Analysis Plan for AspECT

Version 2.0
24th Aug 2017

Based on version 12 of protocol

REVIEW HISTORY				
name		**Version**	**signature**	**date**
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OCTRU is a UKCRC Registered Clinical Trials Unit
OCTRU is a joint venture between the Centre for Statistics in Medicine (CSM) and the Oncology Clinical Trials Office (OCTO) both based at the University of Oxford

Contents

1. Introduction

This document details the proposed presentation and analysis for the main paper(s) reporting results from the CTAAC funded multicentre randomised controlled trial of aspirin and esomeprazole in the prevention of adenocarcinoma or high grade dysplasia in Barrett's metaplasia (AspECT). The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

This analysis strategy should be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.

Version 0.4 of this analysis plan was released for the first DSMC. Since the trial is of a longer duration, it is expected that some of the analysis may be updated with the agreement of the TSC and DSMC. Version 1.0 of the analysis plan was released before the 4 year interim analysis. Version 2.0 is due to be the final version used to analyse the primary aim. Any additional analysis carried out will be mentioned in the published paper of the trial results.

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2. Background Information

2.1 Objectives

Primary OBJECTIVES:

- To assess whether intervention with aspirin results in a decreased rate of all causes of mortality or conversion rate from Barrett's metaplasia (BM) to adenocarcinoma or high grade dysplasia.
- To assess whether high dose PPI therapy results in a decreased rate of all causes of mortality or conversion rate from Barrett's metaplasia to adenocarcinoma or high grade dysplasia.

Secondary OBJECTIVES:

- To assess whether intervention with aspirin results in decreased high-grade dysplasia, in decreased all cause mortality, in decreased oesophageal cancer incidence and in decreased cause-specific mortality when each is considered separately
- To assess whether intervention with high dose PPI results in decreased high-grade dysplasia, in decreased all cause mortality, in decreased oesophageal cancer incidence and in decreased cause-specific mortality when each is considered separately
- To assess whether there are clinical and molecular risk factors which can be identified in BM for the development of Barrett's Adenocarcinoma (BA).
- To assess the cost effectiveness of aspirin and/or PPI treatment in the prevention of BA.
- To assess whether intervention with PPI and/or aspirin induces changes in the expression of molecular markers for BA. To assess the genomics of aspirin sensitivity (efficacy and side effects)
- To assess how quality of life is affected by the different treatments.
- To assess what the biological risk factors are for cardiac disease and aspirin resistance.
- To assess gender differences in outcomes.

Exploratory aims

1. To assess aspirin's role on the development of colorectal adenomas and cancer.
2. To collect and bank samples for use in future ethically approved studies

Exploratory uses of the data

1. Describe Barrett's length over repeat biopsies and investigate a change in length from baseline to 2 years between treatment groups.
2. Can intervention with aspirin result in a lower stage of adenocarcinoma ? Can intervention with PPI result in a lower stage of adenocarcinoma ?
3. Does adjustment for Oesophagectomy, Ablation therapy, Endoscopic mucosal resection change the treatment effect ?
4. Is the event rate different for those previous users of aspirin who were not randomised to it compared to previous users who continued with aspirin as their randomised treatment?
5. Investigation of the hypothesis that aspirin will have a chemopreventive effect on all epithelial cancers using the same mechanism it does to reduce oesophageal cancer**,** however, high dose PPI therapy may arguably increase colon cancer incidence as gastrin levels increase and this hormone is a known stimulant for colon cancer in mice.
6. Look for a dose/time response effect for aspirin and for esomeprazole.

2.2 Study Design and Treatment Interventions

This is a national, multi-centre, phase III, randomised controlled trial of low or high dose PPI (drug name: Esomeprazole) with or without low dose aspirin for 8 years with expected accrual of 5000 patients. The trial sample size was recalculated to 2500 and some patients re-consented to 10 years follow-up or 28Feb2017 (whichever was the sooner).

The trial is a pragmatic, multi-centre, phase III, randomised, open, 2x2 factorial trial.

ARM A: 20mg PPI **Symptomatic treatment only = standard therapy control**	**ARM B:** 80mg PPI **Strong acid suppression**	**No Aspirin (A+B)**
ARM C: 20mg PPI **Symptomatic treatment and aspirin**	**ARM D:** 80mg PPI **Strong acid suppression and aspirin**	**Aspirin (C+D)**
Low dose PPI (A +C)	**High dose PPI (B + D)**	

Patients will receive either continuous PPI 20mg/day or continuous PPI 80mg/day with or without aspirin 300mg/day.

If patients are on low dose aspirin for medical reasons (i.e. previous IHD or CVA's rather than self prescribed) they will be randomised to either continuous PPI 20mg/day or continuous PPI 80mg/day arms. They will be allowed to continue to take their aspirin therapy.

Questionnaires dealing with demographics, family history, drug history, smoking, cardiac disease, alcohol, diet and quality of life measures will be completed at baseline. Quality of Life data collection was not possible in all patients and not at all timepoints.

The majority of patients will have an endoscopy at baseline and thereafter at 2 yearly intervals. A number of patients whose baseline endoscopy was more than 12 months before randomisation should have another endoscopy within 6 months of randomisation. A number of patients will have had an endoscopy within the 12 months prior to randomisation. These patients will not have a baseline endoscopy but may have an endoscopy at 2 years from their last endoscopy or 2 years from randomisation at the PI's discretion. These two groups of patients are called retrospective patients.
NOTE on retrospective patients. It is acceptable for primary outcome investigation not to be linked to date of randomisation since we will be using the actual time of an event. Events linked to endoscopy may occur at screenings or during an endoscopy for change of symptoms. Follow-up forms will be completed at yearly intervals from randomisation and QOL forms posted out at 2 yearly intervals from randomisation (for relevant patients).

At each endoscopy any macroscopic abnormality of the Barrett's metaplasia will be biopsied as per local practice and samples taken for formalin fixation and local histological assessment for dysplasia. DNA and RNA will be extracted from the paraffin embedded material for SNP analysis and micro array analysis. In addition an extra biopsy will be taken at each level and snap frozen for genetic studies. In addition to the endoscopy, blood samples will be taken at baseline, between 4 and 6 years, 8 years and at 10 years if the patient consented to extended follow-up, to assess cardiovascular risk factors and the remainder stored for analysis of circulating genetic mutations as and when reliable assays become available.

Patients will come to the hospital pharmacy every 6 months to drop off unused medication and pick up a new 6 month supply.

The factorial design is efficient due to each patient contributing to the answer to 2 questions. Not only must it be possible for patients to receive both treatments (practically and with consideration of toxicity) but the anticipated treatment effects must be approximately additive. The patients taking part in the PPI only randomisation will be analysed for the PPI primary aim but will not be available for the aspirin primary aim.

2.3 Timings

Date of start of recruitment:	August 2005[@]
Date of end of recruitment:	25February2009
First interim analysis*	April 2011
Second interim analysis*	August 2013
Final analysis start*	September 2017
Actual number of subjects:	2513

* Analyses are due 2 and 4 years after last patient recruitment and at the end of the trial..
[@] A pilot site was open from Mar2005

2.4 Patient eligibility

INCLUSION CRITERIA

- Male between 40 - 75 years; *** AGE LIMIT REMOVED March 2006 now 18 years and over*** *** Planned 500 women entered for generalisability***
- at least 2cm from the gastro-oesophageal junction of circumferential BM (Histologically proven by intestinal metaplasia in at least one sample) *** LATER CHANGED TO 1CM *** *** also changed to or a past history of intestinal metaplasia *** **changed 4th Feb 2008 to no need for IM and tongues of Barrett's are enough for inclusion**
- able to give written informed consent.
- WHO activity profile of 0 or 1 i.e. fully active and self-caring

EXCLUSION CRITERIA

1. those with high grade dysplasia or carcinoma at enrolment;
2. medical conditions which would make endoscopy or completing the trial difficult including:

   a) Frequent transient ischaemic attacks (3 or more) or severe cerebral vascular accident in the previous 6 months*
   b) Severe respiratory disease with arterial oxygen saturation of less than 90% at rest

c) Severe ischaemic heart disease (exercise tolerance less than 100 yards or life expectancy < 4 years) or myocardial infarction in the previous 3 months
d) Severe inflammatory bowel disease requiring at least one hospital admission of 5 days in the last year or bowels open > 6 times/day

* Patients answering yes to this criteria were eligible for the PPI-only (non-aspirin) randomisation.

3. Patients with absolute contraindications to PPIs, aspirin or their excipients i.e. allergies, ulcers, renal impairment or use of oral anticoagulants.

*** added

4. pregnant or lactating women ***

*** amended***

- Up to 100 previous aspirin users will be entered providing they agree to stop aspirin use if not randomised to it.

amended

- Patients not wishing to stop aspirin or who have an absolute contraindication to it can be randomised to the low/high PPI dose arms only and will be analysed for that comparison only

For patients on existing PPI therapy, there is no washout period.

Patients are allowed 6 months of another treatment option or a break from treatment for any 6 month period or 12 months in total over the entire trial length of up to 10 years.

2.5 Sample size

2.5.1 Sample size – estimates used

The conversion rate in the UK is 0.98% (95% CI = 0.67 to 1.39%) (this is higher in the UK than the USA) however to be cautious we have agreed on 0.76%/year (approximately half way between the UK and the USA).
Aspirin has a hazard ratio of 1.5 (33.3% effective) in preventing the colorectal cancer rate. Evidence from epidemiological studies suggests that aspirin may decrease BA rates with a hazard ratio of 1.6 to 10 (40 - 90%). While we believe that current evidence indicates that aspirin is at least equally effective in the prevention of oesophageal adenocarcinoma as colorectal cancer, the role of PPI is more difficult to estimate for the reasons given previously. Therefore we have chosen a hazard ratio of 1.4 (28% effective) to assess weaker therapeutic effects.

2.5.2 Sample Size – original statement

A total of 5000 patients (1250 in each intervention group) will allow us to detect a difference in conversion rates of 0.22% per year between the PPI and aspirin intervention. The sample size required was calculated (pre CSM involvement) using nQuery Advisor® Release 4.0 based on the following:

Patients will be recruited over 2 years and followed up for a further 8 years.
Exponential time to conversion with a constant event rate of at least 0.76% per year
Conversion hazard ratio (drug response) of control to treatment is 1.4
Power = 80% (2-sided test at 5% level of significance).
No interaction between the effects of aspirin and PPI interventions.
A 10% loss to follow-up from Office of National Statistics flagging
A 20% non-compliance with medication
Calculations adjusted for competing causes of mortality. A further proportion may take their medication but be lost to follow-up as they do not return their questionnaire and/or attend for surveillance endoscopy. This will not affect the primary outcome as all cause mortality and conversion to BA will be assessed by the Office of National Statistics, which has almost complete follow-up of registered subjects (we have conservatively assumed a 90% follow-up rate).

2.5.3 Exploratory sample size statement October 2008

Due to protocol amendments and an expected restriction on the number of patients to be recruited, an exploratory sample size is presented at the request of the Funders. Since the trial will recruit until end Dec 2008 in the UK and possibly until the end of June in Canada rather than to the number calculated in the original sample size, this calculation should be considered as a guide to the power and treatment effect size the trial should achieve.

This exploratory sample size is calculated as per the original sample size, but with

- Annual rate of conversion of 1% (allowing for the composite primary endpoint of all cause mortality and conversion to adenocarcinoma and conversion to high grade dysplasia.)

  Justification: Using the National Statistics figures for 2005 based on the age and gender structure of the first 2046 patients recruited to AspECT gives a mortality rate in a year of 1.16%.
  Using the UK Barrett's conversion rate to adenocarcinoma of at least 0.98% per year without treatment for 60% of the sample and 40% with a lower conversion rate of 0.66% (due to being

female, have tongues of Barrett's metaplasia or only 1cm of Barrett's, or being <45 years old. Differing rates are not published for these patients so a rate of two thirds the UK conversion rate has been used) ie 0.85% conversion rate without treatment over the whole sample.
No good estimates of rates for conversion to high grade dysplasia for the UK could be found.
In this population, it is unlikely that the event rate for the composite primary endpoint in the control arm will be less than 1%.

- HR as in colorectal cancer
- No adjustment for compliance
- the follow-up increased to 10 years

Apart from limited recruitment and checking the event rate using the age/sex structure, no information or estimates from the current trial are used.

A total of 2224 patients (196 events) (approximately 556 in each intervention group) will allow us to detect a difference in conversion rates of 0.34% per year between low dose and high dose PPI and aspirin or no aspirin. This sample size was calculated using ARTMENU in STATA release 10.0 based on the following:

- Patients will be recruited over 3 years and followed up for a further 10 years
- Exponential time to conversion with a constant event rate of at least 1% per year without treatment
- Conversion hazard ratio (drug response) of control to treatment is 1.5 (1 year conversion rate 1% no or standard treatment vs 0.66% in the treated patients)
- Power = 80% (2-sided test at 5% level of significance)
- No interaction between the effects of aspirin and PPI interventions
- A 10% loss to follow-up from Office of National Statistics flagging

2.6 Randomisation

Randomisation is by minimisation using age split into four groups and length of Barrett's metaplasia split into three groups. There is a random component, with the patient having an 80% chance of being assigned the treatment that reduces the imbalance.

Age
40-49
50-59
60-69

70-75

Length of Barrett's metaplasia
≤3cm
>3 and ≤8cm
>8cm

Initially the trial accrued only males. From Sept 2005, there are separate minimisation routines running for males and females.

**** Change in 2007
Limit on age to be removed and Barrett's from 1cm to be included***
Then
Age
18-49
50-59
60-69
≥70

Length of Barrett's metaplasia
<2cm
≥2cm and ≤3cm
>3 and ≤8cm
>8cm

2.6.1 * Change 25th Feb 2008 *****
IM to be added as stratification factor
Tongue of Barretts to be added as a level in length
Individuals participating only in the PPI randomisation are randomised in a separate routine (same stratification)
Gender already separate routine
Then
Age
18-49
50-59
60-69
≥70

Length of Barrett's metaplasia
Tongue of Barretts
<2cm
≥2cm and ≤3cm

>3 and ≤8cm
>8cm

IM

Yes
No

2.6.2 Blinding

After randomisation, both the patient and the treating doctor knows what treatment the patient is receiving. There is no blinding of treatments. (The cost of placebo drugs was prohibitively expensive.)

2.7 Definition of Primary and Secondary Outcomes

Primary outcome is

- Time from randomisation date to conversion from Barrett's metaplasia to high grade dysplasia (event), or to adenocarcinoma of the oesophagus (event) or time to death from all causes (event) or time to last follow-up (no event)

I.e. the time is noted as the time from date of randomisation to high grade dysplasia, adenocarcinoma of the oesophagus or death, whichever is the earlier, with the survival time coded as an event. For patients who do not have one of these events of interest, the time from randomisation to date of last follow-up is calculated and the survival time coded as a censored event.

Secondary outcomes are

- Time from randomisation date to conversion from Barrett's metaplasia to high grade dysplasia (event) or time to last follow-up (no event) or event that precludes getting high grade dysplasia (for example death) (no event)
- Time from randomisation date to progression from Barrett's metaplasia to adenocarcinoma of the oesophagus (event) or time to last follow-up (no event)) or event that precludes getting adenocarcinoma of the oesophagus (for example death) (no event)
- Time from randomisation date to death from all causes (event) or time to last follow-up (no event)
- Time from randomisation date to death from oesophageal cancer(event) or time to last follow-up (no event) or death from a cause other than oesophageal cancer (no event)
- Molecular markers to be identified at the end of the trial and used during analyses of collected blood and/or tissue samples
- Cost per oesophageal adenocarcinoma prevented

- Cost per life year saved
- Molecular markers to be identified at the end of the trial and used with samples collected across time along with treatment information
- Cost per quality adjusted life saved
- Cardiac event from SAE (our best data on cardiac disease) and new tumours (a marker of aspirin resistance)
- For gender secondary, same outcome as for primary

2.8 Outcomes Assessment Schedule

		sample collection and CRF completion									
		Baseline	1 month	1 year	2years	3 years	4years	5years	6years	7years	8years
CRFs	Randomisation form	√									
	Patient history	√									√
	Food diary	√									√
	Quality of Life	√			√		√		√		√
	Family history	√									
	Endoscopy form	√			√		√		√		√
	Biopsy sampling form	√			√		√		√		√
	Pathology form	√			√		√		√		√
	1 month fu form		√								
	Follow-up form			√	√	√	√	√	√	√	√
	End of treatment form										√
Sample **collection** [1]	Endoscopy	√			√		√		√		√
	Biopsy samples	√			√		√		√		√
	Blood samples	√									√

Notes: Food Diary, quality of life and family history only collected for first 1000 patients
In later versions endoscopy and pathology form combined

[1] Endoscopy, biopsy and blood samples collected 2 years from previous endoscopy for retrospective patients

3. QC during trial

The quality and completeness of the data will be the responsibility of the data team during the trial. The statistician will feed back any concerns of a specific or general nature to the data team.

1 year into the trial (September 2006), the integrity of the randomization was assessed. The completeness of the data is assessed at each data lock, discussed between the data team and statistician and any necessary action taken.

4. Data Monitoring Committee and Interim Analyses

The DMC met annually to monitor recruitment to the trial and protocol compliance as well as toxicity and serious adverse events. See Charter for full details.

The primary aim was analysed and presented confidentially to the DSMC after 2 years and 4 years of follow-up (interim analyses) and was reported considering a p value of 0.001 as significant. The DSMC were happy for the trial to continue and neither interim analysis was disseminated further.

5. Data Validation

It is expected that much of the data cleaning will be done by the data team. However, before analysis, all fields will be summarised and any possible fields cross referenced. This will improve understanding of the data and allow for feedback of unexpected data to the data team for clarification/correction.

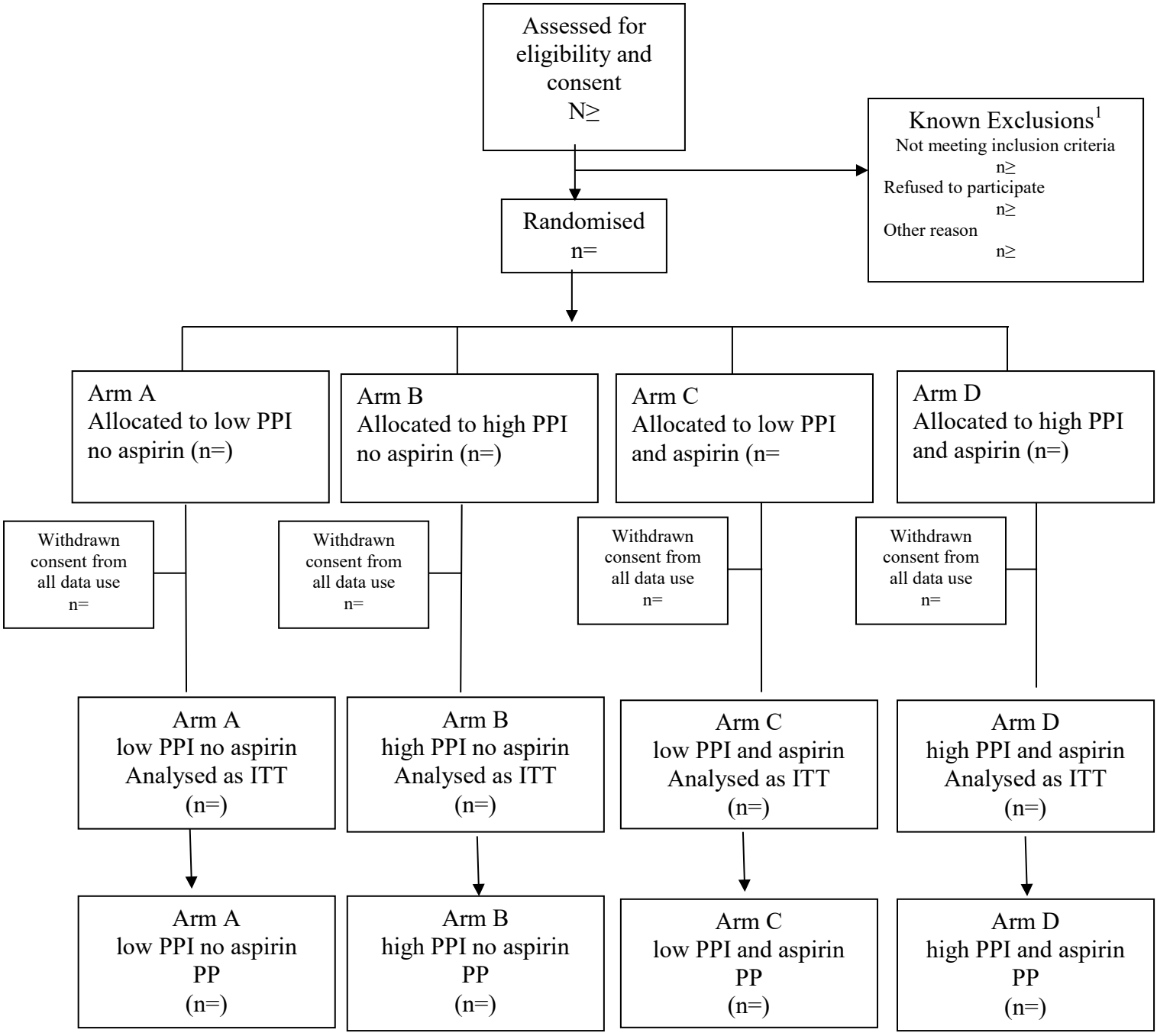
Oesophagectomy, ablation therapy and endoscopic mucosal resection are commonly carried out when high grade dysplasia or oesophageal adenocarcinoma is found. All cases where this treatment is carried out without logging a disease progression will be checked.

The primary analysis and first 2 secondaries will be independently analysed by a second statistician.

6. Descriptive Analyses

A descriptive analysis will be carried out first examining the study sample.

6.1 Representativeness of Study Sample and Patient Throughput



The above diagram was completed for each interim analysis (2 years and 4 years after final recruitment) and will also be completed at the final analyses. Any randomised but ineligible patients will be described. These patients are included in the ITT analysis

The sensitivity analysis will use a per protocol sample of patients given a 'therapeutic' amount of treatment. See section 7 and appendix 1 for a definition of the therapeutic dose.

6.2 Baseline Comparability of Randomised Groups

A table of baseline data for all patients, each of the four intervention groups and the groups for the main comparisons will be given. This will include

Variable at baseline	Low PPI no aspirin	Low PPI and aspirin	High PPI no aspirin	High PPI and aspirin	TOTAL
Length of Barrett's metaplasia at rand (strata for minimisation and median(range), cm)					
Age (strata for minimisation, median(range),years)					
Gender Male Female					
Intestinal metaplasia Yes No					
Retrospective patient Yes No					
Duration of Barrett's pre randomisation Median (range), years					
Alcohol use None some					

Variable at baseline	Low PPI no aspirin	Low PPI and aspirin	High PPI no aspirin	High PPI and aspirin	TOTAL
(For some group, median(iq range),units per week)					
Smoker No never Ex Yes current					
Myocardial Infarction Yes No					
Angina Yes No					
Coronary Intevention Yes No					
Stenosis Yes No					
Cardiac catheterisation Yes No					
Cerebrovascular Yes No					
TIA Yes No					
Peripheral Vascular Disease Yes No					
Diabetes Yes No					

Variable at baseline	Low PPI no aspirin	Low PPI and aspirin	High PPI no aspirin	High PPI and aspirin	TOTAL
Hypertension Yes No					
Hyperlipidaemia Yes No					

This table will be repeated with columns of no aspirin, aspirin, low PPI and high PPI. Those taking part in the PPI only randomisation will not appear in the noaspirin/aspirin columns since they did not take part in the aspirin randomisation.

Numbers (with percentages) for binary and categorical variables and means (and standard deviations), or medians (with range) for continuous variables will be presented. There will be no tests of statistical significance nor confidence intervals for differences between randomised groups on any baseline variable.

6.3 Comparison of Losses to Follow-up

The numbers and distribution of losses to follow-up (defaulters and withdrawals) over the period of the study will be reported overall, for the four randomisation groups and for the primary objective comparison groups. Defaulters are those who attend no further trial related visits.

Note that for conversion to cancer and death from any cause, the NHS digital flagging may still be possible for those lost to active follow-up.
For other endpoints, the baseline characteristics of those lost to follow-up and between those to be analysed will be compared between the two treatment groups. Those randomised may be balanced, but those with data to analyse on the secondary objectives may not be. The consequences of this examination of the data will be described separately for each objective.

6.4 Description of Compliance with Therapy

A summary of the treatment received will be provided. This will include the dosage of drugs in each of the four treatment intervention groups and details of numbers complying with treatment. Note that compliance is assumed when a prescription is collected and patient attends trial visits.

6.5 Reliability of data

Survival time will be calculated from date of randomisation to the first of

date of Barrett's adenocarcinoma
date of high grade dysplasia
date of death from any cause

with a censor variable created coded 1 for an event. If none of the above exist for a patient the survival time will be calculated from date of randomisation to

date of last follow-up

with the censor variable coded 0 to indicate the time does not relate to an event.
This may be done using difference in defined dates in STATA (or similar function/software available at the time of analysis). A random 20 patient manual check will be done to confirm the programming of the calculation.

Other derived variables will have a 20 patient check.

6.6 Description of Available Data

Primary endpoint – survival until adenocarcinoma, high grade dysplasia or death from any cause
There will be a time for every patient. For some of the patients who do not reach an endpoint, the survival time will be less than the trial duration due to loss to follow-up. The endpoint of death and diagnosis of Barrett's adenocarcinoma can be picked up in many cases from NHS digital but the endpoint of high grade dysplasia will be under represented due to those lost to follow-up.
The median follow-up will be described using the median of those without an event and the median using the reverse Kaplan-Meier method.
Kaplan-Meier curves will be drawn for all patients, then split by aspirin intervention and split by PPI dose (low or high).
The analysis of the primary objective involves knowledge of the treatment and then knowledge of the factors used in the minimisation which will be available for everyone from the randomisation.

Secondary endpoints –adenocarcinoma, high grade dysplasia, death
As described for primary but for each event separately.

Secondary endpoints – clinical and molecular risk factors
Available for a varying number of patients

Secondary endpoint - cost effectiveness
This endpoint is not being analysed by CSM

Secondary endpoints - changes in molecular markers and genomics
This endpoint is not being analysed by CSM

Secondary endpoints – quality of life
It was not possible to collect this data across time so this analysis cannot be done

Secondary endpoints – biological risk factors for cardiac disease and aspirin resistance
Cardiac events fulfilling a seriousness criterion will be noted as SAE and this will be used as indicative of cardiac disease. Biological risk factors of hypercholestrol, family history, patient's history, hypertension, diabetes and smoking will be tabled to show availability of this data.

Secondary endpoints – gender effect
Gender data should be available on all from randomisation

Sensitivity analysis – presence of helicobacter will be defined as helicobacter seropostitivity at any point in the trial.

Pathology – local pathology results will be used

7. Patient Groups for Analysis

To address the primary, secondary and exploratory aims, analyses will be conducted on an intention to treat (ITT) sample, defined in this case to mean all patients randomised irrespective of how much or which treatment they actually received.

In addressing the secondary and exploratory aims the ITT sample may be modified (MITT) due to missing data in the variables required for the analysis, i.e. every randomised patient who has the data for the particular analysis will be included using the treatment code to which they were randomised.

For the PPI comparison the ITT sample will be all patients randomised. For the aspirin comparison, the ITT sample will exclude those only taking part in the PPI randomisation.

A sensitivity analysis will be carried out on a per protocol (PP) basis to examine the robustness of the treatment effect conclusions for the primary aim dependent on the actual drugs the patients received. It is accepted that the samples used may be biased since all randomised patients will not be included. The PP sample will be patients who are considered to have received a therapeutic dose of the treatment as defined in Appendix 1. Any patients randomised to no aspirin who take a regular dose of aspirin will be excluded from this analysis. Regular use of other NSAID will not change the inclusion of a patient in the PP analysis
Since this is a trial examining the policy of offering a PPI/aspirin combination, a formal PP analysis is not appropriate.

A sensitivity analysis will be carried out using the corrected Barrett's Length at randomisation – for 60 patients the length given at randomisation was found to be incorrect after the randomisation had occurred. Barrett's is measured as columnar and maximal and reports can also give distance from teeth and confusion between these was not recognised prior to the trial start.

The main analysis will be done on the full sample now expected to be 2500 men and women. The entire analysis may be repeated on the male and female groups separately.

8. Primary Aim Comparative Analyses

8.1 Evaluation/definition of Primary Outcome

See section 6 "reliability of data" for the calculation of survival time for the primary objective.

8.2 Statistical Methods Used for Analysis of Primary Outcome

In the first instance, the primary outcome will be analysed to achieve the primary objectives assuming there is no interaction between treatments.

Kaplan-Meier curves will be produced and logrank analysis will be used to compare the time to primary outcome between those receiving aspirin and those not and also between those receiving low PPI and high dose PPI. A significance level of p=0.05 will be used. An accelerated failure time (AFT)

model will be constructed for each co-primary to adjust the treatment comparisons for age, length of Barrett's metaplasia and presence of intestinal metaplasia (as used in the minimisation procedure). To increase precision, the alternative co-primary will be added into the model. An AFT model was chosen so that the estimates of the effect are in terms of a time ratio (ratio of expected survival times in the aspirin/no aspirin comparison or low PPI/high PPI comparison).

The logrank results will be presented in a table

	total	Number of events	Logrank and p value
Aspirin **Aspirin** **No aspirin**			
PPI **Low PPI** **High PPI**			

The time ratios will be presented with 95% confidence intervals and will form the definitive analysis for the primary aim.

If proportional hazards assumptions are valid, a Cox regression model with just aspirin treatment and then another with just PPI treatment will be presented. A Cox model for each coprimary considering the effect of treatment whilst adjusting for age, length of Barrett's Metaplasia and presence of intestinal metaplasia will be also be presented. To increase precision, the alternative co-primary will be added into the model. These models will give hazard ratios with associated 95% confidence intervals. These are provided for comparison with other research.

All centres will be analysed together. It is expected that either STATA, SAS, SPLUS or other similar statistical software will be used for the analysis.

8.3 Adjustment of P values for Multiple Testing

It is intended that no formal adjustment for multiple significance testing will be made.

8.4 Missing Data

It is expected that all data will be available to make the primary objective analysable on all patients randomised. Nonetheless, patients will be lost to follow-up and this will be summarised as outlined above to allow a reader to adjust their interpretation of the results accordingly.

The secondary endpoints will not all be complete and some analysis will be done on a subgroup of the patients who have the data. In these cases the patients in the analysis and those left out will be described.

We do not expect to impute any data for the analysis in this plan.

8.5 Pre-specified Subgroup Analysis

The analysis applies to males and females (planned to be 500) combined but will be repeated on males only and on just the females.

8.6 Sensitivity analysis

As specified in section 7 using PP sample and variables as randomised.

A Kaplan-Meier plot will be drawn with a line for each of the four treatment groups to look for a visual sign of interaction between the treatments. An interaction term will also be added to AFT and COX models containing both of the treatment main effect to test for significance (although this will have low power). If statistically significant interaction is found, the results will also be presented for aspirin within each PPI treatment group and for PPI within each aspirin group.

The estimate of the treatment effect in AFT and Cox models will be adjusted for presence of helicobacter.

AFT and Cox models will be adjusted for gender as well as the minimisation variables.

AFT and Cox models will be adjusted for NSAID and for Cox Inhibitor use. Use will be defined as more than 1 month continuously and as more than 6 months continuously.

AFT and Cox models with stratifying variables will be redone using actual Barrett's length at baseline.

The primary analysis will be repeated using all oesophageal cancers instead of just adenocarcinoma of the oesophagus.

The primary analysis will be repeated censoring follow-up when patients start taking NSAID.

The primary analysis for PPI will be repeated censoring patients when they start taking another medicine for Barrett's (data in EOT form reasons for withdrawal 'other' and in Follow Up form in 'treatments for Barrett's other than esomeprazole').

8.7 Treatment by Centre Interaction

Consistency of effect will be assessed across the centres by informal examination of the within centre effects. There will be limited capacity to investigate these formally and it is noted that such centre effects are expected by chance.

8.8 Supporting information

The withdrawal rates from aspirin and PPI will be described as well as the dose of drug taken at time points throughout the trial.

9. Analysis to address secondary aims

There are many secondary aims so these will be described under each aim

- To assess whether intervention with aspirin results in decreased high-grade dysplasia, in decreased all cause mortality, in decreased adenocarcinoma oesophageal cancer incidence, and in decreased cause-specific mortality when each is considered separately

ANALYSIS: As for primary using the AFT model. As for primary using COX model for all cause mortality. For the COX model for high grade dysplasia, adenocarcinoma oesophageal cancer incidence and cause-specific mortality a competing risks approach will be used since there are expected to be many competing deaths for these endpoints.
For oesophageal cancer, as a sensitivity, the analysis will be rerun including all oesophageal cancers rather than just adenocarcinomas.

- To assess whether intervention with high dose PPI results in decreased high-grade dysplasia, in decreased all cause mortality, in decreased oesophageal cancer incidence and in decreased cause-specific mortality when each is considered separately

ANALYSIS: As for primary using the AFT model. As for primary using COX model for all cause mortality. For the COX model for high grade dysplasia,

adenocarcinoma oesophageal cancer incidence and cause-specific mortality a competing risks approach will be used since there are expected to be many competing deaths for these endpoints.
For oesophageal cancer, as a sensitivity, the analysis will be rerun including all oesophageal cancers rather than just adenocarcinomas.

- Are there clinical and molecular risk factors than can be identified in BM for the development of BA?

ANALYSIS: Variables to be specified at the end of the trial. These variables of interest will be added to the accelerated failure time model with the event oesophageal adenocarcinoma and those found to be significant will be reported. The variables will also be considered in the competing risk COX approach.;

- What is the cost effectiveness of aspirin and/or PPI treatment in the prevention of BA?

The analysis will not be undertaken by CSM; to be analysed by Prof Paul Moayyedi. Information from protocol:
An economic analysis will be undertaken which compares the mean per patient cost of the alternative trial arms using resource use data collected in the trial and appropriate UK unit costs. Differential costs could then be related to conversion to BA. To increase the value to decision makers, the economic data collected in the trial could be synthesised with other evidence by relating long-term (lifetime) health service costs to long-term quality-adjusted life expectancy. The analysis would be based on a state transition model, which would characterise how patients progress from BM to dysplasia and to BA competing risk of death. The annual cost and health-related quality of life associated with each stage of the disease will be estimated using trial data collected and literature. In addition, some limited data collection in appropriate non-trial patients would be undertaken. Assessments will be completed at baseline and every two years at the time of endoscopy.

- To assess whether intervention with PPI and/or aspirin induces changes in the expression of molecular markers for BA. To assess the genomics of aspirin sensitivity (efficacy and side effects)

ANALYSIS: Based on analysis of the samples, this aim will be carried out after the end of the trial under a separate analysis plan.

- To assess how quality of life is affected by the different treatments.

It was not possible to collect quality of life across time and so this secondary cannot be completed.

- We will also assess what the biological risk factors are for cardiac disease and aspirin resistance.

Cardiac disease will be ascertained from SAE. The risk factors to be tested are hypercholestrol, family history of cardiac disease, patient history of cardiac disease, hypertension, diabetes or smoking. A logistic model predicting cardiac disease using these factors will be built. To consider aspirin resistance, a logistic model predicting cardiac disease in those taking aspirin will be built and described.
These 2 models will be repeated for the data from the time of the new tumour questions using cancer instead of cardiac disease.

- To assess if gender is prognostic for conversion or survival

ANALYSIS: Using the survival time calculated for the primary objective, a logrank test to assess the effect of gender with both males and females in the sample will be reported in a table as for the primary objective. Create an AFT model looking at the gender effect adjusting for age, length of Barrett's and presence of intestinal metaplasia so that there is a time ratio with 95% confidence interval for the gender effect. Repeat with a Cox model if assumptions valid. Redo both of these models with treatment also included.

Exploratory aim
To assess aspirin's role on the development of colorectal adenomas and cancer
Use as outcome the time to colorectal cancer or colorectal adenoma. Using a logrank test, consider if aspirin use is prognostic.

To collect and bank samples for use in future ethically approved studies
To be analysed under other protocols and analysis plans.

And the exploratory uses of the data

- Describe Barrett's length over repeat biopsies and investigate a change in length from baseline to 2 years between treatment groups.

ANALYSIS: For all patients, assess if Barrett's metaplasia length shows a trend with time using repeated measures anova.
Using the baseline and 2 year length, compare the Barrett's length difference between treatment groups using paired ttest.

- Can intervention with aspirin result in a lower stage of adenocarcinoma ? Can intervention with PPI result in a lower stage of adenocarcinoma ?

ANALYSIS: Chi square trend test on the following tables

	No aspirin	Aspirin
None		
Stage I		
Stage II		
Stage III		
Stage IV		

	Low PPI	High PPI
None		
Stage I		
Stage II		
Stage III		
Stage IV		

Note: this ignores the different follow-up times

- Does adjustment for oesophagectomy, ablation therapy, endoscopic mucosal resection change the treatment effect ?

ANALYSIS: Within the multivariate AFT and COX models, add time dependent variables for the above and note the new treatment effect.

- Is the event rate different for those previous users of aspirin who were not randomised to it compared to previous users who continued with aspirin as their randomised treatment?

ANALYSIS: Kaplan-Meier curve and Logrank of the previous users of aspirin who were not randomised to it versus those randomised to aspirin.

- Investigation of the hypothesis that aspirin will have a chemopreventive effect on all epithelial cancers using the same mechanism it does to reduce oesophageal cancer**,** however, high dose PPI therapy may arguably increase colon cancer incidence as gastrin levels increase and this hormone is a known stimulant for colon cancer

ANALYSIS: Define outcome as time to epithelial cancer (oesophageal, colorectal, other gi or skin). For those randomised to aspirin and low PPI or to no aspirin and low PPI, use an AFT model with stratification variables to assess if aspirin is predicting outcome.

Where possible, all these analyses will use the ITT sample.

9.1 Resource Use and Cost Data

The health economics data for this trial will not be analysed by CSM; the analysis will be undertaken by Prof P Moayyedi.

10. Exploratory analyses not specified prior to receiving data

Any analyses not specified in this document will be exploratory in nature.

11. Serious adverse events

Serious adverse events are defined as those that are fatal, life threatening, disabling, a congenital birth defect, another important medical event which require hospitalisation or prolongation of hospitalisation. Any serious adverse event occurring whilst a participant is continuing in the study, until trial medication is stopped will be recorded. All serious adverse events will be described in tables including causality, CTCAEgrade, outcome and seriousness. A comparison of specific serious adverse events between the treatment groups (aspirin vs no aspirin and low PPI vs high PPI) will be assessed by examination of 95% confidence intervals for the difference in incidence. An overall category for any serious adverse event will also be compared using the chi square test. The analysis will be conducted by intention to treat. An overall category for renal impairment (dehydration, renal, obstruction) will be compared using the chi square test.

SAE and death major bleeds will be summed and expressed as number of major bleeds per 1000 person years. 2 major bleeds per 10000 person years is expected.

12. Data from Canada

One site in Canada ran a trial of 50 patients using the same protocol. The entire analysis plan will be re-run including this data when it is available.

Appendix 1: Interpretation of therapeutic dose

Therapeutic dose: 1 year for esomeprazole and 6 months for aspirin.

For the individual treatment groups this means

Treatment	Therapeutic dose
Low ppi no aspirin High ppi no aspirin	1 year of esomeprazole at randomised dose OR event before 1 year and esomeprazole at randomised dose until the event
Low ppi aspirin High ppi aspirin	1 year of esomeprazole at randomised dose and at least 6 months of aspirin at randomised dose OR event before 6 months and esomeprazole and aspirin at randomised dose until the event OR event between 6 and 12 months and esomeprazole at randomised dose until the event and aspirin at randomised dose for at least 6 months

Since this is a trial looking at the policy of offering the drugs (whether the patients take them or not is part of what we need to know), looking at the primary objectives on the patients who received a therapeutic dose of the drugs is a sensitivity analysis rather than a main concern.

We can only classify patients as receiving a therapeutic dose or not if they are a year into the trial and we have received the data through from the pharmacy.

Any patients randomised to no aspirin (first row of the above table) who takes a regular dose of aspirin will be considered not to have received a therapeutic dose irrespective of their esomeprazole dose history.

Appendix 2: Variables to be used in analysis
(Not exhaustive)

To check rand

VARIABLE	FORM
Length of Barrett's at rand or diagnosis	Randomisation or IR patient history
Age at rand	Randomisation
IM	

To describe sample

VARIABLE	FORM
Alcohol consumption	PR Patient history
Smoking history	PR Patient history
Cardiac history	IR Patent history
Duration of Barrett's pre randomisation	IR Patient history
Length of Barrett's at rand or diagnosis	Randomisation or IR patient history
Age at rand	Randomisation

Primary aim

VARIABLE	FORM
Length of Barrett's at rand or diagnosis	Randomisation or IR patient history
Age at rand	Randomisation
Rand date	Randomisation
Last follow-up date Date of death Cause of death	Follow-up form
Date high grade dysplasia	Endoscopy form or follow-up form
Date adenocarcinoma	Endoscopy form or follow-up form

Secondary aims

VARIABLE	FORM
Cardiac history	IR Patient history
Stage of cancer	Endoscopy report

Document History

Document history AspECT statistical analysis plan		
Ver	**Date**	**Comments**
0.1	16th March 2006	First draft by SBL
0.2	21st April 2006	Draft for review by Rachel Waters
0.3	12th July 2006	Draft for review by rest of MSG support and Janusz Jankowski
0.4	18th August 2006	Updated to Jan 2006 version of the protocol Added Info from 20th July meeting with Janusz Added comments/clarifications from 25th July meeting with MSG support Passed to Louise Linsell and Rachel Waters to review and sign. Passed to DSMC
0.5	29th Sept 2006	Added DMC comments Females randomised separately 100 Previous aspirin users to be entered
0.51	15th August 2007	Added gender to the tables in section 6.2 Previous aspirin use deleted from tables in 6.2 since not collected since June 2007 on shortened forms Analysis of effect of high PPI by each event separately Analysis of effect of aspirin use by each event separately Randomisation to PPI arms only Clarification that Barretts length is at rand if available, otherwise it is at diagnosis Addition to appendix 2 – specifying data needed
052	20th Nov 2007	Changed event list to clarify what cause-specific death means.
0.53	Jan 2008	Added version 9 important changes to randomisation
0.6	30thJan2008	Updated to version 9 of the protocol To be submitted to DSMC April 2008 Logo added on front page
0.7	May 2008	
0.8	19th Sep 2008	Name of file changed to CSM policy of statistical analysis plan Updated to version 10 of the protocol Sample size section tidied, now giving only the original and the guidance for 2500 patients
0.81	3rd Oct 2008	Contents page corrected Sample size exploratory calculation changed. Minor changes of text for clarity.
0.82	14th Oct2008	Typo corrected
0.83	6th Jan 2009	Put in analysis for third secondary aim and for last secondary aim
1.0	29Jul2013	Updated to version 10 of protocol All contact details in section1 updated Secondary endpoint split into those within the study and those investigated as substudies Oesophagitis secondary endpoint added Number of events added to sample size information Definition of oesophageal cancer secondary endpoint added Interim analysis, emphasised no plans to disseminate Clarification that trial uses local pathology Describe withdrawal rates Added several sensitivity analyses Added exploratory of dose/time effect Added specific look at renal impairment Express number of major bleeds per 1000 years
1.1	20Aug2013	Changed interim analysis p value to 0.001 as standard
1.2	25Jan2014	Made notes for next version in red on page 1
1.21	6Apr2017	Updated contacts, protocol number and footer
1.22	20Apr2017	Updated to protocol 12
1.31	12Jun2017	Updated after conversation with PI
1.32		Minor edits – each typo corrected as it is seen
1.33		Minor edits
1.34		Minor edits
1.35		Minor edits
1.36		Minor edits
1.37		Minor edits
1.38	15Aug2017	Adelyn's comments added, explicit statement made about Canada data
2.0	24Aug2017	All changes accepted and comments deleted