1 PROTOCOL SYNOPSIS

Trial Title A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway	
Trial code **ALASCCA**	**EudraCT No** **2015-004240-19**
Coordinating Investigator Professor Anna Martling, Department of Molecular medicine and Surgery Karolinska Institutet, Center of Digestive Diseases Gastrocentrum , Karolinska University Hospital	
Trial centre(s) Departments of Surgery in Sweden actively registering patients in the Swedish ColoRectal Cancer Registry (SCRCR)	
Trial period Estimated date of first patient randomized Q3-Q4 2015 Estimated date of last patient completed Q3-Q4 2022	**Phase of development** Phase III

Objectives and endpoints

Primary objective

- To determine whether adjuvant treatment with 160 mg Acetylsalicylic Acid (ASA) once daily for 3 years can improve Time To Recurrence (TTR) in patients with colorectal cancer with somatic alterations in the *PIK3CA* (exon 9 and 20), compared with placebo.

Secondary objective

- To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years can improve Disease-Free Survival (DFS) in patients with colorectal cancer with somatic alterations in the *PIK3CA* (exon 9 and 20) compared with placebo.
- To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years can improve DFS in patients with colorectal cancer with somatic alterations in the *PIK3CA* (other than exon 9 and 20), *PIK3R1* or *PTEN* genes, compared with placebo.
- To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years can improve TTR in patients with colorectal cancer with somatic alterations in the *PIK3CA* (other than exon 9 and 20), *PIK3R1* or *PTEN* genes compared with placebo.
- To compare overall survival (OS) at 5 years from randomization in patients receiving low-dose ASA compared with placebo.
- To assess overall safety and tolerability.

Primary Endpoint

- TTR at 3 years, in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20

Secondary Endpoints

- DFS at 3 years, in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20.
- DFS at 3 years, in patients with tumors harboring *PIK3CA* mutations other than in exon 9 and 20 and in *PIK3R1* and *PTEN*.
- TTR at 3 years, in patients with tumors harboring *PIK3CA* mutations other than in exon 9 and 20 and in *PIK3R1* and *PTEN*.
- OS at 5 years, in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20, other than in exon 9 and 20 and in *PIK3R1* and *PTEN*.

Safety Endpoints

- Frequency and severity of adverse events (AE)
- Cerebral and gastric haemorrhage (for interims analysis)

Exploratory objectives

- Description and presentation of data on patients already on administration with ASA
- In a technical pilot study (50 patients), ensure the handling and shipping of the gene samples

In addition, the study will add clinical value for the patient's future treatment by genetically identifying the tumor's potential mutation in the genes *BRAF*, *KRAS*, *NRAS* and Mismatch Repair (MMR) status (Microsatellite Instability, MSI, alt Immunohistochemistry, IHC, for MMR genes). This is already performed at the hospitals according to clinical praxis.

Trial design

ALASCCA (**A**djuvant **L**ow dose **AS**pirin in **C**olorectal **CA**ncer)
is a randomized, parallel group, double blind, multicenter, placebo-controlled, biomarker-based study of adjuvant treatment with ASA in colorectal cancer. Patients (adult male and female) with colon cancer or rectal cancer pathology stage II-III and somatic alterations in *PIK3CA*, *PIK3R1*, *or PTEN* are considered for the study. The study will compare DFS, time to relapse and OS and will also assess safety and tolerability.

Patients will be randomized to receive 160 mg ASA or placebo. Patients already treated with ASA at inclusion will be included in an observation group. The treatment can be administered alone or in combination with adjuvant chemotherapy. Last date for randomization and start of treatment is 12 weeks postoperatively. The treatment will be administered for 3 years. There will be a follow-up period for two years. Outside the trial, the patient will be treated according to standard care at the site.

A phone contact will be made 3 months after the randomization visit and thereafter every 6th month. The patients will also visit the site 6 months after randomization and thereafter every 6th month i.e. the patients will be in contact with the site every 3rd month. There will be a visit/phone contact at the end of the follow-up period.

An interim analysis will be made on safety, i.e. incidence and type of serious bleeding complication grade > 1 after 12 months. An independent safety data monitoring committee

will be responsible for evaluating and follow-up of the safety.
The primary statistical analysis will be done at 3 years of study treatment and an analysis of overall survival (OS) will be done at 5 years.

Number of patients planned
If 12% of the patients screened have hotspot mutations (exon 9 and 20) in *PI3KCA* a total of 3900 patients will needed to be screened. This also includes approximately 15 % of the patients that will be excluded due to tumor stage 1.

Diagnosis and main eligibility criteria

Inclusion criteria

- Tumor with somatic alterations in *PIK3CA, PIK3R1 or PTEN*
- Colon or rectal cancer tumor stage II-III
- Patient between18-80 years (including)
- Radical surgery according to surgeon and pathologist
- Karnofsky performance status ≥60%
- Platelets ≥ 100 x 10^9 / L
- Clean Colonoscopy or Computed Tomography (CT) colon within 3 months preoperatively or postoperatively but before randomization
- Patient able to swallow tablets
- Patient able to understand and sign written informed consent

Exclusion criteria

- Hereditary colorectal cancer linked to familial colonic polyposis or Lynch syndrome
- Inflammatory bowel disease (Crohn's disease or ulcerative colitis)
- Distant metastases
- Other cancers (excluding colorectal cancer or other skin cancer than melanoma) within 3 years from screening
- Known bleeding diathesis (such as hemophilia)
- Concomitant antiplatelet therapy (eg clopidrogrel or ticlopidine) or anticoagulant therapy (warfarin or low molecular weight heparin). Post-operative treatment with low molecular weight heparin must be withdrawn before administration of study treatment
- Active gastritis or peptic ulcer, or significant surgical post-op bleeding, within the previous three months assessed at screening and randomization
- Ongoing regular use of corticosteroids, Nonsteroidal Anti-Inflammatory Drug (NSAID)
- Uncontrolled hypertension according to Investigator's judgment
- Clinically significant liver impairment according to Investigators judgment
- Existing renal failure according to Investigator's judgment. Renal failure with decreased creatinine clearance <60 should lead to consultation with a nephrologist.
- Significant medical illness that would interfere with study participation
- Pregnancy or breastfeeding females
- Known allergy to NSAIDs or ASA
- Current participation in another clinical trial that will be in conflict with the present study
- Patients who are unlikely to comply with the protocol (e.g. uncooperative attitude,

inability to return for subsequent visits) and/or otherwise considered by the Investigator to be unlikely to complete the study

Patients with regular administration of ASA, defined as >3 doses/week, will be included in the observation group. Other additional regular use of ASA must be withdrawn before randomization.

Investigational Medical Product (IMP)

Acetylsalicylic acid (ASA) 160 mg, orally once daily

Placebo tablet orally once daily

Duration of treatment

The duration of treatment will be 3 years

Duration of patients involvement in the trial

The patients will participate in the study for a total of 5 years. The treatment period will last for 3 years and the follow-up period will be 2 years.

Safety assessments

Safety assessments will be AEs.

An interim analysis will be made on safety i.e incidence and type of serious bleeding complication grade > 1 after 12 months. An independent safety data monitoring committee will be responsible for evaluating and follow-up of the safety.

Statistical methods

The statistical analyses will be described in detail in the Statistical Analysis Plan including a Data Display Plan, which will be in a final draft version prior to study start and finalized and approved before database lock.
All data will be presented using descriptive statistics. Results will be presented in total and by treatment group. Continuous variables will be summarized using mean, standard deviation, median, minimum and maximum. Categorical variables will be summarized using the number and percentage of patients.

The primary efficacy variable is TTR. Subjects who have not experienced loco-regional recurrence, distant metastases or death from colorectal cancer at time of statistical analysis will be censored at the time of the latest assessment. The log-rank test will be applied to test for difference in TTR between the treatment groups.

Kaplan-Meier estimates will be used for the estimation of median times to an event and their confidence intervals. Secondary endpoints will be assessed using the log-rank test or a Cox regression model, with time to event (relapse, secondary malignancy or death) as response variable and treatment group as factor.

All secondary variables will be presented descriptively with point estimate and 95% confidence intervals.

The data from the observation group will only be presented descriptively.

Determination of sample size
Primary endpoint: TTR.

Power was set to 80%, the accrual period to 24 months, total follow-up to 36 months and significance level to 0.05 (two-sided). Assuming an exponential recurrence distribution, a three-year recurrence-free survival among placebo treated patients of 75%, and a recurrence Hazard Ratio (HR) of 0.50 for patients treated with ASA as compared to placebo, 204 patients need to be randomized in each group with an estimated 20% drop-out rate.

If 12% of the patients screened have hotspot mutations (exon 9 and 20) in *PI3KCA* a total of 3900 patients will be needed to be screened. This also includes approximately 15 % of the patients that will be excluded due to tumor stage 1.The estimated number can be changed after the first 1000 patients have been included when better estimated on drop-out and mutation rate in *PIK3CA* are available.