Use of Acetylsalicylic Acid (ASA) for Enhanced Early Detection of Colorectal Neoplasms

EudraCT No.: 2011-005603-32
Internal study code: K357
Statistical Analysis Plan
1 Background

1.1 Objectives

This document provides the statistical analysis plan for the study “Use of Acetylsalicylic (ASA) for Enhanced Early Detection of Colorectal Neoplasms”. The study aims to investigate, according to the study protocol v1.5, diagnostic performance (sensitivity, specificity, positive and negative predictive values, likelihood ratios, area under the curve) of 2 immunochemical Fecal Occult Blood Tests (iFOBTs) for detecting advanced colorectal neoplasms after a single dose of acetylsalicylic acid as compared to placebo.

Secondary objectives, again according to the study protocol v1.5, comprise studying gender-specific performance of the 2 iFOBTs and the possible gain in diagnostic performance by stool sampling on multiple days, as well as studying the safety of single-dose acetylsalicylic acid in the selected population. An optional additional secondary objective is to collect blood samples for additional biomarker analyses.

1.2 Trial design

According to the study protocol v1.5:

The planned study is a randomized, double-blind, placebo-controlled, multicenter study.

Overall, 2,400 eligible participants with an age of 40 to 80 years and with no recent use of acetylsalicylic acid will be recruited into this study when visiting one of the participating gastroenterology private practices or hospitals (outpatient clinics) for an informative appointment, which is routinely scheduled a few weeks before colonoscopy. In addition to participants scheduled for a screening colonoscopy, patients visiting the study centers for a diagnostic colonoscopy but excluding patients with a history of colorectal cancer (see list of in- and exclusion criteria for details) will also be asked to participate. Thus, the colonoscopy procedure is planned and conducted independently from our study.

During this first appointment (day 0), participants will be informed about the study, will receive the printed study information, will have enough time for consideration and will have the opportunity to ask questions. After obtaining written informed consent a detailed medical history and information on the use of medication will be collected and in- and exclusion criteria will be checked. Women who are not postmenopausal have a negative pregnancy test. If in- and exclusion criteria are not met the patient will be considered as a “screening failure” (the reasons will be documented in the patient identification list). If all criteria are met (and the patient consented to the optional blood sampling) four extra tubes of blood (2 EDTA tubes and 2 serum tubes) will be taken for analysis of additional biomarkers (optional). In addition, patients will receive four immunological FOBT-kits for each of two different iFOBTs (FOBGold®Tube Screen, similar test used in our preliminary study (1), as well as the FD Hb/Hp Complex quick test, an internationally well-established test (2,3) (www.frostdiagnostika.de)), so eight kits in total, with instructions on when and how to use them, including a device which is hung in the toilet and aids in easy collection of the stool samples (“Sammelhilfe”). Participants will be randomized to receive either a single dose of 300 mg acetylsalicylic acid or placebo (thereby, a unique participant number is generated and documented). Both participants and their physicians will be blinded with respect to the study medication. Participants will be asked to collect a baseline stool sample on day 0 or on day 1, before taking the study medication. The study medication should be taken on day 1. If the participant cannot collect a stool sample on the first day (e.g. due to severe constipation), this stool sample should be skipped and the study medication should be taken on day 1 without a baseline stool sample. Further stool samples are to be collected from day 3 onwards on 3 different, preferably consecutive days (day 3, 4, and 5). If stool collection on these days is not possible because of
constipation or other reasons, stool collection may be postponed to the subsequent day. It is allowed for the participant to give also only two stool samples (before taking study medications and on day 3). At each time point at which stool samples are collected, 1 kit of each test should be used and the actual day documented. The stool sampling devices ensures collection of defined volumes of stool which are given to a buffer that hinders degradation of hemoglobin. All stool samples should be collected at the participants’ homes and prior to initiation of large bowel preparation for colonoscopy and sent by mail to the coordinating center (Heidelberg) using special pre-paid and addressed mailing devices. Participants will receive a form (Participant diary) on which they have to document when they took the study medication, and the date of stool collection. Finally, participants will be asked to fill out a standardized questionnaire addressing potential determinants of risk of colorectal neoplasms and of test performance, including general participant characteristics, comorbidities, and lifestyle factors. They can either do this during their visit to the study center or at home.

Colonoscopy is planned and conducted independently from our study. However, the investigator will ensure that there are at least 4 acetylsalicylic acid-free days between taking the study medication and the colonoscopy. Although a single dose of acetylsalicylic acid is not considered a contra-indication for elective colonoscopy, this time interval was chosen to minimize any possibility of an increased risk of bleeding at colonoscopy or endoscopic removal of small polyps (see paragraph on safety for further details) (4-6). The colonoscopy is performed in the setting of screening for colorectal cancer or diagnostic workup and as such, it is not part of the study. However, the findings from screening colonoscopy will be collected and used in our analysis.

The maximum time between start of the study and colonoscopy is set at 3 months. At the end of the trial, sensitivity analyses are performed for participants whose colonoscopy is postponed for more than 3 months for any reason. If the colonoscopy does not take place at all, the participant will be considered as a drop-out.

Colonoscopy (and histology) reports will be pseudonymized (i.e. any personal data will be removed and the participant number will be noted) and will be sent from the study center to the coordinating center in Heidelberg following the end of the study for each participant. The relationship between blood samples, stool samples, and colonoscopy results will be established only by the participant number. The information from colonoscopy records will be extracted in a standardized, blinded manner by trained staff.

1.3 Data-sets

The analysis will be performed on data contained in the following documents

- **Main analysis data-set** will contain information relative to the blood and stool sample collection, the participant’s diary, colonoscopy results, iFOBTs results, participant’s questionnaire, demographic and randomization information, and auxiliary variables;
- **eCRF** will contain information regarding compliance with inclusion and exclusion criteria, timing of the planned colonoscopy, demographic and randomization information, vital signs, information regarding the end of the study and about adverse events.
2 Analysis sets

2.1 Definitions

The full analysis set will comprise all recruited subjects complying with the protocol inclusion and exclusion criteria (see section 2.2). This set will be used to tabulate demographic characteristics and compliance to requested timing of stool sample collection.

The per-protocol analysis set will comprise subjects providing at least one stool sample at exactly the expected day of collection, i.e. the first stool sample any time before, or on the same day, of medication intake (this represents a minor deviation from the protocol, which specifies that the first stool sample can be taken at day -1 or 0	extsuperscript{1}), later stool samples at either day 2, 3, or 4. Inclusion and exclusion criteria (see section 2.2), the requirement that the sample provides a valid test result, and that colonoscopy is performed after the stool sample is collected, will apply.

The intention-to-screen analysis set will be formed by participants providing at least one stool sample leading to a valid test result and collected before colonoscopy (or information not available).

2.2 Full list of inclusion and exclusion criteria

The following exclusion and inclusion criteria will apply, according to study protocol v1.5:

Inclusion criteria
- Age 40 to 80 years (both males and females; premenopausal women must have a negative pregnancy test before inclusion into the trial, postmenopausal women are defined as women who have not had menstrual bleeding for at least 12 months, or have been surgically sterilized)
- Planned screening or diagnostic colonoscopy
- Able to speak and understand German sufficiently to be able to give written informed consent and comply with the trial requirements

Exclusion criteria
- Factors potentially influencing the primary endpoint
  - Diseases/symptoms
    - Chronic inflammatory bowel disease (e.g. Crohn’s disease, ulcerative colitis)
    - Colonoscopy due to positive fecal occult blood test
    - History of colorectal cancer
    - Angiodysplasia of the colon
    - Anamnestic or observed blood loss per anum
  - Use of any of the following drugs
    - Within 2 weeks before the trial
      - Anticoagulants (including, but not limited to heparin, vitamin K antagonists [e.g. phenprocoumon, warfarin], direct thrombin inhibitors [e.g. dabigatran], or factor Xa inhibitors [e.g. apixaban, rivaroxaban])

	extsuperscript{1} To improve clarity, the numbering of the days is now sequentially defined with respect to study medication intake, which is assumed to take place at day 0. Consequently day 3, 4, and 5 of section 1.2, will now be referred to as day 2, 3, and 4.
• Antiplatelet drugs (e.g. clopidogrel, prasugrel, ticlopidine)
  ▪ Within 1 week before the trial
  ▪ Acetylsalicylic acid
  ▪ Within 3 days before the trial
    ▪ NSAIDs including selective COX-2 inhibitors

• Factors potentially affecting the safety
  o Any current clinically relevant signs and symptoms, including
    ▪ Signs and symptoms suggesting acute peptic ulcer disease
    ▪ Known clinically relevant thrombocytopenia
    ▪ Acute infection
    ▪ Volume deficit (dehydration)
    ▪ Any currently present allergy with dermal reactions, pruritus, or urticaria
    ▪ Severe or insufficiently controlled asthma
    ▪ Severe kidney or liver diseases (e.g. GFR < 30 ml/min, liver cirrhosis)
    ▪ Severe, not sufficiently treated heart failure (as judged by the investigator)
    ▪ Severe, poorly controlled arterial hypertension
    ▪ Any other unclear symptoms needing further investigation in the opinion of the investigator
  o Any of the following anamnestic findings
    ▪ History of severe gastrointestinal bleeding
    ▪ Known hemorrhagic diathesis, including, but not limited to, hypoprothrombinemia, severe thrombocytopenia, hemophilia
    ▪ Asthma, except for patients who have used acetylsalicylic acid in the past without negative effects
    ▪ Hypersensitivity against salicylic acid or other ingredients of the trial drugs
    ▪ Previous intolerance to NSAIDs including selective COX-2 inhibitors, or antirheumatic medications
    ▪ Severe gout (e.g. recurrent attacks)
    ▪ Hereditary oxaluria
    ▪ Known G6PD or glutathione peroxidase deficiency
    ▪ Known epilepsy with generalized seizures
    ▪ Severe cardiac diseases (including, but not limited to, myocardial infarction in the past 6 months)
  o Intention to use any of the following drugs during the trial
    ▪ Anticoagulants
    ▪ Antiplatelet drugs
    ▪ NSAIDs including selective COX-2 inhibitors
    ▪ Methotrexate ≥ 15 mg/week
    ▪ Systemically administered glucocorticoids
    ▪ Selective serotonin reuptake inhibitors (SSRIs)
    ▪ Valproic acid
  o Planned surgery/dental treatment during participation in the trial

• Other factors
  o Known or suspected relevant alcohol abuse
  o Known or suspected illicit drug abuse
  o Pregnancy
  o Suspected non-compliance with the trial procedures
  o Participation in another clinical trial
3 Analysis variables

3.1 Demography and baseline characteristics

The following demographic variables will be collected and used for stratification purposes or description of the full analysis set:

- **Sex** Categorical variable with two levels: male or female;
- **Year of consent** Quantitative variable, expressed in years.
- **Year of birth** Quantitative variable, expressed in years.
- **Primary indication of colonoscopy** Categorical variable with two levels: screening or diagnostic.
- **Weight** Quantitative variable, expressed in Kg;
- **Height** Quantitative variable, expressed in cm;
- **Smoking status** Categorical variable with two levels: (current) smoker or nonsmoker.

3.2 Primary variables

The primary variable will be represented by the sensitivity of the FOBGold®Tube Screen test to detect advanced neoplasms (colorectal cancer or advanced adenoma). It will be obtained from the following observed variables:

- **Quantitative test outcome of the FOBGold®Tube Screen test** Quantitative variable, expressed in ng Hb/ml Buffer.
- **Categorical test outcome of the FOBGold®Tube Screen test** Categorical variable with three levels: positive, negative or invalid test result. Positivity is defined at the manufacturer’s threshold and a lower threshold (17 μg Hb/g feces and 10.2 Hb/g feces).
- **Most advanced finding at colonoscopy** Categorical variable with eight levels: colorectal cancer, advanced adenoma, sessile serrated polyp greater or equal to 1cm, sessile serrated polyp smaller than 1cm, non-advanced adenoma, hyperplastic polyp, undefined polyp, no finding.

Secondary variables

The secondary variables will include sensitivity of the FD Hb/Hp Complex quick test, the area under the ROC curve for the FOBGold®Tube Screen test, as well as the specificities, the likelihood ratios, and the positive and negative predictive values of both tests, for detection of advanced neoplasms. Number of iFOBTs and number of colonoscopies after a positive iFOBT needed to detect one advanced neoplasm will also be evaluated. The following additional observed variable will be required:

- **Test outcome of the FD Hb/Hp Complex quick test** Categorical variable with four levels: positive, weakly positive, negative, or invalid test result.
3.3 Auxiliary variables

Two additional variables will be used to define the per-protocol and intention-to-screen analysis sets:

- **Difference between the date of stool sample collection and the date of study medication intake** Quantitative variable, expressed in days.

- **Difference between the date of colonoscopy and the date of stool sample collection** Quantitative variable, expressed in days.

4 Handling of missing values

Missing values in the test outcomes, as well as missing values for the day of stool sample collection, will be excluded from the analysis. Invalid test results in any of the test outcome variables will be categorized as missing values and excluded from the analysis. If multiple samples for a given test are collected on the same day, the first valid sample, i.e. the first in the sequence of scheduled iFOBTs, will be used.

5 Statistical analyses / methods

5.1 Subject disposition

A CONSORT flowchart will summarize the process of exclusion of recruited subjects which will not be included in the full analysis set (and consequently the per-protocol analysis set). In particular, the flowchart will show the number of recruited subjects, the number of subjects included in the full analysis set, and the number and reason of exclusion for the discarded subjects.

5.2 Demography and baseline characteristics

The trial population will be described according to demographic characteristics (age, sex), primary indication of colonoscopy and colonoscopy outcomes. Age will be obtained as the difference between year of consent and year of birth, and categorized into 5-year classes. Absolute and percent frequencies for the total population, as well as separately for the control and intervention group population, will be provided. Differences between the control and intervention group will be tested via a chi-square or Fisher’s exact test.

5.3 Exposition to treatment/Compliance

Absolute frequencies displaying the distribution of test samples across days of stool sample collection, as well as of missing and invalid samples will be tabulated. The frequencies will be further stratified by expected day of stool sample collection, test, and intervention group (treatment or placebo).

5.4 Primary analysis

The primary analysis will focus on the sensitivity of the FOBGold®Tube Screen test for the detection of advanced neoplasms.
Sensitivity is defined as the proportion of subjects obtaining a positive test result among the diseased subjects. Manufacturer’s threshold (17 μg Hb/g feces) as well as FD Hb/Hp Complex quick test threshold for positivity (10.2 μg Hb/g feces) will be considered. The analysis will test the null hypothesis of no change in sensitivity after the intake of a single dose of acetylsalicylic acid, as compared to placebo. It will be performed on participants in the per-protocol analysis set providing samples at day 2. Wald adjusted confidence intervals will be computed for sensitivity values (Agresti-Coull adjustment) (7) and the differences between placebo and intervention group (Agresti-Caffo adjustment) (8). Statistical significance will be defined by a two-sided p-value <0.05.

5.5 Secondary analyses

The secondary analyses will evaluate the sensitivity of the FD Hb/Hp Complex quick test, the area under the ROC curve for the FOBGold®Tube Screen test, as well as the specificities, the likelihood ratios, the positive and negative predictive values for the detection of advanced neoplasms for both tests, and number of iFOBTs and number of colonoscopies after a positive iFOBT needed to detect one advanced neoplasm. Positivity of the FD Hb/Hp Complex quick test will be defined by either a positive or weakly positive test result. Specificity is defined as the proportion of subjects obtaining a negative test result among the healthy subjects. Positive predictive value is defined as the proportion of diseased subjects among the subjects obtaining a positive test result, while negative predictive value is defined as the proportion of healthy subjects among the subjects obtaining a negative test result. Likelihood ratio for a given test result is defined as the ratio between the proportion of subjects obtaining the given test result among the diseased subjects, and among the healthy subjects. The ROC curve is obtained by plotting the proportion of subjects obtaining a positive test result among the healthy subjects against sensitivity, for increasing thresholds of test positivity. Number of iFOBTs and number of colonoscopies after a positive iFOBT needed to detect one advanced neoplasm for a given test are defined as the ratio between the total number of performed iFOBTs and the number of subjects obtaining a positive test result among the diseased subjects, and the ratio between the total number of positive iFOBTs (thus leading to colonoscopy) and the number of subjects obtaining a positive test result among the diseased subjects, respectively.

The null hypothesis of no change in sensitivity of the FD Hb/Hp Complex quick test, as well as specificities, positive or negative predictive values after the intake of single dose of acetylsalicylic acid of both tests, as compared to placebo, will be tested. Wald adjusted confidence intervals will be computed for the estimated values (Agresti-Coull adjustment) (7) and the differences between placebo and intervention group (Agresti-Caffo adjustment) (8). Statistical significance will be defined by a two-sided p-value <0.05. Wald adjusted confidence intervals will also be computed for the estimated number of iFOBTs and number of colonoscopies after a positive iFOBT needed to detect one advanced neoplasm (Agresti-Coull adjustment) (7).

The total and partial area under the curve (truncated at specificity equal to 0.8) in ROC analyses for the FOBGold®Tube Screen test will be computed, and their significance tested at level 0.05, using the DeLong et al. (9) method or bootstrap, as implemented in the pROC R package (10). The ROC curve will be plotted. Additionally, a generalized mixed effects model for binomial data with logit link function having sensitivity or specificity as response variables, and intervention group, sex, test and day of stool sample collection as explanatory variables, will be fitted. Replicate measurements on the same participant will be accounted for by a random intercept.

The analyses will be performed on participants in the per-protocol and intention-to-screen analysis set providing samples at day 2. Changes in sensitivity and specificity across days of stool sample collection will be plotted. The specificity and sensitivity values will be computed for the per-protocol analysis set at day “0”
(before study medication), 2, 3 and 4. Differences will be taken between values at each of the days 2, 3, and 4 with respect to values at day 0. Finally, diagnostic performance when test results from multiple days are combined will also be evaluated. Serious adverse events will be evaluated and listed.

5.6 Planned subgroup analyses

Sensitivity and specificity analyses will be stratified by sex, age (as dichotomized in lower than 60 and greater or equal to 60 years old), smoking status and BMI (as obtained from the ratio between the weight and the square of the height in meters, and dichotomized in lower than 25 and greater or equal to 25 Kg/m²). Sensitivity will be additionally stratified by most advanced finding at colonoscopy (sessile serrated polyp>=1cm and advanced adenoma). Interaction with the stratification variable will be assessed by fitting a generalized linear model for binomial data with logit link function, and evaluating the statistical significance of the interaction coefficient at level 0.05.

6 Software

All analyses will be performed with R statistical software. The PropCIs package will be used for the sensitivity and specificity confidence intervals. The pROC package (10) will be used, instead, for the computation and plotting of the ROC curve, as well as for estimating the area under the ROC curve and its significance.

7 Changes to the plan

Primary analysis set

The primary analysis is now performed on participants in the intention-to-screen analysis set providing samples at day 2

Treatment of missing values

Missing values for the day of stool sample collection are included consistently with the intention-to-screen analysis framework. Independence between missing values for the test outcome defining the primary endpoint (FOBGold®Tube Screen test on day 2, intention-to-screen analysis set) and group allocation is tested via Fisher’s exact test. The proportion of missing values is also evaluated. A decision on their treatment is based on the outcomes of the two evaluations.

8 References


