placebo for reduction in colorectal adenoma recurrence.
- 300 mg of aspirin daily is more effective than placebo for reduction in colorectal adenoma recurrence.

### Secondary objectives
The secondary objectives were to assess the tolerability and safety of EPA, as the FFA or as the TG, alone and in combination with aspirin.

## Methods

### Trial design
This was a randomised, blinded, placebo-controlled 2 × 2 factorial trial, which was integrated into the screening and surveillance phases of the NHS Bowel Cancer Screening Programme (BCSP) so that participation did not alter routine clinical practice.

## Eligibility

Recruitment was restricted to BCSP patients aged 55–73 years who were identified as 'high risk' [five or more small (< 10 mm in size) colorectal adenomas or three or more colorectal adenomas, with at least one being ≥ 10 mm, based on endoscopic findings and confirmed later by the histopathology report] at a complete screening colonoscopy. This included patients who were identified as 'high risk' at colonoscopy after faecal occult blood test (FOBt) screening, or who were deemed 'high risk' after a bowel scope flexible sigmoidoscopy (FS) and subsequent screening colonoscopy.

## Interventions

- Four gastro-resistant capsules of 99% EPA-FFA or five soft gelatin capsules of 90% EPA-TG (both equivalent to 2 g of FFA daily), or identical placebos (both containing capric and capryllic acid medium-chain TGs). The capsule investigational medicinal product (IMP) switch was necessitated by cessation of supply of EPA-FFA and its placebo during the trial. Each participant received either the FFA or the TG formulation (or respective placebo), but not both.
- One enteric-coated aspirin tablet (300 mg) or identical placebo.

Both IMPs were provided from randomisation until the day before surveillance colonoscopy, 12 months after the screening procedure.

## Randomisation and blinding

After written informed consent was obtained, the participant was randomised according to a 2 × 2 factorial design (*Table a*).

Internet-based treatment assignment was determined by a computer-generated pseudorandom code using random permuted blocks of randomly varying size (4–12). Trial participants were allocated with equal probability to each treatment group. Stratification was by BCSP site. The sequence of treatment allocations was concealed until recruitment, data collection and all other trial-related assessments had been completed. Allocation was not divulged to researchers or participants.

## The seAFOod trial biobank

Blood [for red blood cells (RBCs), plasma and leucocytes (DNA)], urine and rectal mucosa samples were obtained at baseline and at 6 and 12 months (rectal mucosa samples were obtained at 12 months only). One or more biological samples were received from 95% of participants, with 73% providing a full sample set.

# Outcomes

## Primary outcome

The primary outcome was the number of participants with one or more colorectal adenomas detected at the first BCSP surveillance colonoscopy 12 months after the screening examination [the adenoma detection rate (ADRa)].

TABLE a The Systematic Evaluation of Aspirin and Fish Oil (seAFOod) trial 2 × 2 factorial design

| | |
|---|---|
| • 2 g of EPA-FFA, or equivalent FFA dose of EPA-TG<br>• 300 mg of aspirin | • 2 g of EPA-FFA, or equivalent FFA dose of EPA-TG<br>• Placebo aspirin |
| • Placebo EPA<br>• 300 mg of aspirin | • Placebo EPA<br>• Placebo aspirin |

### Secondary outcomes

The secondary outcomes were as follows:

- Total number of colorectal adenomas per participant at BCSP surveillance colonoscopy [total mean adenomas per participant (MAP)].
- Detection of one or more 'advanced' (i.e. ≥ 10 mm in diameter, high-grade dysplasia or villous histology) colorectal adenomas at the 12-month BCSP surveillance colonoscopy (advanced ADRa).
- Number of 'advanced' colorectal adenomas per participant at the 12-month BCSP surveillance colonoscopy (advanced MAP).
- Detection of one or more conventional adenomas (conventional adenoma end points were defined after database lock) at the first BCSP surveillance colonoscopy (conventional ADRa).
- Number of conventional adenomas (conventional adenoma end points were defined after database lock) per participant at the first BCSP surveillance colonoscopy (conventional MAP).
- Detection of one or more serrated adenomas at the first BCSP surveillance colonoscopy (serrated ADRa).
- Number of serrated adenomas per participant at the first BCSP surveillance colonoscopy (serrated MAP).
- The region of the colorectum (right colon: any part of the colon proximal to the splenic flexure; left colon: the rectum and the colon at/distal to the splenic flexure) in which adenomas are detected at the first BCSP surveillance colonoscopy.
- Reclassification from 'high risk' to 'intermediate risk' after the first BCSP surveillance colonoscopy (BCSP risk stratification at the first surveillance colonoscopy states that any individual who does not continue to fulfil 'high-risk' criteria is classified as 'intermediate risk' for further colonoscopic surveillance at 3 years).
- Detection of CRC prior to, or at, the first BCSP surveillance colonoscopy.
- Dietary fish and other seafood intake at baseline and at the end of the trial.
- Red blood cell EPA and rectal EPA levels at baseline and at 6 months (RBC only) and 12 months from randomisation.
- Absolute RBC fatty acid [docosahexaenoic acid (DHA), arachidonic acid (AA), EPA-to-AA ratio] levels and difference from baseline at 6 and 12 months.
- Rectal mucosal fatty acid (DHA, AA, EPA-to-AA ratio) levels at surveillance colonoscopy.
- Adverse events, including clinically significant bleeding episodes [haemorrhagic stroke or gastrointestinal (GI) bleeding requiring hospital admission or investigation].

## Exploratory outcomes

- Colorectal adenoma size.
- Association between change of RBC EPA level at 12 months and individual number of total colorectal adenomas.
- Association between rectal and RBC EPA levels at 12 months.

### Sample size

It was planned to randomise 853 individuals to detect an 18% relative reduction in ADRa in each two-group comparison, assuming a 10% drop-out rate.

### Statistical methods

The primary analysis was performed on an intention-to-treat basis, without imputation of missing data. The primary end point was analysed 'at the margins', as there was no evidence of an interaction between EPA and aspirin. The log relative risk was estimated using a mixed-effects log-binomial regression model, with site included as a random effect. Both interventions were fitted simultaneously and the analysis was adjusted for repeat colorectal endoscopic procedure within 3 months. Other outcomes were analysed using appropriate regression models depending on outcome type.

# Results

### Recruitment

A total of 3911 'high-risk' individuals were screened for eligibility, of whom 709 (18%) were randomised. Of those individuals not randomised ($n = 3202$), 2179 (68%) met one or more exclusion criteria [regular aspirin/non-steroidal anti-inflammatory drug use, $n = 594$ (19%); need for more than one repeat endoscopy, $n = 328$ (10%); bleeding diathesis or anticoagulant/antiplatelet therapy, $n = 313$ (10%)]. The other 1023 individuals either did not wish to participate or were not randomised for unknown reasons.

### Randomisation

A total of 177 participants were randomised to receive EPA + aspirin, 179 were randomised to receive EPA + placebo aspirin, 177 were randomised to receive placebo EPA + aspirin and 176 were randomised to receive placebo EPA + placebo aspirin. Two participants withdrew immediately after randomisation. A total of 422 (60%) participants were randomised to active or placebo EPA-FFA and 287 (40%) participants were randomised to active or placebo EPA-TG. Of those randomised, 641 (90%) participants underwent surveillance colonoscopy; endoscopic data were available for 640 of these participants.

### Baseline characteristics

Baseline characteristics were well balanced across all four treatment groups with respect to demographic data, medical history, prevalent GI symptoms and total and oily fish intake, as well as baseline colorectal adenoma characteristics. The mean age was 65 years; the male-to-female ratio was 4 : 1. Thirty-eight per cent of participants were obese. Approximately half of the participants were on regularly prescribed drugs at trial entry, which was balanced (including metformin and statin use) between the treatment groups. Compliance with trial medication was uniformly excellent (> 95%). The median time between randomisation and the 12-month surveillance colonoscopy was between 344 and 348 days in the four treatment groups.

### Red blood cell and rectal mucosal polyunsaturated fatty acid levels

Individuals in the active EPA groups had higher RBC EPA levels than placebo EPA users at both time points after the start of the intervention. RBC EPA levels were similar between participants who received either active EPA-FFA or EPA-TG at 6 and 12 months. The increase in RBC EPA level from baseline to the 6- and 12-month time points for all participants was similar across the two EPA formulations. Rectal mucosal EPA levels at the end of the intervention period were higher in those who received EPA-TG than in those who received EPA-FFA, but with substantial overlap between the two groups and no difference in the rectal mucosal EPA-to-AA ratio. As there was no clear difference in RBC or rectal mucosal EPA incorporation between those allocated EPA-FFA and those allocated EPA-TG, it was deemed appropriate to combine the primary and secondary outcome data from those who received either of the capsule investigational medicinal products.

### Primary outcome (adenoma detection rate)

In the EPA + aspirin group, 98 out of 161 (61%) participants had at least one colorectal adenoma at surveillance colonoscopy; in the EPA + placebo aspirin group, 97 out of 153 (63%) had at least one colorectal adenoma at surveillance colonoscopy. The ADRa was 61% (100/163) in the placebo EPA + aspirin group and 61% (100/163) in the placebo EPA + placebo aspirin group. When summarised at factorial margins, the ADRa was similar across interventions, with an ADRa of 62% for those who received active EPA versus 61% for those who did not receive EPA, and an ADRa of 61% for aspirin users versus 62% for those who did not receive aspirin (62%). The risk difference for EPA versus no EPA was –0.9% [95% confidence interval (CI) –8.8% to 6.9%] and for aspirin versus no aspirin was –0.6% (95% CI –8.4% to 7.2%). There was no interaction between EPA and aspirin for the ADRa ($p = 0.85$). Sensitivity analyses were supportive of the primary analysis.

### Secondary colorectal adenoma outcomes

Aspirin use was associated with a reduction in the total MAP [incidence rate ratio (IRR) 0.78, 95% CI 0.68 to 0.90], with preventative efficacy against conventional (IRR 0.82, 95% CI 0.71 to 0.94), serrated (IRR 0.46, 95% CI 0.25 to 0.87) and right-sided (IRR 0.73, 95% CI 0.61 to 0.88) lesions, but not left-sided (IRR 0.85, 95% CI 0.69 to 1.06) adenomas. There was evidence of chemopreventive efficacy of EPA on conventional (IRR 0.86, 95% CI 0.74 to 0.99) and left-sided (0.75, 95% CI 0.60 to 0.94) adenomas, but not on the total MAP (IRR 0.91, 95% CI 0.79 to 1.05) or on the total number of serrated (IRR 1.44, 95% CI 0.79 to 2.60) or right-sided (IRR 1.02, 95% CI 0.85 to 1.22]) adenomas. Overall, colorectal adenoma number was reduced in the EPA + aspirin group (166 adenomas) compared with the other groups (238 in the EPA + placebo group, 209 in the placebo + aspirin group and 231 in the placebo + placebo group), with 794 (94%) of the recurrent lesions being conventional colorectal adenomas.

### Safety and tolerability of eicosapentaenoic acid and aspirin

Dietary fish intake did not change during the trial. There were no safety concerns about either EPA or aspirin. A similar proportion of participants reported at least one adverse event (AE) or adverse drug reaction in all treatment groups (45% in the EPA + aspirin group, 46% in the EPA + placebo group, 39% in the placebo + aspirin group and 44% in the placebo + placebo group). The most commonly reported AEs were GI symptoms, with an excess of mild to moderate GI AEs (i.e. diarrhoea, nausea, abdominal pain) in the EPA + placebo aspirin group. There was no difference in tolerability between EPA-FFA and EPA-TG users. Six significant GI bleeding events were distributed across the treatment groups. No CRCs were detected.

## Conclusions

The Systematic Evaluation of Aspirin and Fish Oil (seAFOod) polyp-prevention trial has found no evidence of an effect of either EPA or aspirin on the primary end point of the proportion of individuals with one or more colorectal adenomas at the 12-month surveillance colonoscopy (the ADRa) in patients deemed 'high risk' in the English BCSP.

However, secondary analyses of the effects of EPA and aspirin on colorectal adenoma number provided evidence of chemopreventive activity of both agents. Aspirin was effective at reducing the total number of colorectal adenomas per participant, but the reduction in the total MAP associated with EPA treatment was not statistically significant. Other secondary analyses suggested that there are colorectal adenoma subtype- and site-selective effects of EPA and aspirin. Participants randomised to EPA had a reduced number (MAP) and ADRa of conventional dysplastic colorectal adenomas in the left colon and rectum compared with those randomised to placebo. Participants randomised to aspirin had a reduced number of adenomas in the right colon, particularly for serrated adenomas, and also reduced risk of conventional colorectal adenomas. Although multiple analyses were undertaken with potential for spuriously significant results, reduction in colorectal adenoma number by aspirin is consistent with published polyp-prevention trial data and the 'right sidedness' of the aspirin effect is in keeping with observational data on CRC risk and mortality. Moreover, the 'left sidedness' of EPA is consistent with efficacy in the familial adenomatous polyposis (FAP) trial of EPA-FFA against conventional rectal adenomas (West NJ, Clark SK, Phillips RK, Hutchinson JM, Leicester RJ, Belluzzi A, Hull MA. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut* 2010;**59**:918–25).

Historically, the ADRa has been used as the primary end point in polyp-prevention trials. However, its use may be confounded by its widespread use as a quality assurance measure of colonoscopist performance. By contrast, colorectal adenoma number has always been used as an end point in Phase II FAP trials. Improved colonoscopy lesion reporting in routine practice now allows MAP to be considered as a more sensitive primary end point in 'sporadic' Phase III trials, with the clinical meaningfulness of an approximate 20% reduction in MAP being supported by the CRC risk reduction from aspirin demonstrated in long-term observational studies.

Safety and tolerability of both EPA and aspirin were excellent. Mild GI symptoms are recognised with ω-3 PUFA use. The excess of GI AEs in the EPA-alone group compared with combined treatment with aspirin should be investigated further. This is the first demonstration that two dose-equivalent formulations of EPA have similar bioavailability and tolerability during long-term (12-month) dosing.

The seAFOod trial should create a paradigm shift in CRC chemoprevention research, whereby:

- Colorectal adenoma number will be introduced and further validated as a primary end point in polyp-prevention trials.
- A stratified approach will be employed for use of the colorectal adenoma as an end point, based on histological type and location.

A key objective should be to identify a predictive biomarker(s) for the type and site of colorectal adenoma recurrence, allowing a precision-medicine approach to the provision of optimal chemoprevention at an individual level. The observation that combination EPA and aspirin treatment was associated with the largest reduction in colorectal adenoma number requires investigation in an appropriately powered study.

The trial biobank will be used to support mechanistic studies into the adenoma selectivity of EPA and aspirin, as well as to explore use of ω-3 PUFA levels as a predictor of conventional colorectal adenoma risk.

## Trial registration

This trial is registered as ISRCTN05926847.

## Funding

# Chapter 1 Introduction

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## Background

### The health burden of colorectal cancer

Colorectal cancer (CRC) continues to represent a huge health burden in the UK. There were approximately 41,300 new cases of CRC (also known as bowel cancer) in the UK in 2014, making it the fourth most common cancer.[2] In the UK, 1 in 14 men and 1 in 19 women will be diagnosed with CRC during their lifetime. CRC is the second most common cause of cancer death in the UK, causing 16,000 deaths in 2014. Worldwide, it has been estimated that nearly 1.4 million cases were diagnosed in 2012.[2] Despite significant advances in the diagnosis and treatment of CRC, overall 5-year survival is currently only 59% (survival figure is for England and Wales for 2010 and 2011).[2]

### Prevention of colorectal cancer

One strategy to reduce CRC incidence and mortality is prevention. The scientific and clinical rationale for prevention of CRC is well established and is based on the following:

- Knowledge of several environmental and behavioural factors that increase CRC risk, including dietary factors (e.g. red and processed meat intake), excess body weight (i.e. obesity), lack of physical activity, tobacco smoking and excess alcohol consumption. The World Cancer Research Fund has estimated that approximately 45% of CRCs are preventable based on modification of these lifestyle factors.[3]
- The long natural history of colorectal carcinogenesis, during which a benign, precursor lesion termed a colorectal adenoma (or polyp) develops and transforms into a malignant neoplasm over a period of years (estimated to be approximately 5–10 years).[4,5]
- Improved outcomes for CRC treatment after diagnosis at earlier stages of CRC (98% 1-year overall survival for stage I CRC compared with 40% for stage IV disease; 95% 5-year survival for stage I compared with 7% for stage IV disease).[6,7]

Colorectal cancer prevention strategies that are currently used, or are under evaluation, include:

- population screening –

  o early CRC diagnosis by a guaiac faecal occult blood test (FOBt) or a faecal immunochemical test (FIT), conferring secondary benefit from colorectal adenoma identification and removal at colonoscopy
  o detection and removal (by polypectomy) of colorectal adenomas by primary screening endoscopy [colonoscopy or flexible sigmoidoscopy (FS)]

- endoscopic surveillance of high-risk groups, for example individuals with long-standing colitis or previous colorectal adenoma(s)
- chemoprevention (a term first coined in 1976 to describe the use of drugs, vitamins or other nutritional agents to try to reduce the risk of, or delay the development or recurrence of, cancer)
- health education, leading to beneficial lifestyle modification and screening uptake
- promotion of awareness and earlier diagnosis of CRC by education aimed at the public and health-care professionals.

## Chemoprevention of colorectal cancer

Despite the undoubted clinical effectiveness of endoscopic polypectomy,[8,9] CRC remains a significant problem in screened populations and high-risk surveillance cohorts because of a combination of factors that include suboptimal screening uptake, poor acceptability of endoscopic procedures and 'interval' CRC (i.e. those cancers that are diagnosed despite FOBt/FIT and/or endoscopy).[10–13] In a Dutch biennial FIT-based CRC screening programme,[14] 23% of individuals developed a FIT-interval CRC. The corresponding interval CRC rate for guaiac FOBt programmes is approximately 50%.[15] Moreover, 18 years' follow-up of the Minnesota guaiac FOBt trial (in which the colonoscopy rate was nearly 40%) found only a 20% reduction in CRC incidence.[13] The UK once-only FS trial[10] demonstrated only a 23% reduction in CRC incidence in the intervention group compared with the control group at 10 years. It is also clear that CRC occurs even in patients under close colonoscopic surveillance (1.7 CRCs per 1000 person-years),[16] with an estimated post-colonoscopy CRC (PCCRC) rate of between 2% and 9%.[17] Overall, only 10% of CRCs in the UK in 2013 were diagnosed within the UK screening programmes.[18] Therefore, there is still an unmet clinical need for safe and effective primary CRC chemoprevention in combination with existing screening and surveillance programmes.

## The natural history and molecular pathogenesis of colorectal carcinogenesis

The molecular pathogenesis of CRC (histopathological term: colorectal adenocarcinoma) has been the subject of several recent reviews.[4,19] In recent years, the original multistage model of cumulative genetic mutations, proceeding through a benign adenoma stage (the so-called adenoma–carcinoma sequence), based on loss of function of the 'gatekeeper' tumour suppressor gene adenomatous polyposis coli (*APC*), which was proposed by Fearon and Vogelstein,[20] has been superseded by widespread acceptance that 'sporadic' (i.e. not occurring on a background of a distinct genetic predisposition syndrome or inflammatory bowel disease) CRC is not 'one disease', but occurs via several pathogenic pathways, which are not mutually exclusive.[21,22]

The chromosomal instability (CIN) pathway is characterised by chromosomal abnormalities, including aneuploidy, usually associated with loss-of-function *APC* mutation and later-stage gain-of-function *KRAS* mutation.[23] It is exemplified by the rare genetic predisposition syndrome familial adenomatous polyposis (FAP), an autosomal dominant condition that occurs in carriers of a heterozygous germline *APC* mutation.[24] The microsatellite instability (MSI) pathway is driven by defective deoxyribonucleic acid (DNA) mismatch repair (MMR), leading to accumulation of further somatic mutations (termed MSI-high) including *BRAF*.[23] It is exemplified by Lynch syndrome, in which carriers of mutations in MMR genes (most commonly *MLH1* and *PMS2*) exhibit increased cancer (including colorectal) risk.[25] Furthermore, a CpG island methylator phenotype (CIMP) pathway is recognised in colorectal adenomas and adenocarcinomas, by which epigenetic changes in DNA methylation lead to altered gene function, in particular silencing of the MMR gene *MLH1* leading to defective MMR, which is associated strongly with *BRAF* mutation.[23] Epigenetic silencing of *MLH1* explains the substantial overlap between MSI and CIMP pathways.[4]

There are limited data on how early during colorectal carcinogenesis the above phenotypes manifest themselves.[4] There are some data to suggest that CIN features are present in adenomas.[4,26] The CIMP pathway is linked strongly to benign serrated lesions (see below).

## Colorectal adenoma

The importance of the benign precursor lesion, which exhibits epithelial cell dysplasia but not invasion of the epithelial basement membrane (termed the adenoma, or adenomatous polyp), as a risk stratification biomarker of future CRC risk but also as a clinically significant lesion (the removal of which is associated with reduced CRC incidence and mortality), has been reviewed in detail.[5]

In parallel with more nuanced understanding of the diverse molecular pathogenesis of CRC, the histopathological classification and terminology of the colorectal adenoma has been revised.[5] In particular, hyperplastic-serrated pathway lesions are now acknowledged as separate entities from the more common (conventional) dysplastic adenoma (which can be tubular, villous or mixed tubulo-villous in morphology). Serrated lesions are recognised to have malignant potential per se and may account for 20–30% of CRCs.[27]

The World Health Organization's (WHO's) *WHO Classification of Tumours of the Digestive System*[28] in 2010 included the term sessile (a term used to recognise that the vast majority of these lesions are 'flat' and not polypoid when viewed endoscopically) serrated adenoma. However, the term sessile serrated polyp is now preferred on the basis that the majority of sessile serrated lesions do not display any dysplasia, which is a prerequisite for pathological classification as an adenoma.[5] The traditional serrated adenoma is a separate, rare, fully dysplastic lesion with classical serrated appearances.[27] For the purposes of this report, the term serrated adenoma will continue to be used as the terminology employed continuously throughout the Systematic Evaluation of Aspirin and Fish Oil (seAFOod) trial from 2009 onwards.

Sessile serrated adenomas are more prevalent in the proximal (also known as right) colon (most commonly defined as proximal to the splenic flexure) than the distal (also known as left) colon.[4] Conventional tubular/tubulo-villous adenomas are more uniformly distributed throughout the right and left colon.[4] Molecular features also distinguish between conventional adenomas and serrated adenomas, with a high prevalence of CIN features in conventional adenomas and serrated adenomas commonly displaying a CIMP-high, *BRAF* mutation-positive, MSI-high phenotype.[4,21,27] Results from a study[29] of CRCs suggest that, in reality, there is likely to be a continuous positive gradient of CIMP-high, MSI-high and *BRAF* mutation frequency in tumours along the distal to proximal colon, rather than an anatomical dichotomy in the distal transverse colon. There is conflicting evidence that a given tumour genotype/phenotype predicts that of synchronous/metachronous lesions, but the majority of data pertain to CRC, not to colorectal adenomas.[4]

As direct precursor lesions of CRC, the removal of which is unequivocally associated with decreased future CRC risk,[8,9] the colorectal adenoma is a clinically important lesion in its own right.[5] It has been estimated (based on cohort prevalence studies) that approximately 1 in 10–20 colorectal adenomas may eventually acquire a malignant phenotype.[5] The features associated with malignant progression are size, grade of dysplasia and 'villousness', namely the degree of villous histological architecture in an individual lesion.[5] On the other hand, the colorectal adenoma can also be considered a biomarker of future CRC risk, regardless of its individual malignant potential.[5] Both colorectal adenoma number and colorectal adenoma size are widely used as the basis for future CRC risk stratification for surveillance after colonoscopy in the UK and elsewhere in the world.[30–33] A common feature of guidelines is the definition of the 'advanced' colorectal adenoma based on size (≥ 10 mm), with or without additional histological (e.g. grade of dysplasia, 'villousness') features.[30,31,33]

### Colorectal adenoma measures

Based on widespread acceptance of the number and size of colorectal adenomas as a CRC risk biomarker, the colorectal adenoma has been used as a surrogate colonoscopic end point of reduced CRC risk in chemoprevention trials following polyp clearance at an index procedure (the 'polyp-prevention trial'). Historically, the presence or absence of any colorectal adenoma [the so-called adenoma detection rate (ADRa)] has been employed as the primary end point in chemoprevention trials, with reliance on this binary end point reflecting the varying quality of colonoscopy between different endoscopists and susceptibility of colorectal adenoma detection to observer variation.[5,34–36] However, this percentage value does not take into account any change in colorectal adenoma number (or size), unlike pre-clinical rodent studies and proof-of-concept clinical studies in FAP patients, in which lesion number and size are routinely measured.[37] More recently, driven by the dramatic improvement in colonoscopy quality and quality assurance (QA) reporting,[38] colorectal adenoma number has begun to be reported as a primary outcome in 'sporadic' polyp-prevention trials as the mean adenomas per participant (MAP).[39] Population-based studies have consistently demonstrated that colorectal adenoma multiplicity predicts future CRC incidence and mortality.[40–42]

### Candidate colorectal cancer chemoprevention agents

The existing literature on several potential CRC chemoprevention agents, including non-steroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy and micronutrients (e.g. folic acid, vitamin D), is well summarised in published reviews.[34,36] The largest body of evidence supports the use of the NSAID aspirin for CRC chemoprevention.[43,44]

## Aspirin

Observational and randomised controlled trial (RCT) data[44,45] suggest primary prevention efficacy of low-dose (usually defined as < 325 mg daily) aspirin; these data are summarised in comprehensive reviews. In brief, observational follow-up studies of historical RCTs of aspirin (variable dose: 75–1000 mg daily) for antithrombotic indications have reported that aspirin reduces the risk of CRC incidence [hazard ratio (HR) 0.75, 95% confidence interval (CI) 0.56 to 0.97] and mortality, with a lag period of approximately 8 years, compatible with the long natural history of 'sporadic' colorectal carcinogenesis.[46,47] A similar phenomenon was also observed in the Women's Health Study,[48] which is the only placebo-controlled RCT of aspirin (i.e. 100 mg of aspirin taken on alternate days) with a primary CRC prevention end point. Original 10-year follow-up did not reveal any effect on CRC incidence, but reduced CRC incidence (HR 0.80, 95% CI 0.67 to 0.97) emerged after longer follow-up.[48] A consistent finding from these RCTs has been the differential effect of aspirin on proximal, as opposed to distal, CRC, with the risk reduction associated with aspirin being primarily for proximal CRC.[47,48]

Consistent with its role as an established biomarker of CRC risk, a random-effects meta-analysis[49] of four previous polyp-prevention RCTs of aspirin (using daily doses varying from 81 mg to 325 mg), using ADRa as the primary outcome measure, reported a pooled risk ratio for 'advanced' colorectal neoplasm or any size of colorectal adenoma in aspirin users of 0.72 (95% CI 0.57 to 0.90) and 0.83 (95% CI 0.72 to 0.96), respectively, an effect that was already apparent at colonoscopy in the first year of follow-up in these studies (risk ratio 0.62, 95% CI 0.48 to 0.81). Three of these aspirin RCTs reported MAP data as a secondary outcome, and all demonstrated a consistent reduction in the MAP value associated with aspirin use.[49]

Aspirin (900 mg) has also been shown to reduce CRC (and other cancer) risk in Lynch syndrome.[50] Chemopreventive efficacy of aspirin in Lynch syndrome was not associated with reduced colorectal adenoma risk during routine surveillance colonoscopy follow-up in a non-screening programme setting.[51]

However, despite the strength of the evidence that regular, long-term aspirin use prevents CRC, aspirin has not yet been widely adopted for primary or secondary CRC chemoprevention because of continuing uncertainty about the optimal daily dose [different trials have reported efficacy of either high- (> 300 mg) or low-dose (< 100 mg) aspirin[49]] and the absence of a clearly defined at-risk population in whom benefit would outweigh the small risk of gastrointestinal (GI) and intracerebral bleeding associated with aspirin.[44,52,53] Nevertheless, the US Preventive Services Task Force[52] has recommended low-dose aspirin use for the primary prevention of cardiovascular disease (CVD) and CRC in some adults aged 50–59 years who have a 10-year CVD risk of $\geq 10\%$.

## Selective cyclooxygenase-2 inhibitors

Consistent with the role of cyclooxygenase (COX)-2-dependent prostaglandin (PG) $E_2$ in the early stages of colorectal carcinogenesis,[54] selective COX-2 inhibitors (i.e. celecoxib and rofecoxib) displayed significant chemopreventive efficacy in RCTs in FAP patients (20–30% reduction in polyp number and size)[55] and in individuals with previous 'sporadic' colorectal adenoma (risk reduction of approximately 20%).[34] However, the unexpected CVD toxicity associated with prolonged selective COX-2 inhibition, which became apparent in the polyp-prevention trials, precludes a role for selective COX-2 inhibitors in primary 'sporadic' CRC chemoprevention.[56]

## Omega-3 polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) are important components of a normal diet. Two classes of PUFAs, ω-6 and ω-3, are classified as essential in that they cannot be readily synthesised in the human body and so must be obtained from dietary sources.[57] The principal bioactive ω-3 PUFAs are C20:5ω-3 [in CX:Yω-Z, X denotes the number of carbon atoms; Y denotes the number of carbon–carbon double bonds and Z denotes the carbon atom from which the first double bond starts from the ω (methyl) end], eicosapentaenoic acid (EPA) and C22:6ω-3 docosahexaenoic acid (DHA), which are found predominantly in oily, cold-water fish such as mackerel, having entered the food chain following synthesis by plankton and algae.[57] In addition, C18:3ω-3 alpha-linolenic acid (ALA) is found in vegetables, but can be converted to EPA and then DHA (by a series of

elongases and desaturases[58]) only very inefficiently (approximately 5%) in humans.[59] In 'Western' diets, ω-6 PUFAs dominate, including C20:4ω-6 arachidonic acid (AA), which is the predominant substrate for the COX enzymes in humans.[57]

### Anticolorectal cancer activity of eicosapentaenoic acid

Eicosapentaenoic acid is an attractive candidate as a 'natural' CRC chemoprevention agent based on several strands of evidence.[60] There is strong pre-clinical evidence that ω-3 PUFAs have anti-CRC activity.[61] However, a systematic review of epidemiological studies has not demonstrated unequivocal benefit from dietary ω-3 PUFA intake on CRC risk.[62] This may be related to the methodological difficulties of measuring ω-3 PUFA or fish intake retrospectively. Alternatively, ω-3 PUFA exposure may not be sufficient for consistent anti-CRC activity in individuals consuming moderate amounts of fish (a portion of oily fish two or three times per week provides only the equivalent of approximately 500 mg per day of EPA and DHA combined). Omega-3 PUFA intake can be increased by 'over-the-counter' fish oil supplements, which contain a complex mix of ω-3 and ω-6 PUFAs.[63] However, many of these supplements are associated with a range of minor, troublesome side effects [e.g. eructation (burping), halitosis]. In the prospective VITAL (VITamin And Lifestyle) cohort study, which has uniquely collected data on fish oil supplement use, as well as dietary fish intake, fish oil supplement users were shown to have a 49% reduced CRC risk compared with non-users, an effect primarily observed in men.[64]

Purified and concentrated EPA is available in several forms and pharmaceutical formulations.[65,66] EPA alone (without DHA) is available as the free fatty acid (FFA), as a triglyceride (TG) conjugate (the predominant natural form of EPA) or as an ethyl ester (EE) conjugate.[65] Dietary EPA-TG is converted to EPA-FFA in the small intestine by the action of pancreatic lipase, which is released in response to (particularly fatty) food intake. It is unclear which form of EPA is absorbed best from the small intestine and has maximal bioavailability, especially during prolonged use.[65,66] Administration of EPA with food maximises absorption of all forms of EPA.[65] A 500-mg gastroresistant capsule formulation of 99% pure EPA as the FFA has been produced (SLA Pharma AG, Liestal, Switzerland). This formulation was used for the administration of 2 g of EPA-FFA daily as four capsules in the RCT in FAP patients described below.[67] Alternative formulations of purified EPA exist, including a 574-mg formulation of 90% EPA-TG (equivalent to 400 mg of EPA-FFA) in a soft gelatin capsule (Igennus Healthcare Nutrition, Cambridge, UK) that can be used to provide the equivalent 2-g daily dose of EPA-FFA in five capsules.

Eicosapentaenoic acid in all three forms (i.e. FFA, TG and EE) has been demonstrated to have chemopreventive activity in several rodent models of colorectal carcinogenesis, including azoxymethane-induced intestinal tumorigenesis and the *Apc*[Min/+] mouse model of FAP.[61,68,69] Preliminary evidence that EPA has chemopreventive efficacy in humans was provided by two separate Phase II studies of 2 g of EPA-FFA daily in patients with previous colorectal adenoma(s), which demonstrated a significant reduction in rectal epithelial cell mitosis frequency (not observed with a 1-g daily dose), which was associated with a fivefold increase in rectal mucosal EPA content.[70,71] These studies led to a Phase III RCT of the effect of 2 g of EPA-FFA daily for 6 months on rectal polyposis in patients with FAP (*n* = 58).[67] This RCT provided the first definitive evidence of chemopreventive efficacy of EPA in humans, with a net decrease in rectal adenoma number and cumulative rectal adenoma size of 22.4% and 29.8%, respectively, between the EPA and placebo groups.[67] The percentage reduction in adenomatous polyp burden was similar to that observed in FAP patients treated with celecoxib,[55] a drug that was subsequently demonstrated to prevent 'sporadic' colorectal adenomas.[34] In 2012, high dietary intake of marine-derived ω-3 PUFAs was associated with reduced colorectal adenoma risk.[72] The protocol for a RCT of 2.7 g of EPA daily for prevention of rectal aberrant crypt foci was published in 2012 (UMIN000008172), but the trial has yet to report results.[73]

### Mechanisms of the antineoplastic activity of eicosapentaenoic acid and aspirin

The precise mechanism(s) by which aspirin and EPA have anti-CRC activity is not fully understood.[43,45,61] However, it is currently accepted that, even though these agents are likely to act via multiple COX-dependent and COX-independent mechanisms, modulation of COX activity plays an important role in their antineoplastic effects. EPA and, particularly, aspirin are both potent inhibitors of COX-1, but they alter COX-2 activity in

different ways, leading to the production of different bioactive lipid mediators, including PGE$_3$ (EPA) and 15$R$-HETE (hydroxyeicosatetraenoic acid) (aspirin).[57] There is some evidence that PGE$_3$ (unlike pro-tumorigenic PGE$_2$) has antitumorigenic activity[74] and it is known that aspirin-triggered lipoxins derived from 15$R$-HETE have antiangiogenic properties.[75]

Aspirin irreversibly acetylates the COX enzymes.[75] When EPA acts as a substrate for aspirin-acetylated COX-2, it leads to synthesis of 18$R$-hydroxyeicosapentaenoic acid (18$R$-HEPE), which can be converted in a 5-lipoxygenase-dependent manner to resolvin (Rv) E1.[75,76] RvE1 has potent anti-inflammatory activity,[76] but it is currently not known whether or not RvE1 has direct antineoplastic activity.[77] Specialised pro-resolving (lipid) mediators, such as resolvins and lipoxins, including RvE1, are technically difficult to measure in biological samples and are likely to exert any biological activity at trace concentrations;[77] therefore, it remains unclear whether or not sufficient quantities are generated in humans to have meaningful antineoplastic activity.[78]

Although RvE1 synthesis provides a hypothesis for a potential interaction between EPA and aspirin, the available clinical evidence suggests that the antiplatelet (COX-1-dependent) effects of EPA and aspirin are simply additive based on the accumulated evidence of extensive use of dual therapy in cardiology patients[79] and the effects of the two agents in ex vivo human platelet aggregation studies.[80,81] Colorectal carcinogenesis and atherosclerosis share common pathophysiological mechanisms and clinical risk factors, including obesity.[82] As a consequence, ischaemic heart disease and stroke are common in elderly populations with colorectal neoplasia.[82] Therefore, an attractive feature of CRC chemoprevention using EPA and/or aspirin is the potential for additional vascular benefit in elderly colorectal adenoma 'formers' at simultaneous risk of occlusive vascular events.[43,79]

### A precision-medicine approach to colorectal cancer chemoprevention

A precision or stratified medicine approach to chemoprevention, whereby the need for chemoprevention and the use of a specific agent is determined based on an individual benefit–risk assessment, has yet to be realised.

The preliminary finding of the APACC polyp-prevention trial,[83] that the pattern of COX-2 expression in an index colorectal adenoma predicted the preventative efficacy of aspirin, suggests that baseline colorectal adenoma characteristics have potential as predictive biomarkers of individual chemoprevention efficacy.

Red blood cell (RBC) membrane ω-3 PUFA levels (as a validated surrogate biomarker of ω-3 PUFA tissue exposure[84,85]) have been long established as a biomarker of dietary ω-3 PUFA exposure in cancer epidemiological studies.[86] Between 2014 and 2016, ω-3 PUFA levels were used in RCTs of ω-3 PUFAs as a biomarker of target tissue ω-3 PUFA exposure (termed 'bioavailability' here), but also as a possible indicator of compliance and/or placebo group 'contamination' by over-the-counter (OTC) ω-3 PUFA use.[67,87,88]

In a RCT of EPA-FFA in patients with CRC liver metastasis, tumour EPA content predicted exploratory survival outcomes.[88] However, there was no relationship between the individual rectal mucosal EPA content and the reduction in rectal polyp number in the small RCT of EPA in FAP patients.[67] Therefore, there is a need for further evaluation of RBC and colorectal mucosal ω-3 PUFA levels, as well as novel biomarkers based on the mechanism of action of EPA, as predictors of individual therapeutic response.

There are no validated biomarkers of aspirin anti-CRC activity. However, all the COX-dependent lipid mediators described earlier are measurable by liquid chromatography–mass spectrometry (LC-MS)[77,89] and may find utility as therapeutic biomarkers.[77]

### Safety and tolerability of eicosapentaenoic acid and aspirin

Aspirin and ω-3 PUFAs are already used widely in patient populations, that are relevant to 'sporadic' CRC prevention, for prophylaxis following myocardial infarction (aspirin and ω-3 PUFAs), hypertriglyceridaemia (ω-3 PUFAs) and stroke (aspirin).[79]

The safety and tolerability of aspirin ($\leq$ 325 mg daily) in previous polyp-prevention trials has been excellent.[34,49] Aspirin use is associated with a dose- and age-dependent increased risk of upper GI and intracranial bleeding.[45,90] Cuzick et al.[53] have put forward the case for a favourable benefit–risk profile for aspirin dosing of $\leq$ 325 mg daily for 10 years for primary CRC (and other adenocarcinoma) prevention in average-risk individuals aged 50–65 years.

There is little doubt about the safety and tolerability of 'nutraceutical' forms of EPA, confirmed by vast experience of intake in healthy human populations.[91–93] Gastroresistant EPA-FFA of 2 g daily has been compared with placebo for up to 6 months in four RCTs, in which tolerability has been excellent.[67,70,71,87] In the RCT involving FAP patients, there was no significant excess of adverse events (AEs) in the EPA-FFA group compared with the placebo group, with only one patient withdrawing from the EPA-FFA group as a result of nausea and epigastric pain.[67] In two Phase II studies of colorectal adenoma patients, there was a slight excess of mild to moderate AEs in the EPA-FFA group compared with the no-treatment[70] and placebo groups.[71] In the latter study,[71] the GI AEs observed in the EPA-FFA 2-g daily group were not apparent in those taking 1 g of EPA-FFA daily.[71] EPA-TG may be associated with fewer GI AEs, particularly diarrhoea, than EPA-FFA.[66] However, a formal comparison of tolerability between different EPA formulations in a RCT has not yet been undertaken.

Although aspirin and ω-3 PUFAs share antiplatelet activity and both agents prolong bleeding time, excess bleeding episodes with their combined use have not been observed in cardiological practice, in which they are widely used together following myocardial infarction.[79,94] However, clinically significant bleeding events associated with treatment with EPA either alone or in combination with aspirin have, to date, not been monitored in a RCT.

### The NHS Bowel Cancer Screening Programme

The NHS Bowel Cancer Screening Programme (BCSP) in England began in 2006. It is currently based on a biennial guaiac FOBt targeted at all individuals aged 60–74 years who are covered by NHS registration data in England (the uptake, based on a returned FOBt kit, is approximately 50–60%).[95] Individuals with an abnormal FOBt ($\approx$2%) are invited for colonoscopy via a specialist screening practitioner (SSPr)-run clinic. All colonoscopy is undertaken by screening-accredited colonoscopists working within a continuous QA framework based on multiple measures, including individual caecal intubation rate, withdrawal time and ADRa.[38,95] Recording of endoscopic findings and subsequent histopathological assessment is also directed by BCSP guidelines and a QA reporting system.[96,97] Any abnormality detected is discussed with the patient at a SSPr follow-up clinic. Detection of a CRC ($\approx$10%, but variable dependent on the number of prevalent vs. incident screening investigations) prompts further management by the local multidisciplinary team (MDT) for CRC. Detection of one or more colorectal adenomas prompts surveillance colonoscopy within the BCSP, as per British Society of Gastroenterology guidelines.[30] Individuals classified as being at 'low risk' (i.e. those having one or two subcentimetre colorectal adenomas) are not offered colonoscopy, but remain in the biennial guaiac FOBt programme. Those individuals with three or four small colorectal adenomas (i.e. < 10 mm in size) or one colorectal adenoma of $\geq$ 10 mm in diameter are classified as being at 'intermediate risk' and are offered another colonoscopy at 3 years from the index procedure. Individuals with five or more subcentimetre colorectal adenomas, or three colorectal adenomas with at least one colorectal adenoma of $\geq$ 10 mm in diameter (i.e. 12% of men and 6.2% of women who undergo screening colonoscopy), are recommended to undergo surveillance colonoscopy 12 months from the screening colonoscopy.[98]

Since 2013, the bowel scope programme has been rolled out across England, whereby, in addition to the biennial guaiac FOBt invitation, a single FS is offered to all individuals aged 55 years.[99] The presence of a colorectal adenoma of $\geq$ 10 mm in diameter, three or more small (i.e. < 10 mm) colorectal adenomas or any adenoma with 'advanced' features prompts an invitation for full colonoscopic evaluation, with the combined colorectal adenoma findings from the FS and colonoscopy directing the subsequent surveillance strategy within the BCSP, as described above.[95]

### The seAFOod polyp-prevention trial

Based on strong proof of concept for primary CRC chemoprevention activity of EPA[67] and aspirin,[49] the National Institute for Health Research (NIHR)/Medical Research Council (MRC) Efficacy and Mechanism Evaluation (EME) programme funded a 2 × 2 factorial RCT of 2 g of EPA-FFA daily and/or 300 mg of aspirin in 'high-risk' individuals identified in the English BCSP. The trial was termed the seAFOod polyp-prevention trial.[100]

## Main research question

Does the ω-3 PUFA EPA prevent colorectal adenomas, either alone or in combination with aspirin?

## Objectives

### Primary objective

The primary objective was to determine whether or not EPA prevents colorectal adenomas, either alone or in combination with aspirin. This was addressed by testing the following hypotheses:

- 2 g of EPA-FFA daily is more effective than placebo for reduction in colorectal adenoma recurrence.
- 300 mg of aspirin daily is more effective than placebo for reduction in colorectal adenoma recurrence.

### Secondary objectives

The secondary objectives were to assess the tolerability and safety of EPA-FFA and EPA-TG alone and in combination with aspirin.

# Chapter 2 Methods

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The trial protocol has been published in open-access form.[100]

## Trial design

The seAFOod polyp-prevention trial was a randomised, blinded, placebo-controlled 2 × 2 factorial trial. The trial was designed to integrate fully into the screening and surveillance phases of the BCSP in England so that participation would not alter routine clinical practice.

Participants were randomised to one of four groups to receive EPA-FFA or EPA-TG (both 2 g of FFA equivalent daily) daily with food, or identical placebo, AND 300 mg of enteric-coated aspirin daily taken with food, or identical placebo, until the day before surveillance colonoscopy (at 12 months). The primary outcome was the number of individuals with one or more colorectal adenomas at the surveillance colonoscopy (the ADRa). Secondary outcomes included the total number of colorectal adenomas per participant, subtype (advanced, conventional, serrated, left and right) of adenomas (ADRa and number), the number of participants reclassified as being at intermediate risk for future surveillance, EPA and other PUFA levels in RBCs and rectal mucosa, dietary fish intake and assessment of the tolerability and safety of EPA (both FFA and TG formulations) alone and in combination with aspirin.

## Trial setting and participants

### NHS Bowel Cancer Screening Programme recruiting sites

The English BCSP is organised into local centres of a variable number (usually 1–3) of individual BCSP sites (hospitals undertaking endoscopy), which receive referrals for screening colonoscopy after guaiac FOBt analysis at five regional hubs. Participants identified as 'high risk' at participating sites were randomised and followed to surveillance colonoscopy at 12 months. The trial was integrated into the BCSP to utilise routine clinical pathways in order to collect quality-assured data from screening and surveillance colonoscopies.

During the trial, 61 BCSP sites were opened. The date of the first participant, first visit (FPFV) was 11 November 2011; the last participant in was 10 June 2016; and the last participant, last visit (LPLV) was 8 June 2017. Despite approval from the BCSP Research Advisory Committee, widespread engagement from SSPrs and Clinical Research Network (CRN)-funded research nurses (RNs) and a trial extension in 2014, the trial did not recruit to target, recruiting 709 participants against a revised target of 755. The sample size calculation remained at 853, based on the predicted effect size of the interventions, giving 80% power. However, recruitment figures achieved prior to the 2014 extension, the limited recruitment period mandated by the funder and the limitations set by the expiry date on the capsule (EPA-TG) investigational medicinal product (IMP) suggested that recruitment of 755 individuals would be feasible in the extended intervention period (see *Statistical methods*).

The original strategy was to open 15 recruiting sites from two BCSP hubs (North-East and Eastern). This was based on data from the national BCSP database that suggested that each BCSP centre could identify approximately 50 high-risk patients per year. Trial screening data supported this assumption; however, the eligibility rate was much lower than expected, primarily because of a higher than expected number

of screened high-risk individuals who were excluded because of the need for repeat colonoscopy or FS to check for adenoma excision within a 3-month window. Analysis of up-to-date BCSP data in early 2012 revealed that the number of cases requiring repeat endoscopy as part of routine BCSP care had increased nationally during the grant application and set-up phases of the trial. It was determined that a second colonoscopy or FS did not significantly alter the overall ADRa at the 12-month surveillance colonoscopy (see *Changes to the protocol*). Therefore, the protocol was amended (version 4.0, dated 24 May 2012)[100] to include these patients, without loss of statistical power.

A trial site expansion strategy was also implemented in 2012, increasing the number of recruiting sites across England, from Cornwall to Cumbria, to 60 (representing ≈50–60% of English BCSP centres). In late 2015, a decision was made to add one further site that had expressed a strong interest to be involved in the trial.

Delays were experienced in gaining NHS trust research and innovation (R&I) approvals as a result of a general lack of Good Clinical Practice accreditation and research training for BCSP staff, many of whom had not previously contributed to a Clinical Trial of an Investigational Medicinal Product (CTIMP). Sites were supported by the Nottingham Clinical Trials Unit (NCTU) and local CRNs to access training. The median time to gain R&I approval at trial sites was 11.5 (range 4–19) weeks. In addition to a site initiation visit, supplementary training was provided to sites by the co-ordinating centre for participating local investigators, RNs and SSPrs via an instructional video.

In February 2014, recruitment was disrupted significantly when the manufacturer of the original capsule IMP (EPA-FFA) was no longer able to provide stock for the trial. Until an alternative capsule IMP could be identified, approved, manufactured and distributed, sites continued to recruit until local stock was exhausted, at which point that site temporarily suspended recruitment. To maximise recruitment during this period, the top eight most active sites were prioritised for allocation of remaining central stock of capsule IMP. Stock management also ensured that all participants completed the intervention phase of the trial using the same EPA formulation (FFA or TG). This strategy enabled the trial to continue recruiting between February and October 2014, after which a new capsule IMP became available. Partly because of this delay, a 36-month extension was approved (in October 2014) by the NIHR EME board in order to complete trial recruitment.

## Identification of participants

Individuals identified as 'high risk' at screening colonoscopy on the basis of colorectal adenoma number and (endoscopic) size, and confirmed later by the histopathology report, were screened for the trial and approached by a member of the site research team to determine whether or not they were interested in trial participation. For those participants who had a bowel scope FS, summated colorectal adenoma findings were used to define individuals as 'high risk'.

All 'high-risk' participants were given written trial information on discharge by a BCSP SSPr or RN. They were provided with a patient information leaflet (PIL), given a verbal explanation of the trial and given the opportunity to ask questions.

Participants were able to discuss the trial with their family, friends and/or health-care professionals before they attended a routine BCSP outpatient follow-up visit 7–14 days after screening colonoscopy. All participants provided written informed consent.

## Eligibility criteria

Patients were eligible for inclusion if they were aged 55–73 years and were a BCSP participant identified as 'high risk' (i.e. they had five or more small colorectal adenomas or three or more colorectal adenomas with at least one being ≥ 10 mm in diameter) at the first complete screening colonoscopy. This included participants who were identified as 'high risk' at colonoscopy after FOBt screening, or who were deemed 'high risk' on the basis of the combined findings from a bowel scope FS and subsequent full colonoscopy. If the first screening colonoscopy was defined as complete, the participant was immediately stratified as 'high risk'. If the first colonoscopy was incomplete, that individual was required to have a second colonoscopy to complete

the initial examination, after which both procedures were added together as the screening colonoscopy result for the purposes of BCSP surveillance and trial eligibility.

Patients were excluded from the trial if:

- They had a requirement for more than one repeat colonoscopy or FS within the BCSP 3-month screening window (see *Changes to the protocol*).
- They had a malignant change in a colorectal adenoma requiring management by a CRC MDT.
- They were regularly (i.e. more than three doses per week) taking prescribed or OTC aspirin or regularly (i.e. more than three doses per week) taking prescribed or OTC non-aspirin NSAIDs. This was not an exclusion criterion if the drug was self-prescribed and not recommended by a doctor and if the individual was willing to stop taking it for the duration of the trial.
- They had aspirin intolerance or hypersensitivity, including aspirin-sensitive asthma.
- They had had active peptic ulcer disease within 3 months or previous peptic ulcer (and were not on proton pump inhibitor prophylaxis).
- They had a fish or seafood allergy.
- They used or were planning to regularly use (i.e. more than three doses per week) fish oil supplements. This was not an exclusion criterion if the supplements were self-prescribed and not recommended by a doctor and if the individual was willing to stop taking them for the duration of the trial.
- They had a known clinical diagnosis or were a gene carrier of a hereditary CRC predisposition (e.g. FAP).
- They had Lynch syndrome, also known as hereditary non-polyposis colon cancer (HNPCC).
- They had a previous or planned colorectal resection.
- They had known bleeding diathesis or concomitant warfarin therapy or use of any other anticoagulant or antiplatelet agent.
- They had severe liver impairment.
- They had severe renal failure (i.e. creatinine clearance of < 10 ml/minute).
- They currently used methotrexate at a weekly dose of ≥ 15 mg.
- They were not able to comply with trial procedures and IMP use.
- They had a serious medical illness interfering with trial participation.
- They were taking part in another interventional clinical trial.
- They failed to give written informed consent.

Responsibilities for checking eligibility and obtaining informed consent could be delegated to the SSPr or RN according to site approvals, but eligibility was confirmed by the local principal investigator (PI) for all participants.

## Changes to the protocol

Soon after FPFV on 11 November 2011, it became apparent from screening log activity at individual BCSP sites that the eligibility rate was ≈15–20%, rather than the 60% that had been predicted in the original recruitment projection. A contributing factor was the higher than expected use of other non-aspirin antiplatelet agents, such as clopidogrel, which was later added as an exclusion criterion in January 2012 (protocol version 3.1, dated 12 January 2012). Another major contributing factor was the larger than expected number of 'high-risk' individuals (≈25%) who required a repeat endoscopy after randomisation. Repeat endoscopy was originally an exclusion criterion because the primary end point might be confounded by colorectal adenoma detection and removal at an extra endoscopic procedure between the screening (index) and 1-year surveillance colonoscopies. However, subsequent analysis in April 2012 of 1189 'high-risk' patients who underwent 1-year surveillance colonoscopy in 2010 in 26 BCSP centres did not support this notion. The overall ADRa at surveillance colonoscopy [including those who underwent repeat partial colonoscopy or FS (ADRa 54%), repeat full colonoscopy (ADRa 67%) or no repeat procedure (ADRa 63%)] was 62%, which was consistent with the value (60%) used in the original sample size calculation. Therefore, the protocol was amended to allow recruitment of those individuals who required no more than one repeat endoscopic procedure [either colonoscopy (full or partial) or FS] within a 3-month screening episode window (version 4.0, dated 24 May 2012).[100] A number of other changes to details contained within the protocol are

documented in the statistical analysis plan (SAP) (version 1.1, dated 24 August 2017). These are listed in *Statistical methods*.

During trial recruitment, the trial was included as part of a larger MRC-funded programme of research [Systematic Techniques for Assisting Recruitment to Trials (START)] to assess web-based recruitment strategies. A separate protocol was approved for the cluster randomised (by site) substudy of a web-based information tool and the PIL was updated accordingly. Details of this study and the seAFOod trial contribution will be reported separately by the MRC-START study team.

## Trial procedures

Trial procedures were performed as shown in *Figure 1*.
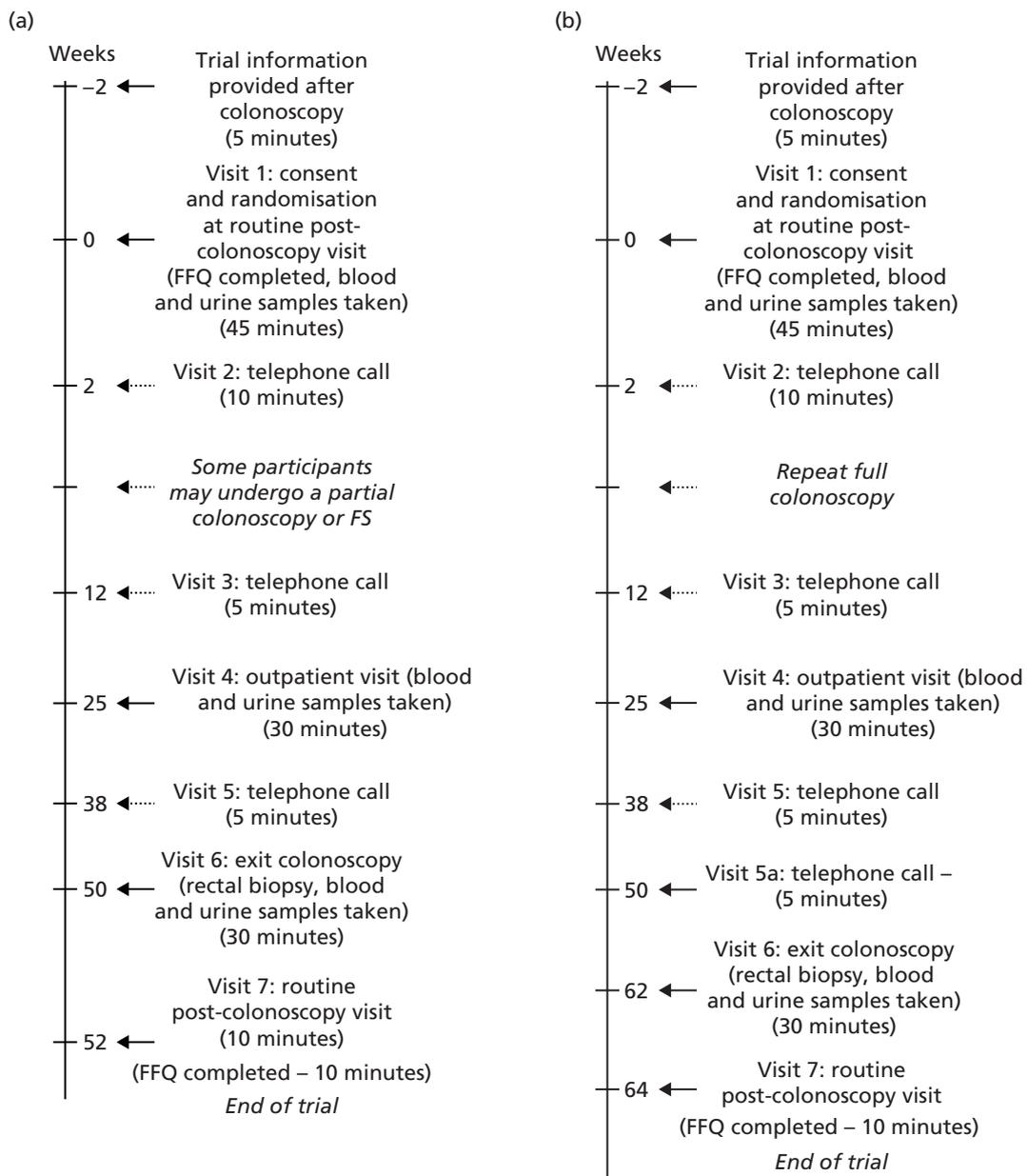


**FIGURE 1** The seAFOod trial participant pathway. (a) Participants whose visit 6 date was scheduled from the first complete colonoscopy; and (b) participants whose visit 6 date was scheduled from a repeat full colonoscopy. FFQ, Food Frequency Questionnaire.

### Baseline visit

Individuals attending a routine outpatient follow-up BCSP appointment to obtain results of the screening colonoscopy were approached. Individuals who were eligible and willing to take part in the trial were asked to provide written informed consent. Demographic information and details of participants' medical histories were collected and participants were randomised. It was preferred that participants were randomised within 4 weeks of the first complete BCSP screening colonoscopy. However, to maximise recruitment, randomisation was allowed outside this time window as long as it was recorded on the protocol deviation log. A prescription was issued for the supply of IMP for 6 months. The local hospital pharmacy dispensed the trial treatment. A second prescription for a further 6 months of IMP was provided at visit 4.

Biological samples were taken, comprising a blood sample [2 × 6-ml $K_2$EDTA (ethylenediaminetetraacetic acid) Vacutainer® tubes; Becton, Dickinson and Company (BD), Franklin Lakes, NJ, USA] and a urine specimen of 5–10 ml. Samples were taken only if the participant provided separate, specific consent for collection of blood and urine.

In addition, participants were asked to complete a pre-treatment Food Frequency Questionnaire (FFQ) so that any change in dietary ω-3 PUFA intake during trial involvement could be determined.

### Repeat endoscopy

Participants were informed whether or not a repeat colorectal endoscopic examination was required at the baseline visit. This was one of the following: a second full colonoscopy, a partial colonoscopy (planned incomplete views of the colorectum) or a FS. Participants undergoing one repeat endoscopy within 3 months of the screening colonoscopy remained eligible for the trial. The results of the colonoscopy were collected at visit 3. These participants were in the intervention period of the trial for a maximum of 15 months, rather than 12 months, so an additional 3-month trial prescription was dispensed to cover this period. If a participant who had already undergone a repeat endoscopy had a further repeat procedure scheduled by the local BCSP team, this made him or her ineligible for the trial.

All participants were asked to commence the IMP immediately following consent at the baseline visit (visit 1). Those participants who were due to undergo a repeat endoscopy procedure temporarily stopped IMP 10 days prior to the endoscopic procedure and restarted IMP 4 days after the endoscopy. Participants who underwent a partial colonoscopy or FS had a surveillance colonoscopy 12 months after the first complete screening colonoscopy, as per BCSP guidelines. Participants who underwent a second full colonoscopy had a surveillance colonoscopy date booked as per BCSP team preference, but preferably dated 12 months after the first screening colonoscopy.

### Visits 2 and 3: telephone calls at 2 and 12 weeks

Participants were contacted by the SSPr/RN by telephone at 2 and 12 weeks after starting trial treatment. Participants were asked about any symptoms or new medical problems since the last contact and were reminded to take the IMP as directed.

Participants who were due to undergo a repeat colorectal endoscopic procedure between visits 2 and 3 were reminded to discontinue IMP temporarily. Colonoscopic findings at the repeat procedure were collected and recorded in the same way as for the baseline visit.

### Visit 4: outpatient visit

At 6 months, participants were invited to attend the BCSP site, at which time mid-treatment blood and urine specimens were collected from those who had provided consent. Participants were asked about any symptoms or new medical problems since the last contact. Any unused trial treatment from the first prescription was collected and counted. Each participant then received a new prescription for trial treatment for a further 6 months.

### Visit 5: telephone call

A third telephone contact was conducted with participants at 38 weeks after starting the trial treatment. The SSPr/RN asked about any symptoms or new medical problems since the last contact and reminded the participant to take trial treatment as directed.

### Visit 5a: telephone call

An extra telephone contact was conducted for participants who had a repeat full colonoscopy between visits 2 and 3 after starting the trial treatment. The SSPr/RN asked about any symptoms or new medical problems since the last contact and reminded the participant to take his/her trial treatment as directed. The SSPr/RN liaised with the hospital pharmacy about delivery of a third dispensing of IMP to these participants. The relevant participants then received a new prescription for trial treatment for a further 3 months.

### Visit 6: surveillance colonoscopy

Participants attended for routine surveillance colonoscopy at 12 months from the date of the screening colonoscopy. Participants took the final dose of trial treatment on the day before surveillance colonoscopy. Blood and urine specimens were obtained, as well as four random biopsies of macroscopically normal rectal mucosa (at least 2 cm from any polyp) at the end of the surveillance colonoscopy.

Colorectal adenoma outcomes at the 12-month surveillance colonoscopy were collected as per usual BCSP practice, including the number, size (maximum dimension in mm from the histopathology report, or the endoscopic size if the adenoma was not retrieved or was removed by hot biopsy), site [proximal to the splenic flexure (right) or at/distal to the splenic flexure (left)], histological type (tubular/tubulo-villous, villous, serrated) and presence of high-grade dysplasia of all colorectal adenomas.

### Visit 7: routine post-colonoscopy visit

Participants were seen after surveillance colonoscopy as part of routine BCSP follow-up, during which a second FFQ was completed. Participants had the option to complete the FFQ at visit 6 or over the telephone if they decided to receive colonoscopy results by telephone.

### Randomisation

Participants were registered in the trial using a secure web-based randomisation system. Randomisation was based on a computer-generated, internet-based treatment assignment determined by a pseudo-random code using random permuted blocks of randomly varying size, created by NCTU. Participants in the trial were allocated with equal probability to either treatment group. It was planned to stratify by BCSP centre. However, after database lock, it was discovered that BCSP site had been used, rather than BCSP centre. As sites could be associated only with an individual BCSP centre, this still ensured balance between centres.

Participants were randomised to a simple 2 × 2 factorial design (*Table 1*) to:

- 2 g of EPA-FFA, or an equivalent FFA dose of 90% EPA-TG (2780 mg), daily by mouth, or their identical placebos (capric and capryllic acid medium-chain TGs for both formulations)

in addition to:

- 300 mg of enteric-coated aspirin daily by mouth (as one 300-mg tablet taken with food) or identical placebo.

The sequence of treatment allocations was concealed until interventions had all been assigned and recruitment, data collection and all other trial-related assessments were completed. The actual allocation was not divulged to either the staff at the BCSP site or the participant. The trial prescription produced by the randomisation system referenced specific trial treatment containers. The trial drug prescription was signed by the local PI or a co-investigator, as defined by the site delegation log.

**TABLE 1** The seAFOod trial 2 × 2 factorial design

| | |
|---|---|
| • 2 g of EPA-FFA, or equivalent FFA dose of EPA-TG<br>• 300 mg of aspirin | • 2 g of EPA-FFA, or equivalent FFA dose of EPA-TG<br>• Placebo aspirin |
| • Placebo EPA<br>• 300 mg of aspirin | • Placebo EPA<br>• Placebo aspirin |

## Interventions

The IMPs used in this trial were gastroresistant capsules of 99% pure EPA in the FFA form (EPA-FFA), 90% EPA as the TG conjugate (EPA-TG) in soft gelatin capsules, enteric-coated aspirin tablets and their identical placebos.

In February 2014, the supplier of the EPA-FFA IMP (SLA Pharma AG) disclosed that it could no longer provide further capsule IMP to the trial. The Trial Management Group (TMG), NIHR EME programme and the Trial Steering Committee (TSC) made the decision to continue the trial using an alternative EPA formulation and identical (medium-chain TG) placebo. Close consultation with the Medicines and Healthcare products Regulatory Agency (MHRA) and the Research Ethics Committee (REC) was also undertaken. A substantial amendment (number 14) was approved by the REC on 26 August 2014 and the trial received a Clinical Trials Authorisation (CTA) from the MHRA on 29 August 2014 for a new capsule IMP (EPA-TG). The new formulation maintained FFA equivalence (2000 mg daily) with the previous EPA-FFA formulation by using 5 × 574-mg 90% EPA-TG capsules per day (a total of 2870 mg), taking into account the percentage weight per weight (w/w) content of EPA and the presence of the glycerol backbone in the re-esterified TG.

The CTA required a specific simplified IMP dossier for the 90% EPA-TG, detailing its multistep manufacture, and also a cover document for the existing investigator brochure, which compared the chemical structure, GI absorption, bioavailability and tolerability profiles of the FFA and TG forms of EPA.

Although the 90% EPA-TG capsules that were proposed as a new IMP were publicly available for purchase as a nutritional supplement (from Igennus Healthcare Nutrition), the MHRA requested a programme of stability testing to meet manufacturing QA requirements for a CTIMP (see *Appendix 1*). A programme of accelerated (30 °C, 65% relative humidity) and standard (25 °C, 60% relative humidity) stability testing of capsules began in July 2014 (performed by ALS Food & Pharmaceutical, Carlisle, UK) and was performed once every 3 months. The core data set comprised the peroxide value (POV), para-anisidine value (pAV) and derived total oxidation (TOTOX) value, as well as a full PUFA analysis, to determine the EPA content. Data from initial accelerated testing at 3 months gave a minimum 12-month shelf life under standard conditions for the trial to continue with the new capsule IMP. Rolling stability testing provided a continuous extension of shelf life until 12 June 2016, when capsule IMP use ceased, as per the maximum approved shelf life (3 years) of the capsule IMP approved by MHRA.

The commercially available 90% EPA-TG capsule was also encapsulated with a differently coloured soft gelatin coat (olive green) to be able to produce an identical placebo because of the difference in appearance of EPA and medium-chain TG oils.

Participants took only one formulation of the EPA, which was either FFA or TG, or its matching placebo. Stocks of the IMP were managed during the transition period from the FFA formulation to the TG formulation to ensure that sites had sufficient stock of EPA-FFA and placebo capsules to manage existing and new participants throughout each individual intervention period.

The trial treatment was taken daily from the date of randomisation to the day before the 12-month surveillance colonoscopy.

## Suppliers

SLA Pharma AG supplied EPA-FFA capsules and an identical placebo free of charge. Igennus Healthcare Nutrition supplied the 90% EPA-TG capsules and an identical placebo at cost price. Aspirin and its identical placebo were supplied by Bayer AG (Leverkusen, Germany) free of charge.

## Eicosapentaenoic acid-free fatty acid dose

Participants took two 500-mg gastroresistant capsules of 99% pure EPA-FFA (or placebo) twice daily with food, giving a total daily dose of 2 g of EPA-FFA. Previous experience suggested that 2 g of EPA-FFA daily is well tolerated; principal side effects are diarrhoea, abdominal pain, nausea and vomiting, which are generally mild in severity and minimised or avoided by dosing with food or dose reduction to 1 g daily. A dose-reduction algorithm was used for participants experiencing side effects and managed by the local RN or SSPr.

## Eicosapentaenoic acid-triglyceride dose

Alternatively, five soft gelatin 574-mg capsules of 90% EPA-TG (or placebo), equivalent to 2780 mg, were taken orally with food each day. It was preferred that three of the EPA-TG capsules were taken with the largest meal of the day and two capsules were taken with a smaller meal. Each capsule contained approximately 516 mg of EPA-TG, which is equivalent to 398 mg of EPA-FFA. Other PUFAs in the formulation included 3.9% (w/w) AA. The 90% EPA-TG did not have pharmaceutical marketing approval [see the project web page: www.journalslibrary.nihr.ac.uk/programmes/eme/0910025/#/ (accessed 25 April 2019)]. Clinical studies have indicated that EPA-TG (usually in a fish oil mixture with other PUFAs) is well tolerated at doses exceeding 2 g per day over periods of up to 6 months. The principal known side effects are the same as indicated for EPA-FFA.[101–105]

### *Aspirin*

Participants were randomised to one 300-mg enteric-coated aspirin tablet (or placebo), taken orally once a day with food. Aspirin (acetylsalicylic acid) was supplied as 300-mg enteric-coated tablets in accordance with the Summary of Product Characteristics for aspirin (see *Appendix 2*). These tablets had marketing approval in the European Union.

## Placebos

The EPA-FFA and EPA-TG placebos consisted of identical capsules of capric and capryllic acid medium-chain TGs that had previously been used in placebo-controlled trials of EPA.[67]

The placebo for aspirin consisted of the same excipients as the active formulation of the drug minus the active ingredient.

## Discontinuation of treatment

In the event of an adverse drug reaction (ADR), either serious or non-serious, the local PI or attending physician was to take direct and appropriate action to provide care for the participant and to decide whether or not the trial treatment should be discontinued. However, unless there was a clear contraindication, trial treatment was continued, or stopped temporarily.

In all cases, the reasons for discontinuation of trial treatment were recorded in the clinical record file and if the investigator had recorded more than one reason, he or she was to indicate the main reason.

Treatment was discontinued permanently if the participant needed treatment with a contraindicated drug: ≥ 15 mg of methotrexate weekly, any dose of warfarin or any other anticoagulant therapy, any other antiplatelet agent such as clopidogrel, or prescription of aspirin for any other indication. These participants were still followed up for the remainder of the trial.

## Prescriptions and accountability

The local PI or delegated pharmacy trial staff member was responsible for ensuring trial treatment accountability, including reconciliation of trial treatment and maintenance of trial treatment records, throughout the course of the trial, in accordance with UK regulatory requirements. On receipt of a delivery of trial treatment, details were checked for accuracy and receipt was acknowledged by signing and dating the documentation provided. In addition, receipt was acknowledged in the web-based system by the local pharmacy team, which had access to the web-based stock control system. Stock did not become available for allocation until it was accepted in the stock control system.

The local hospital pharmacy completed the dispensing process by addition of a participant's name, subject number, date of dispensing and visit number to each allocated container. This process was repeated again at visits 4 and 5a if required.

### Blinding

Participants, SSPr/RNs, local investigators and those assessing the outcomes were all blinded to treatment allocation. The statistical analysis for the trial was also blinded until data were locked, except for independent Data Monitoring Committee (DMC) reports.

## Trial treatment

The trial treatment was packaged and labelled in accordance with UK regulatory requirements. The containers were clearly marked and had a unique identification number.

Bulk supplies of EPA-FFA, EPA-TG, aspirin and placebo capsules and tablets were delivered to Stockport Pharmaceuticals (Stockport, UK) for packaging and labelling to allow preparation of blinded supplies.

Blinded supplies were then stored at Stockport Pharmaceuticals for distribution to participating sites under a web-based stock control system reviewed regularly by the NCTU pharmacist.

### Unblinding

Access to the sequence of treatment allocations was confined to the NCTU data manager and a central pharmacy, in case of out-of-hours unblinding. In the event of the need to break the code, the date and reason were recorded on the web-based unblinding system. The local hospital pharmacy had access to the web-based unblinding system in normal office hours and out-of-hours access was provided via the sponsor at St James's University Hospital. The requirement for unblinding was considered low. All participants were given a trial identification card, containing details of the IMPs, which participants were encouraged to show when seeking advice or management from any health professional. Unblinding did not occur during the trial.

### End of the trial

Participants left the trial when they completed their routine post-surveillance colonoscopy visit (visit 7).

Cases of failure to receive allocated treatment and withdrawal from follow-up were reported, and the reason(s) for withdrawal (if given) were documented. If a participant did not receive allocated treatment but agreed to remain in the trial, outcome data collection continued in accordance with the protocol. Participants were informed at the start of the trial that data collected up to the point of withdrawal would be retained and used in the final analysis.

### Trial withdrawal

Participants could withdraw from the intervention or the trial at any time without giving a reason and without compromising future management. Data collected up to the point of withdrawal were retained for the purposes of the intention-to-treat (ITT) analysis. Participants could withdraw from the intervention only but continue in the trial, thereby completing outcome measures. To maximise primary outcome data collection, BCSP surveillance colonoscopy data were also collected from participants who withdrew from the trial, as per the informed consent.

## Outcome measures

### *Primary outcome*

The primary outcome was the number of participants with one or more colorectal adenomas detected at the first BCSP surveillance colonoscopy 12 months after the screening examination (ADRa).

### *Secondary outcomes*

The secondary outcomes were as follows:

- Total number of colorectal adenomas per participant at BCSP surveillance colonoscopy (total MAP).
- Detection of one or more 'advanced' (i.e. $\geq 10$ mm in diameter, high-grade dysplasia or villous histology) colorectal adenomas at the 12-month BCSP surveillance colonoscopy (advanced ADRa).
- Number of 'advanced' colorectal adenomas per participant at the 12-month BCSP surveillance colonoscopy (advanced MAP).
- Detection of one or more conventional adenomas (conventional adenoma end points were defined after database lock) at the first BCSP surveillance colonoscopy (conventional ADRa).
- Number of conventional adenomas (conventional adenoma end points were defined after database lock) per participant at the first BCSP surveillance colonoscopy (conventional MAP).
- Detection of one or more serrated adenomas at the first BCSP surveillance colonoscopy (serrated ADRa).
- Number of serrated adenomas per participant at the first BCSP surveillance colonoscopy (serrated MAP).
- The region of the colorectum (right colon: any part of the colon proximal to the splenic flexure; left colon: the rectum and the colon at/distal to the splenic flexure) in which adenomas are detected at the first BCSP surveillance colonoscopy.
- Reclassification from 'high risk' to 'intermediate risk' after the first BCSP surveillance colonoscopy (BCSP risk stratification at the first surveillance colonoscopy states that any individual who does not continue to fulfil 'high-risk' criteria is classified as 'intermediate risk' for further colonoscopic surveillance at 3 years).
- Detection of CRC prior to, or at, the first BCSP surveillance colonoscopy.
- Dietary fish and other seafood intake at baseline and at the end of the trial.
- Red blood cell EPA and rectal EPA levels at baseline, 6 months (RBC only) and 12 months from randomisation.
- Absolute RBC fatty acid (i.e. DHA, AA, EPA-to-AA ratio) levels and difference from baseline at 6 months and 12 months.
- Rectal mucosal fatty acid (i.e. DHA, AA, EPA-to-AA ratio) levels at surveillance colonoscopy.
- Adverse events, including clinically significant bleeding episodes (i.e. haemorrhagic stroke or GI bleeding requiring hospital admission or investigation).

### Exploratory outcomes

- Colorectal adenoma size.
- Association between change of RBC EPA level at 12 months and individual number of total colorectal adenomas.
- Association between rectal and RBC EPA levels at 12 months.

## Research governance

The trial was conducted in accordance with (1) the recommendations adopted by the 18th World Medical Assembly, Helsinki 1964, amended at the 48th General Assembly, Somerset West, Republic of South Africa, October 1996 (www.wma.net/wp-content/uploads/2018/07/DoH-Oct1996.pdf); (2) the principles of the International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP) guidelines (https://ichgcp.net); and (3) the Medicines for

Human Use (Clinical Trials) Regulations 2004[106] (UK Statutory Instrument 2004/1031) and any subsequent amendments of the Clinical Trial Regulations.

The National Research Ethics Service (NRES) Trent – Trent REC (reference number 10/H0405/90) gave ethics approval for the trial for NHS participants. First, MHRA CTA approval was obtained on 16 March 2011. The trial was approved by the BCSP Research Committee on 13 October 2009.

This project was funded by the EME programme, a MRC and NIHR partnership (project number 09/100/25). The trial was registered (as ISRCTN05926847) in a publicly available database prior to FPFV.

The final, approved protocol was version 6.0, approved on 11 August 2014. There were a number of administrative and procedural changes made to the protocol during the trial, which are outlined in *Appendix 3*.

### Protocol deviations
The protocol defined a protocol violation as:

- > 50% of trial medication returned in total
- inadvertent use of OTC medication containing aspirin, NSAIDs or fish oil for > 2 weeks in total
- surveillance colonoscopy occurring outside the allowed time windows (48–52 weeks after the last complete screening BCSP colonoscopy, or 60–64 weeks for participants undergoing a repeat full colonoscopy within 3 months of initial screening).

The protocol deviation log collected the above violations and any additional protocol deviations (i.e. any deviation from the protocol that occurred during a participant's time in the trial, whether deliberate or non-deliberate).

### Trial oversight
Oversight committees were assembled to ensure the proper management and conduct of the trial, and to uphold the safety and well-being of participants. The general purpose, responsibilities and structures of the committees were described in the protocol.

### Trial Management Group
The TMG comprised the chief investigator, members of the NCTU and other visiting members of the wider trial team, including the University of Bradford biobank and lipid analysis team and an expert in human nutrition, as required. This group met regularly, with supplementary meetings as required, to oversee the day-to-day operational aspects of the trial, reviewing progress and resolving issues that arose.

### Trial Steering Committee
The TSC was led by an independent chairperson and consisted of members with professional expertise in delivering RCTs, CRC diagnosis and management, primary care gastroenterology and the BCSP. The TSC also had a patient and public involvement (PPI) representative (an individual who had undergone BCSP colonoscopy) during the course of the trial. The TSC monitored, reviewed and supervised the progress of the trial, particularly advising on recruitment and retention, as well as ensuring adherence to the trial protocol. It also monitored blinded data to consider safety and effectiveness indications. The TSC considered reports from the DMC when making recommendations. The chief investigator, trial manager and at least one co-investigator were also in attendance as non-independent members to provide information to the committee. The trial sponsor (University of Leeds) and IMP providers were invited to attend as observers.

The TSC met independently prior to the start of the trial and agreed terms of reference in a charter. The committee met approximately every 6 months thereafter, convening supplementary meetings as required (e.g. related to the capsule IMP switch).

## Data Monitoring Committee

An independent DMC, which met approximately every 6–12 months, was established with access to unblinded data to provide independent review and recommendations in the light of potential treatment effect and harm, as well as to assist and advise the TSC and the TMG. The DMC consisted of a chairperson and members with expertise in colorectal surgery and prevention, early detection and treatment of CRC, and statistics. The DMC met prior to the start of the trial and agreed terms of reference in a charter. Only the DMC had access to unblinded data until the final end-point assessment was completed.

### Risk assessment and safety monitoring

A risk assessment was conducted as part of protocol development and was monitored regularly throughout the trial for new risks. The main risks to the trial were reliance on the BCSP to recruit to a large multicentre RCT, as the BCSP had not previously hosted CTIMP research and staff were not experienced in recruiting participants. Compliance with trial medication and drug accountability was reliant on participants returning medication and patient reports, as well as staff maintaining detailed records of medication logs.

Recruitment sites were supported by the NCTU, which initially provided training through a site initiation visit, as well as supplementary training, as required. Investigators had access to a research area of the trial website (www.seafood-trial.co.uk) and access to the NCTU trial team for day-to-day queries. To obtain high-quality data, regular central monitoring checks were performed according to the monitoring plan, including review of recruitment, retention and data collection rates by the TMG, TSC and DMC.

Data on AEs and serious adverse events (SAEs) were collected. As agreed by the sponsor, REC, DMC and TSC, the DMC was provided with a listing of all AEs and SAEs, including any deaths (if applicable), at each DMC meeting.

As part of the switch from the EPA-FFA formulation to the EPA-TG formulation, a review was undertaken of the Reference Safety Information (RSI) for the trial. Evidence was provided in a supplement, including MHRA-stipulated RSI for the EPA formulations, version 2.0, dated 22 August 2016 (approved by the MHRA on 3 October 2016) to the investigator brochure version 6, dated 10 January 2014, that the FFA and TG formulations were likely to have a similar pharmacological and risk/AE profile, as well as similar anti-CRC efficacy, in the seAFOod trial population [see the project web page: www.journalslibrary.nihr.ac.uk/programmes/eme/0910025/#/ (accessed 25 April 2019)].

### Monitoring

Following an internal assessment of the trial by the quality assurance manager at the NCTU, the trial was assessed as a medium-risk trial requiring low-intensity monitoring. Based on this risk category and the specific risks identified, the monitoring strategy consisted of on-site routine and triggered monitoring visits, central monitoring and review of regular reports by trial oversight committees, as well as assessment and oversight of third-party vendors. On-site visits were conducted by either the trial manager or a trial monitor. Central monitoring was conducted by the NCTU and reviewed regularly by the TMG, the TSC and the DMC. On-site and central monitoring revealed no major common concerns during the trial. Sites that received an on-site monitoring visit at some point during the trial accounted for 68% of randomised participants.

### Patient and public involvement

There was PPI throughout the trial. One PPI member was integral to trial design, grant application and production of participant-facing trial materials. He then took no further part in the trial. Subsequent PPI input on the TSC was provided by a different PPI member. Both PPI representatives brought personal experience of the BCSP pathway to their roles. Two PPI representatives from Independent Cancer Patients' Voice contributed to the production of site and participant results summaries and to the development of the dissemination plan for the trial findings.

## Payments to participants

Participants were not paid to participate in the trial. Visits 1, 6 and 7 were planned to co-ordinate with routine BCSP appointments. However, an additional visit at 6 months (visit 4) was required; therefore, participants were offered a reimbursement of travel expenses up to a maximum of £10.

## The seAFOod polyp-prevention trial biobank

Blood, urine and rectal mucosa sample collection in the seAFOod polyp-prevention trial is described in detail in an appendix of the trial protocol, which has been published.[100]

In brief, a blood sample and a urine specimen were obtained at visits 1, 4 and 6. Blood was immediately separated into plasma, leucocytes and RBCs. Rectal biopsies were obtained only at the surveillance colonoscopy at visit 6.

Sample cryovials were stored in either a Liebherr Underbench –20 °C freezer (Liebherr Group, Bulle, Switzerland) supplied by the seAFOod trial or the existing trust freezer facility at –20 °C or colder, in PathoSeal bags (DGP Intelsius Ltd, York, UK). Storage temperature was monitored using a Hanna HI-141 CH Datalogger (Hanna Instruments Ltd, Leighton Buzzard, UK), or a similar system provided by the trust site, and recorded on sample worksheets.

University of Bradford staff, in close collaboration with the NCTU, organised the collection of samples from each BCSP site approximately every 6 months by a specialist courier (CitySprint Health, London, UK). The original proposal was to transfer samples to the central biobank facility every 3 months. However, the expansion of participating trial sites in 2012–13 meant that the collection strategy was revised. BCSP sites were grouped into 10 individual routes by CitySprint Health so that collection from these sites could be scheduled for the same day. Sites were asked to confirm whether or not they required a collection, and the number of PathoSeal bags at that site, to allow CitySprint Health to supply the correct thermal box size. If sites were unable to accommodate a collection on their scheduled route day, individual collections were arranged. The day before collection, CitySprint Health arranged for pre-addressed thermal boxes, which contained dry ice, to be sent to appropriate BCSP sites. The lid of the thermal box was removed only when necessary for adding samples. All sealed PathoSeal bags collected since the last courier sample collection were placed in the thermal box and dry ice was spread around the bags to ensure that they were completely covered. The thermal boxes were delivered to the University of Bradford on the same day or, in the case of routes involving longer distances, the following morning. No units were received with low dry ice levels and all samples were received frozen.

Each BSCP site completed a shipment form that documented the samples being transported. A carbon copy of the shipment form was kept in the site file with the carbon copy of the completed sample worksheets. The original shipment form and the original completed sample worksheets were sent with the samples.

On receipt of each shipment of transportation units at Bradford, a sample tracking form was completed. Contents were then checked against the sample worksheet. Cryovials were transferred into sample boxes (10 × 10 cell cryoboxes, manufactured by Sarstedt, Nümbrecht, Germany) specific to each sample type, and cryoboxes were kept on ice at all times when removed from the freezer. All details were entered onto a separate sample tracking form for each thermal box. Samples were stored in type-specific cryoboxes and racks, minimising disruption when samples were removed for analysis.

All samples were stored in a dedicated –80 °C freezer (New Brunswick™ U570 –80 °C; Eppendorf, Hamburg, Germany), which was connected to the emergency power supply at the Institute of Cancer Therapeutics, University of Bradford, and supported with a $CO_2$ back-up system (New Brunswick) and Controller AD11+ (Controller, Staines, UK) telephone alarm system. Freezer temperature was monitored daily.

The details from the sample tracking form and the sample worksheets were entered into the seAFOod trial biobank sample database. The database allocated a unique Clinical Trials Pharmacology Laboratory (CTPL)

tracking identification (ID) to each participant-specific set of samples in a PathoSeal bag (i.e. if a participant had samples taken on three visits, they received three CTPL IDs). The CTPL ID was marked on the sample worksheet and the sample tracking form. The database was stored on the University of Bradford server, in the 'Secure' section, accessible only by nominated CTPL staff, and backed up regularly, as described in the University of Bradford's computer policy. The database was the primary source for sample tracking; however, paper documents were available as a secondary source if necessary, stored in participant and shipment folders in the secure Human Tissue Act-approved laboratory.[107]

Samples were obtained at BSCP sites between FPFV (i.e. 11 November 2011) and LPLV (i.e. 8 June 2017). The first samples received at Bradford were delivered on 18 July 2012 and the last sample shipment took place on 27 July 2017. In the intervening time, 332 collections were made from BCSP sites, with 1775 individual PathoSeal bags received.

Samples were stored at BSCP sites for between 1 and 696 days (mean $124 \pm 92$ days, median 115 days). There were 1378 (78%) sample sets stored at BSCP sites for < 6 months. Thirty (2%) sample sets were stored at BSCP sites for > 12 months.

The majority of sample sets ($n = 1021$; 58%) were stored in BCSP sites at –20 °C (range –16 to –24 °C), with 230 (13%) sample sets stored at –40 °C (range –25 to –69 °C) and 524 (30%) sets stored at or below –70 °C.

One or more biological samples were received from 677 of 709 (95%) randomised seAFOod trial participants. Of the 709 participants, 73% (519) of participants provided full sample sets of blood, urine and rectal mucosa from all three visits. There were 76 participants who provided samples at two visits (visits 1 and 4, $n = 49$; visits 4 and 6, $n = 15$; visits 1 and 6, $n = 12$). Eighty-two participants provided samples at only a single visit (visit 1, $n = 75$; visit 4, $n = 2$; visit 6, $n = 5$).

Overall, a total of 7322 biological samples were received (16,258 sample aliquots):

- 1715 plasma samples (6746 aliquots)
- 1714 leucocyte samples (1714 aliquots)
- 1707 RBC samples (3421 aliquots)
- 1664 urine samples (3309 aliquots)
- 522 rectal biopsies (1068 aliquots).

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).

Laboratory protocol deviations were monitored carefully by sample worksheets:

- 80% of blood samples were obtained per protocol (centrifugation within 30 minutes and transfer to the freezer within 60 minutes).
- 2% of sample sets were received without worksheets.
- 1% of samples sets were received with insufficient data to assess timing.
- 8% of blood was separated after 30 minutes, but frozen within 1 hour of collection.
- 9% of blood samples took > 1 hour to separate (often related to split hospital sites for endoscopy and sample handling).

A small number of other protocol deviations were noted, including:

- 1% of sample sets that suffered a temperature deviation (but no thaw).
- 1% of sample sets that defrosted at some point.
- 0.5% of sample sets suffered 'other' deviations (including wrong anticoagulant blood tube used and biopsies placed in formalin).

## Fatty acid measurement and analysis

The methods used are described in detail by Volpato et al.[89] In brief, fatty acids were extracted from washed RBC membranes, or rectal mucosal homogenates, using an isopropanol/chloroform method with acid hydrolysis, in order to measure the total membrane/tissue fatty acid pool.[89] Liquid chromatography, in combination with electrospray ionisation triple quadrupole tandem mass spectrometry (ESI-MS), was performed with a Waters Alliance™ 2695 High Pressure LC module (Waters, Milford, MA, USA) in combination with a Waters Micromass™ Quattro Ultima triple quadrupole mass spectrometer on derivatised samples, in the presence of internal standard (deuterated ALA), as described by Volpato et al.[89] Data are expressed as the percentage of each fatty acid relative to the total fatty acid peak chromatographic area for the $\omega$-3 PUFAs C18:3 (ALA), C20:5 EPA, C22:5 docosapentaenoic acid (DPA) and C22:6 DHA; the $\omega$-6 PUFAs C18:2 linoleic acid (LA) and C20:4 AA; and the monounsaturated C18:1$\omega$-9 oleic acid and saturated fatty acids C18:0 stearic acid and C16:0 palmitic acid.[89]

## Measurement of dietary fish intake

Participants were asked to complete a FFQ at baseline (visit 1) and at the end of the intervention (visit 6 or 7). The validated European Prospective Investigation of Cancer (EPIC) short FFQ was used, which is a semiquantitative, paper-based FFQ that includes 130 food items and supplementary questions about use of fat, milk, cooking methods, salt and supplement use.[108] The FFQ measures an individual's habitual food intake over the preceding 12 months. The primary purpose of the FFQ was to determine if there was any change in dietary marine $\omega$-3 PUFA intake during trial participation. As fish is the primary source of bioactive $\omega$-3 PUFAs EPA and DHA, the reported consumption of fish was analysed before and after intervention with IMP.

Total fish consumption and consumption of oily fish were considered separately. Six FFQ food item variables were used for the analysis of total fish (i.e. fried fish, fish fingers, white fish, oily fish, shellfish, roe). Consumption of oily fish was based on a single food item variable (oily fish). Participants indicated the frequency of food consumption, ranging from 'never or less than once per month' to 'six times per day'. This was recoded into frequency per day (Table 2). The consumption of total fish per day was calculated by summing the reported fish consumption for each of the six fish variables.

The data were used to recode participants into four categories (i.e. never, low, middle or high), based on their weekly consumption of total fish and oily fish (Table 3). Categorisation of weekly fish consumption was based on the Scientific Advisory Committee on Nutrition recommendation to consume two portions of fish per week, one of which should be oily.[109]

Further analysis of the dietary PUFA intake data is planned and will include full nutrient and food group analysis using FETA (FFQ EPIC Tool for Analysis) software (www.srl.cam.ac.uk/epic/epicffq/; accessed April 2018).

**TABLE 2** The FFQ categories

| FFQ category | Frequency per day |
| --- | --- |
| Never or less than once per month | 0 |
| 1–3 times per month | 0.07 |
| Once per week | 0.14 |
| 2–4 times per week | 0.43 |
| 5 or 6 times per week | 0.79 |
| Once per day | 1 |
| 2 or 3 times per day | 2.5 |
| 4 or 5 times per day | 4.5 |
| $\geq$ 6 times per day | 6 |

TABLE 3 Frequency of fish consumption according to the FFQ

| Category | Reported frequency of consumption |
|---|---|
| Total fish | |
|     Never | 0 |
|     Low | < 1 portion per week (0.01–0.13 per day) |
|     Middle | 1–2.99 portions per week (0.14–0.429 per day) |
|     High | ≥ 3 portions per week (0.43–highest per day) |
| Oily fish | |
|     Never | 0 |
|     Low | < 1 portion per week (0.07 per day) |
|     Middle | 1–2.99 portion per week (0.14 per day) |
|     High | ≥ 3 portions per week (0.43–highest per day) |

### *Planned laboratory studies*

The nested laboratory studies originally planned for the EME-funded seAFOod trial project are described in the trial protocol version 6.0, dated 11 August 2014 [see the project web page: www.journalslibrary.nihr.ac.uk/programmes/eme/0910025/#/ (accessed 25 April 2019)]. The switch in capsule IMP that occurred in 2014 required a more detailed PUFA analysis than was originally planned to test equivalence of the bioavailability of FFA and TG formulations measured by RBC and rectal mucosal EPA content. Therefore, funding for much of the planned biomarker work was diverted to a more extensive PUFA analysis.

## Statistical methods

### *Changes from the protocol to the statistical analysis plan*

A number of changes to details contained within the protocol are documented in the SAP (version 1.1, dated 24 August 2017) [see the project web page: www.journalslibrary.nihr.ac.uk/programmes/eme/0910025/#/ (accessed 25 April 2019)]. These are listed in *Table 4*.

In addition, the changes documented in *Table 5* were made after database lock and release of the treatment codes.

TABLE 4 Summary of changes to the SAP from the protocol

| Protocol | SAP | Justification |
|---|---|---|
| The protocol does not include a comparison between the two formulations of EPA used in the trial, EPA-FFA and EPA-TG, as a secondary end point | The SAP includes the prespecified secondary end point 'Red Blood Cell (RBC) EPA and rectal EPA levels at baseline, 6 months (RBC only) and 12 months from randomisation' in section 2.9.2 (Secondary end points). The corresponding statistical analysis is specified in section 6.3 (Secondary analyses) | The formulation of EPA changed during the trial (details in section 2.4). The daily doses of FFA and TG formulations were calculated to be FFA dose equivalent, but there are no available data to determine bioavailability equivalence during clinical use over 6–12 months. Although it is assumed that the two formulations would be approximately bioequivalent, it was deemed important to summarise the EPA levels in participants receiving both formulations. Formal equivalence methods and set margins for non-inferiority are not being used, as the trial was not designed, nor powered, to examine this |

**TABLE 4** Summary of changes to the SAP from the protocol (*continued*)

| Protocol | SAP | Justification |
|---|---|---|
| The protocol does not include information on the secondary end points being derived from the FFQ | The SAP includes the prespecified secondary end point 'Dietary fish and other seafood intake at baseline and at the end of the study' in section 2.9.2 (Secondary end points). The corresponding statistical analysis is specified in section 6.3 (Secondary analyses) | It will be important to determine whether there is an imbalance between groups in the intake of dietary fish and seafood or any change in such intake during trial participation. This variable will also be used as a covariate in a sensitivity analysis of the primary end point |
| The protocol does not include the MAP as an end point. The protocol states 'the total number of adenomas per participant at BCSP surveillance colonoscopy' | The mean adenoma number per person has been included in the secondary end points for adenomas, advanced adenomas and serrated adenomas in section 2.9.2 (Secondary end points). This has also been added to the statistical analyses in section 6.3 (Secondary analyses) | The MAP is now established as a colonoscopic end point, in addition to the ADRa |
| The protocol states that 'If fewer than 5% of participants have missing data for the primary end point then complete-case analysis will be performed'<br><br>The protocol states that 'Losses to follow-up and protocol violations will be treated as missing data for the ITT population' | Section 3 of the SAP (General analysis considerations) provides information about the participant populations being used in the trial and procedures for the investigation and use of missing data. In summary, the primary end point will be analysed for all participants in the ITT population. The proportion of participants who have missing data will be reported, and the interpretation of the primary analysis will take into account this proportion. A per-protocol analysis will be performed as a sensitivity analysis, which will exclude participants with major protocol violations (which will be defined prior to release of treatment allocation) | The primary analyses should be based on the ITT population. In accordance with the ITT principle, all participants should be included in the ITT population (i.e. the data for participants who have violated the protocol should be included) |
| The per-protocol population criteria were stated as 'More than 50% of trial medication returned in total; inadvertent use of OTC medication containing aspirin, NSAIDs or fish oil for more than 2 weeks in total; surveillance colonoscopy occurs outside the allowed time windows (48–52 weeks after the last complete screening BCSP colonoscopy, or 60–64 weeks for participants undergoing a repeat full colonoscopy within 3 months of trial screening)' | Section 3.1 in the SAP (Analysis populations) includes suggestions about what major protocol violations may result in exclusion from the per-protocol population. It also states that specific criteria will be determined in a meeting prior to treatment codes being revealed | Data review prior to treatment unblinding will provide information about what protocol violations and deviations occurred during the trial and will allow judgements about the criteria for exclusion from the per-protocol population to be made |
| Serrated adenomas | There was no specific end point or analysis relating to serrated adenomas detailed in the protocol. The SAP prespecifies end points of 'The number of participants with a recurrence of serrated adenoma at the first BCSP surveillance colonoscopy' and 'The number of serrated adenomas per participant at the first BCSP surveillance colonoscopy' in section 2.9.2 (Secondary end points). The corresponding statistical analysis is specified in section 6.3 (Secondary analyses) | The serrated adenoma is now established as a marker of malignant potential arising from a molecular pathway distinct from the traditional adenoma–carcinoma sequence. Therefore, the serrated adenoma should be analysed separately from the conventional adenoma |

**TABLE 5** Summary of changes to the SAP post database lock

| Change from SAP | Justification |
|---|---|
| Change to planned analysis: recruiting site rather than BCSP centre was used as a covariate in the primary analysis<br><br>An additional sensitivity analysis was performed that treated BSCP centre and site as random effects in a multilevel model | After database lock and release of the treatment codes, it was discovered that the randomisation had stratified by site rather than by BCSP centre. As site was nested in BCSP centre, the balance across treatment groups through stratification was maintained for BCSP centre |
| Additional secondary end point and analyses: the number of participants with at least one conventional colorectal adenoma and the number of conventional colorectal adenomas per participant were analysed in the same way as for serrated adenomas | Because the number of participants with at least one serrated adenoma and the number of serrated adenomas per participant had been analysed, as well as the ADRa and MAP for total colorectal adenomas, it was felt that similar data for conventional colorectal adenomas should be reported |
| Additional sensitivity analyses: the sensitivity analyses for the primary outcome were analysed without adjustment for repeat colonoscopy, as supportive analyses. Similarly, the secondary outcomes were analysed without adjustment as supportive analyses | Data on whether or not participants had had a repeat colonoscopy were not collected from the start of the trial. Therefore, there were no data for the first 61 participants. This meant that the data from these participants were not included in the analysis. It was felt important to investigate the robustness of these results by including the additional participants, although the potential confounding effect of different endoscopic procedures (full colonoscopy, partial colonoscopy or FS) could not be taken into account |
| Additional sensitivity analyses: two further sensitivity analyses were performed. One adjusted for the baseline EPA level and the other adjusted for oily fish intake | It was felt that both of these variables could have an impact on the primary outcome. It was therefore deemed appropriate to perform analyses including these variables in the model |
| Unplanned exploratory end points and analyses: colorectal adenoma size analysed with adjustment for histology type, taking into account multiple adenomas per participant | FAP RCTs have consistently measured and analysed colorectal adenoma number and size. Given the secondary outcome data on colorectal adenoma number, colorectal adenoma size was felt to represent an important exploratory analysis |
| Modification of the definition of advanced adenoma: the definition in the protocol and SAP was changed from '≥ 10 mm diameter, high-grade dysplasia or tubulo-villous/villous histology' to '≥ 10 mm diameter, high-grade dysplasia or villous histology' | Data collected on colorectal adenoma characteristics at surveillance colonoscopy did not allow tubulo-villous adenomas to be distinguished from tubular adenomas so that the 'advanced' definition was based on villous histology alone, not tubulo-villous histology |
| Modification of the definition of the ITT population: the definition of the ITT population included all participants who had been randomised. However, the population analysed excluded two participants who were withdrawn immediately after randomisation | Two participants were randomised and then immediately withdrawn from the trial. They had no trial data (including baseline data collected), and therefore could not contribute to the trial. They were excluded from the ITT population |
| Analysis of safety data: the summaries detailed in the SAP were amended. AEs and ADRs were not summarised by preferred term. GI AEs and ADRs were summarised by preferred term and formulation. In addition, GI ADRs were summarised according to severity and preferred term | GI AEs and ADRs were the most commonly reported. It was therefore judged to be useful to summarise these AEs and ADRs by preferred term. AEs and ADRs for all other system organ classes were not summarised by preferred term, given their low frequency and severity |

## Sample size

The original sample size estimate of 904 participants (to ensure 768 evaluable patients and assuming a 15% loss to follow-up) in trial protocol versions 1–3 was based on a RCT of the same dose and preparation of EPA-FFA in FAP patients,[67] a meta-analysis of aspirin RCTs[49] and detailed 2007–8 audit data from the South of Tyne and Tees BCSP centres.

For the protocol revision in May 2012 (related to inclusion of patients who required a repeat endoscopic procedure during the 3-month screening window), the sample size was re-estimated using audit data on surveillance colonoscopy in 1189 patients classified as 'high risk' in 2010, from the North-East BCSP hub

(nine BCSP centres) and the Southern BCSP hub (17 BCSP centres). A total of 930 (78%) patients went straight to surveillance after a single screening colonoscopy. Colorectal ADRa data at surveillance colonoscopy were available for 738 patients and, of these, one or more colorectal adenomas were detected in 465 (63%) patients. Corresponding figures for 'high-risk' patients having a repeat partial colonoscopy or FS within 3 months of an initial screening colonoscopy showed that one or more adenomas were detected at first surveillance colonoscopy in 59 out of 110 (54%) patients, and for patients having a repeat full colonoscopy, one or more adenomas were detected in 56 out of 83 (67%). The overall ADRa at first surveillance colonoscopy was 62%, which was consistent with the original estimate of 60%; therefore, the sample size remained unchanged.

To detect a minimum 18% relative reduction in adenoma risk in each two-group comparison [less than the 22% reduction in polyp number compared with placebo in the FAP trial[67] and below the absolute reduction in polyp number at 1 year (38%) in aspirin RCTs[49]] from a 60% adenoma recurrence rate at surveillance colonoscopy to 49%, 678 evaluable 'high-risk' individuals were required to be randomised equally to the four treatment groups, with 80% power at a 5% two-sided significance level.

Standard practice for 2 × 2 factorial designs, in the absence of an interaction, bases the sample size estimate on the two-group comparison of treatment versus placebo (and divides the total equally between the four groups). With the sample size of 678 based on this method, there is, in fact, a slight reduction in power (to 75%), which arises if both treatments work, because then the overall comparison for treatment A is not 0.49 versus 0.6, but is 0.445 versus 0.545 (averaging over the placebo and treatment B groups). To keep power at 80% for the above figures, a simulation using Stata® version 10 (StataCorp LP, College Station, TX, USA) and employing the proposed analysis method indicated that 192 individuals were required per group (a total of 768 evaluable 'high-risk' individuals).

In trial protocol versions 1–3, a 15% drop-out rate was assumed. However, feedback from BCSP sites and experience from the first few months of the trial suggested that the drop-out rate of 'high-risk' BCSP patients was < 15%. Allowing for a 10% drop-out rate, the proposed sample size increased to 768/0.9 = 853 individuals.

For the purposes of the trial extension granted by the EME board in 2014, we proposed a revised realistic recruitment target of 755, which provided 71% power to detect the same effect size as above, still assuming a 10% drop-out rate.

### Analysis plan
A SAP was finalised prior to database lock and release of treatment codes to the statistician. All summaries and statistical analyses were conducted using Stata version 15.0.

### Analysis populations

- Intention-to-treat population: all randomised participants with post-randomisation data. Analysis was according to the treatment group to which they were randomised.
- Safety population: all randomised participants who had at least one dose of trial medication. Analysis was according to the treatment they actually received.
- Per-protocol population: all randomised participants not deemed to have a major protocol violation. Major protocol violations that resulted in exclusion from the per-protocol population were –

  - had not taken sufficient medication (participants who had taken ≤ 75% of their expected EPA and/or ≤ 50% of their expected aspirin)
  - found to be ineligible post-randomisation
  - any use of OTC medication containing aspirin, NSAIDs or fish oil for > 2 weeks during the treatment period.

The final composition of the per-protocol population was determined by final data review prior to the treatment codes being revealed.

Analysis of the primary end point was based on the ITT population. The analysis was repeated based on the per-protocol population. This was considered supportive to the primary analysis.

Analyses of all secondary end points, with the exception of AEs, were based on the ITT population.

Summaries of the AEs were based on the safety population.

### Missing data
Sensitivity analyses were performed to support the primary analyses. All secondary analyses assumed that the data were missing at random and no imputation was performed.

### Primary outcome
The primary outcome was the number of participants with one or more colorectal adenomas detected at the first BCSP surveillance colonoscopy 12 months after the screening examination (the ADRa).

The primary outcome was analysed by an 'at-the-margins' approach,[110] after first examining whether or not there was any evidence of an interaction between EPA and aspirin. An 'at-the-margins' approach analyses all participants randomised to EPA (i.e. EPA + aspirin plus EPA + placebo aspirin, combined) versus all participants not randomised to EPA (i.e. placebo EPA + aspirin plus placebo EPA + placebo aspirin, combined), and all participants randomised to aspirin versus all participants not randomised to aspirin. Given that there was no evidence of an interaction, the log relative risk was estimated using a mixed-effects log-binomial regression model, with BCSP site included as a random effect, and the risk differences and ratios presented. Both interventions were fitted simultaneously and the analysis was adjusted for 'repeat colorectal endoscopic procedure within 3 months required' and BCSP site. This was a change to the analyses defined in the SAP, which stated that BCSP centre would be included as a random effect.

### Sensitivity analyses for the primary outcome
The following sensitivity analyses were performed on the primary end point:

- Analysis using the per-protocol population.
- Analysis as a multilevel model. Some BCSP centres comprise multiple hospital sites; therefore, a sensitivity analysis was conducted in which both BCSP centre and site were treated as random effects in a multilevel model.
- Multiple imputation of missing primary end-point data.
- Further adjustment of baseline variables with any marked imbalance, if appropriate.
- Investigation of the effect of treatment adherence using complier-average causal effect (CACE) estimation methods.[111,112] ITT analysis does not represent the treatment effect of non-compliance with treatment; therefore, CACE analysis was deemed to be important if any treatment effect was directly affected by the level of compliance. The percentage of the required total dose taken by participant from randomisation to first surveillance colonoscopy (both in binary and continuous form) was included in the model as an instrumental variable to estimate such an effect.
- Further adjustment for EPA capsule formulation, that is EPA-FFA (or placebo) or EPA-TG (or placebo).
- Further adjustment for oily fish intake.
- Further adjustment for baseline RBC EPA levels.

### Secondary outcomes

- The total number of colorectal adenomas per participant at the 12-month BCSP surveillance colonoscopy was analysed using a Poisson regression model. The incidence rate ratio (IRR) and 95% CIs were presented.
- The number of participants with an 'advanced' colorectal adenoma ($\geq 10$ mm maximum dimension, high-grade dysplasia or villous histology) at the first BCSP surveillance colonoscopy (advanced ADRa) was analysed using a log-binomial regression model. The risk difference and 95% CIs were presented.
- The number of 'advanced' colorectal adenomas per participant at the first BCSP surveillance colonoscopy (advanced MAP) was analysed using a Poisson regression model. The IRR and 95% CIs were presented.
- The number of participants with one or more conventional colorectal adenomas at surveillance colonoscopy (conventional ADRa) was derived from the total and serrated adenoma data and was analysed using a log-binomial regression model. Risk difference and 95% CIs were presented.
- The number of conventional colorectal adenomas per participant at surveillance colonoscopy (conventional MAP) was derived from the total and serrated adenoma data and was analysed using a Poisson regression model. The IRR and 95% CIs were presented.
- The number of participants with one or more serrated adenomas at surveillance colonoscopy (serrated ADRa) was analysed using a log-binomial regression model. The risk difference and 95% CIs were presented.
- The number of serrated adenomas per participant at surveillance colonoscopy (serrated MAP) was analysed using a Poisson regression model. The IRR and 95% CIs were presented.
- The region of the colorectum (right colon: any part of the colon proximal to the splenic flexure; left colon: the rectum and the colon at/distal to the splenic flexure) that colorectal adenomas were detected at the first BCSP surveillance colonoscopy was explored, using a Poisson random-effects model with bivariate response (corresponding to adenoma counts in the left and right colon), in which treatment and a baseline adenoma count were independent variables together with random intercepts corresponding to participant and BCSP site. The IRR and 95% CIs were presented.
- The number of 'high-risk' participants reclassified as 'intermediate risk' after the first BCSP surveillance colonoscopy was analysed using a log-binomial regression model. The risk difference and 95% CIs were presented.
- The number of participants with CRC detected prior to, or at, the first BCSP surveillance were to be summarised descriptively; however, there were no participants with CRC.
- The levels of RBC EPA and rectal mucosal EPA were summarised at baseline (RBC only) and at visits 4 (RBC only) and 6 for those receiving EPA-FFA and those receiving EPA-TG. In addition, the change from baseline to visits 4 and 6 were summarised.
- The levels of DHA, AA and the EPA-to-AA ratio were summarised at baseline (RBC only) and at visits 4 (RBC only) and 6 (RBC and rectal mucosal) for those receiving EPA-FFA and those receiving EPA-TG. In addition, the change from baseline to visits 4 and 6 were summarised for RBC samples.
- Dietary fish and other seafood intake (oily fish and total) at baseline (visit 1) and at the end of the trial (visit 6 or 7) were summarised by treatment group.

### Safety analyses

Adverse events were summarised for the safety population, that is all participants who received at least one dose of trial medication. Summaries were based on the IMP that the participant received, irrespective of randomisation.

Both AEs and treatment-emergent ADRs were summarised by system organ class. GI AEs were also summarised by preferred term and by formulation. ADRs were summarised by severity using the preferred term. The worst case (i.e. severe and/or related to trial treatment) was assumed if severity or causality were missing, unless otherwise stated.

Clinically significant bleeding episodes (i.e. haemorrhagic stroke or acute GI bleeding requiring hospital admission or investigation) were identified by the chief investigator using a manual search of the full list of AEs and SAEs.

Common GI AEs associated with ω-3 PUFA and/or aspirin use were presented separately as clinically meaningful symptom categories, which were defined by the chief investigator.

Serious adverse reactions and ADRs that led to trial discontinuation were summarised by treatment group and preferred term.

All treatment-emergent ADRs were listed.

## Exploratory analyses

- Colorectal adenoma size was estimated using a multilevel model, adjusting for colorectal adenoma histology type and number of colorectal adenomas per individual.
- Rectal and RBC EPA levels were plotted against the individual number of total, conventional, serrated and left- and right-sided colorectal adenomas by treatment group.
- A correlation between rectal mucosal and RBC EPA levels at 12 months was investigated by graph and the correlation coefficient was calculated.

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# Chapter 3 Results

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## Recruitment and follow-up

Between November 2011 and June 2016, 3911 'high-risk' individuals were screened for eligibility, of whom 3202 (82%) were not randomised (*Table 6* and *Figures 2–4*). The mean duration from receipt of local research and development (R&D) approval to FPFV for the 53 sites that randomised patients was 6.3 months. Conversion from screening to randomisation of patients did not appear to be influenced by the size of the site or the number of patients undergoing screening colonoscopy. Sites did report that the time taken to undertake trial screening and recruitment (as well as limited availability of, and changes to, research staff) were reasons for low recruitment.

**TABLE 6** Site set-up, screening and recruitment, by site

| Site number | R&D approval date | Date of FPFV | Time from R&D approval to FPFV (months) | Patients | | |
|---|---|---|---|---|---|---|
| | | | | Screened (*n*) | Recruited (*n*) | Randomised (%) |
| 1 | 18 August 2011 | 7 February 2012 | 6 | 121 | 19 | 16 |
| 2 | 23 September 2011 | 24 November 2011 | 2 | 123 | 14 | 11 |
| 3 | 17 April 2012 | 22 May 2012 | 1 | 92 | 18 | 20 |
| 4 | 31 August 2012 | 29 November 2012 | 3 | 114 | 20 | 18 |
| 5 | 20 September 2011 | 23 April 2012 | 7 | 151 | 37 | 25 |
| 6 | 19 August 2011 | 20 February 2012 | 6 | 77 | 20 | 26 |
| 7 | 5 May 2011 | 31 May 2012 | 13 | 56 | 9 | 16 |
| 8 | 20 July 2011 | 23 March 2012 | 8 | 100 | 30 | 30 |
| 9 | 14 June 2011 | 21 June 2012 | 12 | 147 | 12 | 8 |
| 10 | 25 August 2011 | 10 October 2012 | 14 | 73 | 6 | 8 |
| 11 | 30 June 2011 | 18 January 2012 | 7 | 120 | 23 | 19 |
| 12 | 27 June 2011 | 21 March 2012 | 9 | 53 | 9 | 17 |
| 13 | 8 February 2012 | N/A | N/A | 9 | 0 | 0 |
| 14 | 5 July 2011 | 12 April 2012 | 9 | 107 | 27 | 25 |
| 15 | 13 January 2012 | 15 March 2012 | 2 | 89 | 25 | 28 |
| 16 | 11 April 2011 | 30 January 2012 | 10 | 64 | 11 | 17 |
| 17 | 2 June 2011 | 12 January 2012 | 7 | 66 | 6 | 9 |
| 18 | 24 April 2012 | 27 June 2013 | 14 | 45 | 6 | 13 |
| 20 | 17 February 2012 | 25 April 2012 | 2 | 53 | 21 | 40 |
| 22 | 6 July 2012 | 18 December 2012 | 5 | 26 | 8 | 31 |
| 24 | 29 August 2012 | 9 January 2013 | 4 | 8 | 1 | 13 |
| 25 | 10 April 2012 | 29 May 2012 | 2 | 103 | 12 | 12 |

**TABLE 6** Site set-up, screening and recruitment, by site (*continued*)

| Site number | R&D approval date | Date of FPFV | Time from R&D approval to FPFV (months) | Patients | | |
|---|---|---|---|---|---|---|
| | | | | Screened (*n*) | Recruited (*n*) | Randomised (%) |
| 26 | 7 November 2012 | 10 February 2015 | 27 | 22 | 5 | 23 |
| 27 | 5 May 2011 | 11 November 2011 | 6 | 93 | 24 | 26 |
| 28 | 7 December 2011 | 2 February 2012 | 2 | 177 | 35 | 20 |
| 29 | 27 July 2011 | 9 February 2012 | 6 | 14 | 1 | 7 |
| 30 | 5 July 2012 | N/A | N/A | 8 | 0 | 0 |
| 31 | 18 August 2011 | 23 May 2012 | 9 | 1 | 1 | 100 |
| 32 | 23 August 2011 | 30 August 2012 | 12 | 92 | 11 | 12 |
| 33 | 11 April 2012 | 25 June 2013 | 14 | 51 | 12 | 24 |
| 34 | 22 March 2012 | 29 October 2012 | 7 | 44 | 9 | 20 |
| 35 | 30 March 2012 | 10 July 2012 | 3 | 44 | 12 | 27 |
| 36 | 14 September 2012 | 15 March 2013 | 6 | 95 | 9 | 9 |
| 37 | 8 March 2013 | 22 August 2013 | 6 | 85 | 10 | 12 |
| 38 | 18 September 2012 | 11 January 2013 | 4 | 133 | 10 | 8 |
| 39 | 10 October 2012 | 12 November 2012 | 1 | 28 | 4 | 14 |
| 40 | 5 October 2012 | 26 October 2012 | 1 | 97 | 22 | 23 |
| 41 | 13 September 2012 | 11 March 2013 | 6 | 76 | 12 | 16 |
| 42 | 10 September 2012 | 9 January 2013 | 4 | 30 | 6 | 20 |
| 43 | 23 January 2013 | N/A | N/A | 12 | 0 | 0 |
| 44 | 6 November 2012 | 14 February 2013 | 3 | 89 | 15 | 17 |
| 45 | 12 October 2012 | 17 January 2013 | 3 | 62 | 12 | 19 |
| 47 | 23 January 2013 | N/A | N/A | 9 | 0 | 0 |
| 49 | 6 September 2012 | 20 November 2012 | 2 | 52 | 15 | 29 |
| 50 and 51 | 9 November 2012 | 16 January 2013 | 4 | 231 | 47 | 16 |
| 52 | 16 August 2012 | 11 October 2013 | 14 | 141 | 9 | 6 |
| 53 | 17 August 2012 | 23 January 2013 | 5 | 124 | 29 | 23 |
| 54 | 20 August 2012 | 21 September 2012 | 1 | 48 | 16 | 33 |
| 55 | 3 October 2012 | 4 January 2013 | 3 | 16 | 4 | 25 |
| 56 | 28 August 2012 | 18 September 2013 | 13 | 59 | 8 | 14 |
| 57 | 23 October 2012 | 11 April 2013 | 6 | 59 | 10 | 17 |
| 58 | 19 July 2012 | 11 October 2012 | 3 | 33 | 5 | 15 |
| 60 | 12 November 2012 | 17 June 2013 | 7 | 35 | 6 | 17 |
| 61 | 1 February 2013 | 17 April 2013 | 2 | 19 | 6 | 32 |
| 62 | 5 September 2012 | 10 October 2012 | 1 | 24 | 8 | 33 |
| 63 | 7 September 2015 | 21 April 2016 | 7 | 11 | 2 | 18 |
| Total | | | | 3911 | 709 | |

N/A, not available.
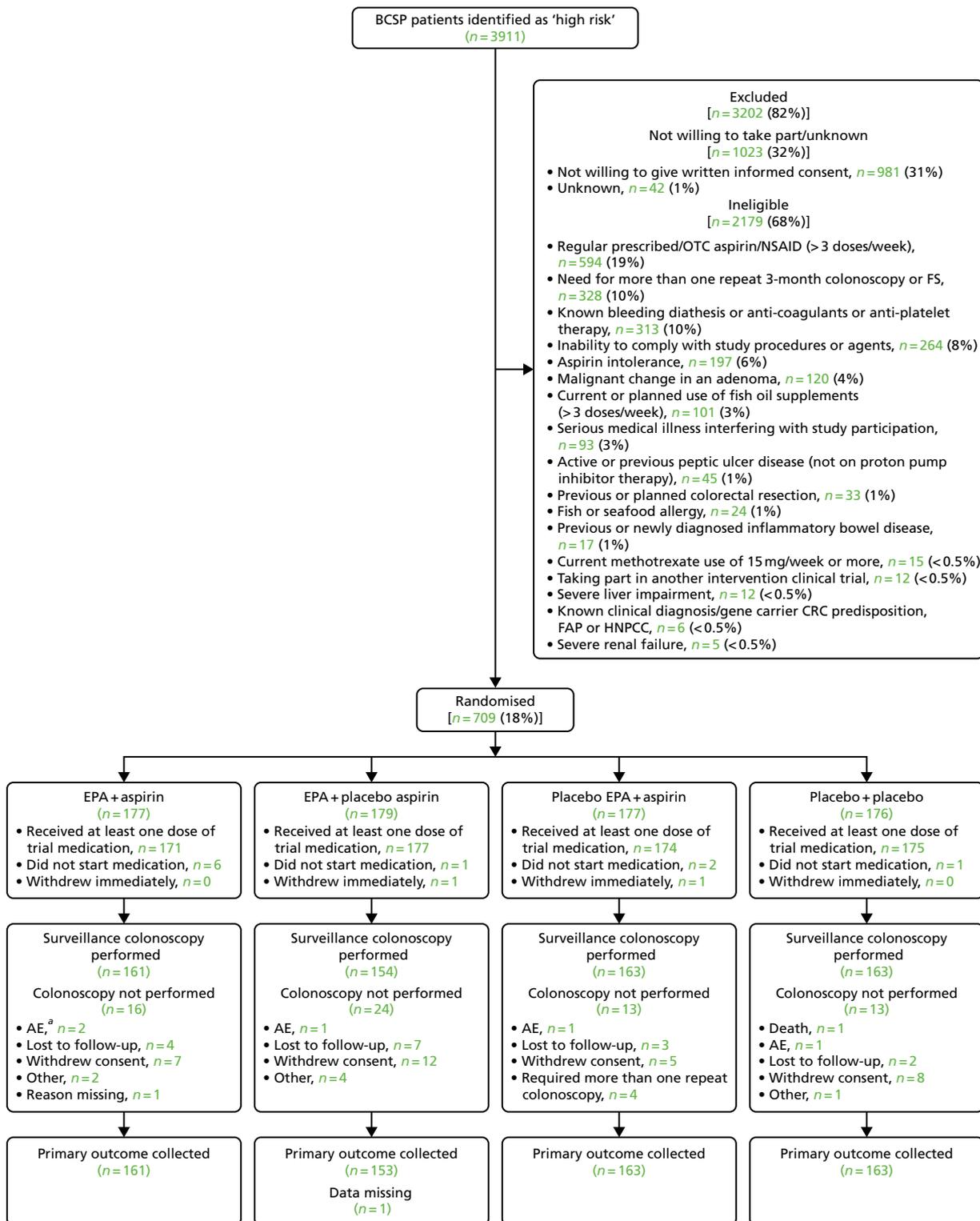
**FIGURE 2** Flow of participants through the trial. a, AE was an option to discontinue from the trial in an earlier version of the CRF; however, this option was removed in later versions. HNPCC, hereditary non-polyposis colon cancer.
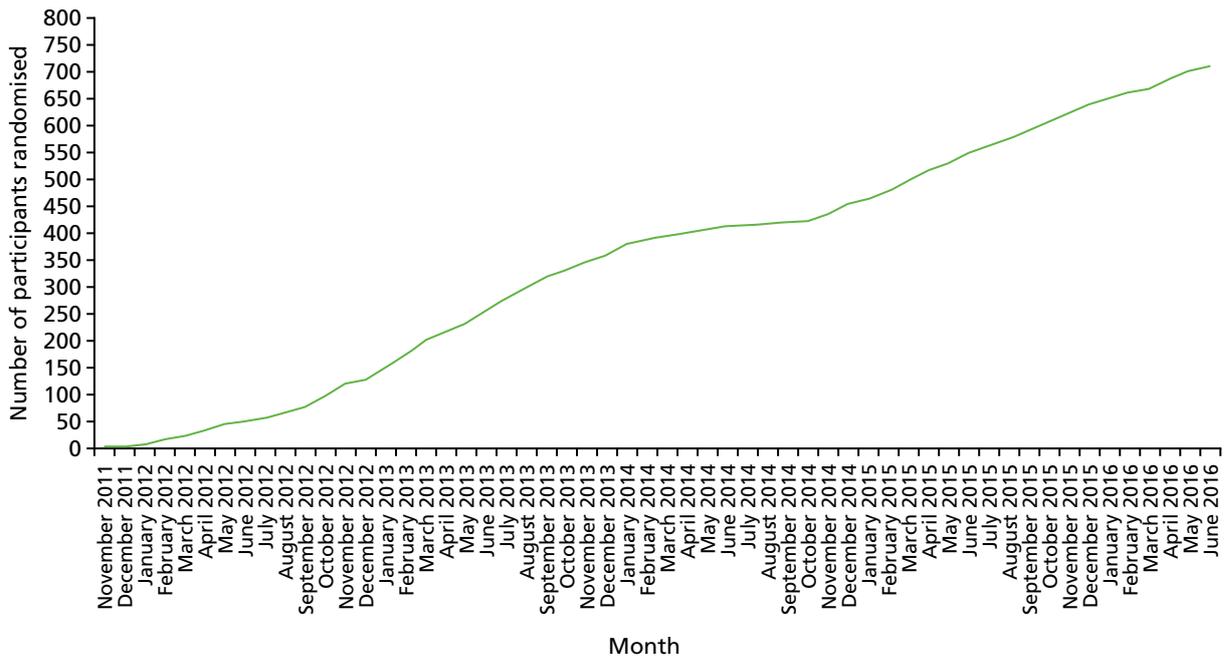
**FIGURE 3** Cumulative trial recruitment by month.
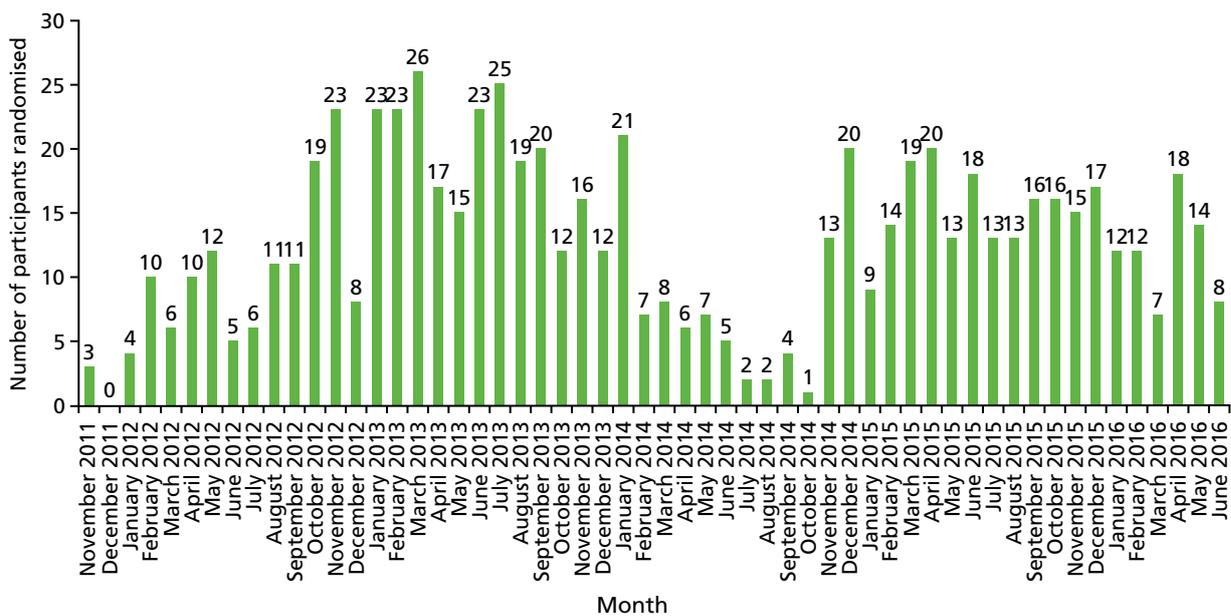


**FIGURE 4** Actual monthly trial recruitment.

Overall, 2179 (56%) patients met one or more of the exclusion criteria (see *Figure 4*). The other 1023 individuals either did not wish to take part in the trial or were not randomised for unknown reasons. The most frequent reasons for no randomisation were not being willing to give written consent [$n = 981$ (25%)], taking more than three doses per week of a prescribed/OTC aspirin/NSAID [$n = 594$ (15%)], the need for more than one repeat colonoscopy or FS [$n = 328$ (8%)] and known bleeding diathesis or anticoagulant use or antiplatelet therapy [$n = 313$ (8%)]. Overall, only 18% ($n = 709$) of screened 'high-risk' individuals were randomised (see *Figure 2*).

By 12 June 2016 (the date stipulated by the MHRA as the end date for randomisation related to the approved shelf life of the second capsule IMP (90% EPA-TG), 709 patients had been randomised, which was 83% of the target (*n* = 853). Reduced recruitment between February and October 2014 (see *Table 6* and *Figures 3* and *4*) was related to diminishing stocks of active and placebo EPA-FFA at sites, prior to the introduction of 90% EPA-TG IMP in November 2014. The mean number of randomisations per month during the whole recruitment phase of the trial was 13. Excluding the run-in period of 12 months, during which sites opened at a variable rate, and the period when capsule IMP stocks were diminishing (February to October 2014), the mean number of randomisations per month was 16 (range 7–26 per month).

Of the 709 participants randomised, 177 participants were randomised to receive EPA + aspirin, 179 were randomised to receive EPA + placebo aspirin, 177 were randomised to receive placebo EPA + aspirin and 176 were randomised to receive placebo EPA + placebo aspirin (see *Figure 2*). Two participants withdrew immediately after randomisation; one was randomised to EPA + placebo aspirin and one was randomised to placebo EPA + aspirin (see *Figure 2*).

A total of 422 (60%) participants were randomised to active or placebo EPA-FFA and 287 (40%) participants were randomised to active or placebo 90% EPA-TG. Participants took only one form of capsule IMP (EPA-FFA or EPA-TG) in all cases.

A total of 641 participants (90% of those randomised) underwent surveillance colonoscopy, with endoscopic data being available for 640 of these participants (see *Figure 2*). The main reasons for not performing surveillance colonoscopy were withdrawal of consent and loss to follow-up (*Table 7* and see *Figure 2*). The 'other' reasons for discontinuation in the trial were the participant was withdrawn because of being randomised in error (*n* = 3), the histology report confirmed a malignant polyp and the participant was referred to MDT for review, failure to comply with trial treatment as a result of ongoing medical problems, the participant being prescribed aspirin and development of cancer (all *n* = 1).

## Baseline characteristics

Baseline characteristics were well balanced across all four treatment groups with respect to demographic data, medical history and baseline colorectal adenoma details (*Table 8*).

**TABLE 7** Discontinuation in the trial

| Reason | Trial group, *n* (%) | | | |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) |
| --- | --- | --- | --- | --- |
| Total discontinued in trial | 16 (9) | 24 (13) | 13 (7) | 13 (7) |
| Death | 0 (0) | 0 (0) | 0 (0) | 1 (1) |
| AE | 2 (1) | 1 (1) | 1 (1) | 1 (1) |
| Lost to follow-up | 4 (2) | 7 (4) | 3 (2) | 2 (1) |
| Withdrew consent | 7 (4) | 12 (7) | 5 (3) | 8 (5) |
| Required more than one repeat colonoscopy | 0 (0) | 0 (0) | 4 (2) | 0 (0) |
| Other | 2 (1) | 4 (2) | 0 (0) | 1 (1) |
| Missing | 1 (1) | 0 (0) | 0 (0) | 0 (0) |

**TABLE 8** Baseline characteristics

| Characteristic | Trial group | | | | |
| --- | --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) | Total (*N* = 707) |
| *Age at enrolment (years)* | | | | | |
| Mean (SD) | 65.6 (4.7) | 65.2 (4.5) | 65.3 (4.5) | 65.2 (4.6) | 65.3 (4.6) |
| Median (IQR) | 66.4 (62.3–68.6) | 64.9 (62.2–68.5) | 64.8 (62.2–68.5) | 64.8 (62.3–68.6) | 65.3 (62.2–68.6) |
| *Sex, n (%)* | | | | | |
| Male | 146 (82) | 138 (78) | 140 (80) | 139 (79) | 563 (80) |
| Female | 31 (18) | 40 (22) | 36 (20) | 37 (21) | 144 (20) |
| *Body mass index (kg/m$^2$), n (%)* | | | | | |
| Underweight (< 18.5) | 0 (< 0.5) | 2 (1) | 1 (1) | 0 (< 0.5) | 3 (< 0.5) |
| Normal (18.5–24.9) | 39 (22) | 29 (16) | 23 (13) | 32 (18) | 123 (17) |
| Overweight (25.0–29.9) | 77 (44) | 77 (43) | 81 (46) | 76 (43) | 311 (44) |
| Obese (≥ 30) | 61 (34) | 70 (39) | 71 (40) | 68 (39) | 270 (38) |
| *Diabetes*<br>*Ever diagnosed?* | | | | | |
| n (%) | 15 (8) | 24 (13) | 18 (10) | 24 (14) | 81 (11) |
| *Years since first diagnosis* | | | | | |
| Mean (SD) | 8.0 (5.3) | 8.5 (5.8) | 9.3 (5.3) | 10.8 (5.5) | 9.3 (5.5) |
| Median (IQR) | 5.0 (5.0–12.0) | 7.0 (5.0–10.5) | 8.5 (5.0–12.0) | 10.0 (6.5–14.0) | 8.0 (5.0–12.0) |
| *Cigarette smoking, n (%)* | | | | | |
| Current smoker | 32 (18) | 13 (7) | 27 (15) | 34 (19) | 106 (15) |
| Ex-smoker | 80 (45) | 96 (54) | 89 (51) | 82 (47) | 347 (49) |
| Never smoked | 65 (37) | 69 (39) | 60 (34) | 60 (34) | 254 (36) |
| *Cigarettes smoked per day (current smoker)* | | | | | |
| None | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 1–10 | 11 (6) | 6 (3) | 12 (7) | 16 (9) | 45 (6) |
| 11–20 | 17 (10) | 6 (3) | 12 (7) | 12 (7) | 47 (7) |
| ≥ 21 | 3 (2) | 1 (1) | 3 (2) | 6 (3) | 13 (2) |
| Missing | 1 (1) | 0 (0) | 0 (0) | 0 (0) | 1 (< 0.5) |
| *Units of alcohol per week, n (%)* | | | | | |
| None | 27 (15) | 28 (16) | 26 (15) | 29 (16) | 110 (16) |
| 1–7 | 51 (29) | 68 (38) | 64 (36) | 51 (29) | 234 (33) |
| 8–21 | 60 (34) | 41 (23) | 58 (33) | 55 (31) | 214 (30) |
| ≥ 22 | 38 (21) | 39 (22) | 28 (16) | 41 (23) | 146 (21) |
| Missing | 1 (1) | 2 (1) | 0 (0) | 0 (0) | 3 (< 0.5) |
| *Medical history,[a] n (%)* | | | | | |
| Abdominal pain/dyspepsia | 41 (23) | 40 (22) | 41 (23) | 36 (20) | 158 (22) |
| Diarrhoea | 9 (5) | 19 (11) | 15 (9) | 19 (11) | 62 (9) |
| Halitosis | 5 (3) | 5 (3) | 5 (3) | 5 (3) | 20 (3) |
| Bleeding | 14 (8) | 20 (11) | 18 (10) | 20 (11) | 72 (10) |
| Stroke | 1 (1) | 0 | 1 (1) | 1 (1) | 3 (< 0.5) |

**TABLE 8** Baseline characteristics (*continued*)

| Characteristic | Trial group | | | | |
| --- | --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) | Total (*N* = 707) |
| ***Colorectal adenoma characteristics*** | | | | | |
| Total number of adenomas | 856 | 892 | 927 | 856 | 3531 |
| Adenomas per participant | | | | | |
|  Mean (SD) | 4.8 (2.3) | 5.0 (2.2) | 5.3 (2.7) | 4.9 (2.6) | 5.0 (2.5) |
|  Median (IQR) | 4 (3–6) | 5 (3–6) | 5 (3–6) | 4 (3–5) | 4 (3–6) |
| 'Advanced'[b] adenomas per participant | | | | | |
|  Mean (SD) | 1.1 (0.9) | 1.3 (1.0) | 1.2 (0.9) | 1.2 (0.9) | 1.2 (0.9) |
|  Median (IQR) | 1 (1–1) | 1 (1–2) | 1 (1–2) | 1 (1–2) | 1 (1–2) |
| Size of largest adenoma (mm) per participant | | | | | |
|  Mean (SD) | 14.3 (7.3) | 14.8 (6.9) | 14.3 (6.9) | 13.7 (5.6) | 14.3 (6.7) |
|  Median (IQR) | 13 (10–17) | 13.5 (11–18) | 13 (10–18) | 12.5 (10–16) | 13 (10–17) |
| At least one adenoma proximal to splenic flexure, *n* (%) | 144 (81) | 146 (82) | 153 (87) | 141 (80) | 584 (83) |
| ***Histology type,[c] n (%)*** | | | | | |
| Conventional | 809 (95) | 844 (95) | 895 (97) | 812 (95) | 3360 (95) |
|  Tubular/tubulo-villous | 803 (94) | 834 (93) | 885 (95) | 807 (94) | 3329 (94) |
|  Villous | 6 (< 1) | 10 (1) | 10 (1) | 5 (1) | 31 (1) |
| Serrated | 21 (2) | 30 (3) | 18 (2) | 22 (3) | 91 (3) |
| Not sent to histopathology | 21 (2) | 16 (2) | 13 (2) | 18 (2) | 68 (2) |
| Missing | 5 (1) | 2 (< 1) | 1 (< 1) | 4 (< 1) | 12 (< 1) |
| ***High-grade dysplasia,[c] n (%)*** | | | | | |
| No | 811 (95) | 841 (94) | 874 (94) | 796 (93) | 3322 (94) |
| Yes | 21 (2) | 33 (4) | 40 (4) | 41 (5) | 135 (4) |
| Missing | 24 (3) | 18 (2) | 13 (1) | 19 (2) | 74 (2) |
| ***Participants with any regular prescribed medication prior to trial entry*** | | | | | |
| *n* (%) | 92 (52) | 93 (52) | 88 (50) | 81 (46) | 354 (50) |
| ***Participants who had any of the following regular prescribed medications,[a] n (%)*** | | | | | |
| Statin | 55 (31) | 54 (30) | 51 (29) | 50 (28) | 210 (30) |
| Calcium | 0 (0) | 3 (2) | 3 (2) | 1 (< 1) | 7 (1) |
| Calcium + vitamin D | 4 (2) | 1 (< 1) | 4 (2) | 2 (1) | 11 (2) |
| Metformin | 9 (5) | 12 (7) | 11 (6) | 14 (8) | 46 (7) |
| Glitazone | 0 (0) | 1 (< 1) | 1 (< 1) | 1 (< 1) | 3 (< 1) |
| Proton pump inhibitor | 20 (11) | 27 (15) | 24 (14) | 19 (11) | 90 (13) |
| Aspirin | 1 (< 1) | 0 (0) | 0 (0) | 0 (0) | 1 (< 1) |
| Fish oil | 2 (1) | 4 (2) | 2 (1) | 1 (< 1) | 9 (1) |
| Non-aspirin NSAID | 5 (3) | 4 (2) | 1 (< 1) | 1 (< 1) | 11 (2) |
| Other | 48 (27) | 34 (19) | 37 (21) | 34 (19) | 153 (22) |

**TABLE 8** Baseline characteristics *(continued)*

| Characteristic | Trial group | | | | |
| --- | --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) | Total (*N* = 707) |
| *Participants who required repeat endoscopy at trial entry,* **n (%)** | | | | | |
| No | 133 (75) | 128 (72) | 133 (76) | 136 (77) | 530 (75) |
| Yes | 34 (19) | 33 (19) | 24 (14) | 25 (14) | 116 (16) |
| Missing | 10 (6) | 17 (10) | 19 (11) | 15 (9) | 61 (9) |

IQR, interquartile range.
a  Not mutually exclusive; some participants reported more than one category.
b  Diameter of ≥ 10 mm, high-grade dysplasia or tubulo-villous/villous histology.
c  Adenoma-level data.

The mean age of participants was 65 years. A male-to-female ratio of approximately 4 : 1 was observed, which reflects the sex distribution of 'high-risk' patients in the English BCSP.[95,99] A total of 581 (82%) participants were overweight; of these, 270 (38%) were obese. Despite the high prevalence of excess body weight, the prevalence of known type 2 diabetes was low, and only approximately half of the participants were on regular prescribed drugs at trial entry; the drug was usually a statin, proton pump inhibitor or metformin. Baseline drug use was balanced across the treatment groups.

There was a slight imbalance across groups with respect to smoking status, with fewer current smokers in the EPA + placebo aspirin group.

Overall, 158 (22%) participants had a medical history of abdominal pain or dyspepsia, which is consistent with the high prevalence of these symptoms in the general population.[113] Ten per cent of participants recorded a prior episode of bleeding and 20 (3%) reported halitosis at baseline. A medical history of symptoms and clinical events at baseline relevant to EPA and/or aspirin therapy was balanced across the treatment groups. Fewer participants reported diarrhoea at trial entry in the EPA + aspirin group than in the other three groups.

'High-risk' participants had a mean of five colorectal adenomas and one advanced colorectal adenoma at entry screening colonoscopy. Of 3531 evaluable colorectal adenomas at baseline, 3360 (97%) were conventional (tubular/tubulo-villous and villous) and 91 (3%) were serrated adenomas. Overall, 584 (83%) 'high-risk' participants had at least one colorectal adenoma proximal to the splenic flexure at screening colonoscopy. Screening colonoscopy findings were balanced across the treatment groups (see *Table 8*).

## Compliance with the allocated intervention

Compliance with both capsule and tablet IMPs was excellent, with mean percentage compliance levels, calculated by capsule/tablet counting, of between 94% and 97%. A total of 10 participants never took any dose of either capsules or tablets and a further seven participants took only one of the treatments (EPA/placebo EPA or aspirin/placebo aspirin) (*Table 9*).

## Concomitant medication during the trial

During the trial intervention phase, approximately one-quarter of participants started regular prescribed medication, in addition to existing drugs, which included a statin, calcium + vitamin D supplement, metformin, proton pump inhibitors, aspirin, fish oil and a non-aspirin NSAID. Slightly more participants started regular, concomitant medication in the placebo + placebo group (*Table 10*).

**TABLE 9** Adherence to trial medication

| Adherence | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin ($N$ = 177) | EPA + placebo aspirin ($N$ = 178) | Placebo EPA + aspirin ($N$ = 176) | Placebo + placebo ($N$ = 176) |
| Participants who never started taking either of capsules or tablets, $n$ (%) | 7 (4) | 2 (1) | 4 (2) | 4 (2) |
| Never started either, $n$ (%) | 6 (3) | 1 (1) | 2 (1) | 1 (1) |
| Never started capsules but started tablets, $n$ (%) | 1 (1) | 0 (0) | 1 (1) | 1 (1) |
| Never started tablets but started capsules, $n$ (%) | 0 (0) | 1 (1) | 1 (1) | 2 (1) |
| Capsules taken as percentage[a] of total expected | | | | |
| Mean (SD) | 96 (15) | 94 (13) | 95 (12) | 95 (14) |
| Median (IQR) | 100 (97–100) | 99 (96–100) | 100 (97–100) | 100 (97–100) |
| Minimum, maximum | 10, 197 | 27, 101 | 40, 123 | 20, 100 |
| Tablets taken as percentage[a] of total expected | | | | |
| Mean (SD) | 97 (8) | 97 (9) | 97 (6) | 97 (9) |
| Median (IQR) | 100 (98–100) | 99 (97–100) | 100 (98–100) | 100 (98–100) |
| Minimum, maximum | 10, 100 | 27, 101 | 55, 100 | 71, 196 |

IQR, interquartile range.
a Percentage compliance with medication was calculated based on the total number of calculated doses when participants were taking the medication as a proportion of the total number of expected doses. The period during which participants should have taken medication was between randomisation and the first surveillance colonoscopy. If participants discontinued and did not have the surveillance colonoscopy, then the period was between randomisation and the last date known to be taking medication.

**TABLE 10** Concomitant medication during the trial

| Concomitant medication | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin ($N$ = 177) | EPA + placebo aspirin ($N$ = 178) | Placebo EPA + aspirin ($N$ = 176) | Placebo + placebo ($N$ = 176) |
| Participants starting regular prescribed medication during the trial, $n$ (%) | 42 (24) | 43 (24) | 42 (24) | 55 (31) |
| Number of participants who started the following regular prescribed medications[a] | | | | |
| Statins | 6 | 12 | 6 | 9 |
| Calcium + vitamin D | 1 | 1 | 0 | 0 |
| Metformin | 1 | 2 | 3 | 3 |
| Proton pump inhibitors | 5 | 13 | 8 | 12 |
| Aspirin | 3 | 6 | 1 | 5 |
| Fish oil | 1 | 1 | 0 | 1 |
| Non-aspirin NSAIDs | 13 | 9 | 16 | 23 |
| Other | 25 | 17 | 17 | 23 |

a Not mutually exclusive as some participants reported more than one.

## Dietary fish intake before trial participation

Most participants completed a FFQ at baseline; there were similar percentages across dietary fish intake categories in each of the treatment groups. The total fish and oily fish intake at baseline was similar across the four treatment groups (*Table 11*).

## Polyunsaturated fatty acid levels in trial participants

Given the importance of the comparison of EPA levels in individuals randomised to either FFA or TG formulations of EPA, to determine if it was appropriate to pool the primary and secondary outcome data for these groups, PUFA data are presented below before the analyses for the primary and other secondary outcomes.

The RBC EPA level was measured as an accepted biomarker of tissue EPA exposure, as well as the rectal mucosal EPA level measured at the end of the intervention period.

Red blood cell EPA and rectal mucosal EPA levels were compared between each treatment group, at each time point and between users of EPA-FFA and EPA-TG formulations. As expected, active EPA groups had higher RBC levels of EPA than placebo EPA users after the intervention began (*Figures 5–7*). RBC EPA levels were similar between participants, who received either active FFA or TG formulations of EPA, at baseline, mid-treatment and at the end of the intervention period (see *Figures 5–7*). Moreover, the increase in

**TABLE 11** Dietary fish and other seafood intake at baseline

| Dietary fish intake | Trial group, *n* (%) | | | |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) |
| --- | --- | --- | --- | --- |
| Participants who completed FFQ at baseline | 177 (100) | 176 (99) | 173 (98) | 174 (99) |
| Total fish intake at baseline | | | | |
| Never | 4 (2) | 7 (4) | 4 (2) | 11 (6) |
| Low | 12 (7) | 13 (7) | 9 (5) | 10 (6) |
| Medium | 97 (55) | 92 (52) | 109 (63) | 97 (56) |
| High | 51 (29) | 52 (30) | 44 (25) | 44 (25) |
| Missing[a] | 13 (7) | 12 (7) | 7 (4) | 12 (7) |
| Oily fish intake at baseline | | | | |
| Never | 48 (27) | 52 (30) | 43 (25) | 42 (24) |
| Low | 54 (31) | 52 (30) | 53 (31) | 69 (40) |
| Medium | 41 (23) | 46 (26) | 52 (31) | 42 (24) |
| High | 32 (18) | 23 (13) | 22 (13) | 20 (11) |
| Missing[a] | 2 (1) | 3 (2) | 3 (2) | 1 (1) |

a  The FFQ was completed; however, the data were not evaluable.

RBC EPA level from baseline to the 6-month and 12-month time points for all participants was similar across the two EPA formulations and for rectal mucosal EPA levels at 12 months (*Table 12*) (see *Figures 5–7*). There was also no clear difference in RBC or rectal mucosal DHA content between EPA-FFA and EPA-TG users (*Table 13*; see also *Table 12*).

Rectal mucosal EPA levels at the end of the intervention period were higher in those who received EPA-TG than in those who received EPA-FFA, but with substantial overlap between the two groups (*Figure 8*). This was not reflected in the rectal mucosal EPA-to-AA ratio at the end of the intervention period, which was similar for both EPA-FFA and EPA-TG users (*Table 14*).
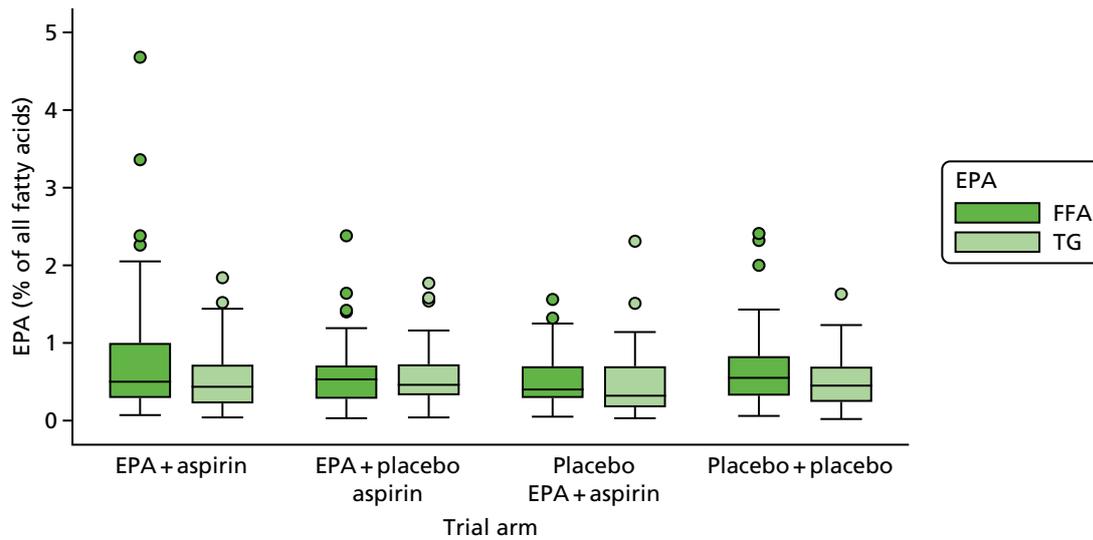


**FIGURE 5** The RBC EPA levels by formulation and by trial group at baseline.
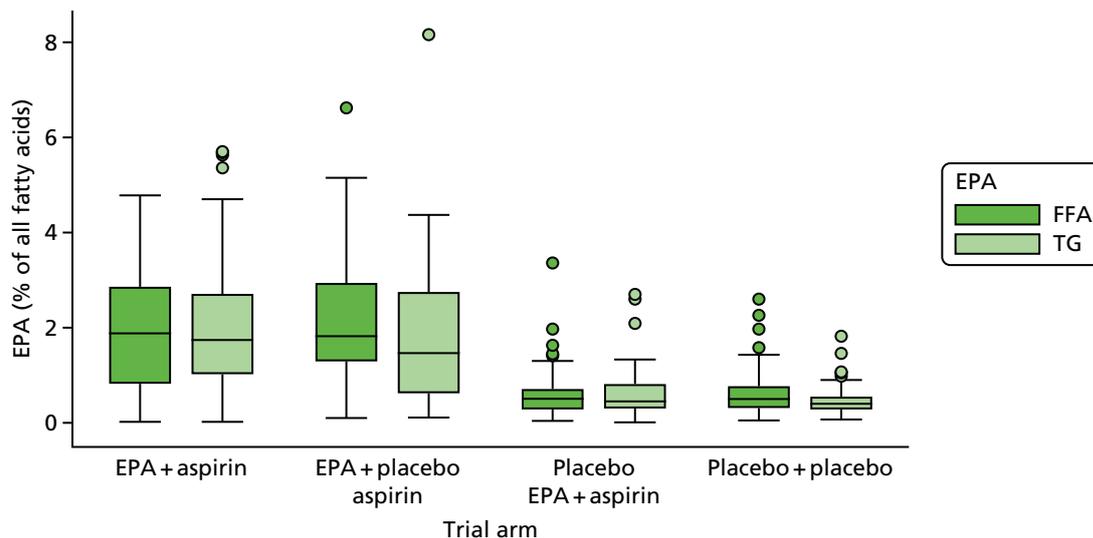


**FIGURE 6** The RBC EPA levels by formulation and by trial group at 6 months.
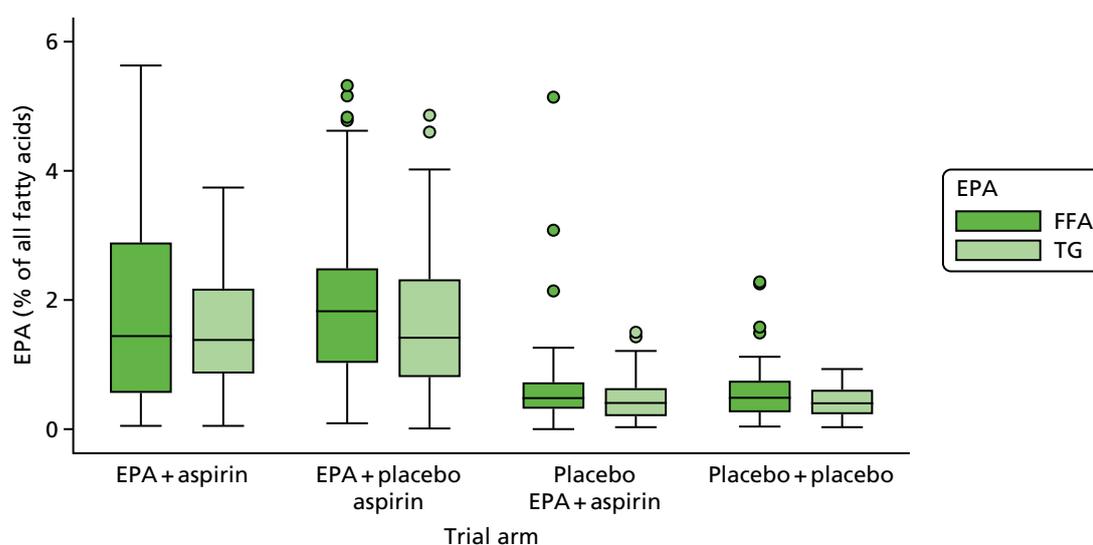
**FIGURE 7** The RBC EPA levels by formulation and by trial group at 12 months.

**TABLE 12** The RBC EPA levels in those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only)

| | EPA | | |
| RBC and rectal mucosal EPA levels[a] | FFA (*N* = 212) | TG (*N* = 143) | Total (*N* = 355) |
|---|---|---|---|
| **_RBC EPA level[a]_** | | | |
| Baseline | | | |
| Mean (SD) | 0.6 (0.6) | 0.5 (0.4) | 0.6 (0.5) |
| Median (IQR) | 0.5 (0.3–0.8) | 0.4 (0.3–0.7) | 0.5 (0.3–0.8) |
| Minimum, maximum | 0.0, 4.7 | 0.0, 1.8 | 0.0, 4.7 |
| *n* | 181 | 128 | 309 |
| 6 months | | | |
| Mean (SD) | 2.0 (1.3) | 1.9 (1.4) | 1.9 (1.3) |
| Median (IQR) | 1.9 (0.9–2.9) | 1.6 (0.9–2.7) | 1.8 (0.9–2.8) |
| Minimum, maximum | 0.0, 6.6 | 0.0, 8.2 | 0.0, 8.2 |
| *n* | 164 | 108 | 272 |
| 12 months | | | |
| Mean (SD) | 1.9 (1.4) | 1.6 (1.0) | 1.8 (1.3) |
| Median (IQR) | 1.7 (0.9–2.8) | 1.4 (0.8–2.2) | 1.6 (0.9–2.4) |
| Minimum, maximum | 0.1, 5.6 | 0.0, 4.9 | 0.0, 5.6 |
| *n* | 157 | 100 | 257 |
| Absolute change in RBC EPA level[a] from baseline at 6 months | | | |
| Mean (SD) | 1.3 (1.4) | 1.3 (1.4) | 1.3 (1.4) |
| Median (IQR) | 1.3 (0.3–2.3) | 1.1 (0.4–2.2) | 1.2 (0.3–2.3) |
| Minimum, maximum | –4.5, 6.1 | –1.7, 7.2 | –4.5, 7.2 |
| *n* | 151 | 103 | 254 |

**TABLE 12** The RBC EPA levels in those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only) (*continued*)

| RBC and rectal mucosal EPA levels[a] | EPA | | Total (*N* = 355) |
|---|---|---|---|
| | FFA (*N* = 212) | TG (*N* = 143) | |
| **Absolute change in RBC EPA level[a] from baseline at 12 months** | | | |
| Mean (SD) | 1.2 (1.4) | 1.1 (1.1) | 1.2 (1.2) |
| Median (IQR) | 1.0 (0.1–2) | 0.9 (0.3–1.6) | 1.0 (0.2–1.9) |
| Minimum, maximum | −3.0, 5.2 | −0.8, 4.2 | −3.0, 5.2 |
| *n* | 143 | 95 | 238 |
| **Rectal mucosal EPA level[a] at 12 months** | | | |
| Mean (SD) | 1.3 (1.0) | 1.8 (1.1) | 1.5 (1.1) |
| Median (IQR) | 1.1 (0.6–1.7) | 1.6 (1.0–2.3) | 1.3 (0.7–2.0) |
| Minimum, maximum | 0.0, 5.2 | 0.4, 5 | 0.0, 5.2 |
| *n* | 153 | 96 | 249 |

IQR, interquartile range.
a  PUFA data are percentage of total fatty acids.

**TABLE 13** The DHA, AA and EPA-to-AA ratio levels for those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only)

| RBC levels[a] | EPA | | Total (*N* = 355) |
|---|---|---|---|
| | FFA (*N* = 212) | TG (*N* = 143) | |
| ***RBC DHA level[a]*** | | | |
| Baseline | | | |
| Mean (SD) | 2.1 (1.4) | 2.3 (1.8) | 2.2 (1.5) |
| Median (IQR) | 2.0 (1.0–2.9) | 2.1 (1.0–3.0) | 2.0 (1.0–2.9) |
| Minimum, maximum | 0.1, 7.7 | 0.1, 9.7 | 0.1, 9.7 |
| *n* | 181 | 128 | 309 |
| 6 months | | | |
| Mean (SD) | 2.0 (1.2) | 1.6 (1.2) | 1.8 (1.2) |
| Median (IQR) | 2.0 (1.0–2.5) | 1.5 (0.6–2.0) | 1.7 (0.8–2.4) |
| Minimum, maximum | 0.0, 7.5 | 0.1, 6.8 | 0.0, 7.5 |
| *n* | 164 | 108 | 272 |
| 12 months | | | |
| Mean (SD) | 2.2 (1.5) | 1.9 (1.1) | 2.1 (1.4) |
| Median (IQR) | 2.2 (1.3–2.8) | 1.9 (1.2–2.3) | 1.9 (1.2–2.7) |
| Minimum, maximum | 0.1, 14.4 | 0.0, 6.6 | 0.0, 14.4 |
| *n* | 157 | 100 | 257 |

**TABLE 13** The DHA, AA and EPA-to-AA ratio levels for those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only) (continued)

| | EPA | | |
| RBC levels[a] | FFA (N = 212) | TG (N = 143) | Total (N = 355) |
|---|---|---|---|
| Change from baseline at 6 months | | | |
| Mean (SD) | –0.2 (1.4) | –0.5 (1.6) | –0.3 (1.5) |
| Median (IQR) | –0.2 (–0.9 to 4.0) | –0.4 (–1.2 to 3.0) | –0.2 (–1.0 to 3.0) |
| Minimum, maximum | –4.5, 5.4 | –8.6, 3.7 | –8.6, 5.4 |
| n | 151 | 103 | 254 |
| Change from baseline at 12 months | | | |
| Mean (SD) | –0.1 (1.4) | –0.3 (1.7) | –0.2 (1.5) |
| Median (IQR) | –0.2 (–0.9 to 8.0) | –0.3 (–1.4 to 7.0) | –0.2 (–1.1 to 7.0) |
| Minimum, maximum | –4.2, 4.3 | –7.7, 2.8 | –7.7, 4.3 |
| n | 143 | 95 | 238 |
| **RBC AA level[a]** | | | |
| Baseline | | | |
| Mean (SD) | 6.3 (3.9) | 7.0 (4.9) | 6.6 (4.4) |
| Median (IQR) | 5.8 (3.7–8.2) | 6.3 (3.9–9.0) | 5.9 (3.7–8.3) |
| Minimum, maximum | 0.4, 27.9 | 0.5, 32.1 | 0.4, 32.1 |
| n | 181 | 128 | 309 |
| 6 months | | | |
| Mean (SD) | 5.6 (2.9) | 5.5 (3.3) | 5.5 (3.1) |
| Median (IQR) | 5.8 (3.5–7.2) | 4.7 (2.7–7.8) | 5.4 (2.9–7.2) |
| Minimum, maximum | 0.1, 18.5 | 0.3, 16.6 | 0.1, 18.5 |
| n | 164 | 108 | 272 |
| 12 months | | | |
| Mean (SD) | 6.1 (3.7) | 6.1 (3.1) | 6.1 (3.4) |
| Median (IQR) | 6 (3.8–7.7) | 5.8 (3.8–7.9) | 5.9 (3.8–7.7) |
| Minimum, maximum | 0.3, 26.1 | 0.0, 13.3 | 0.0, 26.1 |
| n | 157 | 100 | 257 |
| Change from baseline at 6 months | | | |
| Mean (SD) | –0.8 (3.8) | –1.1 (4.8) | –0.9 (4.2) |
| Median (IQR) | –0.9 (–2.6 to 1.0) | –0.9 (–3.2 to 1.9) | –0.9 (–3.0 to 1.3) |
| Minimum, maximum | –14.6, 10.4 | –25.9, 7.3 | –25.9, 10.4 |
| n | 151 | 103 | 254 |
| Change from baseline at 12 months | | | |
| Mean (SD) | –0.3 (4.1) | –0.8 (5.1) | –0.5 (4.5) |
| Median (IQR) | –0.6 (–2.8 to 2.1) | –0.6 (–3.9 to 2.3) | –0.6 (–3.0 to 2.2) |
| Minimum, maximum | –11.9, 13.1 | –22.9, 7.9 | –22.9, 13.1 |
| n | 143 | 95 | 238 |

**TABLE 13** The DHA, AA and EPA-to-AA ratio levels for those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only) (*continued*)

| RBC levels[a] | EPA | | |
| --- | --- | --- | --- |
| | FFA (*N* = 212) | TG (*N* = 143) | Total (*N* = 355) |
| ***RBC EPA-to-AA ratio level[a]*** | | | |
| Baseline | | | |
| Mean (SD) | 0.1 (0.1) | 0.1 (0.0) | 0.1 (0.1) |
| Median (IQR) | 0.1 (0.1–1.0) | 0.1 (0.1–1.0) | 0.1 (0.1–1.0) |
| Minimum, maximum | 0, 0.6 | 0, 0.3 | 0, 0.6 |
| *n* | 181 | 128 | 309 |
| 6 months | | | |
| Mean (SD) | 0.4 (0.2) | 0.3 (0.2) | 0.4 (0.2) |
| Median (IQR) | 0.4 (0.2–5.0) | 0.3 (0.2–4.0) | 0.4 (0.2–5.0) |
| Minimum, maximum | 0, 0.8 | 0, 0.7 | 0, 0.8 |
| *n* | 164 | 108 | 272 |
| 12 months | | | |
| Mean (SD) | 0.3 (0.2) | 0.3 (0.1) | 0.3 (0.2) |
| Median (IQR) | 0.3 (0.2–4.0) | 0.2 (0.2–4.0) | 0.3 (0.2–4.0) |
| Minimum, maximum | 0, 0.8 | 0, 0.7 | 0, 0.8 |
| *n* | 157 | 100 | 257 |
| Change from baseline at 6 months | | | |
| Mean (SD) | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) |
| Median (IQR) | 0.3 (0.1–4.0) | 0.3 (0.1–4.0) | 0.3 (0.1–4.0) |
| Minimum, maximum | −0.5, 0.7 | −0.1, 0.6 | −0.5, 0.7 |
| *n* | 151 | 103 | 254 |
| Change from baseline at 12 months | | | |
| Mean (SD) | 0.2 (0.2) | 0.2 (0.1) | 0.2 (0.2) |
| Median (IQR) | 0.2 (0.1–3.0) | 0.2 (0.1–3.0) | 0.2 (0.1–3.0) |
| Minimum, maximum | −0.3, 0.7 | −0.1, 0.6 | −0.3, 0.7 |
| *n* | 143 | 95 | 238 |

IQR, interquartile range.
a   PUFA data are percentage of total fatty acids.
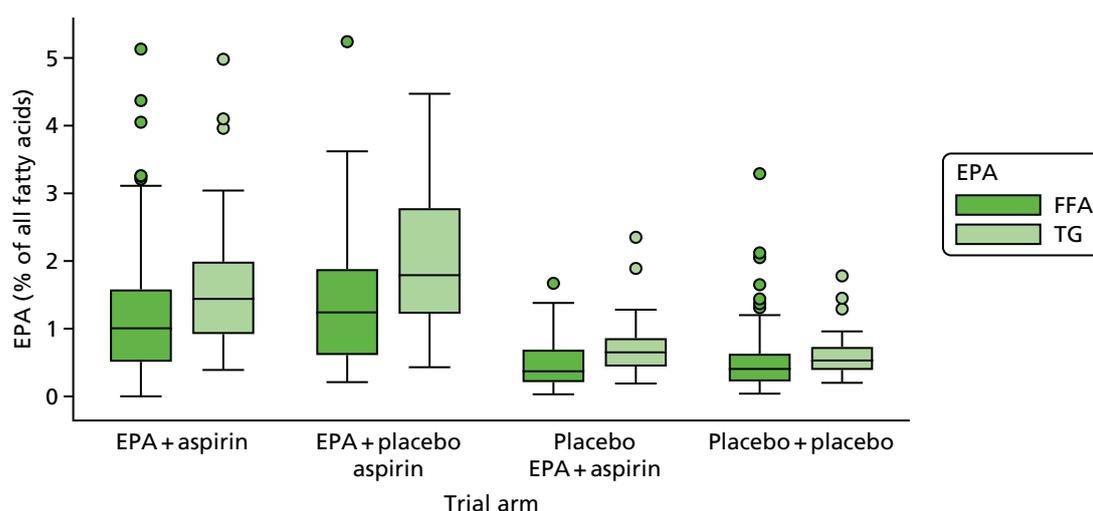
**FIGURE 8** Rectal mucosal EPA levels by formulation and by trial group at 12 months.

**TABLE 14** Rectal mucosal PUFA levels for those who received capsule IMP as EPA-FFA or EPA-TG (active EPA groups only)

| Rectal mucosal levels[a] at 12 months | EPA | | |
| --- | --- | --- | --- |
| | FFA (*N* = 212) | TG (*N* = 143) | Total (*N* = 355) |
| *EPA level[a]* | | | |
| Mean (SD) | 1.3 (1.0) | 1.8 (1.1) | 1.5 (1.1) |
| Median (IQR) | 1.1 (0.6–1.7) | 1.6 (1.0–2.3) | 1.3 (0.7–2) |
| Minimum, maximum | 0.0, 5.2 | 0.4, 5.0 | 0.0, 5.2 |
| *n* | 153 | 96 | 249 |
| *DHA level[a]* | | | |
| Mean (SD) | 0.9 (0.6) | 1.2 (0.4) | 1.0 (0.5) |
| Median (IQR) | 0.7 (0.5–1.4) | 1.2 (1.0–1.4) | 1.0 (0.6–1.4) |
| Minimum, maximum | 0.1, 4.3 | 0.4, 2.1 | 0.1, 4.3 |
| *n* | 153 | 96 | 249 |
| *AA level[a]* | | | |
| Mean (SD) | 4.4 (2.8) | 5.8 (1.6) | 5.0 (2.5) |
| Median (IQR) | 3.5 (2.3–6.3) | 5.5 (4.7–6.5) | 5.0 (2.8–6.4) |
| Minimum, maximum | 0.3, 17.7 | 1.9, 11.4 | 0.3, 17.7 |
| *n* | 153 | 96 | 249 |
| *EPA-to-AA ratio level[a]* | | | |
| Mean (SD) | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) |
| Median (IQR) | 0.3 (0.2–0.4) | 0.3 (0.2–0.4) | 0.3 (0.2–0.4) |
| Minimum, maximum | 0.0, 0.9 | 0.1, 8.0 | 0.0, 0.9 |
| *n* | 153 | 96 | 249 |

IQR, interquartile range.
a PUFA data are percentage of total fatty acids.

As there was no clear difference in RBC or rectal mucosal EPA incorporation, or EPA-to-AA ratio, between those allocated EPA-FFA and those allocated EPA-TG, it was felt appropriate to combine primary and secondary outcome data from those who received either type of capsule IMP.

## Analysis populations

The ITT population was defined as all randomised participants with post-randomisation data (*Table 15*). Participants in the ITT population were analysed regardless of adherence to their allocated group and without imputation for missing data. Although 709 participants were randomised, two participants withdrew immediately and provided no data. Therefore, 707 participants were included in the ITT population (see *Table 15*). All baseline summaries and efficacy analyses were based on this population. Data from surveillance colonoscopy at 12 months was available for 640 participants.

The per-protocol population included all participants who had taken sufficient medication (participants who had taken > 75% of expected capsules and/or > 50% of expected tablets), were not found to be ineligible post randomisation and had not used any OTC medication containing aspirin, NSAIDs or fish oil during the treatment period. The population comprised 659 participants and was used for one of the sensitivity analyses to determine the robustness of the primary analysis.

The safety population was defined as all participants who took at least one dose of allocated treatment. It comprised 697 participants. The 10 participants who did not receive active or placebo EPA or aspirin were excluded from this population.

## Primary outcome

Colorectal adenoma data from the 12-month surveillance colonoscopy were available for 640 participants: 161 (91%), 153 (86%), 163 (93%) and 163 (93%) (percentage of the total number of participants in each group) in the EPA + aspirin, EPA + placebo aspirin, placebo EPA + aspirin and placebo EPA + placebo aspirin groups, respectively. The median time between randomisation and the 12-month surveillance colonoscopy was between 344 and 348 days in the four treatment groups (*Table 16*).

Of 161 participants in the EPA + aspirin group, 98 (61%) had at least one colorectal adenoma (the ADRa) at the surveillance colonoscopy, whereas 97 out of 153 participants (63%) in the EPA + placebo aspirin group had at least one colorectal adenoma (the ADRa) at the surveillance colonoscopy. The ADRa was 61% (100/163) in the placebo EPA + aspirin group and 61% (100/163) in the placebo EPA + placebo aspirin group.

**TABLE 15** Summary of analysis populations

| Participants | Trial group (*n*) | | | | |
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo | Total (*n*) |
| --- | --- | --- | --- | --- | --- |
| Randomised | 177 | 179 | 177 | 176 | 709 |
| Excluded from ITT population as a result of withdrawal immediately after randomisation | 0 | 1 | 1 | 0 | 2 |
| Included in ITT population | 177 | 178 | 176 | 176 | 707 |
| Included in per-protocol population | 156 | 172 | 163 | 168 | 659 |
| Included in safety population | 170 | 177 | 174 | 176 | 697 |

TABLE 16 Summary of the primary outcome data

| Primary outcome data | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) |
| Participants with colorectal adenoma data at 12 months (*n*) | 161 | 153 | 163 | 163 |
| Median (IQR) days from randomisation to surveillance colonoscopy | 348 (337–364) | 349 (333–363) | 348 (335–364) | 344 (334–360) |
| Participants with one or more colorectal adenomas at surveillance colonoscopy (ADRa), *n* (%) | 98 (61) | 97 (63) | 100 (61) | 100 (61) |
| Factorial margins | Active EPA (*n* = 314) | Placebo EPA (*n* = 326) | Active aspirin (*n* = 324) | Placebo aspirin (*n* = 316) |
| Participants with one or more colorectal adenomas at surveillance colonoscopy, *n* (%) | 195 (62) | 200 (61) | 198 (61) | 197 (62) |
| IQR, interquartile range. | | | | |

When summarised according to factorial margins, the ADRa was similar across interventions, with an ADRa of 195 (62%) for those who received active EPA versus an ADRa of 200 (61%) for those who did not receive EPA, and an ADRa of 198 (61%) for individuals who received active aspirin versus an ADRa of 197 (62%) for those who did not receive aspirin (62%) (see *Table 16*).

### Primary outcome analysis

The test of interaction showed that there was no evidence of any interaction between EPA and aspirin for the ADRa ($p = 0.85$). Therefore, primary and secondary outcomes were analysed according to factorial margins, that is the treatment effects for EPA and aspirin were reported separately.

*Table 17* shows adjusted risk differences of the ADRa for the treatment effect of EPA and aspirin. The point estimates and 95% CIs for both EPA and aspirin showed that there was no evidence of a statistically significant difference. These analyses were adjusted for whether or not the participant had a repeat endoscopic procedure (i.e. full colonoscopy, partial colonoscopy or FS) and included BCSP site as a random effect. Supportive analyses that were not adjusted by repeat colonoscopy showed similar results (see *Appendix 4*, *Table 40*).

TABLE 17 Between-group comparisons of ADRa

| Risk difference and ratio | EPA vs. no EPA | Aspirin vs. no aspirin |
| --- | --- | --- |
| Adjusted by site as a random effect and by repeat colonoscopy at baseline (*n* = 588) | | |
| Risk difference (95% CI) (%); *p*-value | –0.9 (–8.8 to 6.9); 0.813 | –0.6 (–8.5 to 7.2); 0.876 |
| Risk ratio (95% CI) | 0.98 (0.87 to 1.12) | 0.99 (0.87 to 1.12) |

The point estimates for EPA and aspirin were –0.9% and –0.6%, respectively, with 95% CIs that included zero, indicating no statistically significant difference from no treatment for both interventions. The trial was designed to detect an absolute ADRa difference of 10%. *Figure 9* and *Table 17* show that the lower limit of the 95% CIs did not reach –10% for either EPA or aspirin.

### Sensitivity analyses for the primary outcome
Several sensitivity analyses were conducted to investigate the robustness of the primary analysis. Although the point estimates and 95% CIs varied between different analyses, they were supportive of the primary analysis (*Table 18* and *Figure 10*).

The multilevel model included both BCSP centre and site as random effects to account for sites embedded within the same BCSP (see *Figure 10*).
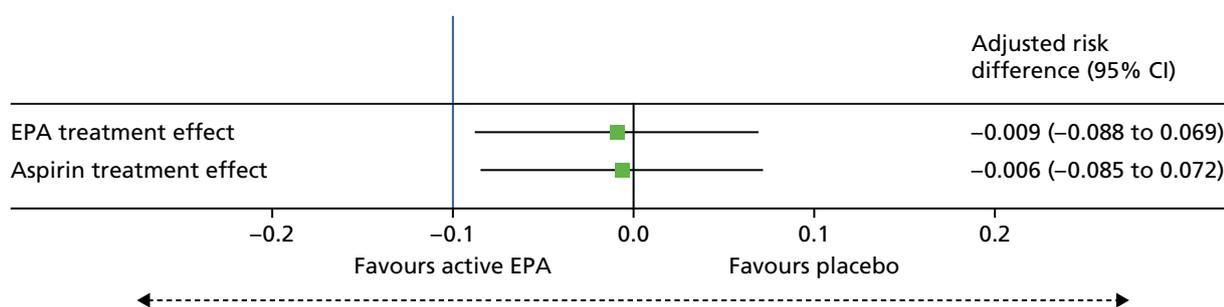


**FIGURE 9** Forest plot of the treatment effect of EPA and aspirin on the ADRa. The blue vertical line represents the prespecified target reduction of ADRa (10%). Adjusted by site as a random effect and by repeat colonoscopy at baseline.

**TABLE 18** Sensitivity analyses of the ADRa with adjustment for repeat colonoscopy

| Analysis | Estimate (95% CI) | |
|---|---|---|
| | EPA vs. no EPA | Aspirin vs. no aspirin |
| Using per-protocol population (risk difference) (%) | –0.6 (–8.7 to 7.4) | –0.6 (–8.7 to 7.4) |
| Multilevel model treating recruiting centre and site as random effects (odds ratio) | 0.96 (0.68 to 1.34) | 0.97 (0.70 to 1.34) |
| Multiple imputation of missing data (risk difference) (%) | 0.1 (–7.3 to 7.6) | –0.7 (–8.3 to 6.8) |
| Adjustment of baseline variables with imbalance[a] (risk difference) (%) | –0.7 (–8.5 to 7.1) | –0.7 (–8.5 to 7.1) |
| Adjustment of oily fish intake during the trial (risk difference) (%) | –2.5 (–11.1 to 6.0) | –2.4 (–11.0 to 6.1) |
| CACE analysis taking account of treatment adherence | | |
| ∙∙∙∙Binary adherence (risk difference) (%) | –1.0 (–9.9 to 7.9) | –0.7 (–8.5 to 7.1) |
| ∙∙∙∙Continuous adherence (risk difference) (%) | –1.2 (–10.0 to 7.5) | –0.8 (–8.8 to 7.1) |
| Adjustment of EPA formulation (risk difference) (%) | –0.9 (–8.8 to 6.9) | –0.4 (–8.3 to 7.4) |
| Adjustment of baseline RBC EPA (risk difference) (%) | –4.2 (–12.6 to 4.2) | 2.3 (–6.0 to 10.6) |

a　Adjusted for sex, whether or not diagnosed with diabetes mellitus, and smoking status.

(a)

Adjusted risk difference (95% CI)

| | |
|---|---|
| Per-protocol analysis | −0.006 (−0.087 to 0.074) |
| Multiple imputation of missing data | 0.001 (−0.073 to 0.076) |
| Adjustment of baseline variables | −0.007 (−0.085 to 0.071) |
| Adjustment of oily fish intake | −0.025 (−0.111 to 0.060) |
| Adjustment of binary adherence | −0.010 (−0.099 to 0.079) |
| Adjustment of continuous adherence | −0.012 (−0.100 to 0.075) |
| Adjustment of EPA formulation | −0.009 (−0.088 to 0.069) |
| Adjustment of baseline RBC EPA | −0.042 (−0.126 to 0.042) |

−0.2    −0.1    0.0    0.1    0.2

Favours EPA        Favours placebo

(b)

Adjusted risk difference (95% CI)

| | |
|---|---|
| Per-protocol analysis | −0.006 (−0.087 to 0.074) |
| Multiple imputation of missing data | −0.007 (−0.083 to 0.068) |
| Adjustment of baseline variables | −0.007 (−0.085 to 0.074) |
| Adjustment of oily fish intake | −0.024 (−0.110 to 0.061) |
| Adjustment of binary adherence | −0.007 (−0.085 to 0.071) |
| Adjustment of continuous adherence | −0.008 (−0.088 to 0.071) |
| Adjustment of EPA formulation | −0.004 (−0.083 to 0.074) |
| Adjustment of baseline RBC EPA | 0.023 (−0.060 to 0.106) |

−0.2    −0.1    0.0    0.1    0.2

Favours aspirin        Favours placebo

(c)

Adjusted odds ratio (95% CI)

| | |
|---|---|
| EPA treatment effect | 0.96 (0.68 to 1.34) |
| Aspirin treatment effect | 0.97 (0.70 to 1.34) |

0.5        1.0        1.5

Favours treatment        Favours no treatment

**FIGURE 10** Forest plots for the sensitivity analyses of the ADRa. (a) EPA vs. placebo; (b) aspirin vs. placebo; and (c) multilevel model. Results using the multilevel model are presented as odds ratios, whereas all other sensitivity analyses are presented using the risk difference. Adjusted by site as a random effect and by repeat colonoscopy at baseline. Some analyses were adjusted by further variables.

## Secondary outcomes

Secondary outcomes were analysed in a similar way to the primary outcome. Results were summarised according to the outcome type, that is the risk difference for the binary outcome (ADRa) and the IRR for count outcomes (*Tables 19–25* and *Figure 17*). Point estimates and CIs reported are according to factorial margins, that is EPA compared with no EPA and aspirin compared with no aspirin.

Summary data on colorectal adenomas reported at surveillance colonoscopy are listed in *Table 19*.

**TABLE 19** Summary statistics of secondary colorectal adenoma outcomes

| Secondary colorectal adenoma outcomes | Trial group | | | |
|---|---|---|---|---|
| | EPA + aspirin (N = 177) | EPA + placebo aspirin (N = 178) | Placebo EPA + aspirin (N = 176) | Placebo + placebo (N = 176) |
| Participants with colorectal adenoma data at 12 months (n) | 161 | 153 | 163 | 163 |
| Overall number of colorectal adenomas | 166 | 238 | 209 | 231 |
| Overall number of advanced colorectal adenomas | 9 | 8 | 11 | 12 |
| Histology of colorectal adenomas (n) | | | | |
| Conventional | 155 | 205 | 194 | 220 |
| Serrated | 4 | 21 | 10 | 8 |
| Missing | 7 | 12 | 5 | 3 |
| Location of colorectal adenomas (n) | | | | |
| Left | 58 | 98 | 101 | 93 |
| Right | 108 | 140 | 107 | 138 |
| Missing | 0 | 0 | 1 | 0 |

**TABLE 20** The analysis results of total colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
|---|---|---|---|---|
| | EPA + aspirin (n = 161) | EPA + placebo aspirin (n = 153) | Placebo EPA + aspirin (n = 163) | Placebo + placebo (n = 163) |
| Total number of colorectal adenomas per participant | | | | |
| Mean (SD) | 1.0 (1.2) | 1.6 (2.1) | 1.3 (1.6) | 1.4 (2.0) |
| Median (IQR) | 1 (0–1) | 1 (0–2) | 1 (0–2) | 1 (0–2) |
| Minimum, maximum | 0, 6 | 0, 10 | 0, 13 | 0, 16 |
| Incidence rate for total number of colorectal adenomas per person per year | | | | |
| Mean (SD) | 1.1 (1.3) | 1.6 (2.2) | 1.3 (1.7) | 1.5 (2.1) |
| Median (IQR) | 1 (0–1.2) | 1 (0–2.1) | 1 (0–2.1) | 1.1 (0–2.2) |
| Minimum, maximum | 0, 6.7 | 0, 10.8 | 0, 13.5 | 0, 16.6 |
| *Incidence rate by margins* | *EPA* | *No EPA* | *Aspirin* | *No aspirin* |
| Mean (SD) | 1.3 (1.8) | 1.4 (1.9) | 1.2 (1.5) | 1.6 (2.1) |
| Median (IQR) | 1 (0–2) | 1 (0–2.1) | 1 (0–2) | 1 (0–2.2) |
| Minimum, maximum | 0, 10.8 | 0, 16.6 | 0, 13.5 | 0, 16.6 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| IRR (95% CI) | 0.91 (0.79 to 1.05) | | 0.78 (0.68 to 0.90) | |

IQR, interquartile range.

**TABLE 21** Analysis of advanced colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Participant with any advanced colorectal adenoma (advanced ADRa), *n* (%) | 8 (5) | 8 (5) | 10 (6) | 11 (7) |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| Risk difference for number of participants with any advanced colorectal adenomas (95% CI) (%) | −0.6 (−4.4 to 3.1) | | −0.3 (−4.1 to 3.5) | |
| Number of advanced colorectal adenomas per participant | | | | |
| Mean (SD) | 0.1 (0.3) | 0.1 (0.2) | 0.1 (0.3) | 0.1 (0.3) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 2 | 0, 1 | 0, 2 | 0, 2 |
| Incidence rate for number of advanced colorectal adenomas per person per year | | | | |
| Mean (SD) | 0.1 (0.3) | 0.1 (0.2) | 0.1 (0.3) | 0.1 (0.3) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 2.1 | 0, 1.2 | 0, 2.1 | 0, 2.1 |
| *Incidence rate by margins* | *EPA* | *No EPA* | *Aspirin* | *No aspirin* |
| Mean (SD) | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 2.1 | 0, 2.1 | 0, 2.1 | 0, 2.1 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| IRR (95% CI) | 0.82 (0.43 to 1.56) | | 0.99 (0.52 to 1.86) | |

IQR, interquartile range.

**TABLE 22** Analysis of conventional colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Participants with any conventional[a] colorectal adenomas (conventional ADRa), *n* (%) | 88 (55) | 83 (54) | 91 (56) | 92 (56) |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| Risk difference for number of participants with any conventional[a] adenomas (95% CI) (%) | −3.3 (−11.2 to 4.7) | | 1.7 (−6.2 to 9.6) | |
| Number of conventional colorectal adenomas per participant | | | | |
| Mean (SD) | 1.0 (1.2) | 1.4 (1.9) | 1.2 (1.6) | 1.4 (2) |
| Median (IQR) | 1 (0–1) | 1 (0–2) | 1 (0–2) | 1 (0–2) |
| Minimum, maximum | 0, 6 | 0, 10 | 0, 13 | 0, 16 |

**TABLE 22** Analysis of conventional colorectal adenomas with adjustment for repeat colonoscopy and BCSP site (*continued*)

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Incidence rate for number of conventional colorectal adenomas per person per year | | | | |
| Mean (SD) | 1.0 (1.3) | 1.4 (2) | 1.2 (1.7) | 1.4 (2.1) |
| Median (IQR) | 1 (0–1.1) | 1 (0–2.1) | 1 (0–2.1) | 1 (0–2.1) |
| Minimum, maximum | 0, 6.7 | 0, 10.8 | 0, 13.5 | 0, 16.6 |
| *Incidence rate by margins* | *EPA* | *No EPA* | *Aspirin* | *No aspirin* |
| Mean (SD) | 1.2 (1.7) | 1.3 (1.9) | 1.1 (1.5) | 1.4 (2) |
| Median (IQR) | 1.0 (0–1.9) | 1.0 (0–2.1) | 1.0 (0–1.9) | 1.0 (0–2.1) |
| Minimum, maximum | 0, 10.8 | 0, 16.6 | 0, 13.5 | 0, 16.6 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| IRR (95% CI) | 0.86 (0.74 to 0.99) | | 0.82 (0.71 to 0.94) | |

IQR, interquartile range.
a Post hoc analysis.

**TABLE 23** Analysis of serrated colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Participants with any serrated colorectal adenomas (serrated ADRa), *n* (%) | 4 (2) | 11 (7) | 6 (4) | 7 (4) |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| Risk difference for number of participants with any serrated adenomas (95% CI) (%) | 0 (–3.2 to 3.2) | | –2.7 (–6.1 to 0.7) | |
| Number of serrated colorectal adenomas per participant | | | | |
| Mean (SD) | 0.0 (0.2) | 0.1 (0.7) | 0.1 (0.4) | 0.0 (0.2) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 1 | 0, 8 | 0, 4 | 0, 2 |
| Incidence rate for number of serrated colorectal adenomas per person per year | | | | |
| Mean (SD) | 0.0 (0.2) | 0.1 (0.8) | 0.1 (0.3) | 0.1 (0.3) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 1.1 | 0, 8.6 | 0, 3.4 | 0, 2.2 |
| *Incidence rate by margins* | *EPA* | *No EPA* | *Aspirin* | *No aspirin* |
| Mean (SD) | 0.1 (0.6) | 0.1 (0.3) | 0.0 (0.3) | 1.0 (0.6) |
| Median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Minimum, maximum | 0, 8.6 | 0, 3.4 | 0, 3.4 | 0, 8.6 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| IRR (95% CI) | 1.44 (0.79 to 2.60) | | 0.46 (0.25 to 0.87) | |

IQR, interquartile range.

**TABLE 24** Analysis of left colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin ($N = 161$) | EPA + placebo aspirin ($N = 153$) | Placebo EPA + aspirin ($N = 163$) | Placebo + placebo ($N = 163$) |
| Participants with any left colorectal adenomas (left ADRa), n (%) | 42 (26) | 58 (38) | 65 (40) | 55 (34) |
| | **EPA vs. no EPA** | | **Aspirin vs. no aspirin** | |
| Risk difference for number of participants with any left colorectal adenomas (95% CI) (%) | −7.8 (−15.5 to −0.2) | | −1.8 (−9.4 to 5.8) | |
| Number of left colorectal adenomas per participant | | | | |
| Mean (SD) | 0.4 (0.7) | 0.6 (10.1) | 0.6 (0.9) | 0.6 (1.0) |
| Median (IQR) | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0 (0–1) |
| Minimum, maximum | 0, 3 | 0, 5 | 0, 5 | 0, 5 |
| Incidence rate for number of left colorectal adenomas per person per year | | | | |
| Mean (SD) | 0.4 (0.7) | 0.7 (10.1) | 0.6 (0.9) | 0.6 (1.0) |
| Median (IQR) | 0 (0–0.9) | 0 (0–1.1) | 0 (0–1.1) | 0 (0–1) |
| Minimum, maximum | 0.0, 3.3 | 0.0, 5.4 | 0.0, 4.4 | 0.0, 5.6 |
| **Incidence rate by margins** | **EPA** | **No EPA** | **Aspirin** | **No aspirin** |
| Mean (SD) | 0.5 (0.9) | 0.6 (1.0) | 0.5 (0.8) | 0.6 (10.1) |
| Median (IQR) | 0 (0–1) | 0 (0–1.1) | 0 (0–1) | 0 (0–1.1) |
| Minimum, maximum | 0.0, 5.4 | 0.0, 5.6 | 0.0, 4.4 | 0.0, 5.6 |
| | **EPA vs. no EPA** | | **Aspirin vs. no aspirin** | |
| IRR (95% CI) | 0.75 (0.60 to 0.94) | | 0.85 (0.69 to 1.06) | |

IQR, interquartile range.

**TABLE 25** Analysis of right colorectal adenomas with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin ($N = 161$) | EPA + placebo aspirin ($N = 153$) | Placebo EPA + aspirin ($N = 163$) | Placebo + placebo ($N = 163$) |
| Participants with any right colon adenomas (right ADRa), n (%) | 69 (43) | 72 (47) | 63 (39) | 66 (40) |
| | **EPA vs. no EPA** | | **Aspirin vs. no aspirin** | |
| Risk difference for number of participants with any right colon adenomas (95% CI) (%) | 6.0 (−1.9 to 13.9) | | −3.1 (−11.0 to 4.7) | |
| Number of right colon adenomas per participant | | | | |
| Mean (SD) | 0.7 (1.0) | 0.9 (1.5) | 0.7 (1.3) | 0.8 (1.7) |
| Median (IQR) | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0 (0–1) |
| Minimum, maximum | 0, 6 | 0, 9 | 0, 13 | 0, 16 |

**TABLE 25** Analysis of right colorectal adenomas with adjustment for repeat colonoscopy and BCSP site (*continued*)

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Incidence rate for number of right colon adenomas per person per year | | | | |
|     Mean (SD) | 0.7 (1.1) | 1.0 (1.6) | 0.7 (1.4) | 0.9 (1.8) |
|     Median (IQR) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) |
|     Minimum, maximum | 0.0, 6.7 | 0.0, 10.4 | 0.0, 13.5 | 0.0, 16.6 |
| *Incidence rate by margins* | *EPA* | *No EPA* | *Aspirin* | *No aspirin* |
| Mean (SD) | 0.8 (1.4) | 0.8 (1.6) | 0.7 (1.3) | 0.9 (1.7) |
| Median (IQR) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) | 0.0 (0.0–1.1) |
| Minimum, maximum | 0.0, 10.4 | 0.0, 16.6 | 0.0, 13.5 | 0.0, 16.6 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| IRR (95% CI) | 1.02 (0.85 to 1.22) | | 0.73 (0.61 to 0.88) | |

IQR, interquartile range.

The median number of colorectal adenomas per participant at the 12-month surveillance colonoscopy was 1 for all four trial groups (see *Table 20*). The median incidence rate was 1 colorectal adenoma per person per year for all four groups. However, fewer colorectal adenomas were detected in the combination EPA + aspirin group (see *Table 19*), with a reduced total MAP value (see *Table 20*).

The factorial margin analysis revealed that the IRR for EPA versus no EPA was 0.91 (95% CI 0.79 to 1.05) in favour of EPA (see *Table 20*). The IRR for aspirin versus no aspirin was 0.78 (95% CI 0.68 to 0.90). The distribution of individual total colorectal adenoma counts was similar across all four groups (see *Figure 11*).

The advanced ADRa was 8 (5%) in participants who received both EPA and aspirin, as well as EPA + placebo aspirin, but was 10 (6%) and 11 (7%) in participants who received placebo EPA + aspirin and placebo EPA + placebo aspirin, respectively (see *Table 21*). Risk differences for the advanced ADRa for EPA and aspirin were –0.6% and –0.3%, respectively. The mean incidence rate was 0.1 advanced colorectal adenomas per person per year. Analysis of the advanced MAP 'at the margins' revealed IRRs of 0.82 for EPA and 0.99 for aspirin, but with 95% CIs crossing unity. The distribution of individual advanced colorectal adenoma counts was similar across all four groups (*Figure 12*).

The conventional ADRa was 55% for EPA + aspirin (88/161), 54% for EPA + placebo aspirin (83/153) and 56% for both placebo EPA + aspirin (91/163) and placebo EPA + placebo aspirin (92/163) (see *Table 22*). Risk differences for EPA and aspirin were –3.3% and 1.7%, respectively. The median incidence rate was 1 conventional adenoma per person per year for all four groups, with a lower mean value for the combined EPA + aspirin group (see *Table 22*). IRRs for the number of conventional colorectal adenomas were 0.86 (95% CI 0.74 to 0.99) for EPA versus no EPA and 0.82 (95% CI 0.71 to 0.94) for aspirin versus no aspirin (see *Table 22*). The distribution of individual conventional colorectal adenoma counts was similar across all four groups (*Figure 13*).

**FIGURE 11** Distribution of the total number of colorectal adenomas per participant at 12 months by trial group. (a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.
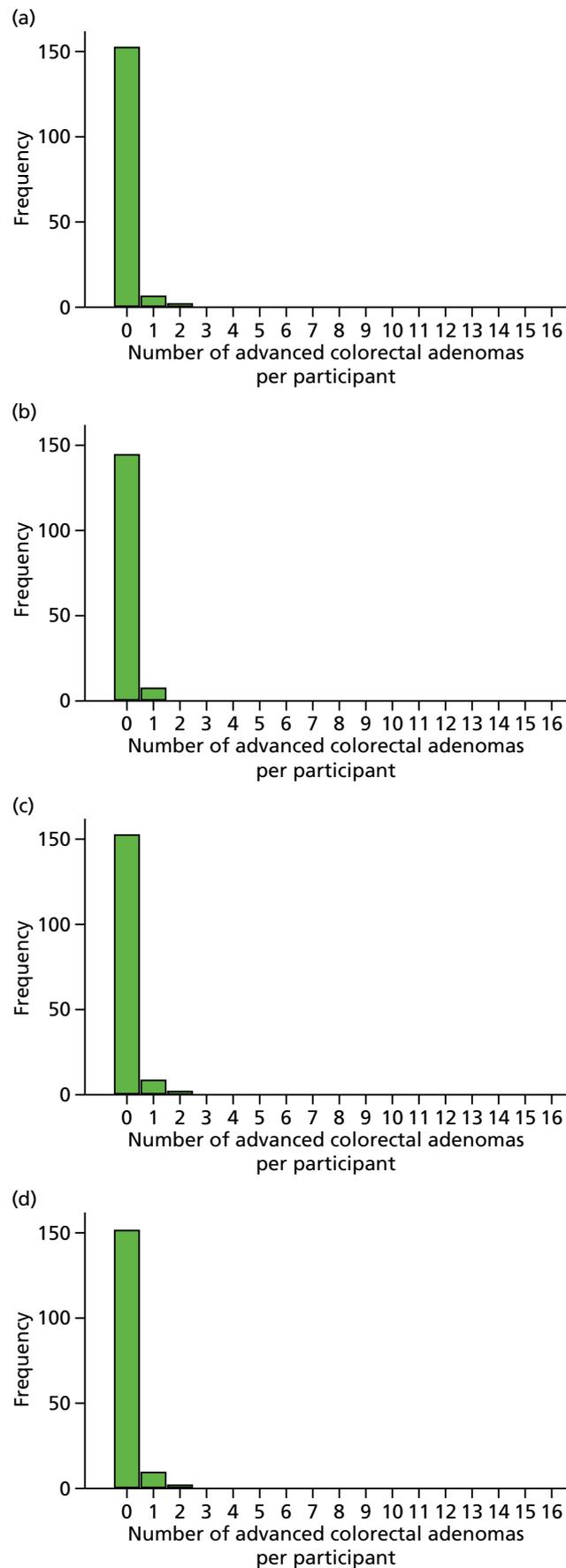
**FIGURE 12** Distribution of the number of advanced colorectal adenomas per participant at 12 months by trial group. (a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.
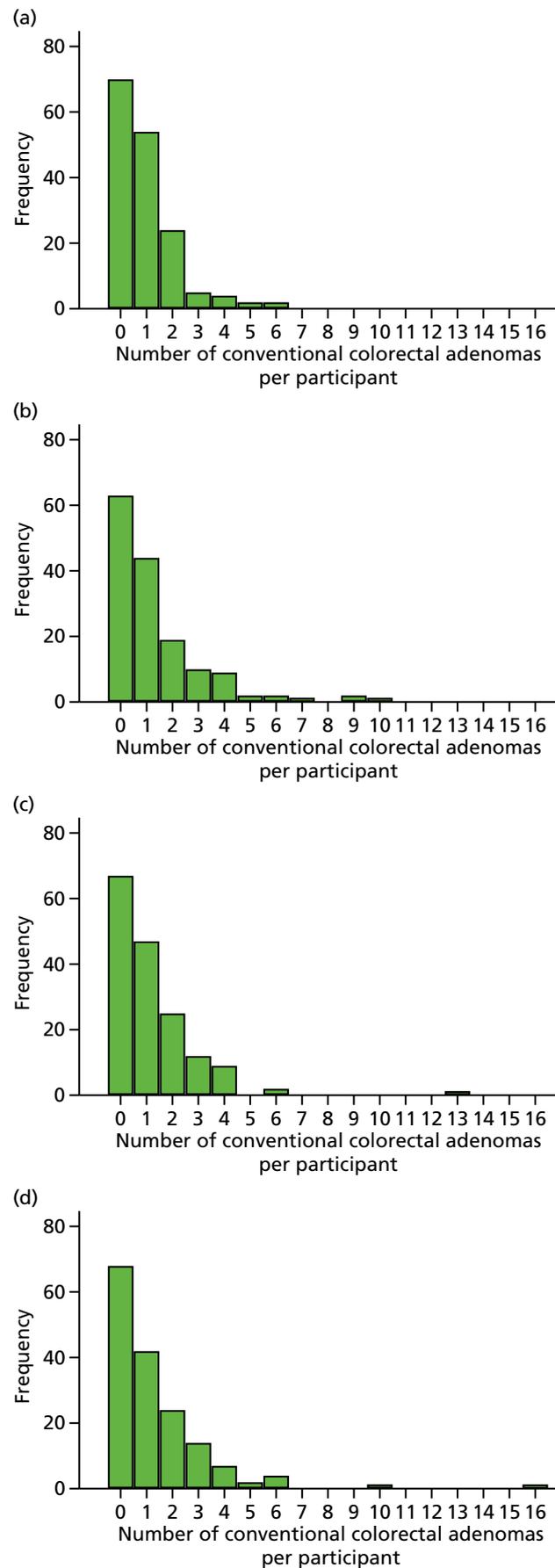
**FIGURE 13** Distribution of the number of conventional colorectal adenomas per participant at 12 months by trial group. (a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.

The serrated ADRa values were small for all treatment groups: 4 (2%) for EPA + aspirin, 11 (7%) for EPA + placebo aspirin, 6 (4%) for placebo EPA + aspirin and 7 (4%) for placebo EPA + placebo aspirin (see *Table 23*). The risk differences for the serrated ADRa were 0% (95% CI –3.2% to 3.2%) for EPA and –2.7% (95% CI –6.1% to 0.7%) for aspirin. The median incidence rate was zero for all four groups and the mean incidence rate was 0.1 serrated adenomas per person per year for all trial groups except the EPA + aspirin group, which was zero. IRRs for the number of serrated adenomas were 1.44 (95% CI 0.79 to 2.60) for EPA versus no EPA and 0.46 (95% CI 0.25 to 0.87) for aspirin versus no aspirin (see *Table 23*). The distribution of individual serrated colorectal adenoma counts was similar across all four groups (*Figure 14*).

The distribution of individual left-sided colorectal adenoma counts in each treatment group is shown in *Figure 15*. Risk differences for the number of participants with at least one left colorectal adenoma (left ADRa) were –7.8% for EPA versus no EPA (95% CI –15.5% to –0.2%) and –1.8% for aspirin versus no aspirin (95% CI –9.4% to 5.8%). The mean incidence rate ranged from 0.4 to 0.7 left colorectal adenomas per person per year. IRRs for left MAP were 0.75 (95% CI 0.60 to 0.94) for EPA versus no EPA and 0.85 (95% CI 0.69 to 1.06) for aspirin versus no aspirin (see *Table 24*).

For the number of participants with at least one right-sided colorectal adenoma (right ADRa), the risk differences were 6% (95% CI –1.9% to 13.9%) for EPA versus no EPA and –3.1% (95% CI –11% to 4.7%) for aspirin versus no aspirin. The mean incidence rate ranged from 0.7 to 1.0 right colorectal adenomas per person per year. IRRs were 1.02 (95% CI 0.85 to 1.22) for EPA versus no EPA and 0.73 (95% CI 0.61 to 0.88) for aspirin versus no aspirin (see *Table 25*). The distribution of individual right-sided colorectal adenoma counts in each treatment group is shown in *Figure 16*.

Following the 12-month surveillance colonoscopy, the majority of BCSP patients were reclassified as being at intermediate risk: 146 (91%) participants were reclassified as being at intermediate risk at follow-up in the EPA + aspirin group, compared with 128 (84%) in the EPA + placebo aspirin group, 140 (86%) in the placebo EPA + aspirin group and 147 (90%) in the placebo EPA + placebo aspirin group (*Table 26*). The risk differences for EPA versus no EPA and aspirin versus no aspirin were small (–0.2% and 0.9%, respectively).

Adjusted IRRs and 95% CIs for the secondary MAP data are summarised in *Figure 17*. Risk differences and 95% CIs for the secondary ADRa data are summarised in *Figure 18*.

Although potential trends may have been observed in the secondary colorectal adenoma data, consideration needs to be given to the large number of analyses that were undertaken (and, therefore, the potential for spuriously significant results), the small sample numbers for some of the colorectal adenoma subtypes and the potential dependencies between the variables.

### *Number of participants with colorectal cancer detected prior to or at first surveillance colonoscopy*
There was no report of any CRC detected at the 12-month surveillance colonoscopy or during the intervention phase of the trial.

## Dietary fish intake during trial participation

Approximately 80% of participants completed a FFQ at 12 months, with similar percentages in each of the four treatment groups (*Table 27*). The total fish and oily fish intakes at 12 months were similar across the four treatment groups (see *Table 27*), as was the proportion of individuals changing intake level between baseline and the end of trial participation (*Table 28*). Approximately 50% of each treatment group remained at the same level of oily fish intake during the trial.
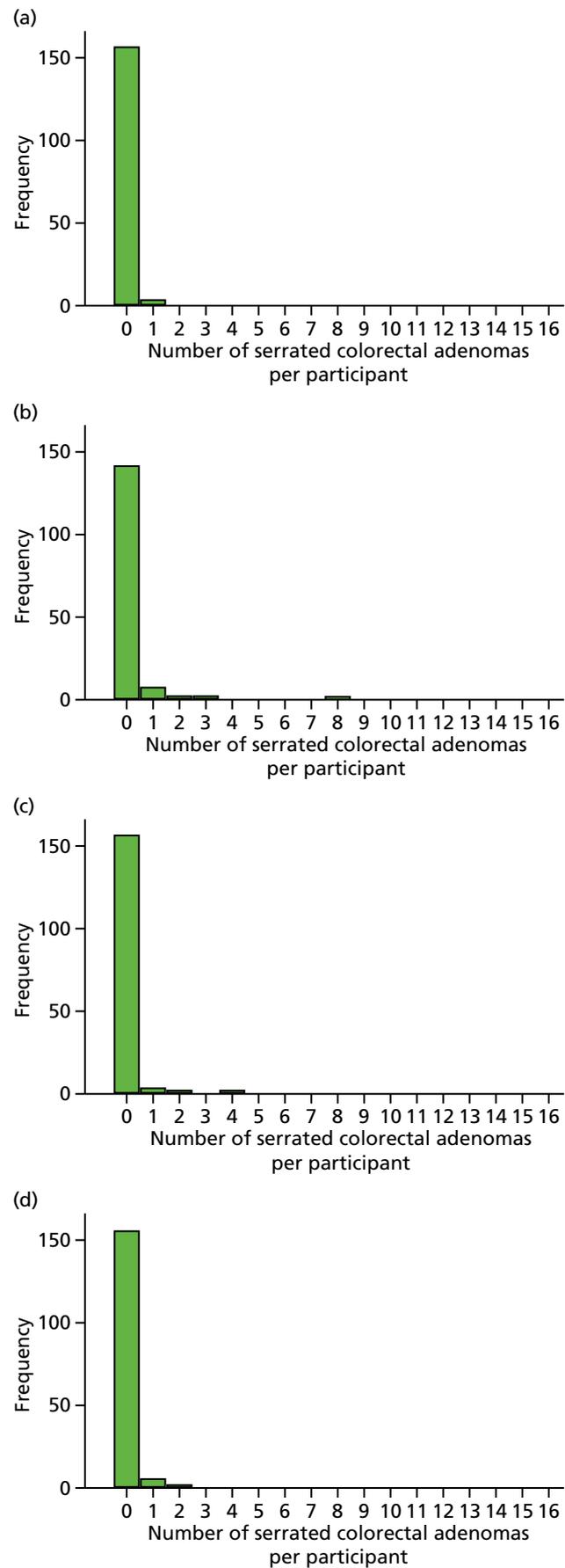
**FIGURE 14** Distribution of the number of serrated colorectal adenomas per participant at 12 months by trial group. (a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.
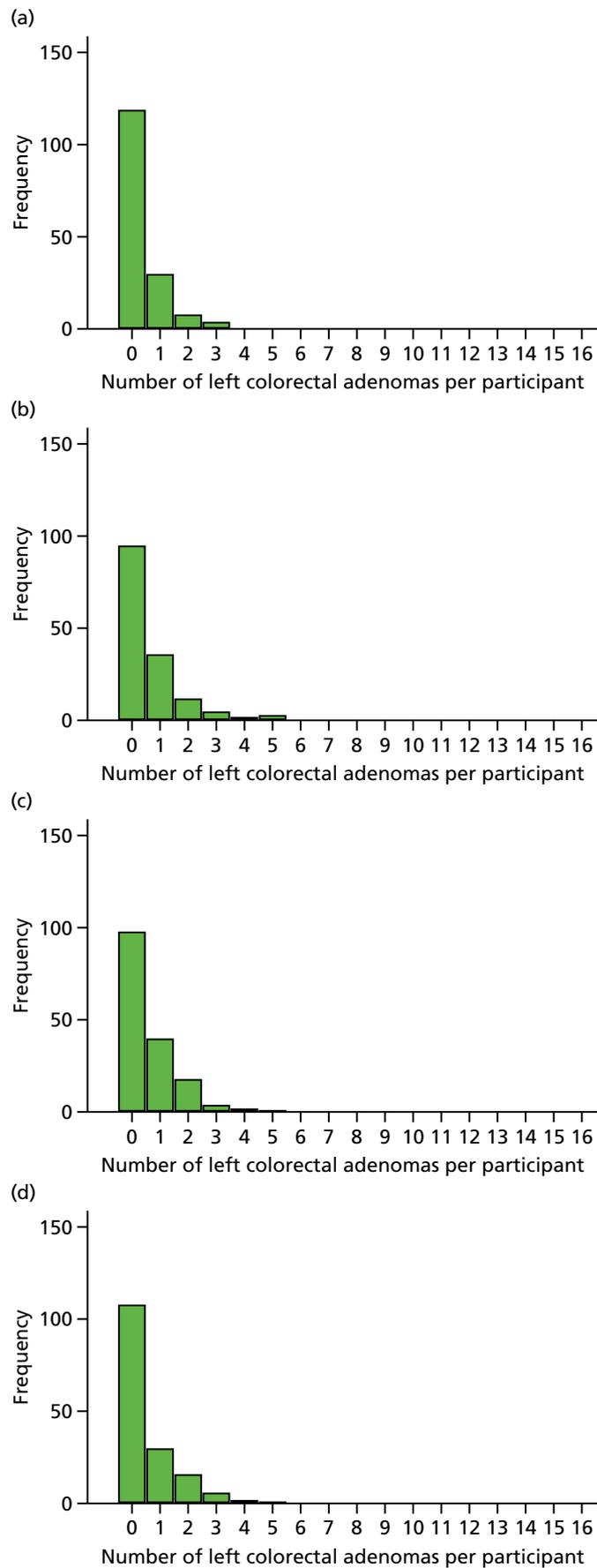
**FIGURE 15** Distribution of the number of left colorectal adenomas per participant at 12 months by trial group.
(a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.
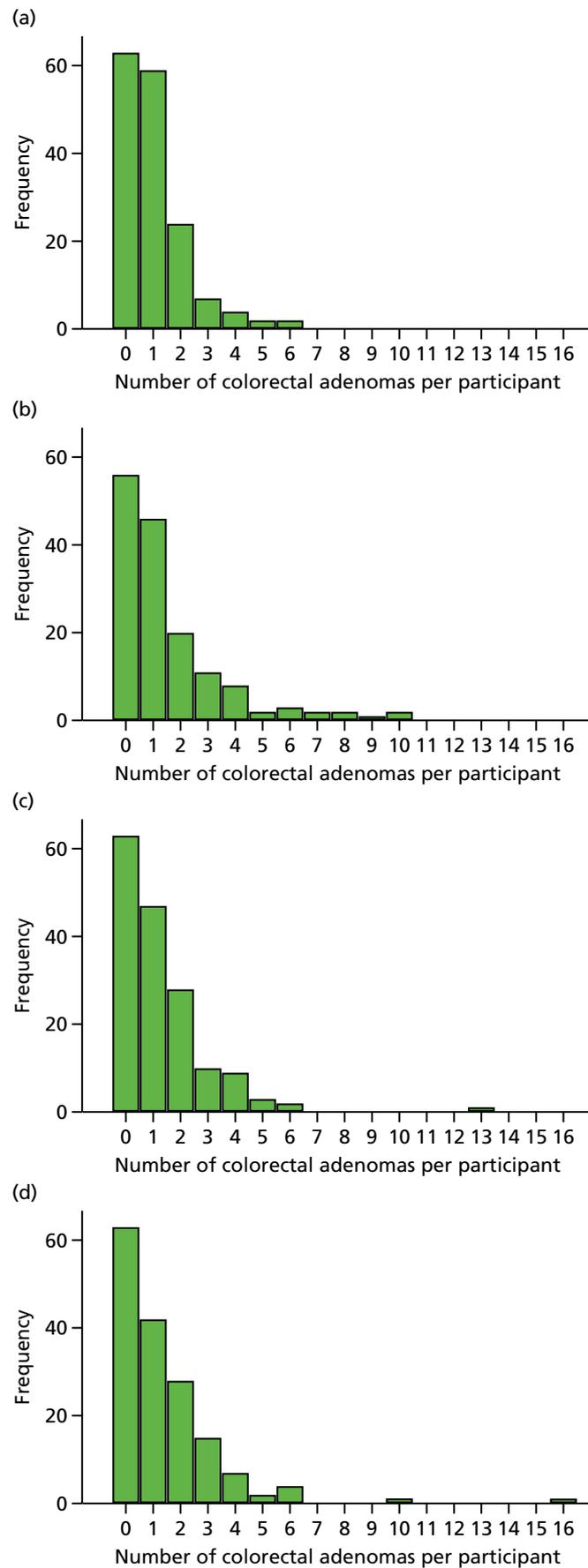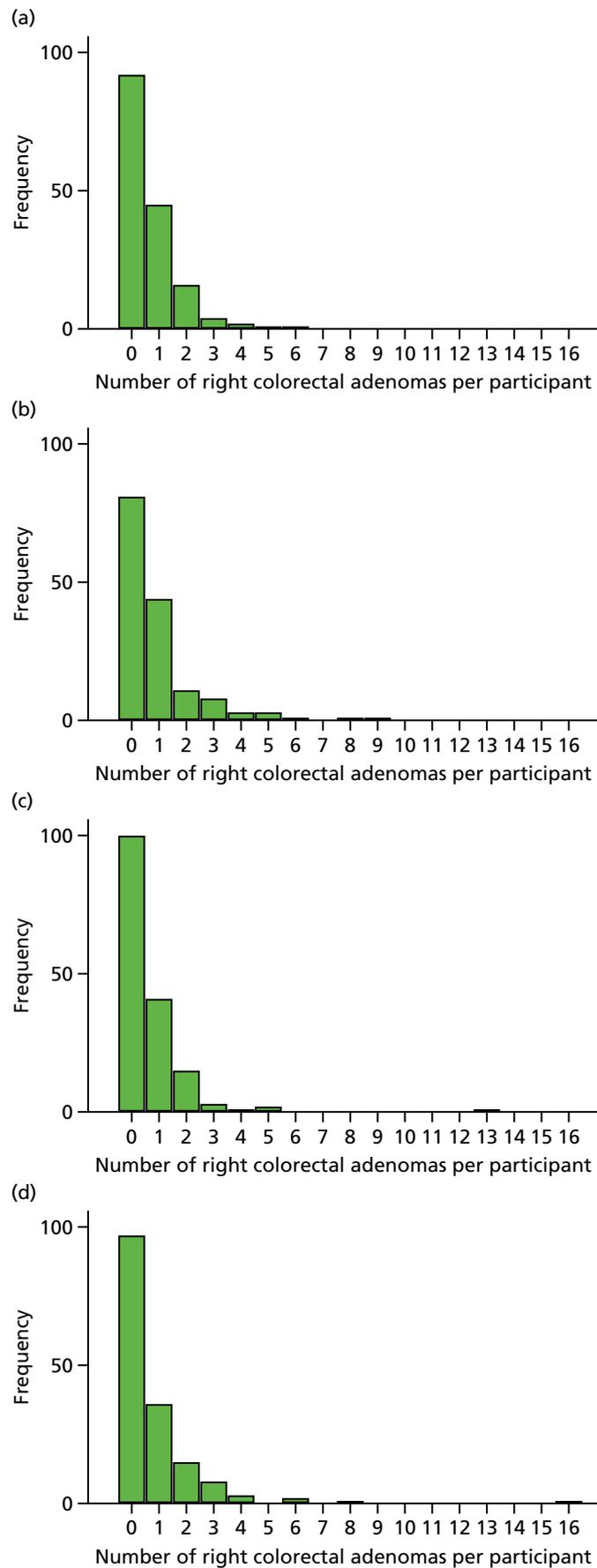
**FIGURE 16** Distribution of the number of right colorectal adenomas per participant at 12 months by trial group.
(a) EPA + aspirin; (b) EPA + placebo aspirin; (c) placebo EPA + aspirin; and (d) placebo + placebo.

**TABLE 26** Analysis of participant reclassification as intermediate risk with adjustment for repeat colonoscopy and BCSP site

| | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 161) | EPA + placebo aspirin (*N* = 153) | Placebo EPA + aspirin (*N* = 163) | Placebo + placebo (*N* = 163) |
| Participants reclassified as being at intermediate risk at follow-up, *n* (%) | 146 (91) | 128 (84) | 140 (86) | 147 (90) |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| Risk difference for number of participants reclassified as being at intermediate risk at 12 months (95% CI) (%) | −0.2 (−5.4 to 5.1) | | 0.9 (−14.1 to 6.2) | |

(a)



| | Adjusted IRR (95% CI) |
| --- | --- |
| Total adenomas per participant | 0.91 (0.79 to 1.05) |
| Advanced adenomas per participant | 0.82 (0.43 to 1.56) |
| Conventional adenomas per participant | 0.86 (0.74 to 0.99) |
| Serrated adenomas per participant | 1.44 (0.79 to 2.60) |
| Left colon adenomas per participant | 0.75 (0.60 to 0.94) |
| Right colon adenomas per participant | 1.02 (0.85 to 1.22) |

(b)



| | Adjusted IRR (95% CI) |
| --- | --- |
| Total adenomas per participant | 0.78 (0.68 to 0.90) |
| Advanced adenomas per participant | 0.99 (0.52 to 1.86) |
| Conventional adenomas per participant | 0.82 (0.71 to 0.94) |
| Serrated adenomas per participant | 0.46 (0.25 to 0.87) |
| Left colon adenomas per participant | 0.85 (0.69 to 1.06) |
| Right colon adenomas per participant | 0.73 (0.61 to 0.88) |

**FIGURE 17** Forest plots for secondary MAP analysis. (a) EPA vs. placebo; and (b) aspirin vs. placebo. Adjusted by site as a random effect and by repeat colonoscopy at baseline.

(a)

Adjusted risk
difference (95% CI)

| | | |
|---|---|---|
| Advanced ADR | | −0.006 (−0.044 to 0.031) |
| Conventional ADR | | −0.033 (−0.112 to 0.047) |
| Serrated ADR | | 0.000 (−0.032 to 0.032) |
| Left colon ADR | | −0.078 (−0.155 to −0.002) |
| Right colon ADR | | 0.060 (−0.019 to 0.139) |
| Reclassified as intermediate risk | | −0.002 (−0.054 to 0.051) |

−0.2    −0.1    0.0    0.1    0.2

Favours EPA          Favours placebo

(b)

Adjusted risk
difference (95% CI)

| | | |
|---|---|---|
| Advanced ADR | | −0.003 (−0.041 to 0.035) |
| Conventional ADR | | 0.017 (−0.062 to 0.096) |
| Serrated ADR | | −0.027 (−0.061 to 0.007) |
| Left colon ADR | | −0.018 (−0.094 to 0.058) |
| Right colon ADR | | −0.031 (−0.110 to 0.047) |
| Reclassified as intermediate risk | | 0.009 (−0.141 to 0.062) |

−0.2    −0.1    0.0    0.1    0.2

Favours aspirin          Favours placebo

FIGURE 18 Forest plots for secondary ADRa analysis. (a) EPA vs. placebo; and (b) aspirin vs. placebo. Adjusted by site as a random effect and by repeat colonoscopy at baseline.

TABLE 27 Dietary fish and other seafood intake during the trial

| Dietary fish intake | Trial group, n (%) | | | |
|---|---|---|---|---|
| | EPA + aspirin (N = 177) | EPA + placebo aspirin (N = 178) | Placebo EPA + aspirin (N = 176) | Placebo + placebo (N = 176) |
| Participants who completed FFQ at 12 months | 138 (78) | 135 (76) | 143 (81) | 146 (89) |
| Total fish intake at 12 months | | | | |
| Never | 3 (2) | 3 (2) | 3 (2) | 11 (8) |
| Low | 6 (4) | 7 (5) | 8 (6) | 12 (8) |
| Medium | 77 (56) | 74 (55) | 85 (59) | 82 (56) |
| High | 45 (33) | 41 (30) | 34 (24) | 32 (22) |
| Missing[a] | 7 (5) | 10 (7) | 13 (9) | 9 (6) |
| Oily fish intake at 12 months | | | | |
| Never | 33 (24) | 38 (28) | 36 (25) | 42 (29) |
| Low | 40 (29) | 37 (28) | 40 (28) | 46 (32) |
| Medium | 35 (26) | 39 (29) | 47 (33) | 38 (26) |
| High | 26 (19) | 16 (12) | 15 (10) | 17 (12) |
| Missing[a] | 4 (3) | 5 (4) | 5 (3) | 3 (2) |

a  FFQ was received; however, data were not evaluable.

**TABLE 28** Change of dietary fish intake during the trial

| Change of dietary fish intake | Trial group, *n* (%) | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) |
| Change of fish intake at 12 months compared with baseline | | | | |
| Stayed the same[a] | 80 (45) | 80 (45) | 86 (50) | 84 (48) |
| Reduced intake | 21 (12) | 18 (10) | 22 (13) | 26 (15) |
| Increased intake | 23 (13) | 18 (10) | 18 (10) | 15 (9) |
| Missing | 53 (30) | 62 (35) | 50 (28) | 51 (29) |
| Change of oily fish intake at 12 months compared with baseline | | | | |
| Stayed the same | 65 (37) | 66 (38) | 78 (45) | 77 (44) |
| Reduced intake | 33 (19) | 32 (18) | 32 (19) | 32 (18) |
| Increased intake | 36 (20) | 30 (17) | 26 (15) | 31 (18) |
| Missing | 43 (24) | 50 (28) | 40 (23) | 36 (20) |

a  Based on intake categories described in *Table 3*.

## Safety

The safety population comprised 697 participants who received at least one dose of EPA or aspirin, or their respective placebos (see *Table 15*).

Overall, there were no safety concerns in participants receiving either EPA or aspirin. There was an excess of AEs and SAEs in participants receiving EPA + placebo aspirin (*Table 29*). In this group, 13 participants reported five or more (87 in total) AEs, which resulted in this imbalance (see *Table 29*). A larger proportion of participants receiving EPA + placebo aspirin reported at least one ADR, with 57 (32%) individuals reporting 119 ADRs. This excess was contributed to by five participants who reported five or more ADRs. Fewer SAEs were reported in the EPA + aspirin group. Five (3%) participants reported at least one SAE, compared with 7% who reported at least one SAE in all the other treatment groups. Only nine SAEs were felt to be related to trial medication.

The majority of AEs and ADRs were mild in severity in each of the treatment groups.

Gastrointestinal disorders were the most frequently reported AE (*Table 30*), with 209 participants reporting at least one GI AE. A larger number of GI AEs were reported in the EPA + placebo aspirin group, contributed by an excess of individuals who reported multiple GI AEs. However, the distribution of GI AEs was similar across the other three groups (*Table 31*).

There was an excess of diarrhoea, abdominal pain and nausea in those allocated active EPA (*Table 32*). The excess of mild to moderate diarrhoea was most prominent in the group receiving EPA alone. There did not appear to be any consistent differences in the reporting of GI AEs between individuals receiving EPA-FFA and those receiving EPA-TG (see *Table 32*).

Post database lock and code release, the chief investigator summarised all GI AEs according to symptoms that are commonly associated with fish oil intake, categorised as diarrhoea, upper GI symptoms, lower abdominal symptoms, eructation/halitosis and other. These were summarised by the treatment group and EPA formulation that the participants received. There were no notable differences between the formulations in each trial treatment group (see *Appendix 5*).

**TABLE 29** Participants who had any AE or SAE from first dose of IMP

| AEs, ADRs and SAEs | Trial group | | | |
|---|---|---|---|---|
| | EPA + aspirin (*N* = 170) | EPA + placebo aspirin (*N* = 177) | Placebo EPA + aspirin (*N* = 174) | Placebo + placebo (*N* = 176) |
| Participants who reported any AE, *n* (%) | 76 (45)[a] | 82 (46) | 68 (39) | 78 (44) |
| Total number of AEs | 129 | 211 | 154 | 160 |
| Severity of all AEs (*n*) | | | | |
| Mild | 110 | 161 | 122 | 119 |
| Moderate | 18 | 47 | 28 | 33 |
| Severe | 1 | 2 | 4 | 5 |
| Missing | 0 | 1 | 0 | 3 |
| AEs per participant, *n* (%) | | | | |
| 0 | 94 (55) | 95 (54) | 106 (61) | 98 (56) |
| 1 | 41 (24) | 35 (20) | 31 (18) | 43 (24) |
| 2 | 24 (14) | 18 (10) | 15 (9) | 10 (6) |
| 3 | 6 (4) | 11 (6) | 10 (6) | 16 (9) |
| 4 | 3 (2) | 5 (3) | 6 (3) | 3 (2) |
| 5 | 2 (1) | 7 (4) | 3 (2) | 2 (1) |
| > 5 | 0 (0) | 6 (3) | 3 (2) | 4 (2) |
| Participants who reported any ADRs, *n* (%) | 43 (25) | 57 (32) | 41 (24) | 38 (22) |
| Total number of ADRs | 63 | 119 | 83 | 63 |
| Severity of ADRs (*n*) | | | | |
| Mild | 50 | 96 | 61 | 44 |
| Moderate | 13 | 22 | 19 | 17 |
| Severe | 0 | 1 | 3 | 0 |
| Missing | 0 | 0 | 0 | 2 |
| Number of ADRs per participant, *n* (%) | | | | |
| 0 | 127 (75) | 120 (68) | 133 (76) | 138 (78) |
| 1 | 28 (16) | 28 (16) | 18 (10) | 23 (13) |
| 2 | 11 (6) | 13 (7) | 13 (7) | 9 (5) |
| 3 | 3 (2) | 7 (4) | 5 (3) | 4 (2) |
| 4 | 1 (1) | 4 (2) | 3 (2) | 1 (1) |
| 5 | 0 (0) | 2 (1) | 1 (1) | 0 (0) |
| > 5 | 0 (0) | 3 (2) | 1 (1) | 1 (1) |
| Participants who reported any SAE, *n* (%) | 5 (3) | 12 (7) | 12 (7) | 13 (7) |
| Total number of SAEs | 6 | 16 | 17 | 16 |
| Number of SAEs per participant, *n* (%) | | | | |
| 0 | 165 (97) | 165 (93) | 162 (93) | 163 (93) |
| 1 | 4 (2) | 9 (5) | 10 (6) | 11 (6) |
| 2 | 1 (1) | 2 (1) | 0 (0) | 1 (1) |
| 3 | 0 (0) | 1 (1) | 1 (1) | 1 (1) |
| 4 | 0 (0) | 0 (0) | 1 (1) | 0 (0) |
| Number of SAEs related to IMP | 1 | 1 | 7 | 0 |

TABLE 30 Summary of AEs by MedDRA system organ class

| AEs | Trial group (n) | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| Participants in the safety population | 170 | 177 | 174 | 176 |
| Participants reporting at least one AE | 76 | 82 | 68 | 78 |
| Cardiac disorders[a] | 1 | 1 | 0 | 2 |
| Congenital, familial and genetic disorders | 0 | 1 | 0 | 0 |
| Ear and labyrinth disorders | 1 | 1 | 0 | 1 |
| Eye disorders | 1 | 0 | 0 | 0 |
| GI disorders | 68 | 146 | 86 | 85 |
| General disorders and administration site conditions | 4 | 2 | 2 | 6 |
| Hepatobiliary disorders | 0 | 2 | 0 | 0 |
| Immune system disorders | 0 | 1 | 1 | 1 |
| Infections and infestations | 14 | 14 | 15 | 12 |
| Injury, poisoning and procedural complications | 7 | 5 | 11 | 6 |
| Investigations | 3 | 3 | 0 | 1 |
| Metabolism and nutrition disorders | 0 | 1 | 3 | 0 |
| Musculoskeletal and connective tissue disorders | 12 | 9 | 10 | 13 |
| Neoplasms: benign, malignant and unspecified (including cysts and polyps) | 1 | 0 | 3 | 3 |
| Nervous system disorders | 4 | 6 | 7 | 6 |
| Psychiatric disorders | 1 | 0 | 0 | 2 |
| Renal and urinary disorders | 4 | 1 | 5 | 2 |
| Reproductive system and breast disorders | 1 | 0 | 0 | 2 |
| Respiratory, thoracic and mediastinal disorders | 3 | 9 | 3 | 3 |
| Skin and subcutaneous tissue disorders | 3 | 4 | 5 | 9 |
| Social circumstances | 0 | 0 | 0 | 1 |
| Surgical and medical procedures | 3 | 2 | 0 | 2 |
| Vascular disorders | 1 | 3 | 0 | 3 |

MedDRA, Medical Dictionary for Regulatory Activities.
a  Event-level data.

TABLE 31 Summary of GI AEs by treatment group

| GI AEs | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| Participants in safety population (*n*) | 170 | 177 | 174 | 176 |
| Number of participants with any GI AE, *n* (%) | 47 (28) | 67 (38) | 44 (25) | 51 (29) |
| Total number of GI AEs | 68 | 146 | 86 | 85 |
| Number of GI AEs per participant | | | | |
| 1 | 30 | 29 | 20 | 32 |
| 2 | 14 | 17 | 13 | 10 |
| 3 | 2 | 9 | 7 | 6 |
| 4 | 1 | 8 | 2 | 1 |
| 5 | 0 | 3 | 1 | 1 |
| > 5 | 0 | 1 | 1 | 1 |

TABLE 32 Summary of GI AEs by preferred term name, treatment group and EPA formulation

| GI AEs | Trial group | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | EPA + aspirin | | EPA + placebo aspirin | | Placebo EPA + aspirin | | Placebo + placebo | |
| | FFA | TG | FFA | TG | FFA | TG | FFA | TG |
| Participants in safety population (*n*) | 99 | 71 | 107 | 70 | 99 | 75 | 109 | 167 |
| Participants reporting at least one GI AE, *n* (%) | 25 (25) | 22 (31) | 43 (40) | 24 (34) | 24 (24) | 20 (27) | 38 (35) | 13 (19) |
| GI AE (*n*) | | | | | | | | |
| Abdominal discomfort | 0 | 0 | 2 | 3 | 0 | 2 | 2 | 0 |
| Abdominal distension | 0 | 2 | 0 | 1 | 0 | 0 | 1 | 0 |
| Abdominal pain | 3 | 2 | 14 | 13 | 6 | 1 | 9 | 3 |
| Abdominal pain lower | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 0 |
| Abdominal pain upper | 2 | 1 | 4 | 1 | 1 | 0 | 3 | 0 |
| Anal haemorrhage | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| Anal inflammation | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Anal pruritus | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Breath odour | 1 | 0 | 3 | 0 | 0 | 1 | 4 | 0 |
| Change of bowel habit | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Constipation | 6 | 2 | 5 | 1 | 2 | 4 | 3 | 0 |
| Defaecation urgency | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 1 |
| Diarrhoea | 8 | 2 | 23 | 15 | 11 | 9 | 6 | 6 |
| Dyspepsia | 5 | 11 | 8 | 7 | 6 | 8 | 14 | 4 |
| Epigastric discomfort | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eructation | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 0 |
| Faeces discoloured | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |

**TABLE 32** Summary of GI AEs by preferred term name, treatment group and EPA formulation (*continued*)

| | Trial group | | | | | | | |
| | EPA + aspirin | | EPA + placebo aspirin | | Placebo EPA + aspirin | | Placebo + placebo | |
| GI AEs | FFA | TG | FFA | TG | FFA | TG | FFA | TG |
|---|---|---|---|---|---|---|---|---|
| Flatulence | 1 | 0 | 2 | 2 | 1 | 1 | 3 | 3 |
| Frequent bowel movements | 1 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| Gastric haemorrhage | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Gastritis | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| GI sounds are abnormal | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gastro-oesophageal reflux disease | 0 | 3 | 1 | 0 | 0 | 4 | 4 | 2 |
| Gingival polyp | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Haematochezia | 0 | 0 | 1 | 0 | 4 | 1 | 0 | 0 |
| Haemorrhoidal haemorrhage | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| Haemorrhoids | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hiatus hernia | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| Melaena | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Mouth haemorrhage | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Nausea | 5 | 2 | 5 | 6 | 2 | 1 | 0 | 3 |
| Oesophagitis | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Proctalgia | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Rectal haemorrhage | 4 | 0 | 2 | 2 | 4 | 3 | 1 | 1 |
| Rectal tenesmus | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Retching | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tongue discolouration | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Tongue eruption | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Tongue haemorrhage | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Vomiting | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 0 |

Similar to the profile of AEs, there were more ADRs reported in the EPA + placebo aspirin group than in the other three groups (*Table 33*). The most commonly reported ADRs across all treatment groups were GI disorders. The distribution of GI ADRs across the four groups was similar (*Table 34*) (see *Appendix 4*, *Tables 41* and *43*).

There were a small number of SAEs reported (*Table 35*) (see *Appendix 4*, *Table 42*). The most frequently reported SAEs were cardiac and GI disorders. Among the nine cardiac disorders, five were episodes of atrial fibrillation (AF) and all of these were reported in the EPA + placebo aspirin group. Three were reported by one participant and two additional participants reported one each. Three myocardial infarctions were reported by participants: one in the EPA + placebo aspirin group, two in the placebo EPA + aspirin group and one in the placebo + placebo group. One participant in the placebo EPA + aspirin group had an arrhythmia.

Eight ADRs led to trial discontinuation for four participants, one from each of the four trial groups. The eight ADRs came from six preferred term names (*Table 36*).

**TABLE 33** Summary of ADRs by MedDRA system organ class

| ADRs | Trial group | | | |
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| --- | --- | --- | --- | --- |
| Participants in the safety population (*n*) | 170 | 177 | 174 | 176 |
| Participants reporting at least one ADR, *n* (%) | 43 (25) | 57 (32) | 41 (24) | 38 (22) |
| Total number of ADRs | 63 | 119 | 83 | 63 |
| GI disorders (*n*) | 46 | 110 | 69 | 58 |
| General disorders and administration site conditions (*n*) | 4 | 0 | 0 | 1 |
| Infections and infestations (*n*) | 1 | 0 | 0 | 0 |
| Injury, poisoning and procedural complications (*n*) | 4 | 1 | 3 | 0 |
| Investigations (*n*) | 1 | 0 | 0 | 0 |
| Metabolism and nutrition disorders (*n*) | 0 | 1 | 1 | 0 |
| Musculoskeletal and connective tissue disorders (*n*) | 0 | 0 | 0 | 1 |
| Nervous system disorders (*n*) | 3 | 3 | 4 | 0 |
| Psychiatric disorders (*n*) | 1 | 0 | 0 | 0 |
| Renal and urinary disorders (*n*) | 2 | 0 | 3 | 0 |
| Respiratory, thoracic and mediastinal disorders (*n*) | 1 | 2 | 2 | 0 |
| Skin and subcutaneous tissue disorders (*n*) | 0 | 2 | 1 | 3 |

MedDRA, Medical Dictionary for Regulatory Activities.

**TABLE 34** Distribution of GI ADRs

| GI ADRs | Trial group | | | |
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| --- | --- | --- | --- | --- |
| Total number of GI ADRs | 46 | 110 | 69 | 58 |
| Number of GI ADRs | | | | |
| 1 | 20 | 26 | 16 | 22 |
| 2 | 11 | 13 | 10 | 7 |
| 3 | 0 | 7 | 4 | 4 |
| 4 | 1 | 4 | 4 | 1 |
| 5 | 0 | 3 | 1 | 0 |
| 6 | 0 | 1 | 0 | 1 |

**TABLE 35** Summary of SAEs by MedDRA system organ class

| SAEs | Trial group | | | |
|---|---|---|---|---|
| | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| Participants in the safety population (n) | 170 | 177 | 174 | 176 |
| Participants reporting at least one SAE, n (%) | 5 (3) | 12 (7) | 12 (7) | 13 (7) |
| Total number of SAEs | 6 | 16 | 17 | 16 |
| Cardiac disorders (n) | 0 | 6 | 2 | 1 |
| GI disorders (n) | 0 | 2 | 5 | 2 |
| General disorders and administration site conditions (n) | 1 | 1 | 0 | 1 |
| Hepatobiliary disorders (n) | 0 | 1 | 0 | 0 |
| Infections and infestations (n) | 1 | 2 | 2 | 3 |
| Injury, poisoning and procedural complications (n) | 0 | 0 | 1 | 0 |
| Investigations (n) | 0 | 0 | 1 | 0 |
| Neoplasms: benign, malignant and unspecified (including cysts and polyps) (n) | 2 | 2 | 0 | 4 |
| Nervous system disorders (n) | 0 | 0 | 2 | 1 |
| Psychiatric disorders (n) | 0 | 0 | 0 | 2 |
| Respiratory, thoracic and mediastinal disorders (n) | 1 | 2 | 1 | 0 |
| Vascular disorders (n) | 1 | 0 | 3 | 2 |

MedDRA, Medical Dictionary for Regulatory Activities.

**TABLE 36** Summary of ADRs that led to trial discontinuation

| ADRs that led to trial discontinuation | Trial group | | | |
|---|---|---|---|---|
| | EPA + aspirin (N = 170) | EPA + placebo aspirin (N = 177) | Placebo EPA + aspirin (N = 174) | Placebo + placebo (N = 176) |
| Participants who discontinued the trial because of an ADR, n (%) | 1 (1) | 1 (1) | 1 (1) | 1 (1) |
| ADR by preferred term name (n) | | | | |
| Abdominal pain | 1 | 0 | 0 | 0 |
| Atrial fibrillation | 0 | 3 | 0 | 0 |
| Deep-vein thrombosis | 0 | 0 | 0 | 1 |
| Lymphoma | 0 | 0 | 1 | 0 |
| Melaena | 0 | 1 | 0 | 0 |
| Skin disorder | 0 | 0 | 0 | 1 |

### Clinically significant acute upper gastrointestinal bleeding episodes

No haemorrhagic strokes were reported during the trial. A manual search of AEs and SAEs revealed six acute upper GI bleeding events that were considered by the chief investigator to be of clinical significance. These were 'oesophageal haemorrhage' (in the placebo EPA + aspirin group), 'gastro-oesophageal reflux disease' (in the placebo EPA + aspirin group) and 'alcohol withdrawal syndrome' (in placebo EPA + placebo aspirin group), which were all reported as SAEs, and 'gastric haemorrhage' (in the EPA + placebo aspirin group) and two cases of 'melaena' (in the EPA + placebo aspirin and placebo EPA + aspirin groups), which were recorded as AEs. It is possible that one of the cases of melaena (in a participant receiving EPA + placebo aspirin) was a SAE because the participant had been hospitalised, but this event was recorded specifically as an AE by the site. All tables reflect this categorisation.

### Deaths

One death was reported during the trial. During contact made to arrange visit 5, site staff were made aware that a participant had died from bladder cancer, which was deemed to be unrelated to the intervention.

### Protocol deviations

Protocol deviations were reported for between 64% and 73% of participants in each treatment group. Most deviations were judged to be minor, with the majority being trial visits outside the time window. The number and types of deviations appeared similar between treatment groups.

There were 11 deviations related to randomisation error (*Table 37*). Ten of these were as a result of participants being ineligible, and one participant was randomised prior to consent. None of these errors occurred in participants who were randomised to the placebo + placebo group. There was no reason to suspect that the deviation was related to the treatment group to which the participant had been randomised.

TABLE 37 Listing of treatment randomisation error deviations

| Treatment group | Details[a] | Included in per-protocol population? | Outcome collected? |
|---|---|---|---|
| EPA + aspirin | Not eligible | No | Yes |
| Placebo EPA + aspirin | Patient not eligible for trial as was intermediate risk | No | Yes |
| EPA + placebo aspirin | Patient does not meet inclusion criteria; withdrawn from trial | No | No |
| Placebo EPA + aspirin | Participant did not fulfil inclusion criteria as had only two polyps > 10 mm, but was still considered a high-risk patient by the BCSP | No | Yes |
| Placebo EPA + aspirin | Patient has three adenomas and all less than the required measurement for patient to be included in the trial | No | No |
| Placebo EPA + aspirin | Randomised in error. Patient had hyperplastic polyps | No | Yes |
| EPA + aspirin | Randomised in error. Patient had four adenomas; however, none was more than 10 mm | No | Yes |
| EPA + placebo aspirin | Patient should not have been included as had had previous BCSP colon – this not first BCSP colon | Yes | Yes |
| EPA + placebo aspirin | Patient randomised prior to consent, as patient prescription needed to be signed by PI, and he [was] not available at site on day of consent. Patient did not want to consent then come back for prescription on another day | Yes | Yes |
| EPA + aspirin | Incorrectly recruited due to confusion of the term 'high risk' | No | No |
| Placebo EPA + aspirin | Patient randomised at age 74 in error due to randomisation database allowing the patient in by accident | Yes | No |

a Verbatim description from trial site staff member.

## Exploratory analyses

### Colorectal adenoma size

Given the decrease in total colorectal adenoma number associated with EPA and aspirin treatment, colorectal adenoma size was analysed consistent with size analysis in previous RCTs of chemoprevention agents in FAP patients.[55,67] Colorectal adenoma size was summarised within each participant across each treatment group. Analyses were based on the within-participant mean value (*Table 38*).

The adjusted mean difference between the EPA and no EPA groups was –0.47 mm (i.e. the mean adenoma size was smaller in those receiving EPA than in those not receiving EPA). The mean adjusted difference between the aspirin and no aspirin groups was 0.42 mm (i.e. the mean adenoma size was bigger in those receiving aspirin than in those not receiving aspirin). There was no statistically significant difference between EPA and placebo users, or between aspirin and placebo users.

### Relationship between individual colorectal adenoma number and eicosapentaenoic acid levels

The relationship between the change in RBC EPA levels at 12 months from baseline and secondary outcomes, according to EPA factorial margin, was also investigated descriptively by plotting individual values. There was no evidence of a clear relationship between individual increase in RBC EPA level and total colorectal adenoma number (*Figure 19*). This was also the case for the rectal EPA level at 12 months. The fact that there were three 'outlier' individuals in the placebo EPA group who had a large increase in RBC EPA level during the intervention phase suggests that 'contamination' by own-use ω-3 PUFA intake may have occurred in these cases (see *Figure 19*).

**TABLE 38** Analysis of colorectal adenoma size

| Adenoma size | Trial group | | | |
| --- | --- | --- | --- | --- |
| | EPA + aspirin (*N* = 177) | EPA + placebo aspirin (*N* = 178) | Placebo EPA + aspirin (*N* = 176) | Placebo + placebo (*N* = 176) |
| Participants with colorectal adenoma data at 12 months (*n*) | 161 | 153 | 163 | 163 |
| Participants with at least one colorectal adenoma (*n*) | 98 | 97 | 100 | 100 |
| Participants with mean colorectal adenoma size available (*n*) | 97 | 97 | 100 | 100 |
| Participants with mean colorectal adenoma size missing (*n*) | 1 | 0 | 0 | 0 |
| Size (mm)[a] of adenoma at 12 months | | | | |
|     Mean (SD) | 3.5 (2.3) | 3.3 (2.2) | 4.2 (3.3) | 3.7 (2.4) |
|     Median (IQR) | 3.0 (2.0–3.5) | 3.0 (2.0–4.0) | 3.5 (2.3–5.0) | 3.0 (2.0–4.4) |
|     Minimum, maximum | 1, 12 | 1, 15 | 1.0, 28.5 | 1, 15 |
| | *EPA vs. no EPA* | | *Aspirin vs. no aspirin* | |
| Adjusted[b] difference in means (95% CI) | –0.47 (–1.04 to 0.98) | | 0.42 (–0.14 to 0.99) | |

IQR, interquartile range.
a   The mean was calculated within participants then the overall mean of the means within participants.
b   Adjusted by histology type (i.e. conventional/serrated) and recruiting site. Estimates were mean difference, taking into account multiple adenomas per participant.
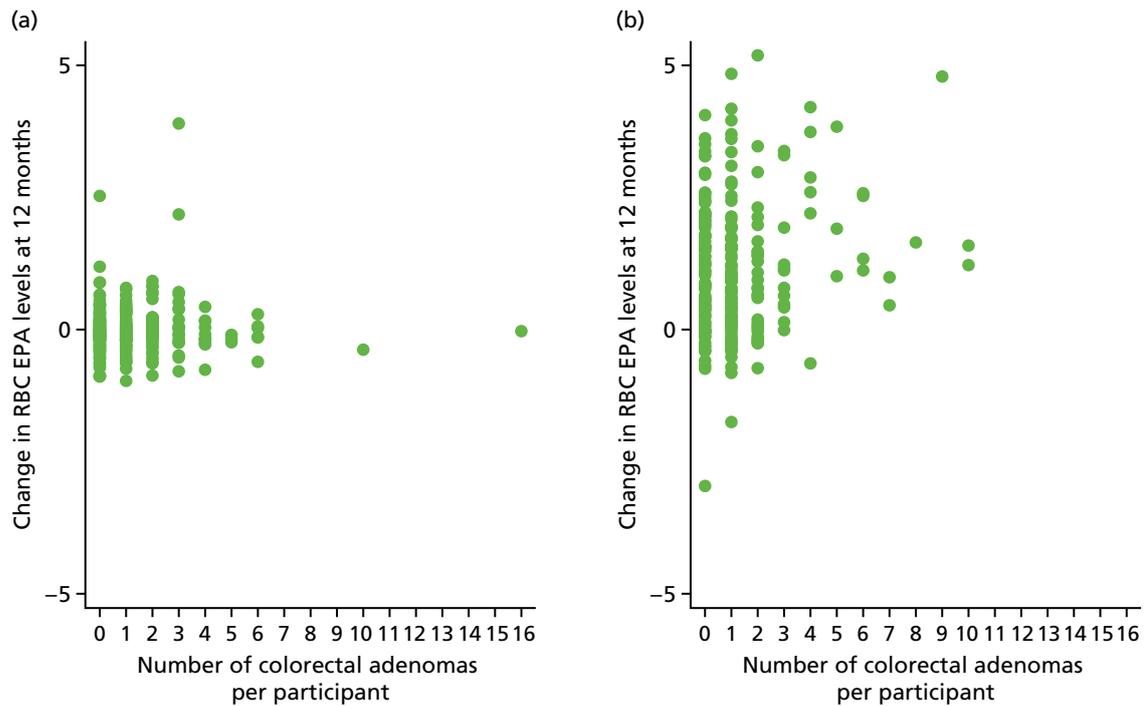
**FIGURE 19** Change in RBC EPA levels at 12 months from baseline against total number of colorectal adenomas per participant by EPA treatment groups. (a) Placebo EPA; and (b) active EPA.

### Relationship between red blood cell and rectal mucosal eicosapentaenoic acid levels in participants

The relationship between RBC and rectal mucosal EPA levels at 12 months was of moderate strength, with a correlation coefficient of 0.455 (*Figure 20*).
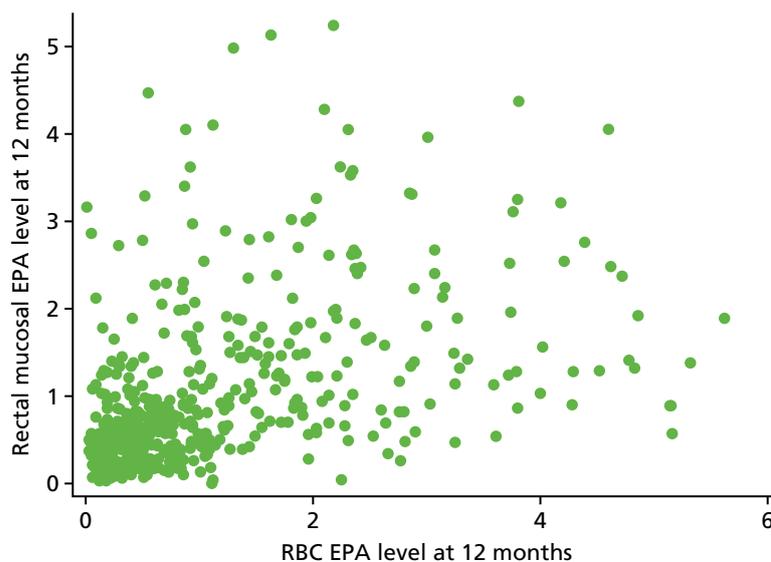


**FIGURE 20** Scatterplot of the individual rectal mucosal EPA level against the corresponding RBC EPA level at 12 months.

# Chapter 4  Discussion

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## Summary of colorectal adenoma findings

The seAFOod polyp-prevention trial found no evidence of an effect of either EPA or aspirin on the primary end point of the proportion of individuals with one or more colorectal adenomas at the 12-month surveillance colonoscopy (the ADRa) in patients deemed 'high risk' in the English BCSP. Overall, ≈60% of trial participants had at least one colorectal adenoma at surveillance colonoscopy. The trial was designed to detect an absolute difference between groups of 10 percentage points, or a relative difference of 18%, assuming an ADRa of 60% in the double placebo group. The 95% CIs for the primary analyses excluded a difference of this magnitude on ADRa for both treatments. Moreover, the primary outcome data were robust to sensitivity analyses.

Secondary analyses of the effects of EPA and aspirin on colorectal adenoma number provided evidence of chemopreventive activity of both agents. Aspirin was effective at reducing the total number of colorectal adenomas per participant, but there was no evidence of an effect on total MAP by EPA. Other secondary analyses suggested that there are colorectal adenoma subtype- and site-selective effects of EPA and aspirin. Participants randomised to EPA had a reduced number (MAP) and ADRa for conventional dysplastic colorectal adenomas in the left colon and rectum compared with those randomised to placebo. Participants randomised to aspirin had a reduced number of adenomas in the right colon, particularly for serrated adenomas, and also a reduced risk of conventional colorectal adenomas. An exploratory analysis found no evidence of any effect of either EPA or aspirin on recurrent colorectal adenoma size.

## Colorectal adenoma end points

The ADRa was chosen as the primary end point, on historical grounds that previous 'sporadic' polyp-prevention trials had all used this binary measure of the presence of any colorectal adenoma at surveillance colonoscopy as the primary measure of colorectal adenoma risk.[49] We expected the high ADRa at the 1-year surveillance colonoscopy of 'high-risk' individuals in the English BCSP compared with previous polyp-prevention trials.[98] The high ADRa in the seAFOod trial population of obese, male-predominant 'polyp formers' will also have been driven by the uniformly excellent colonoscopy quality in the BCSP, in which the ADRa figures prominently as an individual QA measure.[38,95,97] It is noteworthy that those previous aspirin RCTs with the highest placebo ADRa {47.1% [Aspirin and Folate Polyp-Prevention Study (AFPPS)[114]] and 53.4% [APACC[115]]} reported the smallest (statistically insignificant) risk reduction associated with aspirin (risk ratio 0.88 and 0.95, respectively) compared with the other two aspirin RCTs,[49] despite the overwhelming evidence for a CRC chemoprevention effect of aspirin.[43–48] Therefore, the use of the ADRa as a biomarker of chemoprevention efficacy in screening cohorts undergoing high-quality colonoscopic assessment with a high ADRa must be questioned.

By contrast, previous chemoprevention RCTs in FAP patients with a large colorectal adenoma burden have reported efficacy based on colorectal adenoma number and size.[55,67,116] Moreover, three of the four previous aspirin 'sporadic' polyp-prevention trials have reported colorectal adenoma multiplicity as a secondary outcome.[115,117,118] Therefore, adenoma number was stipulated as a secondary end point in the seAFOod trial. Reduction in colorectal adenoma multiplicity is widely accepted as an indicator of anti-CRC activity in pre-clinical studies.[61] More recently, adenoma number has gained credence as a biomarker in 'sporadic' colorectal adenoma prevention RCTs,[39] driven by increasing use of MAP as an outcome measure in colonoscopy QA studies.[38] Moreover, colorectal adenoma number predicts future CRC incidence and

mortality in population-based observational studies.[40–42] We suggest that subsequent chemoprevention RCTs utilise MAP as the primary measure of chemopreventative efficacy, especially in 'high-risk' study populations with a high ADRa, in which the ADRa will be a relatively insensitive measure of colorectal adenoma risk. The recent polyp-prevention RCT of metformin did indeed employ co-primary end points of colorectal adenoma incidence (ADRa) and number (MAP).[39]

## Site and adenoma subtype specificity of the chemopreventive effects of aspirin and eicosapentaenoic acid

All three aspirin RCTs, which reported colorectal adenoma number as a secondary outcome,[115,117,118] demonstrated a significant reduction in total MAP in the aspirin group compared with the placebo group. The APACC trial reported total MAP data at the 12-month colonoscopic follow-up, similar to the seAFOod trial.[115,119] Coupled with the known CRC-preventative properties of aspirin demonstrated during observational follow-up of large-scale aspirin RCTs,[46–48] our data (and those from the previous polyp-prevention trials)[115,117,118] justify colorectal adenoma number as a clinically relevant biomarker of CRC risk in CRC chemoprevention trials, in addition to the ADRa. The similar magnitude decrease in colorectal adenoma number that we observed for aspirin in the seAFOod trial compared with the previous aspirin RCTs suggests that the reductions in colorectal adenoma number that we have reported are likely to be genuine and be clinically meaningful for CRC risk reduction. However, we do acknowledge that the number of secondary comparisons that have been made increases the possibility that findings could be spurious.

With increased understanding that different colorectal carcinogenesis pathways exist, improved insight into differential effects of chemopreventive agents on distinct colorectal adenoma types is of paramount importance. Chemopreventive activity of EPA against conventional colorectal adenomas (but not serrated lesions), based on both MAP and ADRa outcomes, is consistent with the known efficacy of the same dose of EPA-FFA in FAP patients[67] with rectal adenomas, which are conventional, wholly dysplastic lesions occurring on a background of germline mutation of one *APC* allele and tumour initiation after loss of heterozygosity of the other *APC* allele.[120] By contrast, we report that EPA has no chemopreventive activity against serrated adenomas. Based on secondary colorectal adenoma number analysis, a possible signal that EPA use may actually increase serrated lesion risk requires careful examination in future studies of ω-3 PUFA supplementation/dietary intake and CRC risk, in which there is stratification based on location and the different molecular subtypes of CRC.

By contrast, the effect of aspirin on proximal colorectal adenoma multiplicity was partly explained by the strong preventative activity against serrated lesions. The seAFOod trial data add to an existing body of evidence that the reduction in CRC incidence and mortality by aspirin is explained by a dominant effect on proximal CRC, with less preventative efficacy against distal CRC.[47,48] Post hoc analysis of data from the AFPPS trial[114] has revealed that risk of right-sided (but not left-sided) serrated polyps was lower in those allocated aspirin than in those allocated placebo.[121] Moreover, a meta-analysis of observational studies of lifestyle factors and serrated polyp risk has reported that serrated polyp risk is decreased in aspirin users (relative risk 0.81, 95% CI 0.67 to 0.99).[122]

The seAFOod trial data suggest that aspirin also has some antineoplastic activity against conventional adenomas. However, in contrast with EPA, aspirin (100–600 mg daily) has not been demonstrated to have efficacy against rectosigmoid polyps in FAP RCTs,[123,124] which is consistent with the dominant 'right-sidedness' of aspirin chemopreventative activity.[47,48]

Only two previous 'sporadic' polyp-prevention trials have reported an a priori analysis of colorectal adenoma risk related to location in the colorectum.[114,125] In the RCT of the selective COX-2 inhibitor rofecoxib, there was no differential effect on ADRa dependent on location in either the right or left colon.[125] In the AFPPS trial, no differential effects of aspirin on right or left ADRa were observed, similar to the seAFOod trial data.[121]

There has been no location-dependent analysis of colorectal adenoma number in a polyp-prevention trial prior to the seAFOod trial.

The seAFOod trial data, in combination with greater insight into the molecular pathogenesis of early stages of colorectal carcinogenesis,[4] should prompt a paradigm shift in polyp-prevention trials driving data collection on colorectal adenoma subtype (conventional adenoma vs. serrated polyp) and tumour location (proximal/right vs. distal/left), leading to evaluation of stratified chemoprevention. It remains unclear whether or not the biology of the early stages of colorectal carcinogenesis during initiation and growth of a colorectal adenoma differs significantly such that rectal lesions should be classified separately from colonic neoplasia. At the present time, available evidence suggests that the molecular pathologies of colorectal adenomas from the rectum and distal colon do not differ significantly.[126]

## Dose considerations

The seAFOod trial evaluated 300 mg of aspirin daily based on the random-effects meta-analysis of aspirin polyp-prevention RCTs that demonstrated a significant colorectal adenoma risk reduction at doses of 300–325 mg daily, particularly for advanced lesions (risk ratio 0.71, 95% CI 0.56 to 0.92 for advanced ADRa), albeit in subjects approximating to 'intermediate-risk' surveillance populations.[49] Observational data on CRC risk suggest that lower daily doses of aspirin are likely to have 'right-predominant' effects on colorectal adenoma multiplicity similar to those reported herein.[48]

The FFA-equivalent dose of 2 g of EPA daily was based on the same dose of EPA-FFA that demonstrated efficacy in the proof-of-concept RCT in FAP patients.[67] This is a relatively high daily amount of ω-3 PUFA, which would be difficult to provide using more widely available fish oil or re-esterified TG formulations.[65] Restricted supply of the original EPA-FFA formulation, beyond TMG control, meant that we had to switch the IMP to an EPA-TG formulation and matching placebo during the recruitment phase of the trial. This, however, provided a unique (originally unplanned) opportunity to compare 'bioavailability' of two EPA formulations, with the same FFA dose, in the context of prolonged dosing in a RCT. The data suggest that there is no meaningful difference in tissue EPA incorporation, measured by RBC membrane and rectal mucosal EPA levels, between EPA-FFA and EPA-TG. Therefore, colonoscopic outcome data from the two populations receiving either EPA-FFA/placebo or EPA-TG/placebo were combined.

## Mechanistic considerations

We have not yet addressed the mechanistic basis for differential chemopreventive activity of EPA along the colorectum. Conventional dysplastic 'sporadic' colorectal adenomas are found along the length of the colon and can exhibit molecular features compatible with CIN, MSI and CIMP+ pathways, but with proportionally more CIN+ lesions in the distal colon.[4,23] Therefore, differential activity of EPA against the distinct molecular CRC subtypes should be investigated, particularly in view of the absence of any efficacy signal against serrated lesions that are predominantly CIMP+ and often exhibit features of MSI.[4,21,23]

We cannot rule out that differences in mucosal EPA levels explain colorectal site selectivity of the modest chemopreventive activity of EPA. There are no human data describing ω-3 PUFA levels in different regions of the colorectum. However, rodent data suggest that there is no gradient in mucosal EPA exposure along the colon.[127] One clinical study has measured ω-3 PUFA levels in the sigmoid colon (but not the rectum).[128] However, data from this Japanese study cannot be compared with the rectal mucosal PUFA levels measured in the seAFOod trial because of profound differences in baseline characteristics of the two study populations, including dietary ω-3 PUFA intake.[128]

We have recently reported that mixed ω-3 PUFA supplementation is associated with an increase in short-chain fatty acid (SCFA)-producing bacterial genera such as *Lactobacillus* and *Bifidobacteria* in faecal

samples.[129] Although SCFA levels are believed to be higher in the caecum and ascending colon than in the distal colon,[130] the SCFA receptor free fatty acid receptor (FFAR) 2, also known as GPR43, is expressed at higher levels in the left colon.[131] Therefore, a valid hypothesis is that differential antineoplastic activity of EPA may relate to increased antineoplastic SCFA-FFAR2 signalling in the distal versus proximal colon.[132]

Both EPA and aspirin inhibit the COX enzymes COX-1 and COX-2.[57] Independent reports have described how expression of COX-2, which is believed to play a critical role in intestinal tumorigenesis,[54] is higher in distal colonic neoplasms than in the proximal colonic lesions.[133,134] Differential expression and activity of COX-1 in colorectal adenomas in different parts of the colorectum has not been studied. Differences in gene expression in non-neoplastic mucosa and baseline colorectal adenoma characteristics that may predict response to EPA and/or aspirin deserve further investigation.

The mechanism of the CRC-preventative activity of aspirin remains unclear despite intense scrutiny over several years.[44,45] Postulated mechanisms of action include COX-dependent and COX-independent activity, either directly on colorectal epithelial cells or indirectly via the antiplatelet activity of aspirin.[45] More recently, subversion of the host anti-tumour immune response by COX-dependent $PGE_2$ has been reported,[135] which is abrogated by aspirin, thus driving more effective host anti-tumour immunosurveillance. This mechanism of action may explain the preferential antineoplastic activity of aspirin against proximal colonic neoplasms, which are more likely to exhibit a higher neo-antigenic load (and hence potential to stimulate a host anti-tumour immune response) as a result of defective DNA MMR in MSI tumours.[136]

## Combination colorectal cancer chemoprevention

The individual effects of EPA and aspirin on colorectal adenoma number beg the question of the antineoplastic activity of combined treatment, which, given the single agent effects apparent on the 'at-the-margins' analysis, one might expect to be apparent for total colorectal adenoma number. The trial was powered to be able to detect only a major interaction between EPA and aspirin with an 'inside-the-table' analysis of this 2 × 2 factorial RCT. Comparison between the four treatment groups highlighted that total and left-sided colorectal adenoma multiplicity was lower in the combined EPA and aspirin treatment group, consistent with efficacy of both agents on left-sided, conventional colorectal adenomas. A key objective of future work, in order to translate the seAFOod trial findings into clinical application, will be to apply precision/personalised medicine principles to pose the question regarding which individuals might gain most from chemoprevention with one or both agents, based on baseline colorectal adenoma characteristics and/or other mucosal biomarkers.

## Use of the NHS Bowel Cancer Screening Programme for a polyp-prevention trial

The rationale for the study of a 'high-risk' population undergoing 1-year surveillance colonoscopy within the English BCSP was based on data from previous aspirin polyp-prevention trials that suggested that colorectal adenoma risk reduction associated with aspirin use was already evident (and exceeded the risk reduction at 3–4 years) at 1 year, combined with the high ADRa.[49] The relatively short follow-up duration raises the question of how far colorectal adenoma outcomes at 12 months simply reflect missed colorectal neoplasia from the screening examination. However, BCSP data[98,100] and other reports[137,138] have confirmed that the colorectal adenoma yield at 12 months from baseline assessment is significantly higher than the yield from a procedure within 3 months of the index assessment, implying that de novo colorectal adenoma growth contributes significantly to colorectal adenoma 'recurrence' rather than solely 'missed lesions'. In reality, colorectal adenomas detected at a 12-month endoscopic procedure will almost certainly represent a mixed population of 'new' and 'missed' lesions, even after the highest-quality colonoscopic examination, but this does not negate the role of the colorectal adenoma as a biomarker of chemoprevention efficacy (related to

regression of existing colorectal adenomas, as well as inhibition of new growth), as accepted in proof-of-concept FAP RCTs.[37]

The low yield of advanced colorectal adenomas in the seAFOod trial is probably explained by the short surveillance interval and high-quality colonoscopic colorectal adenoma clearance achieved in the BCSP, but also the rather stringent 'advanced' lesion definition, which required complete (100%) 'villousness' of conventional dysplastic adenomas, unlike previous observational and intervention studies that have included lesions with > 25% 'villousness' in the definition of 'advanced' colorectal adenoma.[32,139]

Using the English BCSP as a vehicle for the seAFOod trial provided several advantageous features to the design and performance of the trial, including strict, protocol-driven colonoscopy screening and surveillance procedures, uniform colonoscopy, and histopathology performance and reporting.[95–97] In particular, strict timing of the 12-month surveillance colonoscopy in the seAFOod trial was a major advantage over previous 'sporadic' polyp-prevention trials undertaken in non-screening settings[49,125,141] and was essential for interpretable PUFA biomarker data at 12 months. The trial population had a strong male predominance, mirroring BCSP practice,[95] which limits the generalisability of the data to women. The high prevalence of overweight and obesity in the trial population probably indicates the major contribution of excess body weight to early-stage colorectal carcinogenesis,[140] and is highly relevant to general CRC chemoprevention in non-screening populations given the high prevalence of overweight and obesity in many areas of the world.[142]

A large number (nearly 4000) of individuals stratified as 'high risk' after BCSP screening colonoscopy were identified and screened by participating trial sites, as expected from BCSP data pertaining to the prevalent screening round, which suggested that 'high-risk' features were detected in 9.8–10.3% of screening colonoscopies (Public Health England, 2009, personal communication).[95] However, since the seAFOod trial opened for recruitment, subsequent incident screening rounds have detected less colorectal neoplasia and more recent BCSP data demonstrate that 'high-risk' features make up only 8.3% of screening colonoscopy outcomes (Public Health England, 2009, personal communication). The reduction in incidence of 'high-risk' colorectal neoplasia probably contributed to the slower than expected recruitment during the trial.

Only 18% of 'high-risk' individuals were randomised. This is a higher screen success rate than in several previous polyp-prevention trials that reported a trial screening success rate,[141,143] but lower than we anticipated. Exclusion criteria included concomitant use of anticoagulants or antiplatelet agents, including existing aspirin use (19%). In addition, a higher than expected proportion of patients (31%) declined to be screened for and participate in the trial, despite the fact that the trial design did not entail an additional colonoscopy and that venepuncture for blood sampling was optional. Trial acceptability was lower than expected from a cohort of patients who had already engaged fully with a multistage screening process and who had been informed that they had 'high-risk' colorectal neoplasia necessitating surveillance colonoscopy in 12 months' time. Formal data pertaining to reasons for declining participation were not collected on the basis that any individual did not need to provide a reason for his or her decision. However, an informal review showed that reasons for declining participation at screening included concern about risk associated with the IMP (particularly aspirin) and unwillingness to start medication when previously not taking any regular drugs, but also, conversely, unwillingness to add further medication to an existing drug regimen.

The screen failure rate varied significantly between the BCSP sites despite the strict, uniform inclusion criteria based on BCSP practice and an identical BCSP screen pathway used at all BCSP sites. Several patient (e.g. differential prevalence of comorbidities requiring antiplatelet/anticoagulant use in different regions of England) and research staff factors (e.g. research time available to PI and SSPrs, as well as CRN-funded RN support) contributed to marked differences in recruitment efficiency. In addition, BCSP screening colonoscopy activity varied widely across individual sites, thereby limiting the identification of 'high-risk' individuals at some sites. Recognition of the most-suitable BCSP sites for any similar polyp-prevention trial will be key to improving recruitment efficiency, particularly when 'high-risk' individuals remain a relatively fixed proportion (7–9%) of guaiac FOBt-positive patients. A general theme (but with notable exceptions) was that BCSP sites that utilised CRN-funded research staff best had a higher recruitment rate. This may reflect insufficient time

that many BCSP staff have available for research and/or some SSPrs having limited understanding of clinical trials research and a protectionist approach towards patients; these need to be addressed prior to a future polyp-prevention trial set in the BCSP.

The switch from guaiac FOBt to FIT in the UK BCSP programmes may alter colorectal neoplasia incidence rates relevant to future trial recruitment projections.

## Safety and tolerability of eicosapentaenoic acid and aspirin

Overall, safety and tolerability of both agents was excellent, with no excess of clinically significant bleeding events, even in the combined treatment group. However, we did mandate stopping IMP before and after any planned invasive procedures (including polypectomy at repeat endoscopy) in the unlikely event that the combined antiplatelet activity of EPA and aspirin increased bleeding risk. Excellent tolerability of EPA and aspirin contributed to high compliance levels and trial retention, with limited drop-out.

Eicosapentaenoic acid was associated with an excess of mild to moderate GI AEs, including diarrhoea. Although ω-3 PUFA products have an excellent safety profile and daily doses of up to 4 g are 'generally recognized as safe' (GRAS) by the US Food and Drug Administration (FDA), mild to moderate GI AEs are recognised, commonly diarrhoea, dyspepsia, eructation (burping) and an unpleasant 'fishy' taste.[144] These GI symptoms often occur in the general population unrelated to dietary or 'nutraceutical' ω-3 PUFA intake.[113] Therefore, we confirmed that the treatment groups were well matched for prevalent GI symptoms at baseline. Dose-related ω-3 PUFA-related GI symptoms can occur.[65,144,145] However, an open clinical question has been whether or not prolonged dosing with the different forms of EPA and mixed ω-3 PUFAs (FFA vs. TG vs. EE) available in 'nutraceutical' preparations is associated with differential GI tolerability.[65] The seAFOod trial data do not indicate any major difference in GI tolerability between the same FFA-equivalent dose of the EPA-FFA and EPA-TG formulations in the context of a 12-month intervention trial.

Mild to moderate GI AEs, particularly diarrhoea and abdominal pain, were more common in those allocated to EPA alone, as opposed to EPA plus aspirin. A valid hypothesis is that aspirin co-therapy is causally protective for ω-3 PUFA intolerance. This requires further investigation in the context of subsequent studies of combined antineoplastic activity of both agents.

Five episodes of AF were reported in three participants, all of whom were allocated active EPA alone. Several RCTs have investigated ω-3 PUFA supplementation for primary and secondary prevention of AF that are summarised in recent American Heart Association guidelines on ω-3 PUFA use and prevention of CVD.[146] Overall, there is no supportive evidence for use of ω-3 PUFAs for AF prevention. There has been no signal in any previous RCT of increased AF risk in ω-3 PUFA users.[146]

An important component of the trial was dietary ω-3 PUFA analysis to exclude significant change in dietary ω-3 PUFA intake prompted by participant-facing trial information, prior to consent, that suggested possible anti-CRC activity of EPA. Using the established short-form EPIC FFQ,[108] we confirmed that there was no evidence of increased fish intake during the intervention phase and that the four treatment groups were well matched on dietary fish intake at baseline and at the end of trial participation.

## Biomarkers of omega-3 polyunsaturated fatty acid tissue exposure

The seAFOod trial biobank obtained excellent coverage considering the geographical spread of a large number of trial sites. There was widespread engagement from SSPrs and RNs at sites, with careful sample handling and storage at sites prior to temperature-controlled transport to the biobank in Bradford. The trial protocol outlines the lipid mediator analyses that were originally proposed.[100] At the start of the trial, measurement of RBC PUFA levels was planned in only a small subset of participants. However, the capsule IMP switch necessitated the

measurement of PUFA levels in all RBC and rectal mucosal samples to provide the strongest possible evidence that the trial cohorts exposed to EPA-FFA and EPA-TG gained equivalent exposure to EPA and could be combined for primary and secondary outcome analysis. Therefore, many planned measurements were not carried out, but these should be performed in the future if further funding can be obtained for biomarker analysis.

Baseline RBC PUFA values in the seAFOod trial cohort were comparable with previous cross-sectional UK studies and data from other Western countries with relatively low population ω-3 PUFA levels and dietary fish intake.[147] Despite self-reported compliance being excellent by capsule counting, the increase in RBC EPA levels observed in EPA users was highly variable, a phenomenon that has been seen in several previous ω-3 PUFA studies, in which individual RBC fatty acid profiles have been measured.[88,129,148] The explanation for large variations in RBC membrane EPA levels, despite uniform dosing, remains unclear and requires further investigation in the large trial cohort. There was no evidence that COX inhibition by concurrent aspirin use is associated with higher RBC membrane or rectal mucosal EPA content.

To our knowledge, this is the first large-scale RCT evidence that oral administration of EPA results in incorporation of EPA in the rectum. There was a medium-strength correlation between the RBC % EPA value at 12 months and the rectal mucosal % EPA content. The RBC membrane EPA and DHA content is considered to be the best proxy biomarker of tissue ω-3 PUFA exposure.[84,148] Further exploratory analysis of the seAFOod trial data is required to explore the predictive value of the rectal mucosal EPA level for individual EPA efficacy against left-sided colorectal neoplasia. Integration of dietary ω-3 PUFA intake, baseline PUFA levels and 'nutraceutical' EPA dosing is needed in a treatment-independent analysis of the relationship between EPA level and colorectal adenoma recurrence risk during surveillance. A similar analysis performed in the context of the WELCOME trial[149] of ω-3 PUFA use in patients with non-alcoholic fatty liver disease provided new insights into the role of DHA in treatment of that condition.

There was no evidence of significant conversion of EPA to DHA in either RBC membranes or rectal mucosa, consistent with previous human data from EPA intervention trials.[67,87] Therefore, the trial data do not support the concept of EPA as the 'universal ω-3 PUFA donor', which can substitute for mixed EPA-DHA preparations. The question of whether or not equivalent doses of EPA alone, DHA alone or a EPA-DHA mix have different antineoplastic activity in the intestine has been tested only in rodents, in which equivalent anti-CRC effects have been observed.[61] Equivalent bioactivity of EPA and DHA in a CRC chemoprevention context cannot be assumed from the seAFOod trial; this will be a critical public-facing message from the trial to minimise incorrect assumptions about, and use of, mixed ω-3 PUFA preparations in the context of CRC chemoprevention. The majority of prescription and health supplement ω-3 PUFAs are a mix of EPA and DHA with a smaller proportion of other PUFAs.[150] However, separate EPA formulations are licensed for treatment of severe hypertriglyceridaemia and are marketed as health supplements.[144,151] The low cost and excellent safety/tolerability profile of EPA, combined with existing health claims regarding ω-3 PUFAs, means that, even with modest CRC chemopreventative activity, it may be adopted widely in a cancer-prevention setting by the public.

'Contamination' of the treatment groups by participant use of OTC formulations is an ever-present risk in placebo-controlled trials of agents that are available for purchase by the public. The seAFOod trial fatty acid analysis allows us to speculate about possible placebo contamination by 'own' ω-3 PUFA use by identifying those individuals who were randomised to placebo EPA who had a rise in RBC EPA level. Further analysis of concomitant changes in DHA levels is now required. Individual RBC ω-3 PUFA profiles suggest that placebo contamination, if it did occur, was very rare and did not interfere with the findings pertaining to EPA use.

Aspirin use entails a small, but significant, risk of serious harm from GI and intracranial bleeding.[152] This has hampered translation of the significant body of evidence supporting the CRC chemopreventative properties of aspirin into routine clinical practice. The seAFOod trial data add to this body of evidence and emphasise the chemopreventative activity of aspirin against proximal and serrated neoplasms, which are widely considered to be more 'missable' at colonoscopy and contribute significantly to the burden of interval CRC.

## Concluding remarks

In summary, the seAFOod polyp-prevention trial has demonstrated that the ω-3 PUFA EPA (2 g of FFA daily) and aspirin (300 mg daily) did not reduce colorectal adenoma risk (measured by the ADRa) at a 1-year surveillance colonoscopy in 'high-risk' individuals with colorectal neoplasia in the English BCSP. However, chemopreventative efficacy of both agents was observed, as measured by the reduction in colorectal adenoma number per patient at surveillance colonoscopy. The colorectal adenoma subtype- and location-dependent specificity of EPA and aspirin are consistent with previous observations. Existing data on CRC risk reduction by aspirin suggest that the colorectal adenoma risk reduction observed for both agents is likely to translate into a clinically meaningful decrease in long-term CRC risk. Both agents were safe and well tolerated in patients aged 55–75 years with 'high-risk' colorectal neoplasia, although EPA therapy alone was associated with a slight excess of mild to moderate GI AEs. There was no difference in tolerability of the two EPA formulations used in the trial.

## Results in context

Despite several decades of research into the efficacy of several drug (e.g. aspirin, selective COX-2 inhibitors) and nutrient (e.g. folic acid, vitamin D, ω-3 PUFAs) interventions for chemoprevention of CRC, meaningful translation into clinical practice has yet to occur.[153] The weight of the evidence that long-term (> 5 years) use of aspirin is associated with a ≈25% decrease in the risk of CRC incidence and mortality, particularly marked for proximal CRC (the predominant interval CRC type during colonoscopy screening and surveillance, as well as the CRC type not afforded protection by FS screening),[46–48] makes the lack of 'effectiveness' trials driving clinical use of this agent even more surprising.

One reason for this lack has been the dependency on the polyp-prevention trial, using the colorectal adenoma as an accepted surrogate biomarker of CRC risk,[49] in the absence of direct data regarding how a decrease in either colorectal adenoma incidence (ADRa) or number (MAP) translate into subsequent CRC risk reduction.

Historically, the ADRa has been favoured as the colorectal adenoma end point of choice in polyp-prevention trials. The ADRa may be considered the most relevant end point related to the use of a CRC chemoprevention agent to reduce the frequency of, or need for, endoscopic surveillance of patients with previous colorectal neoplasia, stratification for which is based largely on colorectal adenoma incidence.[30–33] Whether or not the use of a CRC chemoprevention agent will lead to a significant reduction in the amount of surveillance colonoscopy performed is a question that can be addressed only by 'effectiveness' studies in which long-term use of a concurrent chemoprevention agent and surveillance colonoscopy requirement are observed using a 'big-data' linkage approach or perhaps a 'point-of-care' randomised trial.

Reduction in colorectal adenoma multiplicity (MAP) arguably has more biological meaning as a read-out of reduced colorectal neoplastic risk associated with a potential CRC chemoprevention agent, hence its widespread use in FAP trials and pre-clinical studies.[37,61] This read-out is more suited to consideration of a chemoprevention agent for long-term CRC risk reduction, as opposed to more efficient use of colonoscopic surveillance for benign colorectal neoplasia. Consistent with use of colorectal adenoma number as a biomarker of future CRC risk in chemoprevention trials, observational data suggest that colorectal adenoma multiplicity at baseline endoscopy is associated with increased CRC incidence and mortality compared with the general population.[40–42] Moreover, a pooled analysis of eight prospective colonoscopy surveillance studies reported that initial colorectal adenoma number at colonoscopy predicts subsequent advanced colorectal neoplasia (advanced colorectal adenoma and CRC combined), in a stepwise manner with increasing baseline colorectal adenoma number.[139]

The seAFOod trial data on colorectal adenoma number, as well as existing MAP data for aspirin,[115,117,118] should focus more attention on the further validation of colorectal adenoma number as a colonoscopic end point, particularly given that colonoscopy performance and reporting has improved significantly, leading to better routine data quality on colorectal adenoma characteristics.

## Strengths and limitations

### Trial design

A key strength of the seAFOod trial was integration in the English BCSP, which continues colonoscopic surveillance in the BCSP, unlike in Scotland, where ongoing surveillance responsibility is passed to general endoscopy services. This provided several advantages to the trial, including:

- consistent high-quality colonoscopy performance and reporting above that available in general colonoscopy populations
- accurate screening colonoscopy data from the BCSP, with which to predict screening colonoscopy outcomes for recruitment projections
- uniform, protocol-driven care within the BCSP, particularly the small window for the 12-month surveillance colonoscopy in the BCSP, despite high procedural pressure in NHS endoscopy units
- use of a 'high-risk' surveillance cohort and 1-year colonoscopy outcomes, which minimised the intervention period compared with previous polyp-prevention trials,[34–36,141,143] with consequent time and cost savings.

Another strength was the comprehensive seAFOod trial biobank with careful QA control. The biobank will be critical for further biomarker-driven, stratified analysis of the trial data. Availability of dietary and tissue biomarker data for the ω-3 PUFA intervention allowed interpretation of the EPA effect on colorectal outcomes, analysis of which could be confounded by dietary and OTC ω-3 PUFA exposure.

The small number of advanced colorectal adenomas found during the seAFOod trial is a limitation and probably relates to the short surveillance interval, as well as the excellent quality of the clearance screening colonoscopy. Previous polyp-prevention trials have reported an advanced ADRa of ≈10% in the placebo group of patient populations, approximating to combined 'high-risk' and 'intermediate-risk' BCSP patients with more heterogeneous follow-up (1–5 years).[34,36,141,143] Detailed data on advanced ADRa and MAP at surveillance colonoscopy were not available from the BCSP when the seAFOod trial was designed but are now available from the seAFOod trial and other reports[98] in order to determine the feasibility of using advanced neoplasia as a primary or main secondary outcome.

As the first chemoprevention CTIMP in the BCSP, the outcome and performance data from the seAFOod trial will be invaluable for any future polyp-prevention trial (see *Chapter 5*, *Recommendations for research*). The seAFOod trial has established the BCSP as a realistic vehicle for interventional research during the endoscopy phase of the patient pathway.

### Trial performance

#### Failure to reach the recruitment target

Although the BCSP sites provided a large number of potential 'high-risk' trial participants at a constant rate, consistent with well-characterised colonoscopy outcomes after guaiac FOBt screening,[95] recruitment of 'high-risk' individuals after screening colonoscopy (18%) was significantly lower than projected (60%) owing to several factors, which included:

- Exclusion because of increasing use of antiplatelet agents, such as clopidogrel, although concurrent aspirin use (19%) occurred almost as predicted from pilot data (20%).
- Exclusion because of the need for additional endoscopic assessment in the BCSP, which varied significantly across BCSP sites/different BCSP colonoscopists and had not been reported prior to trial design.

- Unwillingness of patients to take part in the trial. Acceptability of the trial was not tested in a pilot study, nor subjected to broader PPI assessment prior to opening, except for the lay input provided by the PPI representative in the application team (a BCSP colonoscopy patient) and the review inherent in both Research Ethics Service and BCSP Research Advisory Committee assessments. Assumptions about a high acceptability level for the trial were based on the necessary prior engagement of 'high-risk' individuals in the BCSP, the recent provision of a diagnosis that stratified potential participants who were at a higher risk of future CRC, and a short intervention period prior to a subsequent routine colonoscopy with no change to normal BCSP practice. Anecdotally, a major reason for unwillingness to join this CTIMP after the screening colonoscopy was patient relief at not having CRC (all patients are informed that there is a 1 in 10 chance of CRC at colonoscopy, prior to the procedure) and a strong desire to 'forget about my colon for a while', at least until surveillance colonoscopy.
- Limited research experience of some BCSP staff, coupled with reluctance to discuss research participation during routine clinical care episodes, may have contributed to a high screen failure rate. There was a lengthy 'lag time' at many sites between local R&D approval and FPFV, which contributed significantly to reduced overall recruitment.

Although recruitment reached only 83% ($n = 709$) of the target (853 randomised participants), we do not believe that the smaller sample size affected the primary results of the trial because the 95% CI for the risk difference in ADRa for both EPA and aspirin did not include –10%. Therefore, assuming no change in the point estimate, a larger sample size would tend only to decrease the width of the CI, thereby moving the lower 95% CI limit further from the hypothesised absolute risk difference of –10%.

## Capsule investigational medicinal product switch

The need to switch capsule IMP during the trial was unforeseen, beyond the control of the TMG and very disruptive to recruitment for at least 12 months, especially for BCSP sites that exhausted IMP stock and had to suspend recruitment for several months.

The potential consequences of a switch in one of the IMPs for analysis of the trial results was given very careful consideration. After extensive discussions between the TMG, oversight committees and the funder (which also sought anonymous peer review), it was concluded that the proposed replacement active IMP (90% EPA-TG) was sufficiently similar to the original active IMP (EPA-FFA) in terms of content, FFA-equivalent dose and probable bioavailability to continue the trial, on the basis that colorectal adenoma outcome data from those who received EPA-FFA or EPA-TG could almost certainly be combined. This potential threat to trial integrity has now become a unique aspect of the trial whereby two different (2-g FFA dose equivalent) EPA formulations have been compared in a RCT, demonstrating no clear difference in either tolerability or EPA bioavailability (as measured by RBC and rectal mucosal EPA content) during dosing over several months.

# Chapter 5 Conclusions

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## Implications for health care

Although there was no difference in the ADRa across treatment groups in the seAFOod trial, the decrease in total colorectal adenoma number associated with aspirin and its selectivity for proximal colorectal adenomas and serrated polyps adds to a substantial body of evidence that, although aspirin use is associated with overall CRC risk reduction in unselected groups,[46–48] there are patient groups for whom, and clinical situations in which, additional benefit from aspirin chemoprevention might be gained. For example, these data are the first randomised trial data to bolster the argument that individuals with the rare, but increasingly recognised (in screening programmes), hyperplastic-serrated polyposis syndrome might benefit from aspirin prophylaxis, as well as those with 'sporadic' serrated polyps.[154] Moreover, further evidence that there is proximal colonic selectivity for aspirin CRC chemoprevention highlights potential uses of aspirin in concert with FS screening and for reduction of clinically important PCCRCs, which are more commonly proximal than screen-detected CRCs.[15]

In the absence of any effect of 2 g of EPA daily on the total colorectal ADRa in the seAFOod trial and numerically small effects on colorectal number (MAP) and incidence rate (ADRa) in the left/distal colorectum only, no firm guidance about 'nutraceutical' EPA use, dietary ω-3 PUFA intake or use of RBC ω-3 PUFA as a predictive risk biomarker, in the context of CRC risk reduction, can be made prior to further exploratory, hypothesis-generating analysis of the seAFOod trial data and biobank samples (see *Recommendations for research*). However, even small chemoprevention benefit from EPA is likely to be accepted and taken up by a significant proportion of the public, who might consider that ω-3 PUFAs have wider health benefits.

## Recommendations for research

The seAFOod trial data available to date will stimulate a debate about how the colorectal adenoma is best used as an 'efficacy' RCT end point. The colorectal adenoma can be viewed solely as an endoscopic biomarker of carcinogenic potential, and hence CRC risk, but also as a clinically important lesion, the presence of which prompts endoscopic removal and stratification for future endoscopy, with implications for endoscopy provision. This distinction is key to how CRC chemoprevention is viewed: as a long-term strategy for reduction in CRC risk and/or as adjuvant therapy for those in endoscopic screening/surveillance programmes with a view to decreasing need for colonoscopy. The lack of effect on total ADRa of either EPA or aspirin (and absence of any short-term reduction in colonoscopy frequency with no shift from 'high' to 'intermediate' risk classification) argues against a role as adjuvant therapy for those in endoscopic screening/surveillance programmes. By contrast, a reduction in MAP suggests chemopreventive activity that may manifest eventually as reduced CRC incidence. It will be important to determine long-term colonoscopy and CRC outcomes in the seAFOod trial participants using routinely collected BCSP data and National Cancer Registration and Analysis Service (NCRAS) data. Trial participants have already provided consent for use of long-term, post-trial data. Although the small sample size of the seAFOod trial cohort is likely to provide insufficient CRC diagnoses to provide a meaningful comparison, analysis of subsequent colonoscopy frequency and colorectal adenoma outcomes should be able to test whether or not short-term treatment provides longer-term chemoprevention of colorectal adenomas (with the weakness that ongoing aspirin or ω-3 PUFA use will not be known). An Office for Data Release request to Public Health England will be made prior to gaining approval from the BCSP Research Advisory Committee for this follow-up study.

**TABLE 39** Summary of MAP data from aspirin polyp-prevention trials

| Study | Dose (mg) | Duration | Total MAP (SD) | | Statistical test |
|---|---|---|---|---|---|
| | | | Placebo | Active | |
| Sandler *et al.*[118] | 325 | Median 12.8 months | 0.49 (0.99) | 0.30 (0.87) | *p* = 0.003 |
| Benamouzig *et al.*[115] | 160/300 | 12 months | 0.86 (0.30) | 0.45 (0.15) | *p* = 0.01 |
| Logan *et al.*[117] | 300 | Mean 3–3.5 years | 0.47 (0.92) | 0.31 (0.70) | *p* = 0.015 |
| The seAFOod trial | 300 | 12 months | 1.6 (2.1) | 1.2 (1.5) | IRR 0.78 (95% CI 0.68 to 0.90) |

Future 'effectiveness' evaluation of the two seAFOod trial interventions will be dependent on whether or not colorectal adenoma number (MAP) is used as a CRC risk biomarker. Including the seAFOod trial, there are now four independent RCTs (*Table 39*) that have reported on colorectal adenoma number associated with aspirin use.[115,117,118] A key piece of work will be to attempt a meta-analysis of the colorectal adenoma number data from these trials to derive an overall colorectal adenoma risk reduction ratio for aspirin use that can be aligned with the observational data on CRC risk reduction, to propose a clinically meaningful MAP reduction value.

The observation that both EPA and aspirin have selectivity for CRC chemopreventive activity based on colorectal adenoma type and location prompts the question as to whether or not a precision-stratified-medicine approach to CRC chemoprevention can be taken whereby baseline colorectal adenoma features can be used to predict the type and/or location of colorectal adenoma recurrence and, therefore, best 'personalised' chemoprevention with a given chemoprevention agent. The detailed trial data set on endoscopic and histological features of baseline colorectal adenomas can be used to investigate the relationship between baseline colorectal adenoma features (location, type, number) and recurrence in the placebo-only group of the seAFOod trial. Other post-polypectomy tissue biomarkers may have utility for prediction of aspirin and EPA efficacy, such as tissue COX-2 expression.[83] A recent study has demonstrated that COX-2 and 15-prostaglandin dehydrogenase (PGDH) expression (combined to provide a surrogate marker of tissue $PGE_2$ content) in baseline colorectal adenomas predicts the chemopreventive efficacy of the selective COX-2 inhibitor celecoxib,[155] building on the previous data from the APACC trial.[83] To this end, formalin-fixed, paraffin-embedded (FFPE) polypectomy specimens from the screening colonoscopy from the seAFOod trial placebo group can be obtained, as detailed in the trial protocol.[100] Other, novel, tissue biomarkers based on the proposed mechanism of action of the type and location specificity of EPA and aspirin can also be tested, for example FFAR2 protein expression in FFPE polypectomy specimens, but also in the non-neoplastic rectal mucosal samples available in the seAFOod trial biobank. Rectal mucosal samples will be invaluable for investigation of any 'field effect' mucosal biomarker with predictive value for colorectal adenoma type recurrence.

The intriguing observation that combined EPA and aspirin use in the seAFOod trial was associated with fewest total and left-sided colorectal adenomas, when comparing across the four treatment groups, suggests that the two agents might have an additive relationship for CRC chemoprevention (with the additional, beneficial feature that aspirin co-therapy may improve tolerability of EPA treatment). In the first instance, an exploratory analysis of the predictive value of the baseline/post-intervention RBC and/or post-intervention rectal mucosal EPA level for the reduction in total and left MAP associated with aspirin should be performed in order to strengthen the case for an 'efficacy' RCT of combined aspirin–EPA (or mixed ω-3 PUFA) treatment or stratified (by baseline EPA level) aspirin use.

Two large RCTs of mixed ω-3 PUFA treatment are due to report in the near future. The ASCEND (A Study of Cardiovascular Events iN Diabetes) trial (NCT00135226)[156] is a 2 × 2 factorial study of long-term (median 7.5 years) ω-3 PUFAs (840 mg of EPA/DHA EEs daily) and aspirin (100 mg daily) treatment for prevention of cardiovascular and cerebrovascular events in patients with diabetes (*n* = 15,480). Cancer outcomes are a secondary end point with the ability to continue with post-trial follow-up. VITAL (VITamin D and OmegA 3

TriaL) (NCT01169259) is a 2 × 2 factorial study of the same dose and formulation of ω-3 PUFAs (840 mg of EPA/DHA EEs) and vitamin D$_3$ (2000 IU daily) in 25,871 participants.[157] Colorectal cancer is a specified secondary outcome. It will be very interesting to interpret the results of the ASCEND trial and VITAL in the light of the seAFOod trial data, particularly in relation to CRC location. It should be noted that both trials tested a smaller dose of mixed ω-3 PUFAs than the daily dose of EPA employed in the seAFOod trial.

The data on recruitment performance and placebo group outcomes will be invaluable for any future CRC chemoprevention RCT set in the BCSP. A key lesson is that the ADRa seems to lack discriminatory power, at least in the 'high-risk' colorectal adenoma cohort investigated in the seAFOod trial, given that the intervention with known chemopreventive efficacy (aspirin) did not demonstrate an ADRa signal.

As discussed earlier, the comprehensive data set of PUFA levels in RBCs and rectal mucosa at baseline and after 'nutraceutical' EPA intervention should prompt an analysis of the predictive value of individual 'EPA status' at baseline and after intervention, independent of allocation to active EPA or placebo, for colorectal adenoma reduction. An understanding of whether or not 'EPA high' status is associated with reduced colorectal adenoma recurrence, regardless of whether or not EPA supplementation was provided, may have important consequences for dietary ω-3 PUFA guidelines and possible measurement of ω-3 PUFA status for CRC risk stratification.

The seAFOod trial biobank is also a rich resource of pre- and post-treatment plasma and urine samples, with which to extend and strengthen the tissue biomarker analysis. For example, parallel measurement of urinary PGE-M levels (the main metabolite of PGE$_2$) may provide further insight into the use of baseline colorectal adenoma COX-2/15-PGDH/PGE$_2$ status[127] and was originally specified in the trial protocol.[100] The possible additive relationship between EPA and aspirin observed for total and left MAP justifies the use of the biobank repository to address the hypothesis that RvE1 is measurable in plasma samples from individuals treated with EPA, with and without aspirin, as described in the trial protocol.[100]

The seAFOod trial data should drive several studies aimed at understanding the mechanism(s) of action of both interventions. Differential preventative activity against conventional and serrated pathway lesions by EPA and aspirin has highlighted the relatively poor characterisation of the molecular phenotype of colorectal adenomas compared with CRC,[4,154] as well as the continuing lack of understanding of mechanism(s) of action of both agents. Further in vitro studies (using human CRC cells) of aspirin and EPA should use representative cellular models of CIN, MSI-H and CIMP+ phenotypes.[158] The tumour immunology of the colorectal adenoma has been largely ignored, perhaps because of the relative difficulty in collecting sufficient, fresh human colorectal adenoma tissue and absence of a suitable animal model.[159] However, regulation of host immune surveillance as a mechanism of primary CRC chemoprevention activity remains a valid hypothesis for both seAFOod trial interventions.[160] We have recently reported that mixed ω-3 PUFA treatment is associated with an increase in abundance of SCFA-producing bacteria in the colon.[129] The hypothesis that the propensity for EPA benefit in the distal colorectum relates to increased anticarcinogenic SCFA-FFAR2 signalling[132] should now be tested by measuring stool SCFA concentrations in human studies, in parallel with FFAR2 expression studies using rectal mucosa from the seAFOod trial biobank.

The seAFOod trial biobank contains a blood leucocyte sample for genomic DNA extraction for nearly all participants, as well as individual participant consent to perform genetic single nucleotide polymorphism studies relevant to CRC risk and both interventions. The results of the ω-3 PUFA biomarker analyses above will generate hypotheses about which genetic polymorphisms that are known to control ω-3 PUFA levels,[161] for example fatty acid desaturase (FADS) genes, should be characterised in parallel with the dietary analysis.

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## Publications

Hull MA, Sandell AC, Montgomery AA, Logan RF, Clifford GM, Rees CJ, *et al.* A randomized controlled trial of eicosapentaenoic acid and/or aspirin for colorectal adenoma prevention during colonoscopic surveillance in the NHS Bowel Cancer Screening Programme (the seAFOod Polyp Prevention Trial): study protocol for a randomized controlled trial. *Trials* 2013;**14**:237.

Hull MA, Sprange K, Hepburn T, Tan W, Shafayat A, Rees CJ, *et al.* Eicosapentaenoic acid and aspirin, alone and in combination, for the prevention of colorectal adenomas (seAFOod Polyp Prevention trial): a multicentre, randomised, double-blind, placebo-controlled, 2 × 2 factorial trial. *Lancet* 2018;**392**:2583–94.

## Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

## Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation.

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# Appendix 1 Simplified investigational medicinal product dossier: investigational medicinal product stability testing

seaFOod Trial : IMPD
E-EPA 90TG and Placebo to Match Capsules
Version No. 1.2 (19 August 2014)

### 2.1.P.8  Stability

Shelf-Life and Storage Conditions:

An initial twelve-month 'shelf-life' for storage below 25°C will be based on three-month stability data at 30°C/65%RH.

Stability Program:

A rolling stability testing programme (POV, p-AV and fatty acid analysis) at 25°C and 30°C was commenced in July 2014.

Stability testing is being performed by ALS Food and Pharmaceutical (http://www.als-testing.co.uk) at their GMP compliant Chatteris facility.

Testing protocol is as follows:

- Product to be stored – 1 batch of concentrated omega-3 EPA fish oil encapsulated and blistered in boxes of 60.

- Product to be stored at 25°C/60%RH for 36 months in accordance with ICH guidelines.

- Product to be stored at 30°C/65%RH for 18 months in accordance with ICH guidelines.

The table below summarises the time point/condition at which product is to be tested:

| Condition/Time Point (months) | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 36 | Spares |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25°C / 60%RH | A,B* | A,B | A,B | A,B | A,B | A,B | A,B | A,B | A,B | A,B | A,B | A,B | 24 |
| 30°C / 65%RH | | A,B | A,B | A,B | A,B | A,B | A,B | | | | | | 12 |
| Minimum number of sample blisters required per batch | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 36 |

The following tests are to be performed, at the time points indicated in the above table:

Suite ID:　　　A = Anisidine value (p-AV), Peroxide value (POV

　　　　　　　 B = Fatty acid Profile (excluding cis / trans breakdown)

Note: B* - sample will be tested after storage for five weeks at 25°C/60%RH. Testing of all following time points will be conducted as per schedule.

seaFOod Trial : IMPD
E-EPA 90TG and Placebo to Match Capsules
Version No. 1.2 (19 August 2014)

| Test | Method | Specification | Result 0M time point, 25'C/60%RH |
|---|---|---|---|
| POV (Peroxide Value) | AM/C/112 (Attachment 12). | Max 5.0meq/kg | 2.5 |
| p-AV (Anisidine Value) | ISO 6885 | Max' 20.0 | 16 |
| TOTOX (2xPOV +p-AV) | (Derived value) | Max' 26.0 | 21 |
| Fatty acid analysis | GOED Omega -3 Voluntary Monograph (v. 4) (Attachment 11) | NLT EPA≥90% | Not yet available. |

0M test certificate provided in Attachment 13 (excluding Fatty acid analysis which is being tested after storage for 5 weeks).

Note: Suite A individual test parameter results will be reported as obtained. TOTOX value will be derived as follows: TOTOX = 2POV + p-AV.

Shelf-Life Extension Program:

A review of the stability programme and supporting data for shelf life assignment will be carried out by the head of R&D, Quality Control North West (QCNW), on behalf of Stockport Pharmaceuticals.

Shelf life extension will be carried out following the receipt of satisfactory data at each time point during the on-going stability test program.

The three-month stability data will be available in mid-October 2014 and will be submitted to the MHRA to gain approval for an initial 12-month shelf-life.

This will be followed by repeat stability testing at 30°C/65%RH every three months allowing extension of the shelf-life in a stepwise manner (see table below), thus taking the maximum shelf-life to 36 months (which will enable the trial to recruit to target and complete the intervention for all participants by July 2017). Ongoing three-month stability testing at 25°C/60%RH will continue through to 36 months and confirm stability stored below 25°C.

| 30°C/65%RH Stability data within specification | Assigned shelf life at 'store below 25°C' |
|---|---|
| 3 months | 1 year |
| 6 months | 2 years |
| 9 months | 30 months |
| 12 months | 3 years |

# Appendix 2 Aspirin Summary of Product Characteristics

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**Prescribing information**
**(Summary of Product Characteristics / SPC)**

1. **Name of drug product**

   **Aspirin® protect 300 mg**
   Enteric-coated tablet
   Acetylsalicylic acid

2. **Qualitative and quantitative composition**

   1 enteric-coated tablet contains: 300 mg acetylsalicylic acid (Ph.Eur.).
   For the full list of excipients, see section 6.1.

3. **Presentation**

   Enteric-coated tablets

4. **Clinical data**

4.1 **Indications**

   Reinfarction prophylaxis.

   Note:
   Aspirin protect 300 mg is not foreseen for the treatment of pain.

4.2 **Posology and method of administration**

   Posology

   *For reinfarction prophylaxis*
   A daily dose of one Aspirin protect 300 mg enteric-coated tablets (equivalent to 300 mg acetylsalicylic acid per day) is recommended.

   Method of administration

   The enteric-coated tablets should be taken with plenty of water, preferably at least 30 min before a meal.

   Enteric-coated tablets should not be crushed, broken or chewed in order to ensure release in the alkaline environment of the intestine.

   For treatment of acute myocardial infarction, the first tablet should be bitten or chewed.

   Aspirin protect 300 mg is intended for long-term use. The attending doctor must decide on the length of the treatment.

### 4.3 Contraindications

Aspirin protect 300 mg must not be used:
- in cases of hypersensitivity to the active ingredient acetylsalicylic acid, other salicylates or any of the other ingredients listed in section 6.1
- by patients with asthma attacks in the history which were caused by salicylates or substances with a similar action, especially nonsteroidal anti-inflammatory drugs;
- by patients with acute gastrointestinal ulcers;
- by patients with haemorrhagic diathesis;
- by patients with liver- or kidney failure;
- by patients with severe heart failure for which they are not receiving adequate treatment;
- in combination with methotrexate at a weekly dosage of 15 mg or more (see section 4.5);
- in the last trimester of pregnancy at dosages above 150 mg acetylsalicylic acid/day (see section 4.6).

### 4.4 Warnings and other precautionary measures

Particularly careful medical supervision is required:
- in cases of hypersensitivity to other analgesic/anti-inflammatory/antirheumatic drugs or other allergenic substances (see section 4.3);
- on concomitant ingestion of some non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen. These may weaken the anti-platelet effect of acetylsalicylic acid. Patients should be advised to talk to their physician if they are taking acetylsalicylic acid and are intending to take NSAIDs ( see section 4.5).
- by patients with other allergies (e.g. with skin reactions, itching, nettle rash);
- by patients with bronchial asthma, hay fever, swelling of the nasal mucosa (nasal polyps), chronic respiratory tract diseases;
- in concomitant therapy with anticoagulant drugs;
- with a history of gastrointestinal ulcers or gastrointestinal bleeding;
- with impaired liver function;
- in patients with impaired renal function or patients with impaired cardiovascular circulation (e.g. renal vascular disease, congestive heart failure, volume depletion, major surgery, sepsis or major hemorrhagic events), since acetylsalicylic acid may further increase the risk of renal impairment and acute renal failure;
- by patients who are about to undergo surgery (including minor surgery such as dental extractions): the bleeding tendency can be increased.
- in patients suffering from severe glucose-6-phosphate dehydrogenase (G6PD) deficiency, acetylsalicylic acid may induce hemolysis or hemolytic anemia. Factors that may increase the risk of hemolysis are e.g. high dosage, fever or acute infections;

**Special instructions:**
At low doses acetylsalicylic acid reduces the excretion of uric acid. This may cause a gout attack in predisposed patients.

Paediatric population
Aspirin protect 300 mg should not be taken by children or adolescents with feverish illnesses unless they have been instructed to do so by a doctor and other therapeutic measures have failed. Prolonged vomiting in conjunction with such illnesses could be a sign of Reye's syndrome, a very rare but life-threatening disease which requires immediate medical attention.

Drugs containing acetylsalicylic acid should not be taken for prolonged periods or at high doses without consulting a doctor.

### 4.5    Interactions with other substances and other forms of interaction

*Enhanced effects ranging up to an increased risk of side effects:*
- Anticoagulants / Thrombolytics: Acetylsalicylic acid can increase the risk of bleeding when taken before thrombolytic treatment. Attention should therefore be paid for signs of external or internal bleeding (e.g. bruising) in patients who are scheduled to undergo thrombolytic treatment.
- Antiplatelet drugs, e.g. ticlopidine, clopidogrel: the bleeding time can be prolonged.
- Other nonsteroidal anti-inflammatory drugs and antirheumatics in general: risk for gastrointestinal ulcers and haemorrhages is increased.
- Systemic glucocorticoids (with the exception of hydrocortisone as replacement therapy for Addison's disease): increased risk for gastrointestinal side effects.
- Alcohol: elevated risk of gastrointestinal ulcers and - bleeding.
- Digoxin: elevated plasma level
- Antidiabetics: the blood glucose level can be reduced.
- Methotrexate: decrease in elimination and displacement from protein binding sites by salicylates.
- Valproic acid: displacement from protein binding sites by salicylates.
- Selective-Serotonin-Re-uptake Inhibitors (SSRIs): elevated risk of gastrointestinal bleeding due to synergistic effects.

*Weakening of effects:*
- Aldosterone antagonists (spironolactone and canrenoate).
- Loop diuretics (e.g. furosemide).
- Antihypertensives (especially ACE inhibitors).
- Uricosuric agents (e.g. probenecid, sulphinpyrazone).
- NSAIDs: Concomitant use (on the same day) of some NSAIDs (except acetylsalicylic acid), such as ibuprofen and naproxen, may weaken the irreversible anti-platelet effect of acetylsalicylic acid. The clinical relevance of this interaction is not known. The treatment of patients who have an elevated cardiovascular risk with some NSAIDs, such as ibuprofen or naproxen, may limit the cardioprotective effect of acetylsalicylic acid (see section 4.4).

Accordingly, patients should not take Aspirin protect 300 mg in conjunction with any of the above-mentioned substances unless expressly instructed to do so by a doctor.

### 4.6    Pregnancy and breast-feeding

Pregnancy
Inhibition of prostaglandin synthesis may adversely affect the pregnancy and/or the embryo/fœtal development. Data from epidemiological studies raise concern about an increased risk of miscarriage and of malformations after the use of a prostaglandin synthesis inhibitor in early pregnancy. The risk is believed to increase with dose and duration of therapy.
Previous experience with administration of ASA at daily doses of 50 to 150 mg in the second and third trimesters of pregnancy has not produced any evidence of inhibition of labour, an elevated tendency to bleed or premature closure of the ductus arteriosus.

No information is available for daily doses between 150 and 300 mg. In the last trimester of pregnancy, administration of analgesic doses of ASA may, as a result of inhibited prostaglandin synthesis, cause prolonged gestation, inhibition of labour and, from the 28th-30th week of pregnancy, premature closure of the ductus arteriosus. At these doses, there may also be an increased tendency to bleeding in both mother and child, as well as an increased incidence of intracranial haemorrhage in preterm babies if ASA is administered shortly before birth.

1st and 2nd trimester
During the first and second trimesters of pregnancy, Aspirin protect 300 mg should only be prescribed at daily doses of up to 300 mg ASA if strictly indicated.

3rd trimester
Administration of a daily dose of up to 150 mg ASA in the 3rd trimester should likewise only be prescribed if urgently indicated. In the last trimester of pregnancy, administration of Aspirin protect 300 mg at daily doses of 150 mg ASA and above is contraindicated (see section 4.3).

Breast-feeding
Small quantities of the active ingredient acetylsalicylic acid and their metabolites pass into breast milk. Detrimental effects on the infant have not been reported to date, it is therefore not necessary to interrupt breast-feeding if the daily dose does not exceed 150 mg. The infant should be weaned if higher doses are taken (more than 150 mg daily).

## 4.7 Effects on the ability to drive and use machines

Acetylsalicylic acid has no influence on the ability to drive and to use machines.

## 4.8. Undesirable effects

The following incidence rating is used to evaluate the frequency of side effects:

| Very common: | $\geq 1/10$ |
|---|---|
| Common: | $\geq 1/100, < 1/10$ |
| Uncommon: | $\geq 1/1,000$ to, $< 1/100$ |
| Rare: | $\geq 1/10,000, < 1/1,000$ |
| Very rare: | $< 10,000$ |
| Not known: | Frequency cannot be estimated from the available data |

**Blood and lymphatic system disorders:**
*Rare* to *very rare* serious bleedings, such as cerebral bleeding, especially in patients with uncontrolled hypertension and/or concomitant treatment with anticoagulants, which in isolated cases may be potentially life-threatening, have been reported.
Hemolysis and hemolytic anemia in patients with severe forms of glucose-6-phosphate dehydrogenase (G6PD) deficiency have been reported.
Bleeding, e.g. nosebleeds, bleeding gums, cutaneous bleeding or urogenital bleedings, possibly with prolongation of the bleeding time (see section 4.4). This effect can persist for 4 to 8 days after use.

**Immune system disorders:**
*Rare:*

- Hypersensitivity reactions of the skin, respiratory tract, gastrointestinal tract and cardiovascular system, especially in asthmatics. Symptoms could be: hypotension, attacks of dyspnoea, rhinitis, nasal congestion, anaphylactic shock or angioneurotic oedema.

**Metabolism and nutrition disorders:**
*Very rare:*
- Hypoglycaemia,
- Acetyl salicylic acid at low dosage reduces uric acid excretion. This may cause a gout attack in predisposed patients.

**Nervous system disorders:**
Headaches, dizziness, impaired hearing ability, tinnitus and mental confusion may be signs of overdose (see section 4.9).

**Gastrointestinal disorders:**
*Common:*
- Gastrointestinal disorders such as heartburn, nausea, vomiting, abdominal pain and diarrhoea.
- Minor blood loss from the gastrointestinal tract (micro haemorrhaging)
*Uncommon*:
- Gastrointestinal ulcers which in very rare cases can lead to perforation.
- Gastrointestinal bleedings.
  Long-term use of Aspirin protect 300 mg may cause iron deficiency anaemia due to occult blood loss from the gastrointestinal tract.
- Gastrointestinale Entzündungen.
If you pass black stools (tarry stools) or vomit blood, both of which are a sign of serious bleeding in the stomach, you must inform your doctor immediately.

**Hepatobiliary disorders:**
*Very rare:*
- Elevated liver values.

**Skin and subcutaneous tissue disorders:**
*Uncommon*:
- Skin reactions (very rare cases ranging up to erythema exsudativum multiforme).

**Renal and urinary disorders:**
*Very rare:*
- Renal impairment and acute renal failure have been reported.

**Reporting of suspected adverse reactions**
Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via
Bundesinstitut für Arzneimittel und Medizinprodukte
Abt. Pharmakovigilanz
Kurt-Georg-Kiesinger Allee 3
D-53175 Bonn
Website: http//www.bfarm.de

### 4.9 Overdose

A distinction is made between chronic acetylsalicylic acid over dosage with predominantly central nervous manifestations such as drowsiness, dizziness, confusion or nausea ("salicylism") and acute intoxication.

The cardinal feature of acute intoxication with acetylsalicylic acid is severe disruption of the acid-base balance. Even in the therapeutic dose range, respiratory alkalosis occurs as a consequence of increased respiration. This is compensated by increased renal excretion of bicarbonate, which normalises the blood's pH value. At toxic dosages, the level of compensation is no longer sufficient and both the pH value and the bicarbonate concentration in the blood drop. The plasma $PCO_2$ value may be temporarily normal. The apparent clinical picture is that of metabolic acidosis. However, the actual condition is a combination of respiratory and metabolic acidosis. The causes are: Respiratory restriction caused by toxic doses, acid accumulation, partially due to decreased renal excretion (sulphuric acid, phosphoric acid, salicylic acid, lactic acid, acetoacetic acid etc.) caused by impairment of carbohydrate metabolism. This is compounded by impairment of electrolyte balance. Major potassium loss occurs.

Symptoms of acute intoxication
Symptoms of milder acute intoxication (200 - 400 µg/ml):
In addition to disruption of the acid-base balance and electrolyte balance (e.g. potassium loss), hypoglycaemia, skin rashes and gastrointestinal haemorrhaging, hyperventilation, tinnitus, nausea, vomiting, disturbed vision and hearing, headache, dizziness and confusion have been observed.

With severe intoxication (above 400 µg/ml), delirium, tremor, difficult breathing, sweating, dehydration, hyperthermia and coma may occur.

In the event of intoxication with a fatal outcome, death usually occurs as a result of respiratory failure.

Treatment of intoxication
The therapeutic measures for treatment of intoxication with acetylsalicylic acid depend upon the extent, stage and clinical symptoms of the intoxication. They comprise the standard measures for decreasing absorption of the active ingredient, monitoring of the water and electrolyte balances, impaired temperature regulation and respiration.

Treatment is focused on measures to accelerate excretion and normalise the acid-base balance and the electrolyte balance. Infusion solutions of sodium hydrogen carbonate and potassium chloride and diuretics are administered. The urine reaction should be alkaline to increase the degree of salicylate ionisation and decrease the rate of back-diffusion to the tubules.

Monitoring of the blood values (pH, $PCO_2$, hydrogen bicarbonate, potassium, etc.) is strongly recommended. In severe cases, haemodialysis may be necessary.

### 5. Pharmacological properties

### 5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antithrombotic agents, Platelet aggregation inhibitors, acetylsalicylic acid.
ATC class: B01AC06

Acetylsalicylic acid has an irreversible platelet aggregation-inhibiting action. This antiplatelet effect is achieved by acetylation of cyclooxygenase, irreversibly inhibiting the formation of thromboxane $A_2$ (a prostaglandin with a platelet aggregation-promoting and vasoconstrictive action) in the platelets. The effect is long-term and usually persists for the entire eight-day lifespan of a platelet.

Paradoxically, acetylsalicylic acid also inhibits the formation of prostacyclin (a prostaglandin with platelet aggregation-inhibiting but vasodilating effects) in the endothelial cells of the vascular walls. This effect is transient.

Once the acetylsalicylic acid has been washed out of the blood, the nucleated endothelial cells resume their production of prostacyclin.

As a consequence, once daily administration of low-dosage (< 300 mg / day) acetylsalicylic acid causes inhibition of thromboxane $A_2$ in the platelets without markedly impairing prostacyclin formation.

Acetylsalicylic acid also belongs to the class of acid-forming nonsteroidal anti-inflammatory drugs with analgesic, antipyretic and anti-inflammatory properties. Its mechanism of action is based on irreversible inhibition of cyclooxygenase enzymes involved in prostaglandin synthesis.

Acetylsalicylic acid is used at higher oral doses to treat mild to moderate pain, elevated temperature and acute and chronic inflammatory diseases (e.g. rheumatoid arthritis).

Experimental data suggest that ibuprofen may inhibit the effect of low dose acetylsalicylic acid on platelet aggregation when they are dosed concomitantly. In one study, when a single dose of ibuprofen 400 mg was taken within 8 h before or within 30 min after immediate release aspirin dosing (81 mg), a decreased effect of ASA on the formation of thromboxane or platelet aggregation occurred. However, the limitations of these data and the uncertainties regarding extrapolation of ex vivo data to clinical situation imply that no firm conclusion can be made for regular ibuprofen use, and no clinically relevant effect is considered to be likely for occasional ibuprofen use.

## 5.2   Pharmacokinetic properties

Acetylsalicylic acid is converted before, during and after absorption into its main metabolite salicylic acid. The metabolites are excreted primarily via the renal route.

In addition to salicylic acid, the main metabolites of acetylsalicylic acid are the glycine conjugate of salicylic acid (salicyluric acid), the ether and ester glucuronides of salicylic acid (salicyl phenylglucuronide and salicyl acetylglucuronide) and gentisic acid, produced by oxidation of salicylic acid, and its glycine conjugate.

Depending on the formulation, absorption of acetylsalicylic acid following oral administration is rapid and complete. The residual acetyl portion of acetylsalicylic acid undergoes partial hydrolytic cleavage during its passage through the mucous membranes oft the gastrointestinal tract.

Peak plasma concentrations are attained after 10-20 min (acetylsalicylic acid) and 0.3-2 h (total salicylate).

The elimination kinetic of salicylic acid is dependent to a great extent on the dose, as the capacity for metabolisation of salicylic acid is limited (elimination half-life fluctuates between 2 and 30 h).

The elimination half-life of acetylsalicylic acid is only a few minutes; the elimination half-life of salicylic acid is 2 h after consumption of a dose of 0.5 g acetylsalicylic acid and 4 h after administration of 1 g; following consumption of a single dose of 5 g, the elimination half-life is extended to 20 h.

Protein binding in human plasma is dependent on the concentration; values ranging from 49 % to over 70 % (acetylsalicylic acid) and 66 % to 98 % (salicylic acid) have been reported.

Salicylic acid has been detected in cerebrospinal fluid and synovial fluid after consumption of acetylsalicylic acid.

Salicylic acid crosses the placental barrier and passes into breast milk.

### 5.3 Preclinical safety data

The preclinical safety profile of acetylsalicylic acid is well documented. In animal tests salicylates caused kidney damage and gastrointestinal ulcers.

Acetylsalicylic acid has been appropriately tested for mutagenicity and carcinogenicity; no relevant evidence of a mutagenic or carcinogenic potential was found.

Salicylates have shown teratogenic effects in a number of animal species. There have been implantation disturbance, embryotoxic and fetotoxic effects, and learning disorders in the young animals after prenatal exposure.

### 6.  Pharmaceutical data

### 6.1 Other ingredients

Maize starch
Cellulose powder
Methacrylic acid – ethylacrylate copolymer 1:1 dispersion 30%
Polysorbat 80
Sodium Dodecylsulfate
Talc
Triethyl citrate

### 6.2 Incompatibilities

None

### 6.3 Shelf-life

5 years.

### 6.4 Special storage instructions

Do not store at temperatures above 25°C.

### 6.5 Type and contents of container

PP aluminium blisters:
Packs of 42 and 98 enteric-coated tablets;
Not all pack sizes may be marketed.

### 6.6 Instructions for use

None

### 7.  Product license holder

Bayer Vital GmbH,
51368 Leverkusen
Germany

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**8.**     **Registration number**
        16854.00.01, 30828.00.01, 33171.01.00


**9.**     **Date of registration/extension of registration**
        16854.00.01: 28.12.1993 / 12.09.2007
        30828.00.01: 28.12.1993 / 25.07.2007
        33171.01.00: 09.10.1995 / 18.11.2013


**10.**    **Date of preparation**
        03.2017


**11.**    **Prescription/pharmacy status**
        Pharmacy only

# Appendix 3 Summary of protocol amendments

| Amendment reference; date | Amendment details | Previous version number and date | New version number and date |
|---|---|---|---|
| **Substantial amendments (SAs)** | | | |
| SA02; 8 August 2011 | Changes to the protocol and PIL to ensure wording is clearer for trial personnel and participants | seAFOod trial protocol version 1.1, dated 14 April 2011 | seAFOod trial protocol version 2.0, dated 8 August 2011 |
| SA04; 30 November 2011 | • Inclusion criteria: lower age limit reduced to 73 years to reflect BCSP surveillance guidelines<br>• Exclusion criteria updated to exclude patients who are taking any non-aspirin antiplatelet therapy | seAFOod trial protocol version 2.0, dated 8 August 2011 | seAFOod trial protocol version 3.0, dated 28 November 2011 |
| SA06; 25 May 2012 | Change to exclusion criteria to include patients who need a second repeat screening endoscopy, allowing inclusion of participants who have a second screening procedure | seAFOod trial protocol version 3.0, dated 28 November 2011 | seAFOod trial protocol, version 4.0, dated 4 May 2012 |
| SA10; 19 June 2013 | Change to inclusion criteria to include patients identified through the bowel scope FS screening programme | seAFOod trial protocol version 4.0, dated 4 May 2012 | seAFOod trial protocol version 5.0, dated 17 June 2013 |
| SA14; 14 August 2014 | Changes to the protocol in line with the introduction of the replacement capsule IMP. This includes additional information on the new EPA-TG formulation | seAFOod trial protocol version 5.0, dated 17 June 2013 | seAFOod trial protocol version 6.0, dated 11 August 2014 |

# Appendix 4 Additional tables, listings and figures

**TABLE 40** Analyses of the primary outcome without adjustment for repeat colonoscopy

| | Estimate (95% CI) (%) | |
| --- | --- | --- |
| | EPA vs. no EPA | Aspirin vs. no aspirin |
| Primary analysis (risk difference) | 0.7 (–6.8 to 8.3) | –1.2 (–8.7 to 6.3) |
| Using per-protocol population (risk difference) | 1.0 (–6.7 to 8.8) | –1.0 (–8.7 to 6.7) |
| Multilevel model treating recruiting centre and site as random effects (odds ratio) | 1.03 (0.75 to 1.42) | 0.95 (0.69 to 1.31) |
| Multiple imputation of missing data (risk difference) | 0.1 (–7.3 to 7.6) | –0.7 (–8.3 to 6.8) |
| Adjustment of baseline variables with imbalance[a] (risk difference) | 1.1 (–6.4 to 8.6) | –1.5 (–9.0 to 5.9) |
| Adjustment of oily fish intake during the trial (risk difference) | –1.3 (–9.5 to 6.9) | –2.7 (–10.9 to 5.5) |
| CACE analysis taking account of treatment adherence | | |
|     Binary adherence (risk difference) | 0.8 (–7.1 to 8.7) | –1.2 (–8.9 to 6.4) |
|     Continuous adherence (risk difference) | 0.4 (–7.3 to 8.1) | –1.3 (–8.6 to 6.4) |
| Adjustment of EPA formulation | 0.8 (–6.7 to 8.3) | –1.1 (–8.3 to 7.4) |
| Further adjustment for baseline RBC EPA (risk difference) | –2.1 (–10.2 to 5.9) | 1.7 (–6.3 to 9.8) |

a  Adjusted for sex, whether or not the participant had diabetes and smoking status.

**TABLE 41** Listing of ADRs

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Heartburn | GI disorders | Dyspepsia | No | Mild | 7 March 2013 | 15 March 2013 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 5 March 2013 | 14 March 2013 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 5 March 2013 | 14 March 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Vomiting | GI disorders | Vomiting | No | Mild | 18 February 2014 | 20 February 2014 | No action taken | Recovered |
| Placebo + placebo | Abdominal wind | GI disorders | Flatulence | No | Mild | 2 July 2013 | 20 August 2013 | No action taken | Recovered with sequelae |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 22 April 2014 | 2 May 2014 | No action taken | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Moderate | 23 August 2015 | 30 August 2015 | Trial medications permanently stopped | Condition improving |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 23 October 2012 | 15 November 2012 | Trial medications reduced | Recovered |
| EPA + aspirin | Gastric irritation | GI disorders | Epigastric discomfort | No | Mild | 15 November 2015 | 15 November 2015 | No action taken | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 22 February 2016 | 22 February 2016 | Trial medication already discontinued | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 8 June 2013 | 9 June 2013 | Unknown | Recovered |
| EPA + placebo aspirin | Halitosis | GI disorders | Breath odour | No | Mild | 16 June 2013 | 23 June 2013 | No action taken | Recovered |
| Placebo + placebo | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 24 November 2013 | 24 November 2013 | No action taken | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Mild | 3 March 2014 | 10 March 2014 | No action taken | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Mild | 20 April 2014 | 21 June 2014 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Halitosis | GI disorders | Breath odour | No | Mild | 3 March 2014 | 10 March 2014 | No action taken | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 1 November 2013 | N/A | No action taken | Condition present and unchanged |
| Placebo EPA + aspirin | Indigestion | GI disorders | Dyspepsia | No | Mild | 25 February 2014 | 15 April 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Indigestion | GI disorders | Dyspepsia | No | Mild | 30 April 2014 | 27 October 2014 | No action taken | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 1 July 2015 | 4 March 2016 | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 6 November 2015 | 23 December 2015 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 5 April 2016 | 16 April 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 5 April 2016 | 16 April 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 5 April 2016 | 16 April 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 24 October 2015 | 1 January 2016 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 28 April 2012 | 4 May 2012 | No action taken | Recovered |
| Placebo EPA + aspirin | Constipation | GI disorders | Constipation | No | Mild | 28 April 2012 | 4 May 2012 | No action taken | Recovered |
| Placebo + placebo | Halitosis | GI disorders | Breath odour | No | Mild | 16 August 2012 | N/A | No action taken | Condition present and unchanged |
| Placebo + placebo | Acid indigestion | GI disorders | Dyspepsia | No | Mild | 2 August 2012 | 15 August 2012 | No action taken | Recovered |

continued

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 19 September 2012 | 15 May 2013 | Unknown | Recovered |
| Placebo + placebo | Rash | Skin and subcutaneous tissue disorders | Rash | No | Mild | 16 November 2012 | 8 April 2013 | Trial medications reduced | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 18 July 2013 | 20 July 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Gastritis | GI disorders | Gastritis | Yes | Mild | 18 October 2013 | 1 November 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Epigastric pain | GI disorders | Abdominal pain upper | No | Mild | 27 November 2013 | 28 November 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 14 September 2013 | N/A | Trial medications reduced | Condition present and unchanged |
| Placebo + placebo | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Mild | 26 January 2014 | 31 March 2014 | Trial medications reduced | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Mild | 26 January 2014 | 31 March 2014 | Trial medications reduced | Recovered |
| Placebo + placebo | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 26 January 2014 | 31 March 2014 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Epigastric pain | GI disorders | Abdominal pain upper | No | Mild | 26 January 2014 | 31 March 2014 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 11 May 2014 | N/A | Trial medications reduced | Condition present and unchanged |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 21 May 2012 | 26 May 2012 | No action taken | Recovered |
| Placebo EPA + aspirin | Oesophageal bleeding | GI disorders | Oesophageal haemorrhage | Yes | Severe | 23 July 2012 | 30 July 2012 | Trial medications reduced | Recovered |

**TABLE 41** Listing of ADRs (continued)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Epistaxis | Respiratory, thoracic and mediastinal disorders | Epistaxis | No | Mild | 5 June 2012 | 5 June 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Anal bleeding | GI disorders | Anal haemorrhage | No | Mild | 15 November 2012 | 15 November 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 08 December 2012 | 6 January 2013 | No action taken | Condition improving |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 27 June 2014 | 26 July 2014 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 25 June 2014 | 26 June 2014 | No action taken | Recovered |
| EPA + aspirin | Medication aftertaste | General disorders and administration site conditions | Product taste abnormal | No | Mild | 26 December 2014 | 29 April 2015 | No action taken | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 30 May 2015 | 31 May 2015 | No action taken | Recovered |
| EPA + aspirin | Head pressure | Nervous system disorders | Head discomfort | No | Mild | 1 April 2015 | N/A | No action taken | Condition present and unchanged |
| EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 2 June 2015 | 3 June 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 14 July 2012 | 14 July 2012 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 7 June 2012 | 15 July 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 7 June 2012 | 15 July 2012 | No action taken | Recovered |
| EPA + aspirin | Bruising | Injury, poisoning and procedural complications | Contusion | No | Mild | 1 September 2016 | N/A | No action taken | Condition present and unchanged |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 7 October 2013 | 30 October 2013 | Trial medications delayed | Recovered |
| Placebo + placebo | Defaecation urgency | GI disorders | Defaecation urgency | No | Na | 1 May 2016 | N/A | Trial medication period completed | Condition present and unchanged |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 30 March 2016 | 25 August 2016 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | Na | Moderate | 1 May 2016 | 6 June 2016 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Nausea | GI disorders | Nausea | No | Mild | 29 June 2012 | 6 July 2012 | Unknown | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 29 June 2012 | 6 July 2012 | Unknown | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 21 March 2013 | 31 March 2013 | Trial medications reduced | Recovered with sequelae |
| EPA + aspirin | Negative thoughts | Psychiatric disorders | Negative thoughts | No | Mild | 19 April 2013 | 19 June 2013 | Trial medications reduced | Recovered with sequelae |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 22 May 2015 | 10 July 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Bruising | Injury, poisoning and procedural complications | Contusion | No | Mild | 26 May 2015 | 11 May 2016 | No action taken | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 2 February 2016 | 1 March 2016 | No action taken | Recovered |
| Placebo EPA + aspirin | Halitosis | GI disorders | Breath odour | No | Mild | 15 December 2015 | 30 January 2016 | No action taken | Recovered |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 26 May 2015 | N/A | No action taken | Condition improving |
| Placebo EPA + aspirin | Headache | Nervous system disorders | Headache | No | Moderate | 20 May 2015 | 22 July 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Urinary frequency | Renal and urinary disorders | Pollakiuria | No | Mild | 20 May 2015 | N/A | No action taken | Condition present and unchanged |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Mild | 17 January 2013 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Moderate | 10 January 2013 | 17 January 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Moderate | 7 May 2013 | 16 August 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 10 September 2014 | N/A | No action taken | Condition improving |
| Placebo + placebo | Halitosis | GI disorders | Breath odour | No | Mild | 28 March 2013 | 1 June 2013 | Trial medications reduced | Recovered |
| EPA + aspirin | Nausea | GI disorders | Nausea | No | Mild | 1 August 2013 | 14 August 2013 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Nausea | GI disorders | Nausea | No | Moderate | 3 July 2015 | 18 September 2015 | Trial medications permanently stopped | Condition present and unchanged |
| Placebo + placebo | Nausea | GI disorders | Nausea | No | Moderate | 19 May 2015 | 2 June 2015 | Trial medications permanently stopped | Recovered with sequelae |
| Placebo + placebo | Nausea | GI disorders | Nausea | No | Mild | 6 June 2015 | 26 June 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Moderate | 22 June 2015 | 29 June 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Moderate | 20 May 2015 | 22 June 2015 | Trial medications reduced | Condition deteriorated |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 9 April 2015 | 20 May 2015 | No action taken | Condition deteriorated |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 16 April 2012 | 25 May 2012 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 15 February 2015 | 14 May 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Moderate | 30 January 2015 | 10 February 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 25 December 2014 | 30 January 2015 | Trial medications reduced | Condition deteriorated |
| Placebo EPA + aspirin | Excessive flatulence | GI disorders | Flatulence | No | Mild | 10 May 2015 | 5 June 2015 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Loose motions | GI disorders | Diarrhoea | No | Mild | 10 May 2015 | 12 May 2015 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 10 May 2015 | 2 June 2015 | Trial medications reduced | Recovered |
| EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 18 May 2015 | 30 May 2015 | No action taken | Recovered |
| EPA + aspirin | Burping | GI disorders | Eructation | No | Mild | 6 August 2015 | 9 August 2015 | No action taken | Recovered |
| EPA + aspirin | Burping | GI disorders | Eructation | No | Mild | 5 August 2015 | 31 July 2016 | No action taken | Recovered |
| EPA + placebo aspirin | Exacerbation of asthma | Respiratory, thoracic and mediastinal disorders | Asthma | No | Moderate | 24 July 2016 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Flatulence | GI disorders | Flatulence | No | Mild | 28 February 2016 | N/A | No action taken | Condition improving |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Flatulence | GI disorders | Flatulence | No | Mild | 27 October 2015 | 11 January 2016 | No action taken | Recovered |
| EPA + placebo aspirin | Loose stools | GI disorders | Diarrhoea | No | Moderate | 27 October 2016 | N/A | No action taken | Condition present and unchanged |
| Placebo + placebo | Wind | GI disorders | Flatulence | No | Mild | 19 November 2015 | 7 December 2015 | No action taken | Recovered |
| Placebo + placebo | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 1 July 2016 | 27 October 2016 | No action taken | Recovered |
| EPA + placebo aspirin | Bloating | GI disorders | Abdominal distension | No | Mild | 1 June 2016 | 4 July 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 5 July 2012 | 3 August 2012 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 5 July 2012 | 3 August 2012 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Loose stools | GI disorders | Diarrhoea | No | Mild | 16 April 2013 | 30 July 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Excessive flatulence | GI disorders | Flatulence | No | Mild | 16 April 2013 | 30 July 2013 | No action taken | Recovered |
| Placebo + placebo | Oesophageal reflux | GI disorders | Gastro-oesophageal reflux disease | No | Na | 20 February 2013 | N/A | Unknown | Na |
| EPA + placebo aspirin | Defaecation urgency | GI disorders | Defaecation urgency | No | Mild | 7 October 2012 | 15 June 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Hyperhidrosis | Skin and subcutaneous tissue disorders | Hyperhidrosis | No | Mild | 14 March 2013 | 15 June 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Loose stools | GI disorders | Diarrhoea | No | Mild | 7 October 2012 | 15 June 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 5 November 2012 | 10 December 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Halitosis | GI disorders | Breath odour | No | Mild | 7 October 2012 | 15 June 2013 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Dizziness | Nervous system disorders | Dizziness | No | Mild | 14 March 2013 | 15 June 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 20 May 2013 | 21 May 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 3 February 2013 | 4 February 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 17 June 2013 | 18 June 2013 | No action taken | Recovered |
| Placebo + placebo | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 15 July 2013 | N/A | No action taken | Condition present and unchanged |
| Placebo EPA + aspirin | Duodenitis | GI disorders | Duodenitis | Yes | Moderate | 7 September 2013 | 13 September 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 1 October 2013 | 20 January 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 5 November 2013 | 8 November 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Stool discolored | GI disorders | Faeces discoloured | Yes | Severe | 30 August 2013 | 13 September 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Reflux oesophagitis | GI disorders | Gastro-oesophageal reflux disease | Yes | Moderate | 7 September 2013 | 13 September 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 12 May 2014 | 9 September 2014 | No action taken | Recovered |
| EPA + placebo aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 21 December 2014 | 28 December 2014 | No action taken | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 13 January 2014 | N/A | No action taken | Condition improving |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 10 September 2014 | 27 November 2014 | No action taken | Recovered |
| EPA + aspirin | Borborygmi | GI disorders | Gastrointestinal sounds abnormal | No | Mild | 7 April 2014 | N/A | No action taken | Condition present and unchanged |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + aspirin | Halitosis | GI disorders | Breath odour | No | Mild | 7 April 2014 | N/A | No action taken | Condition present and unchanged |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 8 June 2014 | N/A | No action taken | Condition improving |
| EPA + placebo aspirin | Gastric bleeding | GI disorders | Gastric haemorrhage | No | Mild | 19 September 2014 | N/A | Trial medications permanently stopped | Condition present and unchanged |
| EPA + placebo aspirin | Defaecation urgency | GI disorders | Defaecation urgency | No | Mild | 31 March 2014 | 12 December 2014 | No action taken | Recovered |
| EPA + placebo aspirin | Oesophagitis | GI disorders | Oesophagitis | No | Mild | 19 September 2014 | N/A | Trial medications permanently stopped | Condition present and unchanged |
| EPA + placebo aspirin | Flatulence | GI disorders | Flatulence | No | Mild | 31 March 2014 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Gastritis | GI disorders | Gastritis | No | Mild | 19 September 2014 | N/A | Trial medications permanently stopped | Condition present and unchanged |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 26 August 2014 | N/A | Trial medications reduced | Condition present and unchanged |
| Placebo + placebo | Stomach ache | GI disorders | Abdominal pain upper | No | Mild | 11 August 2014 | 13 August 2014 | No action taken | Recovered |
| EPA + aspirin | Bruising of leg | Injury, poisoning and procedural complications | Contusion | No | Mild | 12 February 2015 | 28 February 2015 | No action taken | Recovered |
| EPA + aspirin | Bruising of leg | Injury, poisoning and procedural complications | Contusion | No | Mild | 10 September 2014 | 1 October 2014 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 1 October 2015 | 31 December 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Dizzy | Nervous system disorders | Dizziness | No | Mild | 1 July 2016 | 31 July 2016 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 1 July 2016 | 31 July 2016 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 1 July 2016 | 31 July 2016 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Burping | GI disorders | Eructation | No | Mild | 1 September 2012 | 1 December 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 1 September 2012 | 1 December 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Constipation | GI disorders | Constipation | No | Mild | 7 August 2012 | 12 August 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Indigestion | GI disorders | Dyspepsia | No | Mild | 7 August 2012 | 12 August 2012 | No action taken | Recovered |
| Placebo + placebo | Stool discoloured | GI disorders | Faeces discoloured | No | Mild | 1 March 2013 | 1 April 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Bruising of hand | Injury, poisoning and procedural complications | Contusion | No | Mild | 20 July 2013 | 27 July 2013 | No action taken | Recovered |
| Placebo + placebo | Loose stools | GI disorders | Diarrhoea | No | Mild | 1 September 2014 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 11 April 2015 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Loose stools | GI disorders | Diarrhoea | No | Mild | 23 April 2015 | N/A | No action taken | Condition present and unchanged |
| Placebo EPA + aspirin | Gastritis | GI disorders | Gastritis | No | Mild | 27 June 2016 | 1 July 2016 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Hiatus hernia | GI disorders | Hiatus hernia | No | Mild | 27 June 2016 | 1 July 2016 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Moderate | 5 March 2016 | 1 July 2016 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Change of bowel habit | GI disorders | Change of bowel habit | No | Mild | 11 November 2015 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Epigastric pain | GI disorders | Abdominal pain upper | No | Mild | 14 January 2012 | 28 May 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Belching | GI disorders | Eructation | No | Mild | 14 January 2012 | 28 May 2012 | No action taken | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 1 February 2015 | 30 September 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 1 September 2015 | 30 September 2015 | No action taken | Recovered |
| EPA + aspirin | Constipation | GI disorders | Constipation | No | Mild | 9 April 2013 | 13 April 2013 | No action taken | Recovered |
| EPA + aspirin | Constipation | GI disorders | Constipation | No | Mild | 14 February 2013 | 18 February 2013 | No action taken | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 25 December 2012 | 27 December 2012 | No action taken | Recovered |
| EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 4 January 2013 | 6 January 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Constipation | GI disorders | Constipation | No | Mild | 17 March 2013 | 28 March 2013 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Change of bowel habit | GI disorders | Change of bowel habit | No | Moderate | 1 August 2013 | 23 August 2013 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Nauseous | GI disorders | Nausea | No | Moderate | 3 July 2015 | 3 July 2015 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + aspirin | Retching | GI disorders | Retching | No | Moderate | 24 April 2015 | 12 May 2015 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Loose stools | GI disorders | Diarrhoea | No | Moderate | 16 March 2016 | 22 March 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Faeces discoloured | GI disorders | Faeces discoloured | No | Moderate | 16 March 2016 | 22 March 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Abdominal cramps | GI disorders | Abdominal pain | No | Moderate | 16 March 2016 | 22 March 2016 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Gout | Metabolism and nutrition disorders | Gout | No | Mild | 8 May 2017 | 18 May 2017 | Study med. Period completed | Recovered |
| Placebo EPA + aspirin | Headache | Nervous system disorders | Headache | No | Moderate | 9 August 2012 | 28 August 2012 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Anal mucositis | GI disorders | Anal inflammation | No | Moderate | 12 October 2015 | 30 July 2016 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 19 December 2015 | 19 December 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Angioedema | Skin and subcutaneous tissue disorders | Angioedema | No | Moderate | 22 December 2015 | 23 December 2015 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 28 November 2011 | 5 March 2012 | No action taken | Recovered |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Moderate | 20 March 2012 | 29 March 2012 | Trial medications reduced | Recovered |
| EPA + aspirin | Indigestion | GI disorders | Dyspepsia | No | Mild | 27 February 2012 | 9 May 2012 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 23 January 2016 | 22 March 2016 | Trial medications reduced | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 21 August 2015 | 23 August 2015 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Gout | Metabolism and nutrition disorders | Gout | No | Mild | 14 June 2013 | 27 June 2013 | Trial medications delayed | Recovered with sequelae |
| Placebo + placebo | Frequent bowel movements | GI disorders | Frequent bowel movements | No | Moderate | 18 December 2012 | 19 December 2012 | Trial medications reduced | Recovered |
| Placebo + placebo | Excessive flatulence | GI disorders | Flatulence | No | Moderate | 18 December 2012 | 19 December 2012 | Trial medications reduced | Recovered |
| Placebo + placebo | Excessive flatulence | GI disorders | Flatulence | No | Mild | 1 December 2012 | 19 December 2012 | Trial medications permanently stopped | Condition improving |
| Placebo + placebo | Heartburn | GI disorders | Dyspepsia | No | Moderate | 21 March 2014 | 12 April 2014 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 24 May 2012 | 16 June 2012 | Trial medications reduced | Recovered with sequelae |
| Placebo + placebo | Medication aftertaste | General disorders and administration site conditions | Product taste abnormal | No | Mild | 26 March 2013 | 26 March 2014 | No action taken | Condition present and unchanged |
| Placebo + placebo | Abdominal distension | GI disorders | Abdominal distension | No | Mild | 26 March 2013 | 26 March 2014 | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 2 March 2015 | 10 March 2015 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Vomiting | GI disorders | Vomiting | No | Severe | 2 March 2015 | 5 March 2015 | Trial medications reduced | Recovered |
| EPA + aspirin | Indigestion | GI disorders | Dyspepsia | No | Moderate | 16 July 2015 | 20 July 2015 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Belching | GI disorders | Eructation | No | Mild | 15 November 2012 | 21 November 2012 | Trial medications reduced | Recovered |
| Placebo + placebo | Constipation | GI disorders | Constipation | No | Mild | 15 November 2012 | 21 November 2012 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Vomiting | GI disorders | Vomiting | No | Mild | 16 May 2013 | 20 July 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 16 May 2013 | 20 July 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Halitosis | GI disorders | Breath odour | No | Mild | 16 May 2013 | 1 July 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 16 April 2013 | 16 May 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Taste abnormality | Nervous system disorders | Dysgeusia | No | Mild | 16 May 2013 | 1 July 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 16 May 2013 | 20 July 2013 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 12 November 2013 | 13 November 2013 | No action taken | Recovered |
| Placebo + placebo | Stomach pain | GI disorders | Abdominal pain upper | No | Mild | 3 September 2014 | 4 September 2014 | No action taken | Recovered |
| Placebo + placebo | Perianal bleeding | GI disorders | Anal haemorrhage | No | Mild | 24 December 2013 | 24 December 2013 | No action taken | Recovered |
| Placebo + placebo | Knee pain | Musculoskeletal and connective tissue disorders | Arthralgia | No | Mild | 13 January 2014 | 24 February 2014 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Bleeding postoperatively | Injury, poisoning and procedural complications | Post procedural haemorrhage | No | Mild | 20 December 2012 | 3 January 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Haematuria | Renal and urinary disorders | Haematuria | No | Mild | 13 August 2014 | 19 August 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Haematuria | Renal and urinary disorders | Haematuria | No | Mild | 24 December 2013 | 26 December 2013 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 17 January 2015 | 19 January 2015 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 2 February 2015 | N/A | Trial medications permanently stopped | Na |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 14 November 2014 | 16 November 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Bleeding PR (excluding gut haemorrhage and piles) | GI disorders | Rectal haemorrhage | No | Mild | 20 December 2015 | 22 December 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Bleeding PR (excluding gut haemorrhage and piles) | GI disorders | Rectal haemorrhage | No | Mild | 25 June 2015 | 24 July 2015 | No action taken | Recovered |
| EPA + aspirin | Pustule | Infections and infestations | Rash pustular | No | Mild | 12 December 2013 | 8 January 2014 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Loose motions | GI disorders | Diarrhoea | No | Mild | 1 July 2013 | N/A | No action taken | Condition present and unchanged |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 1 July 2013 | N/A | No action taken | Condition present and unchanged |
| Placebo EPA + aspirin | Generalised itching | Skin and subcutaneous tissue disorders | Pruritus generalised | No | Mild | 24 January 2014 | 25 July 2014 | Trial medications delayed | Recovered |
| EPA + placebo aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Moderate | 28 April 2014 | 12 May 2014 | Trial medications delayed | Recovered |
| EPA + placebo aspirin | Constipation | GI disorders | Constipation | No | Mild | 28 November 2012 | 29 November 2012 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Stomach pain | GI disorders | Abdominal pain upper | No | Mild | 28 November 2012 | 29 November 2012 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Stomach pain | GI disorders | Abdominal pain upper | No | Mild | 17 November 2012 | 28 November 2012 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Constipation | GI disorders | Constipation | No | Mild | 2 November 2012 | 28 November 2012 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Constipation | GI disorders | Constipation | No | Mild | 2 November 2012 | N/A | No action taken | Condition present and unchanged |
| Placebo + placebo | Lower abdominal pain | GI disorders | Abdominal pain lower | No | Moderate | 8 January 2013 | 10 January 2013 | Trial medications delayed | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Moderate | 13 January 2013 | 22 January 2013 | Trial medications delayed | Recovered |
| Placebo + placebo | Indigestion | GI disorders | Dyspepsia | No | Moderate | 25 January 2013 | 10 February 2013 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 13 January 2013 | 22 January 2013 | Trial medications delayed | Recovered |
| Placebo + placebo | Constipation | GI disorders | Constipation | No | Moderate | 8 January 2013 | 10 January 2013 | Trial medications delayed | Recovered |
| Placebo + placebo | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 25 January 2013 | 10 February 2013 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Dizziness | Nervous system disorders | Dizziness | No | Mild | 30 April 2013 | 27 January 2014 | No action taken | Recovered |
| EPA + aspirin | Dizziness | Nervous system disorders | Dizziness | No | Mild | 1 April 2013 | 14 April 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 25 September 2013 | N/A | No action taken | Recovered |
| EPA + placebo aspirin | Flatulence | GI disorders | Flatulence | No | Mild | 25 September 2013 | N/A | No action taken | Recovered |
| EPA + aspirin | Constipation | GI disorders | Constipation | No | Moderate | 16 March 2014 | 18 March 2014 | Trial medications permanently stopped | Condition present and unchanged |
| EPA + aspirin | Constipation | GI disorders | Constipation | No | Moderate | 25 January 2014 | 28 January 2014 | Trial medications delayed | Recovered |
| Placebo EPA + aspirin | Constipation | GI disorders | Constipation | No | Severe | 1 May 2014 | 1 July 2014 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Stomach pain | GI disorders | Abdominal pain upper | No | Moderate | 1 May 2014 | 1 July 2014 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 31 July 2014 | 14 August 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 24 March 2014 | 3 April 2014 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Moderate | 3 May 2015 | N/A | Trial medications delayed | Condition improving |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 15 May 2013 | 15 May 2013 | No action taken | Recovered |
| Placebo + placebo | Lower abdominal pain | GI disorders | Abdominal pain lower | No | Mild | 13 October 2013 | 15 October 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Blood in stool | GI disorders | Haematochezia | No | Mild | 7 September 2015 | 11 September 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Stomach discomfort | GI disorders | Abdominal discomfort | No | Mild | 8 August 2015 | 11 September 2015 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 1 August 2015 | 30 May 2016 | No action taken | Recovered |
| EPA + aspirin | Bruising | Injury, poisoning and procedural complications | Contusion | No | Mild | 1 December 2015 | N/A | No action taken | Condition improving |
| EPA + aspirin | Burping | GI disorders | Eructation | No | Mild | 7 July 2015 | 29 May 2016 | No action taken | Recovered |
| Placebo + placebo | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Moderate | 20 April 2016 | 2 May 2016 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Medication aftertaste | General disorders and administration site conditions | Product taste abnormal | No | Mild | 7 February 2016 | N/A | No action taken | Condition present and unchanged |
| EPA + aspirin | Flatulence | GI disorders | Flatulence | No | Mild | 10 February 2013 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Lower abdominal pain | GI disorders | Abdominal pain lower | No | Mild | 1 July 2013 | 1 September 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Lower abdominal pain | GI disorders | Abdominal pain lower | No | Mild | 24 June 2013 | 27 June 2013 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Tongue eruption | GI disorders | Tongue eruption | No | Mild | 24 June 2013 | 30 August 2013 | No action taken | Recovered |
| EPA + aspirin | Macroscopic haematuria | Renal and urinary disorders | Haematuria | No | Moderate | 1 February 2014 | 20 May 2014 | No action taken | Recovered |
| Placebo EPA + aspirin | Vomiting | GI disorders | Vomiting | No | Moderate | 4 December 2013 | 16 December 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Nausea | GI disorders | Nausea | No | Moderate | 4 December 2013 | 16 December 2013 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Heartburn | GI disorders | Dyspepsia | No | Mild | 21 November 2013 | 23 November 2013 | Trial medications reduced | Recovered with sequelae |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 21 November 2013 | 23 November 2013 | Trial medications reduced | Recovered with sequelae |
| Placebo + placebo | Black stools | GI disorders | Faeces discoloured | No | Moderate | 12 June 2013 | 12 July 2013 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 1 May 2013 | 12 June 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 20 April 2013 | 02 May 2013 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 20 April 2013 | 20 June 2013 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 20 April 2013 | 20 June 2013 | Trial medications reduced | Recovered |
| EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Mild | 29 May 2015 | N/A | Trial medications permanently stopped | Condition improving |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + aspirin | Acid reflux (oesophageal) | GI disorders | Gastro-oesophageal reflux disease | No | Moderate | 15 July 2015 | N/A | Trial medications permanently stopped | Condition deteriorated |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 16 September 2015 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 7 March 2016 | 16 March 2016 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 3 July 2015 | 11 August 2015 | No action taken | Condition deteriorated |
| Placebo EPA + aspirin | Melaena | GI disorders | Melaena | No | Moderate | 3 August 2015 | 11 August 2015 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 31 May 2016 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 27 May 2016 | 31 May 2016 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Mild | 1 February 2013 | 25 March 2013 | Trial medications reduced | Recovered |
| Placebo + placebo | Frequent bowel movements | GI disorders | Frequent bowel movements | No | Mild | 1 April 2013 | 18 April 2013 | No action taken | Recovered |
| Placebo + placebo | Frequent bowel movements | GI disorders | Frequent bowel movements | No | Mild | 1 July 2013 | 30 July 2013 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 16 December 2013 | 22 December 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 28 August 2013 | 2 September 2013 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Mild | 31 May 2014 | 4 June 2014 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Increased frequency of bowel movements | GI disorders | Frequent bowel movements | No | Mild | 20 January 2013 | N/A | No action taken | Condition improving |
| EPA + aspirin | Increased frequency of bowel movements | GI disorders | Frequent bowel movements | No | Mild | 6 February 2013 | 2 April 2013 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Indigestion | GI disorders | Dyspepsia | No | Mild | 3 April 2014 | N/A | No action taken | Condition present and unchanged |
| Placebo + placebo | Dry skin | Skin and subcutaneous tissue disorders | Dry skin | No | Mild | 13 January 2014 | N/A | No action taken | Condition improving |
| EPA + aspirin | Constipation | GI disorders | Constipation | No | Moderate | 21 August 2014 | 29 August 2014 | No action taken | Recovered |
| Placebo + placebo | Itchy skin | Skin and subcutaneous tissue disorders | Pruritus | No | Moderate | 1 July 2015 | 13 October 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Nose bleed | Respiratory, thoracic and mediastinal disorders | Epistaxis | No | Mild | 8 January 2016 | 11 April 2016 | No action taken | Recovered |
| EPA + aspirin | Weight loss | Investigations | Weight decreased | No | Mild | 9 July 2013 | N/A | Trial medications reduced | Condition present and unchanged |
| EPA + aspirin | Nausea | GI disorders | Nausea | No | Mild | 9 July 2013 | N/A | Trial medications reduced | Condition present and unchanged |
| EPA + aspirin | Stomach cramps | GI disorders | Abdominal pain upper | No | Mild | 9 July 2013 | N/A | Trial medications reduced | Condition present and unchanged |
| EPA + aspirin | Nausea | GI disorders | Nausea | No | Mild | 14 August 2013 | 1 May 2014 | Trial medications reduced | Condition improving |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Loose stools | GI disorders | Diarrhoea | No | Mild | 7 January 2015 | 2 April 2015 | Trial medications delayed | Recovered |
| Placebo + placebo | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 7 January 2015 | 2 April 2015 | Trial medications delayed | Recovered |
| Placebo + placebo | Constipation | GI disorders | Constipation | No | Mild | 7 January 2015 | 2 April 2015 | Trial medications delayed | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 6 January 2015 | 24 February 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 23 January 2015 | 30 January 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 2 December 2015 | 3 January 2016 | Trial medication period completed | Recovered |
| Placebo EPA + aspirin | Constipation | GI disorders | Constipation | No | Mild | 2 December 2015 | 3 January 2016 | Trial medication period completed | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 21 April 2015 | 28 April 2015 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 8 February 2015 | 17 February 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 1 September 2015 | 8 October 2015 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 1 September 2015 | 8 October 2015 | Trial medications permanently stopped | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 22 August 2015 | 29 August 2015 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 26 February 2015 | N/A | Trial medications permanently stopped | Condition present and unchanged |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 22 August 2015 | 29 August 2015 | No action taken | Recovered |
| Placebo EPA + aspirin | Constipation | GI disorders | Constipation | No | Mild | 16 October 2015 | N/A | No action taken | Condition improving |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo EPA + aspirin | Bruising | Injury, poisoning and procedural complications | Contusion | No | Mild | 16 October 2015 | N/A | No action taken | Condition improving |
| EPA + aspirin | Bloating | GI disorders | Abdominal distension | No | Mild | 1 December 2015 | 7 December 2015 | Trial medication already discontinued | Recovered |
| EPA + aspirin | Feeling unwell | General disorders and administration site conditions | Malaise | No | Mild | 1 December 2015 | 7 December 2015 | Trial medication already discontinued | Recovered |
| Placebo EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 5 June 2013 | 9 June 2013 | No action taken | Recovered |
| EPA + aspirin | Stomach pain | GI disorders | Abdominal pain upper | No | Mild | 14 March 2015 | 18 March 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Taste abnormality | Nervous system disorders | Dysgeusia | No | Mild | 22 January 2013 | 5 February 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Epistaxis | Respiratory, thoracic and mediastinal disorders | Epistaxis | No | Moderate | 6 January 2014 | 7 January 2014 | Trial medications reduced | Recovered |
| Placebo EPA + aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 10 October 2013 | 25 November 2013 | No action taken | Recovered with sequelae |
| Placebo EPA + aspirin | Taste abnormality | Nervous system disorders | Dysgeusia | No | Mild | 14 March 2013 | 20 March 2013 | Trial medications reduced | Recovered |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 25 August 2016 | 27 August 2016 | Trial medications permanently stopped | Recovered |
| Placebo + placebo | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 28 August 2016 | 30 August 2016 | Trial medications permanently stopped | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 1 June 2016 | 6 June 2016 | Trial medications reduced | Condition improving |
| EPA + placebo aspirin | Abdominal discomfort | GI disorders | Abdominal discomfort | No | Mild | 1 November 2016 | N/A | No action taken | Condition present and unchanged |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Mild | 3 March 2015 | N/A | Trial medications reduced | Condition improving |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 15 July 2015 | 30 September 2015 | No action taken | Recovered with sequelae |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 7 July 2015 | 8 June 2016 | Trial medications reduced | Recovered |
| EPA + placebo aspirin | Loose stools | GI disorders | Diarrhoea | No | Mild | 7 February 2013 | 10 February 2013 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Macroscopic haematuria | Renal and urinary disorders | Haematuria | No | Mild | 12 September 2015 | 15 September 2015 | Trial medications permanently stopped | Recovered |
| Placebo EPA + aspirin | Bleeding mouth | GI disorders | Mouth haemorrhage | No | Mild | 21 August 2013 | 21 August 2013 | No action taken | Recovered |
| EPA + aspirin | Dyspepsia | GI disorders | Dyspepsia | No | Mild | 23 August 2013 | 25 August 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Moderate | 19 November 2013 | 22 November 2013 | Trial medications permanently stopped | Recovered with sequelae |
| EPA + placebo aspirin | Nausea | GI disorders | Nausea | No | Moderate | 19 November 2013 | 22 November 2013 | Trial medications permanently stopped | Recovered with sequelae |
| EPA + placebo aspirin | Defaecation urgency | GI disorders | Defaecation urgency | No | Mild | 19 September 2013 | 22 September 2013 | No action taken | Recovered |
| EPA + placebo aspirin | Abdominal pain | GI disorders | Abdominal pain | No | Mild | 19 September 2013 | 22 September 2013 | No action taken | Recovered |

**TABLE 41** Listing of ADRs (*continued*)

| Treatment group | Description | System organ class name | Preferred term name | SAE | Severity | Start date | End date | Action taken | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Placebo + placebo | Abdominal pain | GI disorders | Abdominal pain | No | Moderate | 30 January 2014 | 10 March 2014 | Trial medications permanently stopped | Recovered |
| EPA + aspirin | Chest pain | General disorders and administration site conditions | Chest pain | Yes | Moderate | 29 July 2013 | 31 July 2013 | No action taken | Recovered |
| Placebo EPA + aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 12 October 2012 | 14 October 2012 | No action taken | Recovered |
| EPA + placebo aspirin | Diarrhoea | GI disorders | Diarrhoea | No | Mild | 22 November 2012 | 26 November 2012 | No action taken | Condition improving |
| Placebo + placebo | Halitosis | GI disorders | Breath odour | No | Mild | 22 February 2013 | 22 August 2013 | No action taken | Recovered |
| EPA + aspirin | Epistaxis | Respiratory, thoracic and mediastinal disorders | Epistaxis | No | Mild | 4 June 2014 | 12 June 2014 | No action taken | Recovered |

N/A, not available; PR, per sectum.

**TABLE 42** Listing of SAEs

| Treatment group | System organ class name | Preferred term name |
|---|---|---|
| Placebo + placebo | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Renal cancer |
| EPA + placebo aspirin | GI disorders | Gastritis |
| Placebo + placebo | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Lung neoplasm malignant |
| Placebo EPA + aspirin | GI disorders | Oesophageal haemorrhage |
| Placebo EPA + aspirin | Vascular disorders | Thrombosis |
| Placebo EPA + aspirin | Vascular disorders | Thrombosis |
| Placebo EPA + aspirin | Infections and infestations | Lower respiratory tract infection |
| EPA + aspirin | Infections and infestations | Pharyngeal abscess |
| Placebo EPA + aspirin | Investigations | Blood glucose increased |
| Placebo + placebo | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Lung neoplasm malignant |
| Placebo + placebo | GI disorders | Abdominal pain |
| EPA + placebo aspirin | GI disorders | Small intestinal perforation |
| EPA + aspirin | Vascular disorders | Deep-vein thrombosis |
| Placebo EPA + aspirin | Vascular disorders | Deep-vein thrombosis |
| EPA + placebo aspirin | Infections and infestations | Lung infection |
| EPA + placebo aspirin | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Oesophageal carcinoma |
| Placebo + placebo | Infections and infestations | Labyrinthitis |
| Placebo EPA + aspirin | GI disorders | Gastro-oesophageal reflux disease |
| Placebo EPA + aspirin | GI disorders | Duodenitis |
| Placebo EPA + aspirin | GI disorders | Hiatus hernia |
| Placebo EPA + aspirin | GI disorders | Faeces discoloured |
| Placebo + placebo | Vascular disorders | Femoral artery occlusion |
| EPA + placebo aspirin | Respiratory, thoracic and mediastinal disorders | Pulmonary embolism |
| Placebo EPA + aspirin | Infections and infestations | Post procedural infection |
| EPA + placebo aspirin | Hepatobiliary disorders | Liver disorder |
| EPA + placebo aspirin | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Lung neoplasm malignant |
| Placebo + placebo | Vascular disorders | Deep-vein thrombosis |
| Placebo + placebo | General disorders and administration site conditions | Chest pain |
| Placebo EPA + aspirin | Nervous system disorders | Syncope |
| Placebo EPA + aspirin | Respiratory, thoracic and mediastinal disorders | Respiratory failure |
| EPA + placebo aspirin | Cardiac disorders | AF |
| EPA + placebo aspirin | Cardiac disorders | AF |
| EPA + placebo aspirin | Cardiac disorders | AF |
| EPA + placebo aspirin | Cardiac disorders | AF |
| Placebo EPA + aspirin | Nervous system disorders | Transient ischaemic attack |

**TABLE 42** Listing of SAEs (*continued*)

| Treatment group | System organ class name | Preferred term name |
|---|---|---|
| Placebo + placebo | Infections and infestations | Colonic abscess |
| EPA + placebo aspirin | Cardiac disorders | Acute myocardial infarction |
| Placebo + placebo | Cardiac disorders | Acute myocardial infarction |
| Placebo + placebo | Infections and infestations | Lower respiratory tract infection |
| EPA + aspirin | Respiratory, thoracic and mediastinal disorders | Chronic obstructive pulmonary disease |
| EPA + placebo aspirin | Cardiac disorders | Atrial fibrillation |
| EPA + placebo aspirin | Infections and infestations | Cellulitis |
| EPA + aspirin | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Prostate cancer |
| EPA + aspirin | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Metastases to bone |
| EPA + placebo aspirin | General disorders and administration site conditions | Chest pain |
| Placebo EPA + aspirin | Injury, poisoning and procedural complications | Laceration |
| Placebo EPA + aspirin | Cardiac disorders | Myocardial infarction |
| Placebo + placebo | Psychiatric disorders | Confusional state |
| Placebo + placebo | Neoplasms: benign, malignant and unspecified (including cysts and polyps) | Bladder squamous cell carcinoma stage unspecified |
| EPA + placebo aspirin | Respiratory, thoracic and mediastinal disorders | Chronic obstructive pulmonary disease |
| Placebo + placebo | Psychiatric disorders | Alcohol withdrawal syndrome |
| Placebo + placebo | Nervous system disorders | Encephalopathy |
| Placebo + placebo | GI disorders | Faeces discoloured |
| EPA + aspirin | General disorders and administration site conditions | Chest pain |
| Placebo EPA + aspirin | Cardiac disorders | Arrhythmia |

**TABLE 43** Summary of GI ADRs by preferred term name and by severity

| Preferred term name | Severity | Trial group (*n*) | | | |
|---|---|---|---|---|---|
| | | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| Abdominal discomfort | Mild | 0 | 3 | 1 | 2 |
| | Moderate | 0 | 0 | 1 | 0 |
| Abdominal distension | Mild | 1 | 1 | 0 | 1 |
| Abdominal pain | Mild | 2 | 16 | 5 | 2 |
| | Moderate | 1 | 5 | 2 | 3 |
| Abdominal pain lower | Mild | 0 | 2 | 0 | 1 |
| | Moderate | 0 | 0 | 0 | 1 |
| Abdominal pain upper | Mild | 2 | 4 | 0 | 3 |
| | Moderate | 0 | 0 | 1 | 0 |
| Anal haemorrhage | Mild | 0 | 1 | 0 | 1 |
| Anal inflammation | Moderate | 0 | 0 | 1 | 0 |
| Breath odour | Mild | 1 | 3 | 1 | 4 |
| Change of bowel habit | Mild | 0 | 0 | 1 | 0 |
| | Moderate | 0 | 0 | 1 | 0 |
| Constipation | Mild | 2 | 5 | 3 | 2 |
| | Moderate | 3 | 0 | 0 | 1 |
| | Severe | 0 | 0 | 1 | 0 |
| Defaecation urgency | Mild | 0 | 3 | 0 | 0 |
| Diarrhoea | Mild | 4 | 18 | 12 | 5 |
| | Moderate | 3 | 10 | 1 | 0 |
| Duodenitis | Moderate | 0 | 0 | 1 | 0 |
| Dyspepsia | Mild | 11 | 10 | 11 | 9 |
| | Moderate | 1 | 0 | 3 | 5 |
| Epigastric discomfort | Mild | 1 | 0 | 0 | 0 |
| Eructation | Mild | 3 | 2 | 0 | 1 |
| Faeces discoloured | Mild | 0 | 0 | 0 | 1 |
| | Moderate | 0 | 1 | 0 | 1 |
| | Severe | 0 | 0 | 1 | 0 |
| Flatulence | Mild | 1 | 4 | 2 | 3 |
| | Moderate | 0 | 0 | 0 | 1 |
| Frequent bowel movements | Mild | 1 | 1 | 0 | 2 |
| | Moderate | 0 | 0 | 0 | 1 |
| Gastric haemorrhage | Mild | 0 | 1 | 0 | 0 |
| Gastritis | Mild | 0 | 2 | 1 | 0 |
| GI sounds abnormal | Mild | 1 | 0 | 0 | 0 |
| Gastro-oesophageal reflux disease | Mild | 2 | 0 | 3 | 2 |

**TABLE 43** Summary of GI ADRs by preferred term name and by severity (*continued*)

| Preferred term name | Severity | Trial group (*n*) | | | |
| | | EPA + aspirin | EPA + placebo aspirin | Placebo EPA + aspirin | Placebo + placebo |
| --- | --- | --- | --- | --- | --- |
| | Moderate | 1 | 1 | 2 | 1 |
| Haematochezia | Mild | 0 | 1 | 5 | 0 |
| Hiatus hernia | Mild | 0 | 0 | 1 | 0 |
| Melaena | Moderate | 0 | 0 | 1 | 0 |
| Mouth haemorrhage | Mild | 0 | 0 | 1 | 0 |
| Nausea | Mild | 3 | 8 | 1 | 1 |
| | Moderate | 1 | 3 | 1 | 2 |
| Oesophageal haemorrhage | Severe | 0 | 0 | 1 | 0 |
| Oesophagitis | Mild | 0 | 1 | 0 | 0 |
| Rectal haemorrhage | Mild | 0 | 0 | 2 | 0 |
| Retching | Moderate | 1 | 0 | 0 | 0 |
| Tongue eruption | Mild | 0 | 1 | 0 | 0 |
| Vomiting | Mild | 0 | 2 | 0 | 0 |
| | Moderate | 0 | 0 | 1 | 0 |
| | Severe | 0 | 1 | 0 | 0 |

# Appendix 5 Summary of gastrointestinal adverse events according to the categories defined by the chief investigator

| GI AE | Trial group | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | EPA + aspirin | | EPA + placebo aspirin | | Placebo EPA + aspirin | | Placebo + placebo | |
| | FFA | TG | FFA | TG | FFA | TG | FFA | TG |
| Number in safety population | 99 | 71 | 107 | 70 | 99 | 75 | 109 | 167 |
| Participants reporting at least one GI AE, *n* (%) | 25 (25) | 22 (31) | 43 (40) | 24 (34) | 24 (24) | 20 (27) | 38 (35) | 13 (19) |
| Diarrhoea[a] (*n*) | 9 | 2 | 27 | 15 | 11 | 9 | 9 | 7 |
| Upper GI symptoms (*n*) | 11 | 18 | 21 | 15 | 11 | 15 | 19 | 9 |
| Lower abdominal symptoms (*n*) | 4 | 5 | 20 | 17 | 7 | 3 | 18 | 3 |
| Eructation/halitosis (*n*) | 1 | 3 | 5 | 0 | 0 | 1 | 5 | 0 |
| Other (*n*) | 12 | 3 | 19 | 7 | 15 | 14 | 11 | 4 |

a  Data are at event level.
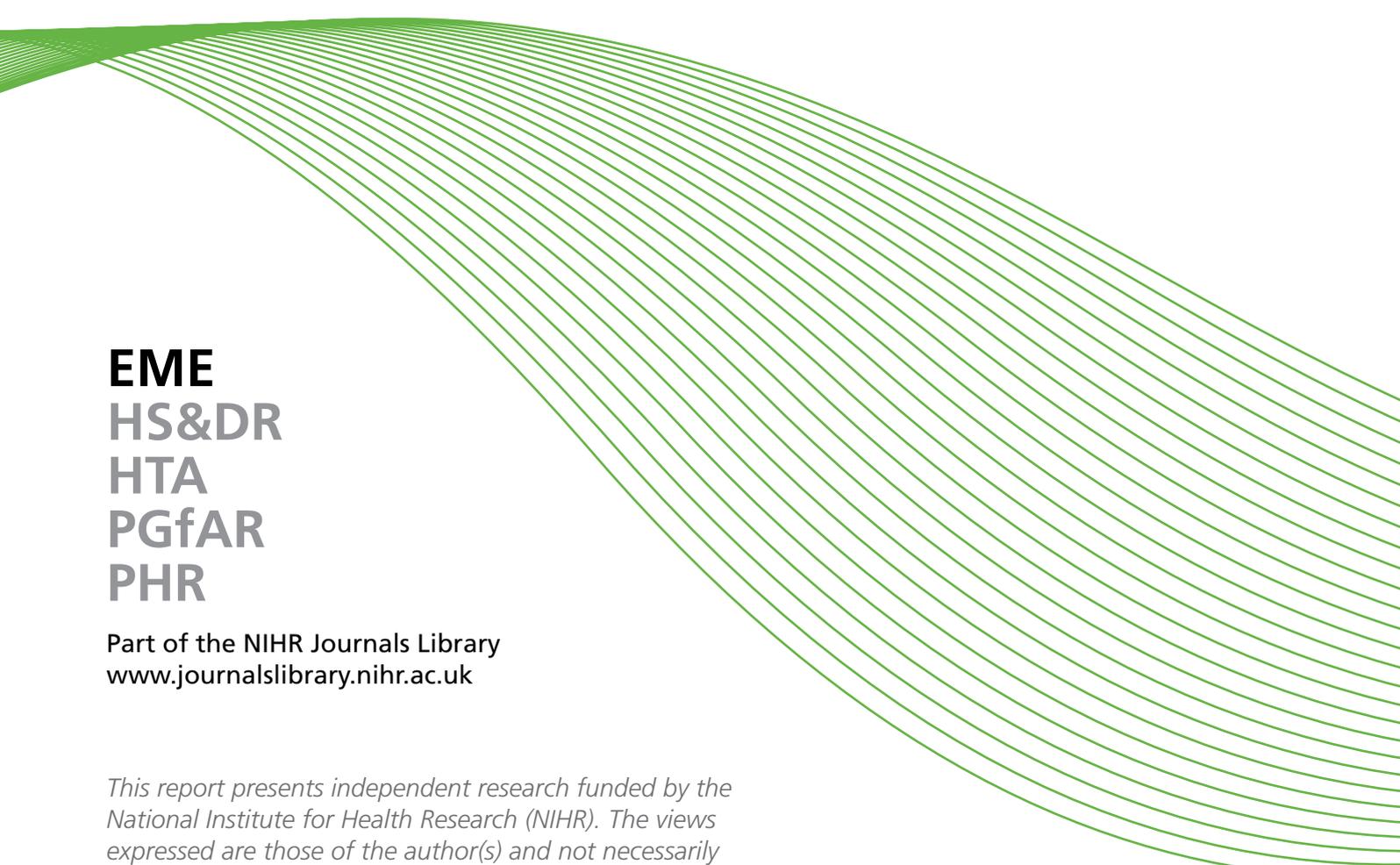
# EME
## HS&DR
## HTA
## PGfAR
## PHR

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