A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway

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1 PROTOCOL SYNOPSIS

<table>
<tr><td colspan="2">Trial Title
A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway</td></tr>
<tr><td>Trial code
ALASCCA</td><td>EudraCT No
2015-004240-19</td></tr>
<tr><td colspan="2">Coordinating Investigator

Professor Anna Martling, Department of Molecular medicine and Surgery
Karolinska Institutet, Center of Digestive Diseases Gastrocentrum , Karolinska University Hospital</td></tr>
<tr><td colspan="2">Trial centre(s)

Departments of Surgery in Sweden actively registering patients in the Swedish ColoRectal Cancer Registry (SCRCR)</td></tr>
<tr><td>Trial period

Estimated date of first patient randomized Q3-Q4 2015
Estimated date of last patient completed Q3-Q4 2022</td><td>Phase of development

Phase III</td></tr>
<tr><td colspan="2">Objectives and endpoints

<u>Primary objective</u>
• 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.

<u>Secondary objective</u>
• 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.

<u>Primary Endpoint</u>
• TTR at 3 years, in patients with tumors harboring PIK3CA mutations in exon 9 and 20</td></tr>
</table>

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.

2 REVISION HISTORY

Number	Document date	Revision (main changes from previous version)
1.0	24 March 2015	
2.0	28 January 2016	Synopsis: EudraCT No added. Synopsis clarification added: 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. Inclusion criteria: Karnofsky performance status changed from ≥70% to ≥60%. This allows for participation of patients with lower Karnofsky score. Section 10.5 The patients will be carefully informed that they only can take the tablets included in the study and no additional ASA. Compliance will be checked every third months. Section 10.8.3: In addition, 2x4 ml (EDTA) plasma samples for biobank storage is added, to allow for future analyses. Clarification regarding statistical analysis section 10.13: 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. Inclusion criteria: adding of birth control methods. Section 11.7.7 Emergency unblinding, is rephrased to clarify the unblinding process.

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4 PROTOCOL SIGNATURE PAGE

Sponsor and Coordinating Investigator

Trial title:

A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway

Sponsor

Professor Anna Martling	*Signature*	*Date*

Coordinating Investigator

Professor Anna Martling	*Signature*	*Date*

INVESTIGATOR SIGNATURE PAGE

Investigator

Trial title:

A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway

"I agree to the terms of this trial protocol. I will conduct the study in accordance with the procedures specified in the protocol, the ethical principles in the latest version of the Declaration of Helsinki, ICH Good Clinical Practice and applicable regulatory requirements".

Investigator Name	*Signature*	*Date*

5 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
AE	Adverse Event
ALASCCA	**A**djuvant **L**ow dose **AS**pirin in **C**olorectal **Ca**ncer
ASA	Acetylsalicylic Acid
CEA	Carcinoembryonic Antigen
COX-2	Cyclooxygenase-2
CT	Computed Tomography
DFS	Disease-Free Survival
eCRF	electronic Case Report Form
FAP	Full Analysis Population
GCP	Good Clinical Practice
HR	Hazard Ratio
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IXRS	Interactive Response System
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Mismatch Repair
NSAID	Nonsteroidal Anti-Inflammatory Drug
OS	Overall Survival
PP	Per Protocol
QC	Quality Control
RA	Regulatory Authorities
SAE	Serious Adverse Event
SCRCR	Swedish ColoRectal Cancer Registry
SUSAR	Suspected Unexpected Serious Adverse Reaction
TTR	Time To Recurrence

6 ETHICS

6.1 Ethical and Regulatory review

Necessary approvals of the Trial Protocol, the Patient Information and Informed Consent Form (ICF) must be obtained before enrolment of any patient into the trial. Furthermore, it is the responsibility of the Sponsor to keep the applicable Independent Ethics Committee (IEC) informed of any Suspected Unexpected Serious Adverse Reactions (SUSARs) and any substantial amendments to the protocol during the trial period. The written approval from the IEC, including a trial identification and the date of approval, will be filed at the Sponsor representative and at the trial site(s) together with a list of the IEC members, their titles or occupation, and their institutional affiliations.

6.2 Ethical conduct of the trial

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human patients that were adopted in 1964 by the 18th World Medical Assembly, in Helsinki, Finland, with later revisions.

6.3 Patient information and consent

It is the responsibility of the Investigator to give each patient (or the patient's legal representative), adequate verbal and written information regarding the objectives and the procedures of the trial as well as any risks or inconvenience involved before including the patient in the trial. The patient (or the patient's legal representative) should be informed that by signing the ICF he/she authorizes monitor(s), auditor(s), the IEC and the Regulatory Authorities (RA) to have direct access to the patient's medical records for verification of clinical trial procedures. The patient must be informed about the right to withdraw from the trial at any time. The patient should be allowed sufficient time for consideration of the proposal.

It is the responsibility of the Investigator to obtain signed informed consent from all patients before including them in the trial. The ICF must be signed and dated before any trial-specific procedures are performed, including screening procedures. The signed ICFs must be filed by the Investigator for possible future audits and/or inspections.

The final version of the Patient Information and ICF is submitted to the Ethics Committee(s) and concerned RA and must not be changed without permission from the Sponsor and the local IEC.

6.4 Patient data protection

The Investigator must file a patient identification list which includes sufficient information to link records, i.e. the Case Report Form (CRF) and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

The patients will be informed that the data will be stored and analyzed by computer, that Swedish regulations for the handling of computerized data will be followed and that identification of individual patient data will only be possible for the Investigator.

The patients will be informed about the possibility of inspections/audits of relevant parts of the clinical records by representatives of the Sponsor, and/or RA. Authorization to direct access to the patient's clinical records, as described above, is given by signing the ICF.

7 INVESTIGATOR(S) AND TRIAL ADMINISTRATIVE STRUCTURE

This is an Investigator initiated study where Professor Anna Martling will be the Sponsor representative and the coordinating Investigator. The Sponsor is ALASCCA Study group, that includes the members listed in the table below. Departments of surgery in Sweden with a defined high volume of colorectal resections and that are actively registering patients in the Swedish ColoRectal Cancer Registry (SCRCR) will be asked to participate in the study.

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Bengt Glimelius	Sponsor	Professor of Oncology, Department of Immunology, Genetics and Pathology, Uppsala University	Department of Oncology, Akademiska Sjukhuset, 85185 Uppsala	+46 18 611 55 13	Bengt.glimelius@igp.uu.se
Scandinvian CRO	CRO		Skolgatan 8 Box 15027 750 15 Uppsala	+46 18 100 5500	

8 INTRODUCTION

Colorectal cancer annually affects 6000 individuals in Sweden.[1] Refined surgery, addition of radiotherapy (in rectal cancer) and novel medical treatments have improved survival. Despite a localized primary tumor, 20-40% of patients with colorectal cancer stage II-III develop metastatic disease with a high risk of death from the disease.[2, 3] More efficient adjuvant therapies thus have a strong potential to improve survival in colorectal cancer.

Colorectal cancer represents a model system for how mutations in cancer-associated genes accumulate during progression from adenoma to cancer. These mutations have been of great importance for the diagnosis of hereditary forms of colorectal cancer and to predict of treatment response. Inherited mutations in the APC gene cause familial colonic polyposis and mutations in mismatch repair genes *MLH1, MSH2, MSH6* and *PMS2* cause Lynch syndrome.[4, 5] Individuals with hereditary predisposition are offered surveillance or preventive procedures to decrease morbidity and mortality from colorectal cancer. Acquired mutations in the genes *KRAS, NRAS* and *BRAF* are in metastatic colorectal cancer used to predict response to and benefit from targeted inhibitors of *EGFR*.[6] Hence, there are currently diagnostic markers for hereditary colorectal cancer and treatment predictive markers for advanced disease, but a lack of markers to predict benefit from adjuvant therapy.

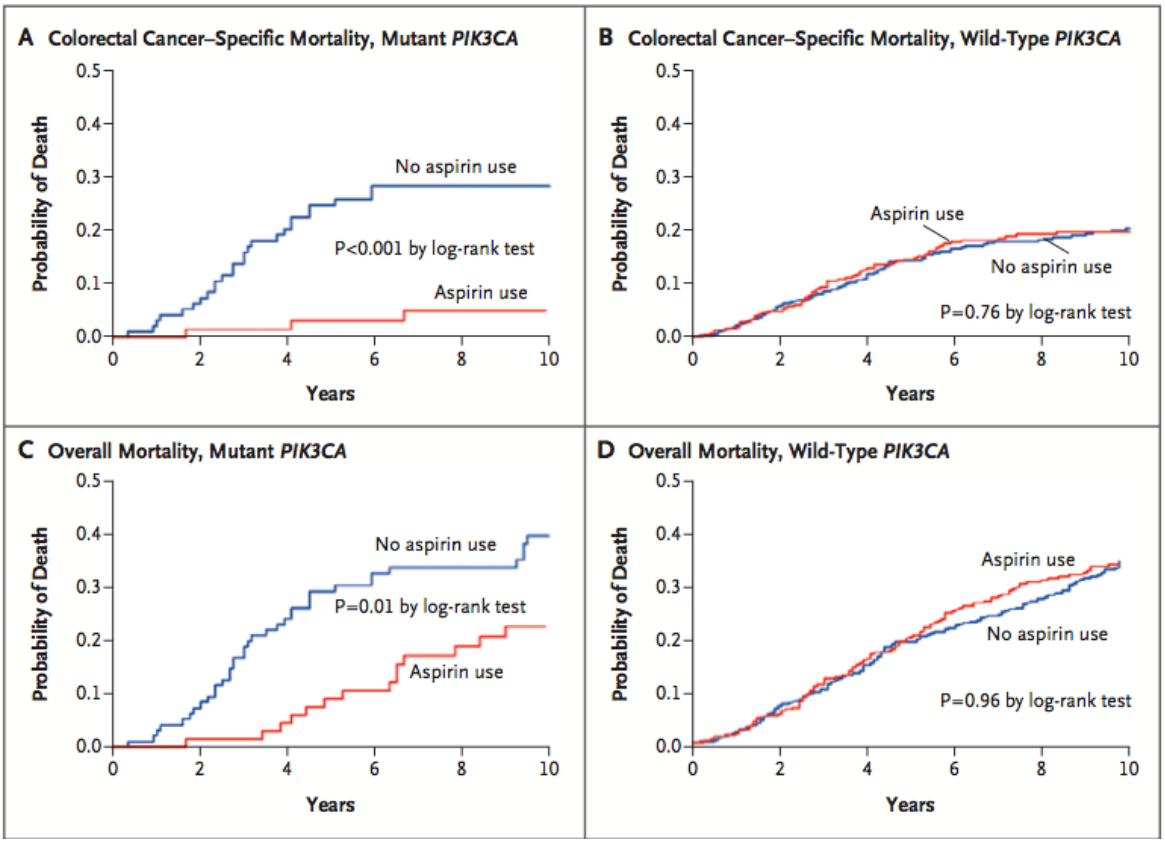
Low-dose acetylsalicylic acid (ASA) therapy reduces the risk of colorectal cancer, reduces the incidence of adenomas in patients with previous adenomas or cancer and decreases the incidence of metastases after diagnosis of colorectal cancer. [7, 8, 9] These findings have been confirmed in both animal studies and in human studies. ASA works by inhibiting the enzyme Cyclooxygenase-2 (COX-2) that is overexpressed in colorectal cancer.[10] Several observational studies have also suggested that treatment with ASA after a colorectal cancer diagnosis improves disease-free and/or overall survival (OS). These observations have been made in unselected patient populations and have shown a benefit equivalent to a Hazard Ratio (HR) of about 0.55 for colorectal cancer-specific survival.[11]

The PI3K pathway is frequently altered in colorectal cancer e.g. through mutations/inactivation of genes including *PIK3CA, PIK3R1 IGF2, IGR2* and *PTEN*. Recently, acquired mutations in the *PIK3CA* gene were shown to predict benefit from treatment with ASA.[12, 13] While patients whose tumors did not carry *PIK3CA* a mutation had no benefit from aspirin therapy, patients whose tumors carry *PIK3CA* mutations had an HR of 0.11-0.18 for colorectal cancer-specific death. These data are based on retrospective analyzes and require confirmation in prospective randomized trials to establish treatment recommendations with ASA in patients with colorectal cancer. The connection between *PIK3CA* mutations and prediction of response to ASA fits well with the effect that *PIK3CA* mutation have in terms of increased AKT signaling, which in turn up-regulates COX-2. Inhibition of COX-2 in tumor cells with mutant *PIK3CA* will inhibit tumor cell survival.

Liao et al. (2012) [12] used a retrospective cohort (Nurses' Health Study) to study 964 patients with colorectal cancer. *PIK3CA* mutations were detected in 17% of the tumors. Patients whose tumors lacked mutations showed no difference in prognosis in relation to ASA use, whereas patients whose tumors carried *PIK3CA* mutations showed longer cancer-specific survival (HR for cancer-related death 0.18). Only 3/66 patients with *PIK3CA* mutated tumors died of the disease. Patients who took aspirin before the cancer diagnosis, as those who started after diagnosed had the benefit of treatment - if the tumor had a *PIK3CA* mutation.

Domingo et al. (2013) [13] analyzed retrospectively the 896 patients enrolled in a study of COX-inhibitor rofecoxib (Vioxx). *PIK3CA* mutations were identified in 12% of the tumors and did not

correlate with the Vioxx treatment. In the ASA-treated group, the risk of relapse correlated with *PIK3CA* mutations. Of the patients whose tumors did not have *PIK3CA* mutations, 23/90 (26%) relapsed, compared to 0/14 ASA-treated patients with *PIK3CA* mutant tumors. This finding corresponds to a HR for relapse at 0.11.



From Liao et al. N Engl J Med 367;17, 2012

Acquired changes in the PI3K signaling pathway are important for cell proliferation and cell survival and are estimated to be existing in one-third of colorectal cancers.[14] In addition to activating mutations in *PIK3CA*, mutations and deletions of *PIK3R1* or *PTEN* genes are present and it may be hypothesized that these changes may also have an effect related to benefit from ASA treatment though these genes were not investigated in the studies by Liao et al and Domingo et al. [12, 13]

The two retrospective studies point in the same direction and show a highly significant reduction in the risk of relapse in patients with *PIK3CA*-mutated colorectal cancer who for some reason were treated with ASA, whereas patients with wild-type tumors did not benefit from the treatment. [12, 13] On the other hand, two recent publications with similar design and sample size did not show any effect of low dose ASA in patients with PIK3CA mutations.[15, 16]

The role of mutations in the other PI3K genes, *PIK3R1* or *PTEN*, in relation to effect form ASA is unknown. These genes can, because of their central location in the PI3K pathway, be hypothesized to have the same effect on ASA benefit, but this remain to be demonstrated. The ALASCCA study design is based on power calculations in relation to *PIK3CA* mutations in exon 9 and 20 (hotspots), but will include an additional 12% patients with mutations in PIK3CA (mutations outside exon 9 and 20) *PIK3R1 (*mutations*)* or *PTEN* (mutations and homozygote deletions).

Adjuvant chemotherapy is administrated to patients with high risk of relapse. The treatment has side effects and reduces the risk of relapse in stage II by about 20% and about 35-40% in stage III.

Since ASA has well-known and limited side effects it is not necessary with an extensive toxicity study. Adjuvant use of ASA can be used as monotherapy or in combination with adjuvant chemotherapy.

The current study will focus at patients with stage II and III colorectal cancer, who are at >10% risk of recurrence and will evaluate clinical benefit from adjuvant treatment with ASA in patients with mutations in the PI3K signaling pathway. The study will also introduce multi-gene molecular diagnostics in colorectal cancer, which benefits patients irrespective of mutation in relation to treatment with targeted drugs in case of disease recurrence.

9 TRIAL OBJECTIVES AND ENDPOINTS

9.1 Primary objective

- To determine whether adjuvant treatment with 160 mg 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.

9.2 Primary endpoint

- Time To Recurrence (TTR) at 3 years in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20

9.3 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 versus placebo.
- To assess overall safety and tolerability.

9.4 Secondary endpoint

- Disease-Free Survival (DFS) at 3 years, in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20.
- Disease-Free Survival (DFS) at 3 years, in patients with tumors harboring *PIK3CA* mutations other than in exon 9 and 20 and in *PIK3R1* and *PTEN*.
- Time To Recurrence (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*.

9.5 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.

9.6 Safety Endpoints

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

10 INVESTIGATIONAL PLAN

10.1 Overall trial design

ALASCCA is a randomized, parallel group, double blind, multicenter, placebo-controlled, biomarker-based study of adjuvant treatment with ASA in colorectal cancer. The study will compare DFS, time to relapse and OS and will assess safety and tolerability.

Patients (adult male and female) with colorectal cancer clinical stage I-III with localized disease are considered for the study. Patients will be screened for inclusion at the time of surgery of the tumor (at time of routine patient visit before elective surgery or postoperatively within 12 weeks in case of emergency procedure or if screening was missed preoperatively). After inclusion and when surgery is performed, patients with *PIK3* mutations and stage II and III tumors will be randomized to receive 160 mg ASA or placebo orally. Last date for randomization and start of treatment is 12 weeks postoperatively. The treatment can be administered alone or in combination with adjuvant chemotherapy. The choice of any adjuvant chemotherapy is made by the Investigator and should follow the guidelines in the National Care Program (www.cancercentrum.se). 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 also be a visit/phone contact at the end of the follow-up period.

A total of 3900 patients will be screened in order to include 408 patients with *PIK3CA (Exon 9 and 20)* mutated tumors in each treatment arm (Group A). With an estimated 20 % drop-out rate, 204 patients will be randomized in each arm. This also includes approximately 15 % of the patients that will be excluded due to tumor stage 1.

An additional 408 patients with mutations in other PI3K pathway genes *PIK3CA* (other than exon 9 and 20*), PIK3R1 or PTEN* will also be randomized in each arm and will be treated as a separate group in the analyses (Group B). With an estimated 20 % drop-out rate, 204 patients will be randomized in each arm.

The randomization process is expected to take 24 months.

Patients already treated with ASA at inclusion will be included in an observation group.

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.

For details see figure 1 and figure 2.

10.2 Study procedures

Patients planned for surgery of a colon or rectal cancer (clinical stage I-III) will be screened for this study. The investigations and assessments performed according to standard care of the surgery of the primary cancer in the colon or the rectum will not be part of the documentation of the study.

10.2.1 Screening (Visit 1)

Screening Part 1

The purpose with screening visit part 1 is to be able to select patients with alterations in *PIK3CA, PIK3R1 or PTEN* and colon or rectal cancer tumor stage II-III.

Pre-operation;

The screening visit will take place in connection of surgery of the primary tumor in the colon or rectum. The visit could preferably be at the same time as the pre-operative visit. Randomization will take place within 12 weeks after surgery.

Post-operation;

In case of emergency procedures or if screening was missed preoperatively, patients can be screened postoperatively. The screening visit can not be performed later than allowing randomization 12 weeks after surgery.

The Investigator will explain to the patient the nature and aim of the study, its procedures, possible side effects, requirements and restrictions. The patient will sign the written informed consent before any study related procedures are performed.

The following assessments will be performed during the screening visit;

- Obtain written informed consent

Post-operative characterization of;

- tumor tissue for mutations in the genes
- 4 ml (EDTA) blood samples for extraction of germline DNA that will be used for the molecular profiling. In addition, 2 x 4 ml (EDTA) plasma sample for biobank storage for any future analysis. The sample will be taken in connection with screening part 1.
- tumor stage

Patients without somatic alterations in *PIK3CA, PIK3R or PTEN* or tumor stage I or IV will be withdrawn from the study.

Screening Part 2 (Visit 2)

Patients with somatic alterations in *PIK3CA, PIK3R1 or PTEN* and tumor stage II-III are able for screening part 2. This visit will take place after screening visit part 1 when the result of the mutation analysis and tumor stage is available. The following assessments will be performed;

- Karnofsky performance status
- Documentation of platelets; if results are older than 60 days from visit 2, a sample will be taken.
- Documentation of Carcinoembryonic antigen (CEA), if results are older than 30 days from visit 2, a sample will be taken
- Colonoscopy or Computed Tomography (CT) colon available within 3 months preoperatively
- Obtain medical and surgical history
- Record demographic information
- Record prior and concomitant medication
- Distant recurrence investigation available within 3 months preoperatively or for emergency cases immediately postoperatively
- Pregnancy test (urine dipstick)
- Assess of all inclusion and exclusion criteria

10.2.2 Randomization (Visit 3)

Patients with somatic alterations in *PIK3CA, PIK3R1 or PTEN* and tumor pathological stage II-III that fulfil the inclusion criteria and none of the exclusion criteria will be randomized to one of the treatment arms. If all data is available from screening visit part 2, the randomization visit can be performed at the same time. The treatment period begins at the Randomization visit and ends after 3 years.

The following assessments will be performed during the randomization visit;

- Randomization of the patient
- Provide the patient with IMP for 6 months + 1 week

10.2.3 Phone calls (Visit 4, 6, 8, 10, 12 and 14)

The patient will be contacted every 3rd months by a study nurse. A visit window of ± 21 days is allowed. The following will be reviewed every 3rd months;

- Assess AEs
- Compliance check
- Record concomitant medication

10.2.4 Treatment visits (Visit 5, 7, 9, 11, 13, 15)

The patient will visit the site every 6 months. A visit window of ± 21 days is allowed. Follow-up is otherwise according to standard national guidelines.

The following assessments will be performed during the treatment visits;

- Assess AEs
- Record concomitant medication
- Compliance and drug accountability check
- Provide the patient with IMP for 6 months + 1 week (not applicable for visit 15)

In addition to the items that will be performed during the treatments visits, the following will be performed at the time of visit 7 and 15 (after 1 and 3 years);

- CT/MRI (Magnetic Resonance Imaging) thorax and abdomen
- CEA

In case of any recurrence the patient will be taken care of according to standard treatment at the site and will be withdrawn from further study treatment.

10.2.5 Follow-up period (Final visit 16)

The follow-up period will start after the final treatment visit and ends after 2 years. The patient will visit the site or be contacted by phone at the end of the follow-up period i.e. after 2 years. A visit window of ± 30 days is allowed. Any other assessment e.g. endoscopic investigation that will be performed according to standard care will not be documented in the study.

The following assessments will be performed during the follow-up visit;

- Documentation of CEA, if not available a sample will be taken
- Review if there is any clinical suspicion of recurrence

10.3 Observation group

Patients already on ASA treatment, > 3 doses per week, at the screening visit will be included in the observation group. These patients will be handled according to standard care and will only be followed through the Swedish ColoRectal Cancer Registry.

Figure 1 Study design

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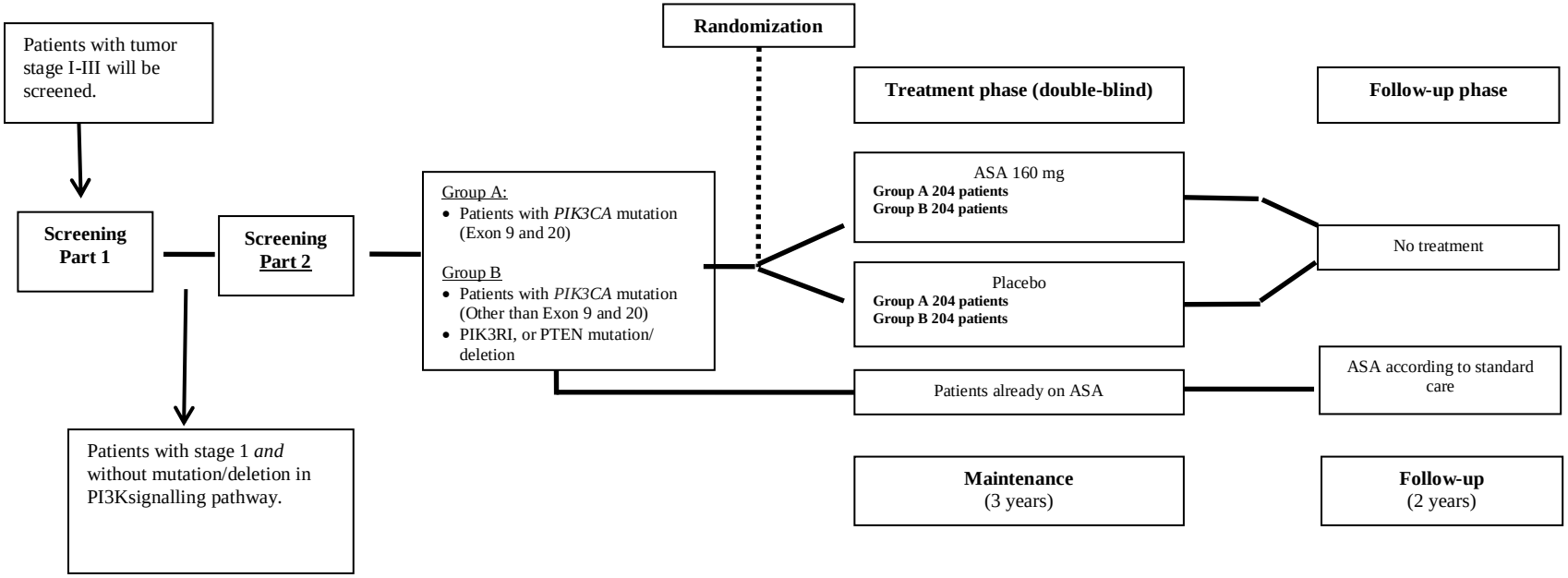

Figure 2 Schedule of activities

Activities	Screening		Randomiza-tion[1]	Treatment Period												F-U period
Visits	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V16**
Weeks	≤-12	X[2]	0													
Months				3	6	9	12	15	18	21	24	27	30	33	36	60
Phone call				X		X		X		X		X		X		X
Visit window (days)				±21	±21	±21	±21	±21	±21	±21	±21	±21	±21	±21	±21	±30
Informed consent	X															
Demographics		X														
Medical and surgical history		X														
Prior. and concom. Medication		X		X	X	X	X	X	X	X	X	X	X	X	X	
Inclusion/Exclusion Criteria		X														
Karnofsky status		X														
Distant recurrence investigation		X														
Colonoscopy or CT colon		X														
CT thorax and abdomen							X								X	
Characterizing of tumor stage	X															
Characterizing of tumor tissue for mutations	X															
Blood sampling for germline DNA extraction	X															
Randomization		X[3]	X													
Platelets		X														
CEA		X					X								X	X
Pregnancy test urine dipstick (female patients)		X														
IMP administration			X		X		X		X		X		X			
Compliance and drug accountability check				X	X	X	X	X	X	X	X	X	X	X	X	
AEs				X	X	X	X	X	X	X	X	X	X	X	X	X[4]

1) Randomization will take place within 12 weeks from surgery.
2) V2 will take place when the result of the mutation analysis and tumor stage is available.
3) If all results are available with regards to inclusion and exclusion criteria the randomization visit can take place at visit 2.
4) Review of clinical recurrence

10.4 Rationale for trial design, doses and control group

In order to evaluate the ASA effect on AEs and efficacy, a placebo control group is added. To avoid bias the study is double-blinded to participants, Investigator and sponsor.

Rationale for 3 years of treatment: Previous studies on ASA have described effect after 3 years of treatment and this treatment time is relevant also in relation to the standardized follow-up for patients with colorectal cancer who in the standard program undergo medical and radiologic evaluations after 1 and 3 years following surgery.

Rationale for the dose selected: Studies that have described an effect from ASA treatment in colorectal cancer have detected effect at doses of 0.5-5 tablets of 325 mg/week (HR 0.57) and a stronger effect (HR 0.47) at doses of >6 tablets. A dose of 650 mg ASA/week has been defined as the lower limit. [12] Studies of the primary preventive effect from ASA have demonstrated an effect from daily doses of 300 mg. [17,] 9 There is thus evidence for an effective dose between 100-300 mg/day, which led us to choose the 160 mg daily regimen.

10.5 Risk/benefit assessment

Regular ASA is associated with an increased risk of gastrointestinal bleeding of around 0.1% per year and a risk of intracranial haemorrhage that is estimated to be around 0.01%. [18] The risk is estimated to be dose-dependent and the risk of intracranial haemorrhage is probably linked to platelet inhibition.[19] The dose administrated in the study, 160 mg once daily, is considered so low that the risk of bleeding is small.

ASA uses is not recommended in pregnancy and may in rare cases cause sensitivity reactions and exacerbate asthma.

The side-effects of ASA are well documented and are estimated not to outnumber the potential benefit in relation to a reduced risk of death from cancer. Moreover, ASA use has preventive effects related to cardiovascular disease and thromboembolic disease.[19]

Due to the risk of adverse reactions and a possible interference with the study drug, patients are advised not to use Nonsteroidal Anti-Inflammatory Drug (NSAID), but to choose paracetamol in case of pain or fever. The patients will be carefully informed that they only can take the tablets included in the study and no additional ASA. Compliance will be checked every third months.

An independent Safety Monitoring Committee will be responsible for evaluating and follow-up of the safety.

10.6 Selection of trial population

The target group is patients with localized colon cancer or rectal cancer, stages II-III with tumor mutations in *PIK3CA* or related genes, regardless of adjuvant chemotherapy. The

patients will be selected from the surgery clinic. Each clinic will keep a log of all patients screened and included. The reason for screen failure should be stated for all patients screened but not included. The reason for withdrawal should be stated for all patients included but not completed.

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 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
- If a woman, before inclusion she must be:
- Postmenopausal, defined as: >45 years of age with amenorrhea for at least 24 months or

- If menstrual, surgically sterile (have had a hysterectomy or bilateral oophorectomy, tubal ligation, or otherwise be incapable of pregnancy), or

- If heterosexually active, practicing a highly effective method of birth control, including combined (estrogen and progestogen containing) hormonal prescription oral, intravaginal, transdermal contraceptives, or progestogen containing hormonal prescription oral, injectable, implantable contraceptives or intrauterine device (IUD), or intrauterine hormone-releasing system (IUS), or male partner sterilization, or

- If not heterosexually active (if refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the preferred and usual lifestyle of the subject).

Women must agree to continue using these methods of contraception throughout the study and up to the last dose of study treatment.

10.6.2 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, 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

10.6.3 Patients treated with ASA before randomization

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

10.6.4 Removal of trial patients from treatment or assessments

A patient should be withdrawn from the trial treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the expressed wish of the patient. Patients are free to discontinue their participation in the trial at any time. Irrespective of the reason for withdrawal and when ever possible, data from the Swedish colorectal registry can be collected.

The electronic case report form (eCRF) should be completed as far as possible and collected by the Monitor.

A withdrawn patient is not allowed to re-enter into the trial. A withdrawn patient will not be replaced. The criteria for withdrawal are the following:

- Patient wishes to discontinue
- Unacceptable AE, including laboratory values
- In case the patient experience a relapse
- Non-compliance with the study protocol
- Patient refuses to cooperate
- Other medical reasons
- Pregnancy
- Unblinding of treatment

10.7 Treatments

10.7.1 Identity of Investigational Medicinal Product (IMP)

The Investigator must ensure that the IMP will only be used in accordance with the protocol.

The IMPs for this study are;

- Acetylsalicylic acid 160 mg tablet
- Placebo tablets matching Acetylsalicylic acid 160 mg

10.7.2 Packaging, labeling and storage of IMP

The IMP will be manufactured and packed according to Good Manufacturing Procedure by Pfizer. The package will be labeled with the following information:

- Route of administration, quantity of dosage, pharmaceutical dosage form
- Directions for use
- Storage conditions
- Batch number
- Limited to investigational use
- Name of Sponsor
- Protocol Number
- Patient Number
- Name of Investigator
- Expiration date
- "Keep out of reach of children"

The IMP will be stored in room temperature (15-25°C).

10.7.3 Doses and treatment regimens

The patient will receive IMP in a package covering a period of 6 months+1 week. The patient will receive one package at each treatment visit. One tablet will be administered once daily. The tablet will be swallowed with a glass of water.

The randomized schedule will make sure that 408 patients (204 patients from Group A and Group B) will receive 160 mg ASA and 408 patients (204 patients from Group A and B) will receive a matching placebo tablet.

10.7.4 Investigational Medicinal Product accountability

The IMP will be released to the trial site(s) after approvals of the trial protocol have been received from the IEC and the RA and the trial agreement has been finalized and signed by the site.

The Investigator is responsible for keeping detailed records, which show the quantity of IMP that is received, stored, delivered to and taken out from the place of storage. Any discrepancies between dispensed and returned IMP must be explained and documented.

Products deliberately and/or accidentally destroyed by the Investigator/ Hospital Pharmacy or the patient must also be accounted for.

The Monitor will perform IMP accountability and make sure that all unused IMP is adequately destroyed/returned and documented.

10.7.5 Method of assigning patients to treatment groups

Randomization will be performed centrally using an Interactive Response System (IXRS). At time for randomization the Investigator or study personnel will contact or access the IXRS to register the patient. When the patient is confirmed as eligible, the Investigator will obtain the identification number of the IMP that should be dispensed to the patient.

The randomization schedule will be generated by an independent statistician.

10.7.6 Blinding

This is a double-blind study i.e. nor the patient or the Investigator will know which treatment that will be administrated to the patient. Placebo tablets matching the ASA will make sure that the blinding is kept.

10.7.7 Emergency unblinding for safety reason

In case of a rare emergency where, in the opinion of the Investigator, discontinuation of the study treatment is not sufficient and the study treatment must be unblinded in order to evaluate further course of action, the Investigator will access the IXRS system to initiate patient unblinding.
In the event the blind is broken, the sponsor should be informed as soon as possible. The date, time, and reason for unblinding must be documented by the IXRS, in the appropriate section of the eCRF, and in the source document. The documentation received from the IWRS indicating the code break must be retained with the subject's source documents in a secure manner.
Information about the treatment assignment must be restricted to designated study site staff who are providing immediate care to the patient.

As a backup system, the investigator or other treating physician can contact the sponsor staff according to instructions that will be provided to the investigators, to unblind the treatment for an individual patient in a manual way. The sponsor is not entitled to stall or reject unblinding.

Once the study treatment has been unblinded, the patient will complete the final visit of the treatment period.

10.7.8 Prior and concomitant therapy

Adjuvant chemotherapy or other therapy that are considered necessary for the patient's welfare may be given at the discretion of the Investigator.

Post operative treatment with low dose heparin must be withdrawn before administration of study treatment.

Administration of IMP will be temporary discontinued during enterostomy closure or any other procedure requiring administration of low molecular weight heparin. The IMP will be stopped the day before administration of the low molecular weight heparin. The IMP will be administered again the day after the treatment with low molecular weight heparin has stopped. The temporary discontinuation of IMP cannot be longer than 30 days.

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

All concomitant therapy used during the trial period must be recorded in the eCRF. Adjuvant chemotherapy administrated prior to the trial period will also be documented.

No other drug under investigation may be used concomitantly with the trial medication.

10.7.9 Treatment compliance

Treatment compliance will be made by medication count by the study personnel at the site.

10.8 Trial assessments

The following will be assessed during the study.

10.8.1 Demographic and baseline characteristics

- Demographics such as gender and age will be recorded.
- Karnofsky status will be assessed by the Investigator according to the following;
    - 100%, normal no complaints, no evidence of disease
    - 90% able to carry on normal activity, minor signs or symptoms of disease
    - 80% normal activity with effort, some signs or symptoms of disease
    - 70% cares for self, unable to carry on normal activity or to do active work
    - 60% require occasional assistance, but is able to care for most of their personal needs
- All relevant medical and surgical history with in the last 3 years must be documented in the eCRF.

- A colonoscopy or CT colon performed within 3 months from surgery demonstrating a clean colon i.e. no tumors available apart from the tumor that will be removed during surgery.
- A distant recurrence investigation according to local practice available within 3 months before surgery will be collected.

10.8.2 Molecular profiling of the tumors

Patients with stage I-III will be included in the screening process preoperatively or short after surgery. The pathology result will classify the tumor and patients with tumor stage II-III will be randomized. Patients with clinical tumor stage IV will be excluded before screening Part 1.

From all patients that are pre- or postoperatively eligible to be included in the ALASCCA study, a standardised piece (approximately 10x20x5 mm) is routinely cut from the primary tumor and fixated in formalin at the local pathology department. At the regional or local pathology department, this piece is sectioned, stained and the tumor content in the selected piece is estimated. This selected tumour piece is then sent to the selected laboratory of choice.

The following somatic alterations in the tumours are defined as inclusion criteria in the ALASCCA study:

- Somatic mutations affecting the function of the *PIK3CA*, *PIK3R1* or *PTEN* genes
- Homozygous deletion of the *PTEN* gene

In addition, mutation analysis of the *BRAF*, *KRAS* and *NRAS* genes and Mismatch Repair (MMR) status (Microsatellite instability, MSI, alt Immunohistochemistry, IHC, for MMR genes) are also performed on the selected tumour piece/sections and forwarded back to both the treating doctor and to the local pathology lab. These genomic alterations are already used in clinical routine today and will add value to the clinic.

The molecular analyses can either be performed at a regional pathology department or at the national reference laboratory at Science for Life Laboratory in Stockholm.

10.8.3 Laboratory measurements

If CEA and platelets are not available according to normal praxis, the samples will be taken. The handling of the samples will be made according to the routines at each site. Analysis will be made by the local lab at the sites.

- CEA- 3.5 ml (serum) will be taken for analysis of CEA.
- Platelets- 4 ml (EDTA) ml blood sample will be taken for analysis of platelets.
- A urine pregnancy test- will be taken on all female patients with child-bearing potential unless they
    - have undergone surgical sterilization
    - are post menopausal

  The pregnancy test will be a commercially available urine test.

- A 4 ml (EDTA) blood sample is sent directly to Karolinska Institutet Biobank for extraction of germline DNA to be used for the molecular profiling.
- Plasma- 2 x 4 ml (EDTA) blood will be taken for biobank storage for any future analysis.

10.8.4 Efficacy assessments

Primary clinical efficacy assessments

The primary clinical efficacy assessment will be CT thorax and abdomen. The CT will be performed, year 1 and 3 during the treatment phase.

Secondary clinical efficacy assessments

OS will be reviewed during the study. In case the patient dies during the study the cause of death will be recorded.

Time until relapse will also be documented.

10.8.5 Safety assessments

The safety assessment will include AEs. Especially focus will be on bleeding complications.

10.8.6 Safety Monitoring committee

An independent Safety Monitoring Committee will be responsible for evaluation of safety. The committee will consist of independent physicians. A separate document will be written describing the process in details

10.8.7 Adverse Events (AEs)

10.8.7.1 Definitions

Adverse Event
Any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

Serious Adverse Event (SAE)
An SAE is any untoward medical occurrence or event, at any dose, that:

- results in death
- is life threatening
- requires hospitalization or prolongation of existing hospitalization

- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect.

or

- other important medical event (example of such events are convulsions that do not result in hospitalization)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient's health or may require intervention to prevent one of the other outcomes listed in the definitions above. These AEs should also usually be considered as *serious.*

Suspected Unexpected Serious Adverse Reaction (SUSAR)
A SUSAR is a **s**uspected **u**nexpected **s**erious **a**dverse **r**eaction, potentially causally related to the investigational medicinal product, and will be reported to the appropriate IEC and RA.

10.8.7.2 Eliciting and recording of AEs

The method for collecting and recording AEs will be spontaneous reporting and open question e.g. "How have you felt since previous visit" during the treatment and follow-up period.

All AEs, serious and non-serious, and pre-existing conditions, should be recorded in the eCRFs. If no AE has occurred during the trial period, this should also be recorded.

Exception

Events directly connected to expected chemotherapeutics side effects e.g. nausea, diarrhoea neutropenia and infections related to neutropenia will not be reported as AEs.

Planned enterostomy closure performed after administration of IMP and complications after the closure will not be reported as AE/SAE.

In case of any bleeding events related to the above, the event should be reported as an AE/SAE.

The following evaluations are to be done by the Investigator in connection with the AE:

- *description of the AE*
  - If possible the Investigator uses the diagnosis for description of the event. If not the diagnosis is decided, report the primary sign or symptom as the AE or SAE term
- *seriousness*
  - Yes or No, see definition of a SAE for seriousness, as of above.
- *intensity*
  - Mild - Symptoms which are easily tolerated (acceptable)
  - Moderate - Symptoms which interfere with usual activity (disturbing)
  - Severe - Symptoms which give incapacity to do usual activity (unacceptable)

  If the intensity changes during the period of the AE the maximum intensity should be recorded.

- *duration of the AE (start - end)*
- *action taken* due to the AE
  - None
  - IMP stopped
- *causality rating with investigational medicinal product*
  - Not related: Indicates that the AE is definitely not related to the study drug.
  - Related: .
    - The AE could have been produced by the patient´s clinical status or the medicinal product.
    - The AE follows a reasonable temporal sequence from the time of administration of the medicinal product, and can not be reasonably explained by the known characteristics of the patient´s clinical status.
- *outcome of the* AE
  - Recovered/Resolved
  - Recovering/Resolving
  - Not recovered/Not resolved
  - Recovered/Resolved with sequelae
  - Fatal
  - Unknown

10.8.7.3 Pre-existing condition:

Events available at the first administration of IMP are part of baseline information (pre-existing conditions). These events will be considered as AE, only if they worsen after first administration of IMP.

Elective procedures or surgery planned before first administration of IMP is not considered as an AE/SAE.

10.8.7.4 Reporting of SAEs

All SAEs must be reported by the Investigator using phone, mail or fax within 24 hours from the Investigator's knowledge of the event to the Monitor or other members of the staff at the Sponsor, regardless of the time that may have elapsed from the time the event occurred to when the Investigator first learns of it. The initial report should contain as a minimum the following information:

- patient identification
- treatment specification
- diagnosis or symptoms
- time specification for the SAE

- name of the original reporter

A SAE report form must also be completed, signed by the Investigator and submitted to the monitor or the Sponsor no later than five working days after the initial information was received. Apart from the information above, this follow-up report should also contain the following information:

- assessment of intensity
- assessment of causality

No distinction should be made between the tested investigational product and the reference/comparator product regarding reporting of SAEs as long as the code is not broken.

Only SAEs that are both unexpected and assessed as related to IMP(s), i.e. SUSARs, are patient to expedited reporting to the appropriate IEC and RA, by the Sponsor.

10.8.7.5 Follow-up of unresolved AEs

If a patient is withdrawn due to an AE, or if an AE persists at the end of the trial treatment period, this should be followed up until the condition has ceased or until the patient is under professional medical care and a potential causality between IMP and the AE has been penetrated. An outcome assessment should be performed when an AE persists.

10.8.7.6 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the trial treatment must be stopped immediately, and the patient discontinued from participation in the trial. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the patient was discontinued from the trial.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. All outcomes of pregnancy must be reported to the Sponsor on the pregnancy report forms; Part I (at confirmed pregnancy) and Part II (pregnancy outcome).

10.8.7.7 Coding of AEs

All AEs will be coded according to Medical Dictionary for Regulatory Activities (MedDRA). All SAEs are coded upon reporting to the Sponsor. The coding will be performed by the Sponsor.

10.9 Data Management

Data from the study will be collected from the medical records and from the Swedish Colorectal Cancer Registry (SCRCR). Part of the information from the medical records will be entered into eCRF e.g. date of visits, study treatment, concomitant medication and AEs. Other parts will be imported to the study data base from the SCRCR e.g. tumor characteristics, surgical procedure, adjuvant treatment and long term follow-up.

Other external data e.g. laboratory data will be entered or transferred into the study database.

10.10 Swedish Colorectal Cancer Registry (SCRCR)

Patients included in the study are also part of the SCRCR. The register was launched in 1995 for rectal cancer and since 2007 it also includes patients with colon cancer. The database is continuously validated and updated and >98 % of the patients are included in the register. The SCRCR contains information such as patient demographics, (neo) adjuvant treatment, surgical procedure, tumor characteristics and short and long term follow-up.

10.11 Quality Control

A quality control (QC) of data will be performed to ensure that data entry and verification have been performed correctly in accordance to pre-defined instructions. The QC will be performed as described in the Data Management Plan. The QC will be performed before data is declared clean.

10.12 Data Quality Assurance

10.12.1 Case Report Forms (eCRFs)

The Monitors will review and verify the data collected in the eCRFs and SCRCR against the source documents during monitoring visits. The Monitors will address the discrepancies found in the data and will ensure that corrections to the data are properly made and documented by the site personnel. All corrections will be documented in an audit trail.

10.12.2 Monitoring

The trial site will be visited by the Monitor and/or the Clinical Trial Manager (co-monitoring) periodically at times agreed with the Investigator. It is the function of the Monitor to ascertain that all aspects of the protocol are complied with and that the conduct of the trial conforms to applicable regulatory requirements and established rules for Good Clinical Practice (GCP).

Preferably at the time of each monitoring visit, the Monitor will

- review the completed eCRFs to ascertain that items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol.
- verify that the data in the eCRF and SCRCR register are consistent with the clinical records or other relevant record (Source Data Verification) and that trial results are recorded completely and correctly.
- verify compliance with the procedures for reporting of SAEs, product accountability and record keeping.

For this purpose the Monitor must be given direct access to clinical records, original laboratory data, etc., as far as these relate to the trial and without jeopardizing patient

integrity. The Investigator and other relevant personnel should be available during the monitoring visit and should devote sufficient time.

10.12.3 Training of staff

All Investigators and staff carrying out observations of primary or other major efficacy variables involved in the trial should provide a curriculum vitae. The Investigator will keep a list of all personnel involved in the trial together with their function and trial related duties delegated. He/she will ensure that appropriate trial related training is given to all of these staff, and that any new information of relevance to the performance of this trial is forwarded to the staff involved.

Before inclusion of patients the Monitor will perform a trial initiation visit to inform and train relevant trial staff.

10.12.4 Audit and inspections

The trial site may be patient to quality assurance audit by the Sponsor or someone appointed for this task by the Sponsor. A RA may request to make an inspection of the trial site. The procedures of such a visit would be similar to those of a monitoring visit, and data already checked by the Monitor may be checked again. The Investigator is required to inform the Sponsor immediately of an inspection requested by a RA. The Investigator and other relevant personnel must be available during the audit/inspection and must devote sufficient time.

10.12.5 Changes in the approved Trial Protocol

Trial procedures must not be changed without the mutual agreement of the Investigator and the Sponsor.

Any substantial change to the approved Final Trial Protocol will be documented in a written and numbered Protocol Amendment. Any proposed substantial change to the Final Trial Protocol must be discussed with and approved by the Sponsor before submitted to IEC and Regulatory Authority for approval, according to applicable national regulations.

10.13 Statistical methods and determination of sample size

The statistical analysis 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.

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.

10.13.1 Demographics and baseline data

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.

10.13.2 Endpoints

10.13.2.1 Primary Endpoint

Primary endpoint TTR at 3 years will be analyzed in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20.

TTR is defined as time from randomization to occurrence of any of the following; loco-regional recurrence, distant metastases or death from colorectal cancer.

10.13.2.2 Secondary Endpoint

Secondary endpoint DFS at 3 years will be analyzed in patients with tumors harboring *PIK3CA* mutations in exon 9 and 20.

DFS and TTR at 3 years will be analyzed in patients with tumors harboring *PIK3CA* mutations other than in exon 9 and 20 and in *PIK3R1* and *PTEN*.

DFS is defined as time from randomization to occurrence of any of the following events; loco-regional recurrence, distant metastases, second primary colorectal cancer, second primary other cancer, death from colorectal cancer, death from other cancer, non-cancer related death and treatment-related death whichever comes first.

OS at 5 years will be analyzed in patients receiving low-dose ASA versus placebo.

10.13.2.3 Safety Endpoints

Safety endpoints will be frequency and severity of AEs.

The safety endpoints for the interim analysis will be the bleeding complications cerebral and gastric haemorrhage will.

10.13.3 Analysis of efficacy

The primary efficacy variable is TTR. Subjects who have not experienced loco-regional recurrence, distant metastases or death from colorectal cancer at time of 3- and 5- years 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 treatment failure and their confidence intervals. Secondary endpoints will be assessed using the log-rank test or a Cox regression model, with time to treatment failure (relapse 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.

10.13.4 Analysis of safety

AEs will be classified into standardized medical terminology from the verbatim description (Investigator term) using MedDRA. AEs will be presented by preferred term nested within System Organ Class. Verbatim description and all MedDRA level terms, including the lower level terms, for all AEs will be contained in the data listings of the clinical study report for this protocol.

AEs will be summarized by presenting, for treatment group the incidence of AEs. The incidence of AEs will be based on the numbers and percentages of patients with AEs. Although a MedDRA term may be reported more than once for a subject, that subject will be counted only one time in the incidence count for that MedDRA term.

10.13.5 Interim analyses and data monitoring

Interim analyses on the safety endpoints, will be performed when data are available for all patients still included in the study after one years of treatment. The objective of the interim analyses is to follow up safety.

No adjustment of the significance level will be made due to the interim analyses.

10.13.6 Analysis data sets

The Full Analysis Population (FAP) consists of all patients who were randomized and had eligible measure at baseline and at least one eligible measure post baseline. Patients withdrawn after randomization and with follow-up data will also be included.

The Per Protocol Population at 3 year (PP3) consists of all patients in the FAP who had complete at least 3 years in the study, 80 % compliance and no significant protocol violations.

The Per Protocol Population 5 year (PP5) consists of all patients in the FAP who had completed at least 5 years in the study, 80 % compliance and no significant protocol violations.

The analyses will be performed on both FAP and PP.

The Safety Population which consists of all patients who were included into the study and met all inclusion criteria and met no exclusion criteria and had at least one exposure to the study interventions.

10.13.7 Determination of sample size

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.

Three year survival					
Placebo	ASA	Ratio of median survival	Hazard ratio	Total number of patients	Total number of events

0.25	0.80	1.29	0.78	1907	484
0.25	0.85	1.77	0.56	447	96
0.25	**0.866**	**2.00**	**0.50**	**326**	**71**
0.25	0.90	2.73	0.37	189	31

With an estimated 20% drop-out rate, 204 patient needs to be randomized in each group.

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. This number can be changed after pilots and first 1000 patients have been included when better estimated on drop-out and mutation rate in *PIK3CA*.

11 TRIAL MANAGEMENT

11.1 Trial time table

The study will start during Q2 2015 with a technical pilot. The process for handling of the gene sampling will be investigated. No IMP will be administrated. The estimated time for randomization of the first patient taking IMP will be Q3 2015. The recruitment period is estimated to 2 years. Included the treatment period and follow-up period the study will end approximately during 2022.

11.2 Insurance/indemnity

The trial patients are covered by the Swedish Patient Insurance and the Swedish Drug Insurance.

The Sponsor agrees to indemnify (legal and financial coverage) and hold the Investigator free of harm from any claim, whether based on legal principles or on generally accepted liability standards within the pharmaceutical industry, made against him by reason of personal injury, including death, to any person arising out of or connected with the performance of the trial to the extent that the injury is not caused by:

1. failure by the Investigator to adhere to the terms of the Protocol;
2. failure by the Investigator to comply with any applicable governmental regulations;
3. malpractice, negligence or willful malfeasance by the Investigator.

The Investigator agrees to notify the Sponsor whenever he/she becomes aware of a claim or action, and to co-operate with and to authorize the Sponsor to carry out sole management of such claim or action.

The Sponsor's responsibility is covered by LFF Läkemedelsförsäkring via an association agreement with Judicial Board (Kammarkollegiet). The insurance covers ALASCCA study group's liability under law and generally accepted liability standards within the pharmaceutical industry towards any third parties, including participating women, as Sponsor of the study.

11.3 Trial agreements

The Principal Investigator at the investigational site must comply with all the terms, conditions, and obligations of the Clinical Trial Agreement (CTA) for this trial.

11.4 Criteria for termination of the trial

The Sponsor reserves the right to discontinue the trial at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must call in all participating and inform the patients as well as perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused trial products and other trial materials must be returned and all CRFs completed as far as possible.

Investigator(s) will be reimbursed for reasonable expenses incurred, in the event this becomes necessary.

11.5 Reporting of results and publication policy

A clinical trial report, in compliance with ICH E3; Guideline for Industry, will be prepared by the Sponsor describing the conduct of the trial, the statistical analysis performed and the obtained results.

The data generated by this trial are considered confidential information and the property of the Sponsor. Said confidential information may be published only in collaboration with participating personnel from the Sponsor. The Sponsor reserves the right to review and comment on the proposed publication prior to being submitted and/or published."

11.6 Record retention

The Investigator must arrange for retention at the investigational site of a list of the patients and their identifying code, patient files and other trial documents. The archiving period must be adapted to regulations in force but should not be shorter than ten years after the termination of the trial and the presentation of the final report.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

11.7 Disclosure and confidentiality

All unpublished information concerning the IMP and research carried out by ALASCCA study group, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and the sole property of ALASCCA study group. Disclosure to third parties must be limited to those undertaking legitimate peer review of the scientific and

ethical aspects of the trial and to those participating, including the recipients of drugs, so that customary medical care and informed consent can be achieved.

12 EMERGENCY PROCEDURES

12.1 Emergency contacts

In case of a medical emergency, contact Sponsor representative;

Professor Anna Martling

+46 8 517 728 02
+46 76 050 57 97

12.2 Procedures in case of medical emergency

The Investigator is responsible for ensuring that there are procedures and expertise available to cope with medical emergencies during the trial.

12.3 Procedures in case of overdose

Very high doses of ASA can cause severe acidosis with fatal multiorgan failure. In these cases the patients will be taken care of according to standard treatment.

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