ALASCCA

STATISTICAL ANALYSIS PLAN

Version 1.1

Date: August 28, 2024

Trial full title	A randomized double-blind placebo-controlled study with ASA treatment in colorectal cancer patients with mutations in the PI3K signaling pathway
Protocol version (SAP associated with)	5.2
Trial principal investigator	Anna Martling, PhD, professor, Karolinska Institutet, Stockholm, Sweden

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Revisions

Version	Date	Reason
1.1	August 28, 2024	Description of collection of oncological treatment data added, with appropriate changes to patient characteristics table. Exploratory endpoints edited. Clarifications on withdrawal of consent, censoring due to loss of follow-up and death, incorrectly entered mutation status, and per-protocol populations analyses. Cumulative incidence curves replacing Kaplan-Meier curves as main figures. Figure templates added. Patient flowchart template edited. Version 1.1 drafted and approved before unblinding of the trial.
1.0	September 27, 2023	Version 1.0 drafted and approved before unblinding of the trial.

1 Abbreviations

AE	Adverse event
ALASCCA	Adjuvant Low dose ASpirin in Colorectal CAncer
ASA	Acetylsalicylic acid
BMI	Body mass index
CEA	Carcinoembryonic antigen
CI	Confidence interval
CIF	Cumulative incidence function
CRC	Colorectal cancer
CRT	Chemoradiotherapy
CT	Computed tomography
DFS	Disease-free survival
eCRF	Electronic case report form
EoS	End of Study
FAP	Full analysis population
HR	Hazard ratio
IMP	Investigational medical product
IPCW	Inverse probability of censoring weight
IPTW	Inverse probability of treatment weight
IQR	Inter-quartile range
KM	Kaplan-Meier
KTA	Karolinska Trial Alliance
MEB	Department of Medical Epidemiology and Biostatistics
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
NSAID	Nonsteroidal anti-inflammatory drug
OS	Overall survival
PDR	Swedish Prescribed Drug Register
PHA	Proportional hazards assumption
PHM	Proportional hazards model
PI	Principal investigator
PP3	Per protocol population at 3 years
PP5	Per protocol population at 5 years
pTNM	Pathological tumor-node-metastasis
RCT	Randomized clinical trial
RR	Risk ratio
RT	Radiotherapy
SAE	Serious adverse event
SAP	Statistical analysis plan
SCRCR	Swedish Colorectal Cancer Registry

SMC	Safety Monitoring Committee
SP	Safety population
TTR	Time to recurrence
UAS	Uppsala Akademiska Sjukhus

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3 Introduction

Observational studies have shown a possible effect of acetylsalicylic acid (ASA) on the risk of recurrence of colorectal cancer (CRC) in patients with a primary tumor mutation in the PI3K signaling pathway. Whether this association is causal, and what the size of the effect is, have yet to be shown in a randomized clinical trial (RCT). The ALASCCA trial is an RCT designed to answer the question of whether adjuvant ASA reduces the risk of CRC recurrence in patients with a tumor mutation in the PI3K signaling pathway.

Throughout this statistical analysis plan (SAP), randomized individuals will be referred to as *subjects*, and screened individuals will be referred to as *patients*.

4 Trial overview

4.1 Trial description

The ALASCCA trial is a randomized, 2-arm, parallel group, double blind, multicenter, placebo-controlled, superiority clinical trial. CRC patients with a stage I-III (rectal cancer) or II-III (colon cancer) localized tumor with somatic alterations in the PIK3CA, PIK3R1, or PTEN genes are invited to participate in the randomized part of the trial.

CRC patients are screened for mutations in the PI3K signaling pathway, randomized, and provided with the investigational medical product (IMP) – ASA 160 mg or placebo – within three months from surgery. The IMP is to be taken daily for three years after randomization. Subjects treated with adjuvant chemotherapy, who, for medical reasons, should not receive ASA are allowed to start taking the IMP after termination of chemotherapy. Subjects are

allowed to pause treatment for up to 30 consecutive days to enable surgical procedures that should not be combined with ASA.

Subjects are contacted every three months until three years post randomization, with an additional contact five years after randomization. Computed tomography (CT) or magnetic resonance imaging (MRI) of the thorax and abdomen to detect CRC recurrence is performed at 1 year and 3 years after randomization.

Patients that are being treated regularly with ASA (>3 doses per week) at inclusion will not be randomized. However, these patients will be included in an observational arm – regardless of mutation status. Aims, endpoints, data collection, and analyses for the observational arm will be described in an amendment to this SAP.

The ALASCCA trial includes 33 hospitals across Sweden, Denmark, Finland, and Norway.

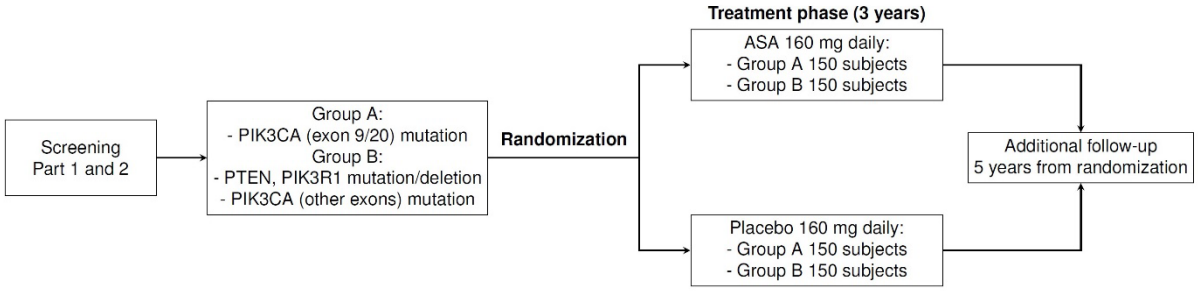

Figure 1. Study design of the randomized part of the ALASCCA trial

4.2 Objectives

4.2.1 Primary objective

To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve time to recurrence (TTR) in CRC patients with tumors harboring somatic alterations in the PIK3CA (exon 9 and/or 20) gene.

4.2.2 Secondary objectives

1. To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve disease-free survival (DFS) in CRC patients with tumors harboring somatic alterations in the PIK3CA (exon 9 and/or 20) gene.
2. To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve TTR in CRC patients with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
3. To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve DFS in CRC patients with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
4. To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve overall survival (OS) in CRC patients with tumors harboring somatic alterations in the PIK3CA (exon 9 and/or 20) gene.
5. To determine whether adjuvant treatment with 160 mg ASA once daily for 3 years compared with placebo can improve OS in CRC patients with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
6. To assess overall safety and tolerability.

4.3 Endpoints

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

DFS is defined as time from randomization to occurrence of any of the following events: loco-regional recurrence, distant metastases, second primary CRC, second primary other cancer, death from any cause, whichever comes first.

OS is defined as time from randomization to death from any cause.

4.3.1 Primary endpoint

The primary endpoint is TTR assessed at 3 years from randomization, in patients with tumors harboring PIK3CA mutations in exon 9 and/or 20.

4.3.2 Secondary endpoints

1. DFS assessed at 3 years from randomization, in subjects with tumors harboring somatic alterations in the PIK3CA (exon 9 and/or 20) gene.
2. TTR assessed at 3 years from randomization, in subjects with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
3. DFS assessed at 3 years from randomization, in subjects with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
4. OS assessed at 5 years from randomization, in subjects with tumors harboring somatic alterations in the PIK3CA (exon 9 and/or 20) gene.
5. OS assessed at 5 years from randomization, in subjects with tumors harboring somatic alterations in the PIK3CA (not exon 9 or 20), PIK3R1 and/or PTEN genes.
6. Frequency and severity of adverse events (AE). Cerebral and gastric hemorrhage (for interim analyses).

4.4 Eligibility

4.4.1 Inclusion criteria

All of the below criteria have to be fulfilled for a patient to be included into the randomized part of the trial.

- Tumor with somatic alterations in the PIK3CA, PIK3R1 and/or PTEN genes
- Colon cancer pathological tumor stage II-III or rectal cancer pathological tumor stage I-III
- Patient aged minimum 18 years and maximum 80 years
- Radical surgery according to surgeon and pathologist
- Karnofsky performance status ≥60%
- Platelets ≥ 100 x 10^9 / L
- Clean colonoscopy or CT colon planned for within 3 months preoperatively or postoperatively but before randomization
- Patient able to swallow tablets
- Patient able to understand and sign written informed consent

4.4.2 Exclusion criteria

Fulfilment of at least one of the below criteria is enough for a patient not to be included into the randomized part of the trial.

- Known hereditary CRC linked to familial colonic polyposis or Lynch syndrome
- Inflammatory bowel disease (Crohn's disease or ulcerative colitis)
- Distant metastases (Stage IV)
- Other cancers within 3 years before screening, with the exception for CRC, other skin cancer than melanoma, and benign thyroid tumor
- Known bleeding diathesis (such as hemophilia)
- Concomitant antiplatelet therapy (e.g. clopidogrel 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-operative bleeding, within the previous three months assessed at screening and randomization
- Ongoing regular use of corticosteroids, and/or 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 mL/minute should lead to consultation with a nephrologist.
- Significant medical illness that would interfere with study participation
- Pregnancy or breastfeeding
- Known allergy to NSAIDs or ASA
- Current participation in another clinical trial conflicting 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
- Regular use of ASA (>3 doses weekly) – such patients will be included in an observational arm but not randomized or provided with IMP

4.5 Sample size

Sample size calculations were based on the primary endpoint of 3-year TTR in subjects with a tumor mutation in the PIK3CA (exon 9 and/or 20) gene.

4.5.1 Initial calculation in protocol version 2

Power was set to 80 %, two-sided significance level alpha to 0.05, the accrual period to 24 months, and a minimal follow-up of 36 months for all subjects. Underlying exponential distributions of CRC recurrence for both groups were assumed, with a three-year cumulative incidence of recurrence of 25 % in the placebo arm. Assuming 20 % drop-out, a hazard ratio (HR) of 0.5 for the ASA arm compared to the placebo arm can be detected (with 80 % power) if 204 subjects in each arm are recruited. If 12% of the patients screened have hotspot mutations (exon 9 and 20) in the PIK3CA gene, a total of 3900 patients will need to be screened. This also includes approximately 15% of the patients that will be excluded due to tumor stage I. Details of the initial sample size calculation are listed in Table 1.

Table 1. Details of the initial sample size calculation in protocol versions 2-5.1.

Power (%)	**80**
Two-sided alpha (%)	5
Minimal follow-up (months)	36

Accrual period (months)	24
Mutation rate (%)	12
3-year cumulative incidence of recurrence in the placebo arm (%)	25
HR	0.5
Number of subjects in each arm (incl. 20 % drop-out)	204
Number of subjects in each arm (excl. 20 % drop-out)	163
Number of patients needed to be screened	3900

4.5.2 Updated calculation in protocol version 5.2

An updated sample size calculation was included in the ALASCCA Clinical Study Protocol Version 5.2 after 3206 patients had been screened. The three-year cumulative incidence of recurrence in the placebo arm was estimated to 18 % based on the Stage/Risk factor-specific estimates in Osterman and Glimelius (2018)[1] , and a 10 % drop-out rate was assumed. A total of 150 subjects in each arm would have to be included to detect a risk ratio (RR) of 0.38 for comparison between the ASA and placebo arms with 80 % power and a two-sided alpha of 0.05. Assuming 15 % of screened patients have somatic alterations in the PIK3CA (exon 9 and/or 20) gene, a total of 3750 patients will have to be screened. The estimate of a 15 % mutation rate was based on the 3206 first screened patients. The sample size calculation was based on a two-sample test for proportions. Details of the initial sample size calculation are listed in Table 2.

Table 2. Details of the updated sample size calculation in protocol version 5.2.

Power (%)	**80**
Two-sided alpha (%)	5
Minimal follow-up (months)	36
Mutation rate (%)	15
3-year cumulative incidence of recurrence in the placebo arm (%)	18
RR	0.38
Number of subjects in each arm (incl. 10 % drop-out)	150
Number of subjects in each arm (excl. 10 % drop-out)	135
Number of patients needed to be screened	3750

4.6 Randomization

The randomization module is built into the electronic case report form (eCRF) system. When a patient is eligible for randomization, the study personnel clicks the randomization button in the eCRF system and receives a subject specific randomization ID, and the identification number of the IMP that should be dispensed to the subject.

An adaptive randomization method is used, meaning when a new subject is to be randomized, the treatment allocations of the previously entered subjects affect the allocation probability of the new subject.

Randomization is stratified for the factors listed in Table 3.

Table 3. Stratification factors in the randomization.

Variable	**Levels**
Location	- Colon - Rectum
Stage	- I - II - III
Mutation type	- PIK3CA exons 9/20 - PIK3CA (other exons)/PIK3R1/PTEN

The first subject in a specified stratum is allocated to the ASA or placebo arm with equal probability (0.5). A succeeding subject in a specified stratum is allocated with probability 1 to the treatment arm that has the smallest number of allocated subjects within the same stratum. If the two arms in a specified stratum have equal number of subjects the next subject in that stratum is allocated to the ASA or placebo arm with equal probability (0.5).

It is impossible for the principal investigator (PI) or study personnel to predict the allocation of the next subject in a specified stratum, since neither the subject specific randomization ID generated by the eCRF, nor the identification number of the IMP, contain information on which arm the subject was randomized to. In addition, there is no stratification by site, so the next randomized subject is likely to be at another site, giving even more protection against any theoretical possibility to deduce randomization order. The PI or study personnel do not have access to any subject list specifying randomization arm.

4.7 Blinding

ALASCCA is a double-blind trial, meaning neither subjects nor investigators have knowledge of which treatment (ASA or placebo) a certain subject is receiving. In case of an emergency, the blinding for a certain subject can be broken. Any emergency unblinding will be carefully documented.

In addition to the blinding of treatment allocation, the type of somatic alteration is concealed from subjects and investigators to minimize any potential bias arising from subjects' and/or investigators' knowledge of the pre-specified hypotheses connected to each somatic alteration type. The somatic alteration type is reported to trial sites as "A" or "B", omitting information on which coding refers to which specific somatic alteration. See Section 4.9.6 for details on the procedure of investigating and reporting somatic alterations.

4.8 Trial assessments

Written informed consent is given by the patient in the first screening visit, which takes place before or shortly after surgery (allowing for randomization to be performed within 12 weeks from surgery).

In the second screening visit, which takes place after surgery, inclusion and exclusion criteria are assessed in patients with tumors harboring somatic alterations in the PIK3CA, PIK3R1 or PTEN genes. Patients without such somatic alterations, and patients not fulfilling all inclusion criteria, or fulfilling at least one exclusion criteria are excluded from the trial. However, patients with regular use of ASA (>3 doses/week) fulfilling all other eligibility criteria, are included in an observational arm.

Randomization takes place in the third visit, in which the subject is also provided with IMP for 200 days. The treatment period begins at the randomization visit and ends after 3 years.

A phone contact is made with the subject three months from randomization, and thereafter every sixth month. Also, subjects visit their respective site every sixth month from randomization, i.e,. the subjects are in contact with the site every third months for three years. At the end of the follow-up period, five years from randomization, there is an additional visit or phone contact for subjects who have completed three years in the trial (up to and including Visit 15). The trial assessments, starting from randomization, are visualized in Figure 2, and described in detail in Table 4.

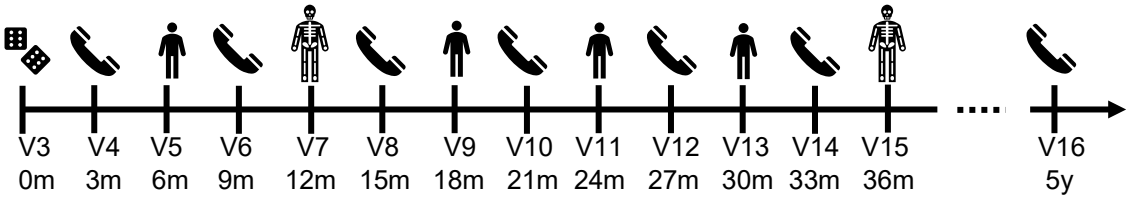

Figure 2. Time-line of trial assessments. The dice icon refers to Randomization, the phone icon to Phone contact, the human icon to Clinic visit, and the skeleton icon to clinic visit including examination with CT/MRI.

Table 4. Overview of trial assessments

Assessment/Visit	**Random-ization (R)**	**First phone call**	**First site visit**	**Subsequent phone calls**	**Subsequent site visits**	**Final visit/phone call**
Visit no.*	3	4	5	6, 8, 10, 12, 14	5, 7, 9, 11, 13, 15	16
Timepoint	0	3mo after R	6mo after R	Every 6mo after first phone call	Every 6mo after first site visit	5y from R
Visit/call window	-	±21 days	±21 days	±21 days	±21 days	±30 days
Provide with IMP	Yes		Yes		Yes	
Assess AEs		Yes	Yes	Yes	Yes	
Subject asked about compliance		Yes	Yes	Yes	Yes	
Remaining pill count			Yes		Yes	
Record concomitant medication		Yes	Yes	Yes	Yes	
CT/MRI of thorax and abdomen					Yes (visit 7 and 15 – 1y and 3y after R)**	
Measure carcinoembryonic antigen (CEA)					Optional, visit 7 and 15 – 1y and 3y after R	Optional
Subject asked about regular use of ASA after end of trial intervention						Yes
CRC recurrence/Death (and corresponding dates)						Yes

*Visits 1 and 2 are screening visits.
**Two Finnish sites do not include CT/MRI of thorax and abdomen as part of their standard of care.

Subjects leave the IMP part of the trial at Visit 15, or at fulfillment of an end of study (EoS) reason. EoS reasons are

- Death,
- CRC recurrence,
- Severe unexpected AE,
- Pregnancy,

- Other medical reason (including other cancer),
- Wanting to leave the study,
- Lack of compliance with study protocol,
- Refusal to cooperate,
- Other reason (specified in the eCRF by study personnel).

4.8.1 Trial assessments during the COVID-19 pandemic

From April 4th, 2020, most clinic visits are switched to phone contacts, and subjects are provided with IMP by mail. Subjects are asked about their compliance at each phone contact, and remaining pills are counted whenever the subject has a possibility to visit the clinic. Remaining pill count is then retrospectively entered into the eCRF for each clinic visit that has been replaced with a phone contact. Study personnel have been encouraged to go back to clinic visits as the COVID-19 spread has decreased.

4.8.2 Treatment compliance

Every sixth month, subjects are provided with two boxes of IMP, each containing 100 pills. Subjects are to take one pill per day. Subjects treated with adjuvant chemotherapy, who, for medical reasons, should not receive ASA during said treatment are allowed to start taking the IMP after termination of chemotherapy. Subjects are also allowed to pause taking the IMP for up to 30 days in a row during the trial. All treatment pauses, for any reason, are entered with a start and end date in the eCRF.

At each phone contact and clinic visit, the subject is asked how often they forget to take the IMP, with response options

- Never/Rarely
- Once in a while
- Sometimes
- Often
- Always.

At each clinic visit, the subject brings the IMP packages provided to them at the preceding clinic visit, the remaining pills are counted mechanically and the sum is entered into the eCRF.

Compliance until the last clinic visit (C_{LCV}) will be calculated as

$$C_{LCV}\ \% = \frac{\text{DP before the final clinic visit - RP up to and including the final clinic visit}}{\text{TD until the final clinic visit - number of days on allowed break before the final clinic visit}} \cdot 100,$$

where DP refers to the total number of dispensed pills, RP to the total number of remaining pills, and TD to the total number of potential treatment days. The number of days on break before the final clinic visit only includes breaks that are maximum 30 days or related to adjuvant chemotherapy, i.e. breaks that are allowed by the study protocol.

Subjects with an EoS event before the first clinic visit, in which the first pill count takes place, but after the first phone contact, will not have any pill counts in the eCRF. However, they will have been asked at the first phone contact (Visit 4) how often they forget to take the IMP. Compliance until Visit 4 (C_{V4}) for these subjects will be defined as 80 % if the response is “Never/Rarely” or “Once in a while”, and 0 % if the response is “Sometimes”, “Often”, or “Always”. C_{LCV} (or C_{V4}) for subjects with visits outside of the allowed time-window of ±21 days will be calculated similarly as if their visits had been within the allowed time-window.

Subjects leaving the trial for any reason between two clinic visits, or between the first phone contact and the succeeding clinic visit, will not have a compliance measurement for the time between the last clinic visit (or Visit 4) and trial exit. For these subjects, an assumption on how long C_{LCV} (or C_{V4}) is assumed valid must be made. Denote the last date for which C_{LCV} (or C_{V4}) is assumed valid $D_{LCV/V4}$. For subjects experiencing recurrence, death, or other cancer, $D_{LCV/V4}$ will be defined as the date of last IMP intake, if such a date is registered in the eCRF, or the event date (see Section 6.2.1 for definition), whichever comes first. For subjects leaving the trial before an endpoint or before trial completion, $D_{LCV/V4}$ will be defined as the date of last IMP intake, if such a date is registered in the eCRF, or the Visit 15 date/EoS date, whichever comes first. For subjects completing 3 years in the trial, $D_{LCV/V4}$ will be defined as the date of Visit 15 (the 3-year follow-up clinic visit). The number of days for which C_{LCV} (or C_{V4}) will be assumed valid will be calculated as days from randomization until $D_{LCV/V4}$, subtracting the number of days on treatment breaks (before $D_{LCV/V4}$) *not* allowed by the protocol (see above), for each randomized subject having received the IMP at least once.

For subjects reaching a trial endpoint, days from $D_{LCV/V4}$ until the event date will be defined as having 0 % compliance. Similarly, for subjects leaving the trial before completion, days from $D_{LCV/V4}$ until 3 years from randomization will be defined as having 0 % compliance. For all subjects, regardless of trial completion or exit, days on treatment breaks (before $D_{LCV/V4}$) not allowed by the study protocol will be defined as having 0 % compliance. Overall compliance (OC) will be calculated as a weighted average between C_{LCV} (or C_{V4}) and 0, as follows

$$\text{OC \%} = \frac{\text{Valid days} * C_{\text{LCV or V4}} + \text{Zero-compliance days} * 0}{\text{Valid days} + \text{Zero-compliance days}}.$$

Subjects with an EoS event before the first phone contact will not have answered the question on frequency in taking the IMP, or have any pill counts in the eCRF, hence, it is not possible to calculate compliance for such subjects.

See 6.2.2 for derivation of the variables needed for compliance calculation.

4.9 Data collection

Data collection is performed partly through the eCRF, partly through the Swedish Colorectal Cancer Registry (SCRCR) and other Swedish national registries (for Swedish subjects), and partly through separate data collections directly from trial sites (subjects in other Nordic countries). Baseline characteristics that are collected from the SCRCR for Swedish subjects are collected separately from each site for the Nordic countries outside of Sweden. Table 5 contains information on data sources for subjects in Sweden and other Nordic countries. Each data source is described in the below subsections (4.9.1, 4.9.2, 4.9.3, 4.9.4, and 4.9.6). Note that some variables are collected from several data sources, e.g. the pathological tumor-node-metastasis (pTNM) stage and cancer location. For such variables, data from the eCRF will be preferred, with the exception of data from the Department of Medical Epidemiology and Biostatistics (MEB) (see subsection 4.9.6 for details), since the eCRF is regularly monitored (see Section 4.10), but data from other sources can be used if there are data missing from the eCRF.

Table 5. Data sources for the ALASCCA clinical trial, for randomized subjects.

Variable	Sweden	Denmark, Finland, Norway
- Inclusion and exclusion criteria - Mutation type (blinded) (PIK3CA (exon 9/20) or PTEN/PIK3R1/PIK3CA (other exons)) - Randomized group - Age at screening visit 2 - Date of surgery - Sex - pTNM stage - Cancer location (colon/rectum) - Compliance - Concomitant medication - CT/MRI at 12 and 36 months - AEs	eCRF	eCRF
Endpoint variables assessed at 3 years for subjects reaching a study endpoint or completing 3 years in the trial: - CRC recurrence within 3 years (local and distant) - Other cancer within 3 years - Death within 3 years - CEA level (if applicable) - Date of 3-year follow-up CT	eCRF	eCRF
Additional endpoint variables assessed at 3 years for all subjects: - CRC recurrence, and corresponding date - Location(s) of CRC recurrence (local/liver/lung/other) - Other cancer, and type of other cancer, within 3 years, and corresponding date - Death within 3 years, and corresponding date - Date of last known to be alive - CEA level (if applicable) - Date of 3-year follow-up CT	Collected separately from sites/SCRCR	Collected separately from sites
Endpoint variables assessed at 5 years for subjects reaching who completed 3 years in the trial: - CRC recurrence within 5 years (local and distant) - Death within 5 years - ASA treatment after end of trial (assessed at 5 years) - CEA level (if applicable)	eCRF	eCRF
Endpoint variables assessed at 5 years for subjects leaving the trial before study completion/death: - CRC recurrence yes/no and date of recurrence or date of last known to be without CRC recurrence	SCRCR/Collected separately from sites	Collected separately from sites

Variable	Sweden	Denmark, Finland, Norway
- Death yes/no and date of death or date of last known to be alive		
ASA treatment after end of trial (assessed at 5 years)	PDR	Not collected
SAEs	eCRF (and reported on paper directly to trial coordinator)	eCRF (and reported on paper directly to trial coordinator)
Treatment and cancer specific variables at baseline: - Date of CRC diagnosis - Body mass index (BMI) - ASA Score - Tumor location - Tumor location in colon cancer - Height from anal verge (rectal cancer) - Neoadjuvant radiotherapy (RT) (yes/no) - Neoadjuvant chemotherapy (yes/no) - Neoadjuvant chemoradiotherapy (CRT) (yes/no) - Elective/emergency surgery - Type of surgery - pTNM stage - Tumor differentiation - Adjuvant chemotherapy (yes/no)	SCRCR	Collected separately from sites
Oncological treatment - Type of neoadjuvant and adjuvant treatment (None, Only RT, CRT, RT+Chemotherapy, CRT+Chemotherapy, Only Chemotherapy) - Total dose, number of fractions, and start date of neoadjuvant and adjuvant RT - Start date and type of neoadjuvant and adjuvant chemotherapy	SCRCR/Collected separately from sites	Collected separately from sites
Other tumor mutations (MSI-status, BRAF, KRAS, NRAS)	Extracted from MEB database	Extracted from MEB database

4.9.1 Electronic Case Report Forms (eCRFs)

The eCRF is integrated with INCA – a national IT platform for Swedish quality registries. Study personnel at the study sites have secure login credentials to access and enter subject data into the eCRF system.

4.9.2 Swedish Colorectal Cancer Registry (SCRCR)

All Swedish CRC cases are reported to the SCRCR, which includes patient, tumor, and treatment specific factors. Several baseline variables, and some endpoint variables (see Section 4.9.4), for the randomized Swedish subjects will be collected from the SCRCR, see Table 5.

4.9.3 Additional baseline data for Nordic countries outside of Sweden

Data on patient, tumor, and treatment specific variables from subjects from the Nordic countries outside of Sweden are not part of the SCRCR. These data are collected separately from each included site using standardized forms in xlsx-format. The data will be merged with the Swedish data extracted from the SCRCR.

4.9.4 Additional collection of endpoint variables

Endpoint variables assessed at 3 years from randomization for subjects leaving the trial before trial completion, CRC recurrence, other cancer, or death, are not entered into the eCRF. Date and location(s) (local/liver/lung/other) of CRC recurrence, other cancer and death, or dates for which a subject is last known to be without such events, will be collected separately from all sites, including the Swedish sites. For subjects experiencing CRC recurrence, sites will be asked to provide information on date and location(s) (local/liver/lung/other) of CRC recurrence. For subjects completing the trial without any registration of CRC recurrence/other cancer/death in the eCRF or SCRCR (for Swedish subjects), sites will be asked to review the medical charts and provide any information on endpoint variables that has not been entered into the eCRF. Each site will be asked to provide the additional 3-year endpoint data when 3 years have elapsed since their last subject was randomized.

Endpoint variables assessed at 5 years from randomization for subjects leaving the trial before trial completion are not entered into the eCRF. Date of CRC recurrence, other cancer, and/or death, or dates for which a subject is last known to be without such events, will be collected from SCRCR for Swedish subjects, and from each included site for subjects in Nordic countries outside of Sweden. Data on ASA treatment after the IMP part of the trial until 5 years after randomization will be collected from the Swedish Prescribed Drug Register (PDR) for Swedish subjects.

4.9.5 Additional collection of oncological treatment

Type and details of neoadjuvant and adjuvant oncological treatment, including start dates of RT and chemotherapy, RT total doses and number of fractions, and substances given in chemotherapy, are collected from the SCRCR (if registered) and separately from each trial site.

4.9.6 Data on tumor mutations

Tumor cell DNA is extracted from tumor samples at the Karolinska Institutet Biobank, and a pre-specified set of genes are sequenced at Clinical Genomics, SciLife Lab. The sequencing data are analyzed by bioinformaticians at MEB, KI. Data containing study ID, somatic alterations in PIK3CA exon 9/20 (Mutation A: PIK3CA exon 9 and 20 hotspot small mutations), other PIK3CA/PIK3R1/PTEN (Mutation B: other PIK3CA, PIK3R1 and PTEN non-silent small mutations and focal/homozygous deletion of PTEN), microsatellite instability (MSI), and KRAS, BRAF, NRAS mutation status will be extracted from the database at MEB. Whether a subject has somatic alterations in PIK3CA (Mutation A), PIK3CA/PIK3R1/PTEN (Mutation B), or no such alterations is reported (blinded for specific mutation) to the respective site of each subject. One trial site, Uppsala Akademiska Sjukhus (UAS), with three randomized subjects, has performed the tumor DNA sequencing and bioinformatics without assistance from Clinical Genomics or MEB. To enable randomization of a subject, each site enters the mutation status into the eCRF, which is regularly monitored (see Section 4.10), making errors in the mutation status variable highly unlikely. Data extracted from

MEB/UAS and from the eCRF on mutation status in the PI3K pathway will be compared, and in case of differences, the mutation status from MEB/UAS will be used in all analyses, even though randomization is based on mutation status from the eCRF (see Section 4.6 for details on the randomization procedure).[2] In case multiple tumor samples from the same patient have been analyzed, several reports are sent to site. If a subject has somatic alterations in PIK3CA (Mutation A) in one tumor sample, and other PIK3CA/PIK3R1/PTEN (Mutation B) in another, the PIK3CA (Mutation A) mutation status will be used in all analyses. Any discrepancies between mutation status in the eCRF and in data extracted from MEB/UAS will be clearly described.

4.9.7 Data on subjects withdrawing written informed consent

For subjects who, during the trial, withdraw their written informed consent, data collected up to the date of withdrawal will be used in descriptive tables and analyses, but no further data will be collected.

4.10 Monitoring

Each site is monitored at regular time-intervals to ensure the site's compliance with the protocol, adherence to good clinical practice, completeness of the eCRFs, and consistency of the eCRF to the clinical records. The monitoring is performed by the Karolinska Trial Alliance (KTA) – a regulatory unit of the Karolinska University Hospital in Stockholm. When each randomized subject at a specific site has reached EoS and/or has 3 years of follow-up, KTA will close the site. The closing of most sites will occur during 2024.

4.11 Trial reporting

Results from the trial will be reported according to the CONSORT statements.[3]

5 General analysis considerations

5.1 Timing of analyses

The last subject to be randomized was entered into the trial July 19, 2021, meaning all subjects will have at least 3 years of follow-up on August 9, 2024, allowing for the visit window of 21 days. Analyses of the primary endpoint, and of secondary endpoints 1-3 and 6 will be performed as soon as possible after August 9, 2024. All sites need to be closed by KTA before any analyses can be performed, see Section 4.10 for details.

The analysis of secondary endpoints 4 and 5 (OS assessed at 5 years from randomization) will be possible from August 19, 2026, allowing for the 30-day visit window of Visit 16. Vital status and death date (if any) for subjects having reached EoS before visit 15 will have to be collected from sites before the analyses of secondary endpoints 4 and 5 can be conducted.

5.2 Analysis populations

Each subject's inclusion/exclusion status with regards to each analysis population will be assigned before breaking the blind.

5.2.1 Full analysis population

The full analysis population (FAP) consists of all randomized subjects, with the sole exception of subjects that were randomized by mistake and never included into the trial. Subjects with follow-up data, who were randomized but did not fulfil all eligibility criteria will

be included. Subjects who withdrew their written informed consent will be included until the date of consent withdrawal (see Section 4.9.7 for details).

5.2.2 Per protocol populations

The per protocol population at 3 years (PP3) consists of all subjects in the FAP who had at least 80 % compliance, fulfill all eligibility criteria, no significant protocol violations, and have either reached a study endpoint (recurrence, other cancer, or death) within 3 years, or completed at least 3 years in the trial.

The per protocol population at 5 years (PP5) consists of all subjects in the FAP who had at least 80 % compliance, no significant protocol violations, fulfill all eligibility criteria, and have either reached a study endpoint (recurrence, other cancer, or death) within 5 years, or completed at least 5 years in the trial.

Subjects who are thought to fulfil all eligibility criteria at randomization, but later are found not to fulfil all eligibility criteria will be excluded from the PP3 and PP5, for example subjects where Lynch syndrome is discovered after randomization.

Significant protocol violations include more than 12 weeks from surgery until randomization, leaving the study because of pregnancy, lack of compliance with the study protocol, wanting to leave the study, refusal to cooperate, other medical reasons, e.g. initiating another treatment not compatible with ASA, or leaving the trial because of an SAE/AE.

5.2.3 Safety population

The safety population (SP) consists of all randomized subjects who have received the IMP at least once.

5.3 Handling of trial sites

The ALASCCA trial includes subjects from Sweden, Denmark, Finland and Norway. In total, 33 hospitals have screened and recruited subjects, see Table 6 for the number of sites per country.

Table 6. Number of sites per country

Country	**Number of sites**
Sweden	24
Denmark	3
Finland	2
Norway	4
TOTAL	**33**

Due to the low number of randomized subjects per site, and, for the Nordic countries outside of Sweden, per country, no adjustment or stratification for site/country will be made in the analyses of study endpoints.

5.4 Missing data

The amount of missing data in the eCRF will likely be very small, due to the following efforts:

- Regular monitoring of all sites making sure the completeness of registration into the eCRF, and
- Regular contact between the central trial administration and local sites to request missing data.

If, despite the above efforts, baseline variables are still missing for some subjects, these subjects will be compared to subjects with non-missing baseline variables with regards to their non-missing characteristics. The number of missing values for each baseline characteristic will be clearly presented in the patient characteristics table, stratified by randomization and mutation group.

Only factors used in the randomization are adjusted for in endpoint analyses, and these variables have to be entered into the eCRF for a subject to be randomized. Hence, adjustment variables cannot be missing for any randomized subject. If there is a need to adjust for other variables in the analyses, and there are missing values, multiple imputation using chained equations will be used, using non-missing baseline variables to estimate the missing values, and using Rubin's rules to combine the imputed datasets. Fifty imputed datasets will be generated, and the seed will be set to *87228*. Baseline characteristics that have a risk of containing missing values are platelet count, BMI, CEA, ASA score, tumor location in colon cancer, height from anal verge, neoadjuvant radiotherapy, neoadjuvant and adjuvant chemotherapy, emergency/elective surgery, specific type of surgery, and tumor differentiation. See Section 6.1 for a listing of baseline variables.

5.5 Multiple testing

No adjustment for multiple testing will be applied.

6 Summary of trial data

All data will be presented using descriptive statistics, stratified by randomized group and type of somatic alteration. Continuous variables will be summarized using median, inter-quartile range (IQR), minimum and maximum. Categorical variables will be summarized using the number and percentage of subjects. See Table 14 for a table template of how the baseline variables will be presented.

6.1 Demographic and baseline variables

The demographic and baseline variables of the ALASCCA trial are listed in Table 7.

Table 7. Demographic and baseline variables in the ALASCCA trial

Variable	**Type**	**Range/categories**	**Unit of measurement**
Age at screening visit 2	Continuous	18-80	Years (truncated to integer)
Sex	Dichotomous	Female/Male	-
Country	Categorical	Sweden/Norway/Denmark/Finland	
Date of randomization	Date	2016-07-07 – 2021-07-19	YYYY-MM-DD
Date of CRC diagnosis	Date	-	YYYY-MM-DD
Date of CRC surgery	Date	-	YYYY-MM-DD
Mutation	Categorical	- PIK3CA (exon 9 and/or 20) - PIK3CA (not exon 9 or 20), PIK3R1 or PTEN	
BMI	Continuous		kg/m^2
Platelet count	Continuous	≥0	x10^9/L
CEA	Continuous	≥0	µg/L

Variable	Type	Range/categories	Unit of measurement
ASA Score	Categorical	1-5	-
Tumor location	Dichotomous	Colon/Rectum	
Tumor location in colon cancer	Categorical	Caecum/Ascendens/Flexura hepatica/Transversum/Flexura lienalis/Descendens/Sigmoideum	
Height from anal verge (rectal cancer)	Continuous	≥0	cm
Colon tumor stage	Dichotomous	II, III	-
Rectal tumor stage	Categorical	I, II, III	-
Neoadjuvant RT	Dichotomous	Yes/No	-
Neoadjuvant chemotherapy	Dichotomous	Yes/No	-
Neoadjuvant CRT	Dichotomous	Yes/No	-
Type of neoadjuvant treatment	Categorical	RT only/CRT/RT+Chemo/CRT+Chemo/Chemo only/No neoadjuvant treatment	-
Start of neoadjuvant RT	Date		YYYY-MM-DD
Start of neoadjuvant chemo in CRT	Date		YYYY-MM-DD
Start of neoadjuvant chemo	Date		YYYY-MM-DD
Neoadjuvant RT dose	Categorical	5 Gy/2 Gy/1.8 Gy/Other	-
Neoadjuvant RT fraction	Categorical	5 fractions/25 fractions/28 fractions	-
5-FU in neoadjuvant CRT	Dichotomous	Yes/No	-
Capecitabin in neoadjuvant CRT	Dichotomous	Yes/No	-
Other chemotherapy in neoadjuvant CRT	Dichotomous	Yes/No	-
Specification of other chemotherapy in neoadjuvant CRT	Text	-	-
5-FU in adjuvant chemo	Dichotomous	Yes/No	-
Capecitabin in neoadjuvant chemo	Dichotomous	Yes/No	-
Oxaliplatin in neoadjuvant chemo	Dichotomous	Yes/No	-

Variable	Type	Range/categories	Unit of measurement
Other neoadjuvant chemotherapy	Dichotomous	Yes/No	-
Specification of other neoadjuvant chemotherapy	Text	-	-
Surgery	Dichotomous	Elective/emergency	-
Type of surgery	Categorical	Ileocecal resection/Right-sided hemicolectomy/Transversum resection/Left-sided hemicolectomy/Sigmoideum resection/Total colectomy/Anterior resection/Rectum amputation/Hartmann's procedure	-
pTNM stage	Categorical	I, II, III, IV	-
Tumor differentiation	Dichotomous	Low grade/High grade	-
MSI	Categorical	MSI-high/MSI-low/Uncertain	
BRAF-mutation	Categorical	Yes/No/Uncertain	
KRAS-mutation	Categorical	Yes/No/Uncertain	
NRAS-mutation	Categorical	Yes/No/Uncertain	
Adjuvant chemotherapy	Dichotomous	Yes/No	-
Type of adjuvant treatment	Categorical	RT only/CRT/RT+Chemo/CRT+Chemo/Chemo only/No adjuvant treatment	-
Start of adjuvant RT	Date		YYYY-MM-DD
Start of adjuvant chemo in CRT	Date		YYYY-MM-DD
Start of adjuvant chemo	Date		YYYY-MM-DD
Adjuvant RT dose	Categorical	5 Gy/2 Gy/1.8 Gy/Other	-
Adjuvant RT fraction	Categorical	5 fractions/25 fractions/28 fractions	-
5-FU in adjuvant CRT	Dichotomous	Yes/No	-
Capecitabin in adjuvant CRT	Dichotomous	Yes/No	-
Other chemotherapy in adjuvant CRT	Dichotomous	Yes/No	-
Specification of other chemotherapy in adjuvant CRT	Text	-	-
5-FU in adjuvant chemo	Dichotomous	Yes/No	-
Capecitabin in adjuvant chemo	Dichotomous	Yes/No	-
Oxaliplatin in adjuvant chemo	Dichotomous	Yes/No	-
Other adjuvant chemotherapy	Dichotomous	Yes/No	-

Variable	Type	Range/categories	Unit of measurement
Specification of other adjuvant chemotherapy	Text	-	-

6.2 Derived variables

6.2.1 Endpoint variables

For the primary endpoint TTR assessed at 3 years, subjects will be censored at the date for which they are last known to be free of CRC recurrence. Subjects who are alive and free of CRC recurrence at 3 years plus 21 days (allowing for the 21-day visit-window) will be censored at that time-point. Subjects who have withdrawn their written informed consent during the trial will be censored at the withdrawal date. Subjects who have relocated, and where information on endpoints is not collectable, will be censored at date of relocation. Subjects who have died will be censored at the date of death. For subjects experiencing CRC recurrence, TTR will be calculated as the number of days from randomization until date of CT/MRI, or EoS date if the aforementioned date is missing, or recurrence date collected separately from sites if recurrence has not been entered into the eCRF (see Section 4.9 for details on data collection). For subjects not experiencing CRC recurrence, TTR will be calculated as the number of days from randomization until date of censoring (see above). The TTR event indicator will be defined as 1 for subjects who have experienced CRC recurrence with a CT/MRI/EoS/recurrence date within 3 years plus 21 days from randomization, and 0 for those that have not. The time variable will be named *ttr3y*, and the event indicator will be named *rec_status_3y*.

TTR will also be assessed at 5 years (see Section 8.3). The variables *ttr5y* and *rec_status_5y* will be created similarly as described above, with censoring at 5 years plus 30 days.

For the secondary endpoint DFS assessed at 3 years, subjects who are free of CRC recurrence, second primary CRC, other cancer, or death from any cause will be censored at the date for which they are last known to be free of such events. Subjects who are free of the above events at 3 years plus 21 days (allowing for the 21-day visit-window) will be censored at that time-point. DFS will be calculated as the number of days from randomization until recurrence date (see definition in the above paragraph), EoS date, death date or date of other cancer collected separately from sites (see Section 4.9), or the date of censoring (see above). The DFS event indicator will be defined as 1 for subjects who have a DFS event within 3 years plus 21 days from randomization, where DFS events are defined as:

- Having EoS reasons CRC recurrence or death, or
- Having EoS reasons “other medical reason” or “other reason”, with other cancer or second primary CRC specified as reason, or
- Having a recurrence/other cancer/death collected separately from sites (see Section 4.9),

and 0 for subjects who have not reached any of the above endpoints. The time variable will be named *dfs3y*, and the event indicator will be named *dfs_status_3y*.

For the secondary endpoint OS assessed at 5 years, subjects who have not died will be censored at the date for which they are last known to be alive. Subjects who are alive at 5 years plus 30 days (allowing for the 30-day visit-window for visit 16) will be censored at that

time-point. For subjects who have died within 5 years plus 30 days from randomization, OS will be calculated as the number of days from randomization until one of the following:

- EoS date, where “Death” is entered as reason for EoS, or
- death date entered at Visit 16, or,
- death date collected separately from sites (see Section 4.9),

and the OS event variable will be defined as 1 for such subjects. For other subjects, the OS event variable will be defined as 0, and OS will be calculated as the number of days from randomization until date of censoring (see above). The time variable will be named *os5y*, and the event indicator will be named *os_status_5y*.

6.2.2 Variables for compliance calculation

See Section 4.8.2 for the definition of overall compliance.

The number of treatment days until the last clinic visit will be calculated as days from the date of randomization (*A_randomiseringsdatum*) until the date of the last clinic visit (*V_Visitdatum* for the latest clinic visit, where clinic visits are *V_Visit* V5, V6, V7, V8, V9, V10, V11, V13, V15).

The number of dispensed pills at a certain clinic visit will be calculated as 100 times the number of unique package numbers (*S_Package_Number*) for packages that are not shredded (*R2465T32612_MAKULERAD)* or not found at the clinic (*S_Not_Found*).

The total number of dispensed pills will be calculated as the sum of the dispensed pills at each visit.

The total number of remaining pills will be calculated as the sum of the number of remaining pills for each clinic visit (*S_returned_number*). If the package was lost by the subject (S_Lost_Drug=”True”), and *S_returned_number* equals 0 or “NA”, the number of remaining pills for that package will be defined as 100, to not overestimate compliance.

The number of days on break will be calculated as the sum of days from *V_UppehallFromDatum* until *V_UppehallTomDatum*, for all visits where *V_Uppehall*=1. Treatment breaks that are longer than 30 days and unrelated to adjuvant chemotherapy will be defined as non-compliant with the study protocol. Similarly, breaks that are at most 30 days of related to chemotherapy will be defined as compliant with the study protocol. Days on treatment break that are overlapping with a later registered treatment break will be subtracted.

Compliance for subjects with an EoS event after the first phone contact but before the first clinic visit will be based on the variable *V_LMIntag* for *V_Visit*=V4.

The date for which C_{LCV} (or C_{V4}) is considered valid until ($D_{LCV/V4}$) is *E_sistaintag, dfs_event_date, E_endofstudydtm*, or *v15_visitdatum*. The number of valid days will be calculated as the number of days from randomization (*A_randomiseringsdatum*) until $D_{LCV/V4}$, subtracting the number of days on non-allowed treatment break (see above). The number of zero-compliance days will be calculated as days from $D_{LCV/V4}$ until *E_endofstudydtm* or 3 years from randomization, adding the number of days on non-allowed treatment break.

The compliance variable will be named *overall_compl*, and the indicator variable for compliance of at least 80% will be named *overall_compl_80*.

6.3 Subject disposition

A skeleton CONSORT flowchart is shown in Figure 3. The layout of the flowchart may be updated in accordance with the guidelines of the selected journal(s).

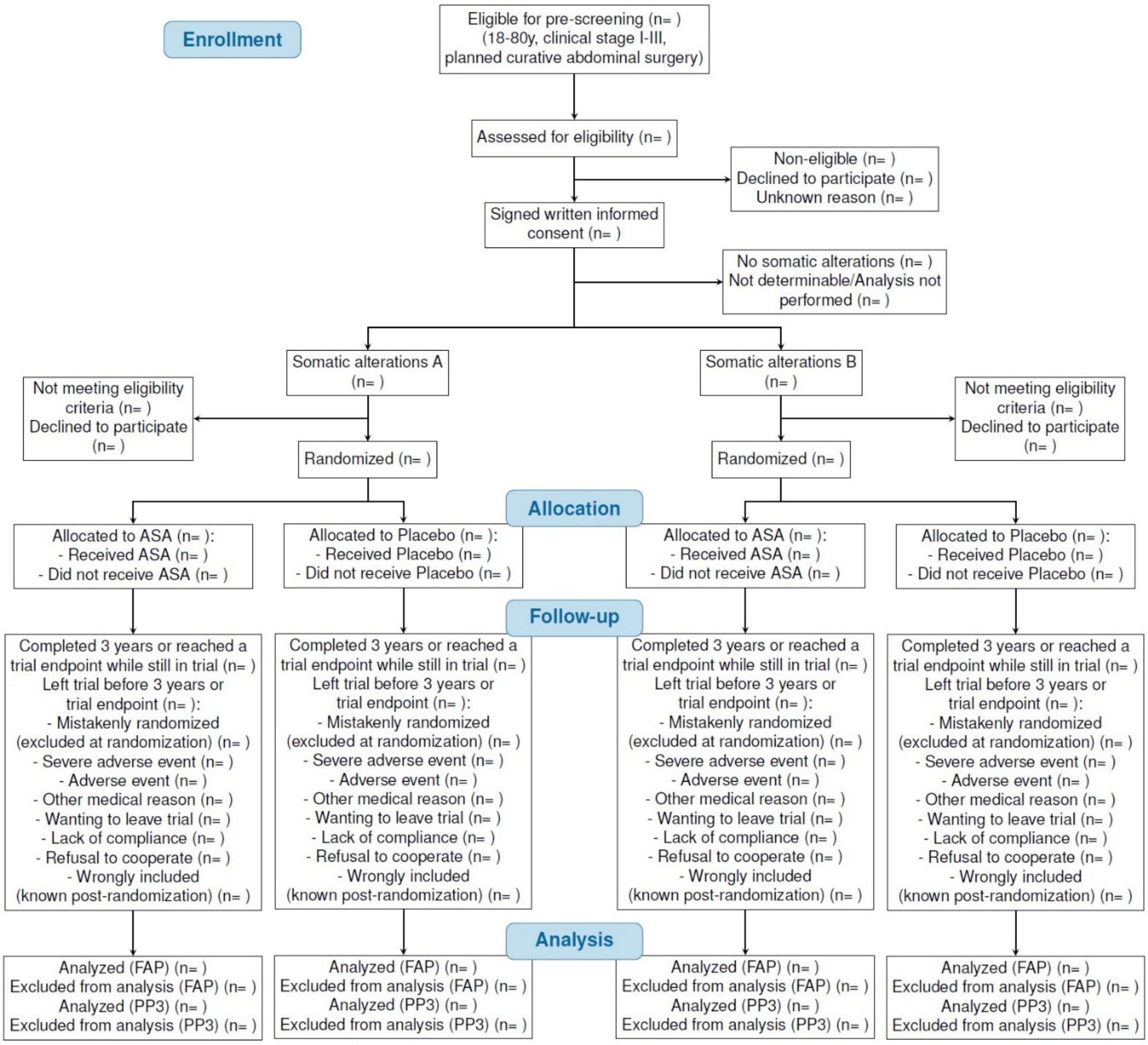

Figure 3. Skeleton CONSORT flowchart

7 Safety interim analyses

7.1 Safety interim analysis 2018

Data was extracted from the eCRF on March 27th, 2018, including subjects that were randomized up to and including March 12th, 2018, with follow-up up to and including March 27th, 2018. The safety report was sent to the Safety Monitoring Committee (SMC) on April 13th, 2018. The frequency of serious adverse events (SAEs) and AEs per randomized arm, visit, and type of SAE (see Table 8 for categories) was presented. The frequency of AEs at the first visit and in all visits combined, in total and stratified by intensity (see Table 8 for categories), was compared between the randomized arms using Fisher's exact test. Information on all SAEs and AEs were listed, see Table 8 for details.

7.2 Safety interim analysis 2020

Data was extracted from the eCRF on January 17th, 2020, including subjects that were randomized up to and including October 21st, 2019, with follow-up up to and including January 17th, 2020. The safety report was sent to the SMC on March 5th, 2020.

The number of SAEs were summarized by randomized arm and visit. Fisher's exact test was used to test differences in frequencies of SAEs between randomized arms for each visit. Information on all SAEs were listed, see Table 8 for details. Similarly, the total number of AEs, and number of AEs per AE type (using the categories listed under "Type of SAE" in Table 8), were summarized by randomized arm and visit, and compared between arms for each visit using Fisher's exact test. The number of subjects with multiple AEs was also summarized by randomized arm, visit, and type of AE.

Table 8. SAE and AE details included in the 2018 and 2020 safety reports to the SMC

SAE variable	**Categories**
Visit number	4-9 (2018 report), 4-15 (2020 report)
SAE day	Day in relation to randomization date
Randomized arm	0/1 (not disclosing associated treatment)
Type of SAE	– Hematological – Cancer – Cardiovascular – CNS/Neurological – Dermatological – Endocrine – Eyes, ears, nose, throat – Gastrointestinal – Urogenital – Hormonal – Immunological – Musculoskeletal – Respiratory – Other
SAE description	– Leads to death – Is life threatening – Other important medical event – Requires hospitalization for more than 24 hours or extended medical care – Causes permanent or significant invalidity/functional impairment
Intensity	– Mild – Moderate – Severe
Treatment modification	– Unchanged – Stopped – Other, e.g., treatment pause
Result	– Restored – Under improvement – Not Restored, with sequels – Death

8 Analysis of trial endpoints

All tests and confidence intervals (CIs) will be two-sided. The significance level will be 5 %, and CIs will have 95 % confidence level. The endpoints and their respective variables and populations are summarized in Table 9.

Table 9. Specification of variables and populations for the primary endpoint or secondary endpoints 1-5 in the ALASCCA trial. Mutation A: PIK3CA exon 9 and 20 hotspot small mutations, Mutation B: other PIK3CA, PIK3R1 and PTEN non-silent small mutations and focal/homozygous deletion of PTEN.

Endpoint	**Specification**	**Time variable**	**Event indicator**	**Population**	**Subgroup**
Primary endpoint	TTR assessed at 3 years between randomized arms	ttr3y	rec_status_3y	FAP	Mutation A
Secondary endpoint 1	DFS assessed at 3 years between randomized arms	dfs3y	dfs_status_3y	FAP	Mutation A
Secondary endpoint 2	TTR assessed at 3 years between randomized arms	ttr3y	rec_status_3y	FAP	Mutation B
Secondary endpoint 3	DFS assessed at 3 years between randomized arms	dfs3y	dfs_status_3y	FAP	Mutation B
Secondary endpoint 4	OS assessed at 5 years between randomized arms	os5y	os_status_5y	FAP	Mutation A
Secondary endpoint 5	OS assessed at 5 years between randomized arms	os5y	os_status_5y	FAP	Mutation B

8.1 Primary efficacy analysis

The analysis of the primary endpoint TTR assessed at 3 years from randomization in subjects with somatic alterations in the PIK3CA gene (exons 9/20) will be performed on a subset of the FAP containing only subjects with such somatic alterations.

The primary efficacy analysis will consist of a Cox proportional hazards model (PHM), including the covariate *Treatment* (ASA vs Placebo, defined by randomization). The baseline hazard will be stratified by the covariates used in the randomization – location (colon/rectum), and stage (I/II/III, included as a categorical variable). The strata will thus be the following

- Stratum 1: Colon, Stage II
- Stratum 2: Colon, Stage III,
- Stratum 3: Rectum, Stage I,
- Stratum 4: Rectum, Stage II,
- Stratum 5: Rectum, Stage III.

The Breslow method will be used to handle tied event-times. The model is given by

$$h_j(t)=h_{0,j}(t)\cdot\exp(\beta_{Treat}\cdot Arm),$$

where $h_j(t)$ and $h_{0,j}(t)$ denote the hazard function and baseline hazard function, respectively, for stratum *j, j=1,…,5*, and *Arm* denotes randomized arm (ASA=1, Placebo=0).

A HR=exp(β_{Treat}) comparing the hazard rates of the ASA and placebo arms will be estimated, along with a two-sided 95 % CI, and a two-sided p-value for testing the null hypothesis

H_0: $\beta_{Treat} = 0$

against the alternative hypothesis

H_1: $\beta_{Treat} \neq 0$.

The proportional hazards assumption (PHA) of the Treatment variable will be assessed using the Grambsch-Therneau test with a significance level of 5 %. If the PHA is violated, the overall HR will still be reported as the primary efficacy estimate, but an additional analysis will be added where follow-up is divided into two intervals, 0-1.5 years, and 1.5-3 years from randomization, and one HR for Treatment will be estimated for each time interval by adding an interaction term between Treatment and the time intervals.

In addition to the Cox PHM, TTR in the two treatment arms (ASA and Placebo) will be visually inspected by the cumulative incidence function (CIF) using Aalen-Johansen estimates, including 95% CIs, from time of randomization until three years of follow-up. Death will be considered a competing event. The 1- and 3-year Aalen-Johansen estimates of cumulative incidence of CRC recurrence in each arm, along with 95 % CIs, will also be presented. Kaplan-Meier (KM) curves estimating the net proportion of CRC recurrence, including 95% CIs, from time of randomization until three years of follow-up will be included as a supplementary figure.

8.2 Secondary efficacy analyses

8.2.1 Secondary endpoints 1-5

The secondary endpoints 1-5 will be analyzed as described in Section 8.1. DFS and OS will be visualized using Kaplan-Meier estimates. For OS endpoints, the KM estimates for the net proportion or survival will be made at 5 years, and any analyses of OS endpoints will be performed earliest in 2026 when each randomized subject has a full 5-year follow-up. See Table 9 for specifications on endpoint variables, event indicators and subsets for secondary endpoints 1-5.

8.2.2 Safety and tolerability

SAEs and AEs in the SP will be listed and summarized, stratified by randomized arm. All SAEs will be coded by authorized study personnel according to the most recent version of MedDRA. SAEs and AEs will be reported in EudraCT and clinicaltrials.gov.

8.3 Exploratory analyses

In addition to the primary efficacy analysis and the analyses of secondary endpoints, some or all of the exploratory analyses listed in Table 10 (3-year endpoints) and Table 11 (5-year endpoints) may also be performed. All exploratory endpoints on the FAP will be analyzed as described in Section 8.1, however, for analyses in only colon/rectum/left-sided/right-sided colon subjects, the strata will only be defined by stage. For analyses with subgroups based on adjuvant treatment, an interaction-term between randomization arm and a time-varying treatment indicator will be used considering that adjuvant treatment for some subjects starts post-randomization. In result tables for exploratory analyses, 95 % CI's, unadjusted for multiple testing will be presented, but not p-values.

For the analyses on the PP3 and PP5 populations, stabilized inverse probability of treatment weights (IPTW) will be used to estimate the effect on the randomized population. The stabilized IPTW for each subject in the PP3 and PP5 populations will be calculated as the ratio between the probability of receiving the treatment that was received (ASA or Placebo) estimated from a logistic regression model without covariates, and the probability of receiving the treatment that was received (ASA or Placebo) estimated in a logistic regression model including the covariates age, sex, country, BMI, tumor location (colon/rectum), type of neoadjuvant treatment, ASA score, time from surgery until randomization, calendar time of randomization, platelet count at baseline, CEA at baseline, MSI status, BRAF V600E status, KRAS status, and tumor differentiation. The use of inverse probability of censoring weights (IPCW), in addition to the IPTW's, for the PP analyses has been considered, but deemed unsuitable for the ALASCCA trial data for several reasons: 1) The time-point for which a subject is to be considered non-compliant is not well-defined, and therefore an IPCW approach would demand strong assumptions and/or looking into the future for certain subjects, 2) Compliance (described in Section 4.8.2) is defined as a weighted average of the visit-specific compliance measurements over the whole study period, and censoring at the first visit-specific non-compliance would result in the censoring of a substantial number of subjects with an overall compliance of >80%, and 3) There are no relevant time-dependent covariates measured during follow-up to be included for capturing treatment-confounder feedback. Given the above considerations, the limitations of the suggested PP analysis approach must be highlighted when presenting the trial results.

A secondary or exploratory analysis involving the Mutation B subgroup may be subjected to sensitivity analysis to assess the impact of observations that may not represent true driver (cancer-relevant) variants. This may include repeating the analysis using a subset containing only high-confidence driver variants.

Table 10. Specification of variables and populations for the exploratory analyses in the ALASCCA trial for 3-year endpoints. Mutation A: PIK3CA exon 9 and 20 hotspot small mutations,

Mutation B: other PIK3CA, PIK3R1 and PTEN non-silent small mutations and focal/homozygous deletion of PTEN.

Specification	**Time variable(s)**	**Event indicator(s)**	**Population**	**Subgroup**
TTR between randomized arms	ttr3y	rec_status_3y	PP3	Mutation A
TTR between randomized arms	ttr3y	rec_status_3y	PP3	Mutation B
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A + Mutation B
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Clonal high-confidence drivers: Mutation A + Mutation B
TTR between randomized arms	ttr3y	rec_status_3y	FAP	Active treatment Mutation A vs Placebo Mutation A + Mutation B
TTR starting 6 months from randomization	ttr3y	rec_status_3y	FAP	Mutation A
TTR starting 6 months from randomization	ttr3y	rec_status_3y	FAP	Mutation B
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, rectal cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, left-sided colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, right-sided colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, neoadjuvant and/or adjuvant treatment received

Specification	Time variable(s)	Event indicator(s)	Population	Subgroup
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, neoadjuvant and/or adjuvant treatment not received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, rectal cancer, any neoadjuvant treatment received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, rectal cancer, no neoadjuvant treatment received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, MSI-H
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation A, MSS/MSI-L
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, rectal cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, left-sided colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, right-sided colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, neoadjuvant and/or adjuvant treatment received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, neoadjuvant and/or adjuvant treatment not received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, rectal cancer, any neoadjuvant treatment received

Specification	Time variable(s)	Event indicator(s)	Population	Subgroup
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, rectal cancer, no neoadjuvant treatment received
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, MSI-H
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Mutation B, MSS/MSI-L
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Rectal cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Left-sided colon cancer
TTR and DFS between randomized arms	ttr3y, dfs3y	rec_status_3y, dfs_status_3y	FAP	Right-sided colon cancer

Table 11. Specification of variables and populations for the exploratory endpoints in the ALASCCA trial for 5-year endpoints. Mutation A: PIK3CA exon 9 and 20 hotspot small mutations, Mutation B: other PIK3CA, PIK3R1 and PTEN non-silent small mutations and focal/homozygous deletion of PTEN.

Specification	Time variable	Event indicator	Population(s)	Subgroup
TTR between randomized arms	ttr5y	rec_status_5y	FAP and PP5	Mutation A
TTR between randomized arms	ttr5y	rec_status_5y	FAP and PP5	Mutation B
TTR and OS between randomized arms	ttr5y, os5y	rec_status_5y, os_status_5y	FAP and PP5	Mutation A + Mutation B
OS between randomized arms	os5y	os_status_5y	PP5	Mutation A

Specification	Time variable	Event indicator	Population(s)	Subgroup
OS between randomized arms	os5y	os_status_5y	PP5	Mutation B

9 Quality assurance of statistical programming

At the start of any code file there will be a comment specifying the author, date of file creation, date(s) and details of any file updates, reference to any parent code file that runs the child code file, references to input and output files.

A second statistician will review the code producing the data processing, and the primary and secondary efficacy analyses.

The statistical software R (R Foundation for Statistical Computing) will be used, in a version released less than a year before the time of analysis of the primary and secondary endpoints.

10 Listing of tables and figures

Note that the table and figure numbering in the below sections does not necessarily reflect the actual numbering in future publications. Some of the tables and figures may be published separately and in different order. Table formatting, e.g. number of decimals/significant digits, headings, row ordering etc. may be changed to follow the specifications of the selected journal(s).

Table 12. List of tables.

Table	Description	Population	Columns
Table 1.	Patient characteristics for randomized subjects in the ALASCCA trial.	FAP	Randomized to ASA Randomized to Placebo
Table 2.	Results from analysis of primary endpoint, and secondary endpoint 1-3. If the PHA is violated, one HR per time-interval (in addition to the overall HR).	FAP	N per randomized arm, no. of events per randomized arm, HR, 95% CI, p-value
Table 3.	Results from analysis of secondary endpoints 4-5.	FAP	N per randomized arm, no. of events per randomized arm, HR, 95% CI, p-value
Table 4.	Results from analyses of exploratory endpoints.	FAP/PP3	N per randomized arm, no. of events per randomized arm, HR, 95% CI
Table 5.	SAEs per randomized arm.	SP	Number of SAEs per randomized arm
Table 6.	AEs per randomized arm.	SP	Number of AEs per randomized arm
Supplementary Table 1.	Number of AEs per randomized arm and visit, stratified by type of AE.	SP	Number of AEs per randomized arm

Table 13. List of figures. Mutation A: Mutation in PIK3CA exons 9/20, Mutation B: Mutation in PIK3CA (other exons), PIK3R1, PTEN.

Figure	Description	Population	Axis labels (if applicable)
Figure 1.	CONSORT flowchart showing number of screened patients, exclusions, randomized subjects.		
Figure 2a.	CIF curves, with 95% CIs, comparing TTR assessed at 3 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (exons 9/20).	FAP, Mutation A	X-axis "Months from randomization" Y-axis "Cumulative incidence of CRC recurrence (%)"
Figure 2b.	CIF curves, with 95% CIs, comparing TTR assessed at 3 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (other exons), PIK3R1, or PTEN.	FAP, Mutation B	X-axis "Months from randomization" Y-axis "Cumulative incidence of CRC recurrence (%)"
Figure 3a.	KM curves, with 95% CIs and risk table, comparing DFS assessed at 3 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (exons 9/20).	FAP, Mutation A	X-axis "Months from randomization" Y-axis "Net proportion of DFS (%)"
Figure 3b.	KM curves, with 95% CIs and risk table, comparing DFS assessed at 3 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (other exons), PIK3R1, or PTEN.	FAP, Mutation B	X-axis "Months from randomization" Y-axis "Net proportion of DFS (%)"
Figure 4.	CIF curves, with 95% CIs, comparing TTR assessed at 3 years between ASA and Placebo.	FAP, Mutation A + Mutation B	X-axis "Months from randomization" Y-axis "Cumulative incidence of CRC recurrence (%)"
Figure 5a.	KM curves, with 95% CIs and risk table, comparing OS assessed at 5 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (exons 9/20).	FAP, Mutation A	X-axis "Months from randomization" Y-axis "Net survival (%)"
Figure 5b.	KM curves, with 95% CIs and risk table, comparing OS assessed at 5 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (other exons), PIK3R1, or PTEN.	FAP, Mutation B	X-axis "Months from randomization" Y-axis "Net survival (%)"
Figure 6.	KM curves, with 95% CIs and risk table, comparing OS assessed at 5 years between ASA and Placebo.	FAP, Mutation A + Mutation B	X-axis "Months from randomization" Y-axis "Net survival (%)"
Supplementary Figure 1.	KM curves, with 95% CIs and risk table, comparing TTR assessed at 3 years between ASA and Placebo in subjects with somatic alterations in PIK3CA (exon 9/20).	FAP, Mutation A	X-axis "Months from randomization" Y-axis "Net proportion of CRC recurrence (%)"

10.1 Table templates

Note that, in the patient characteristics table (Table 14), if there are missing values in a certain variable, a row specifying the number of missing values will be added for that variable.

Table 14. Template for Table 1: Patient characteristics for randomized subjects in the ALASCCA trial.

Characteristic		**Randomized to ASA n (%)**	**Randomized to Placebo n (%)**	**Mut. group A (ASA) n (%)**	**Mut. group A (Placebo) n (%)**	**Mut. group B (ASA) n (%)**	**Mut. group B (Placebo) n (%)**
Age at baseline (years)	Median (IQR, min-max)						
Sex	Female						
	Male						
Country	Sweden						
	Norway						
	Denmark						
	Finland						
Time from CRC surgery to randomization (weeks)	Median (IQR, min-max)						
BMI at baseline (kg/m^2)	Median (IQR, min-max)						
Platelet count at baseline (x10^9/L)	Median (IQR, min-max)						
CEA at baseline (µg/L)	Median (IQR, min-max)						
ASA score	1						
	2						
	3						
	4						
	5						
Tumor location	Right colon						
	Left colon						
	Rectum						
Hight from anal verge in rectal cancer (cm)	0-5						
	6-10						
	≥11						
Neoadjuvant treatment in rectal cancer	RT only						
	CRT						
	RT+Chemo						
	CRT+Chemo						
	Chemo only						
	No neoadjuvant treatment						

Characteristic		Randomized to ASA n (%)	Randomized to Placebo n (%)	Mut. group A (ASA) n (%)	Mut. group A (Placebo) n (%)	Mut. group B (ASA) n (%)	Mut. group B (Placebo) n (%)
Neoadjuvant RT dose (Gy) and fraction in rectal cancer	5x5						
	2x25						
	1.8x28						
	Other						
Surgery	Elective						
	Emergency						
Type of surgery	Ileocecal resection						
	Right-sided hemicolectomy						
	Transversum resection						
	Left-sided hemicolectomy						
	Sigmoideum resection						
	Total colectomy						
	Anterior resection						
	Rectum amputation						
	Hartmann's procedure						
	Other procedure						
pTNM stage in colon cancer	II						
	III						
pTNM stage in rectal cancer	I						
	II						
	III						
Tumor grade	Low grade						
	High grade						
	Unknown						
MSI	MSI-high						
	MSI-low						
	Uncertain						
BRAF mutation[1]	Yes						
	No						
	Uncertain						
NRAS mutation[2]	Yes						
	No						
	Uncertain						

Characteristic		Randomized to ASA n (%)	Randomized to Placebo n (%)	Mut. group A (ASA) n (%)	Mut. group A (Placebo) n (%)	Mut. group B (ASA) n (%)	Mut. group B (Placebo) n (%)
KRAS mutation[2]	Yes						
	No						
	Uncertain						
Adjuvant chemotherapy in colon cancer	Fluorouracil/capecitabin						
	Fluorouracil/capecitabin + oxaliplatin						
	Other adjuvant chemotherapy						
	No adjuvant chemotherapy						
	Missing						
Adjuvant chemotherapy in rectal cancer	Fluorouracil/capecitabin						
	Fluorouracil/capecitabin + oxaliplatin						
	Other adjuvant chemotherapy						
	No adjuvant chemotherapy						
	Missing						

[1]V600E
[2]Exons 12, 13, 59, 61, 117 and/or 146

Table 15. Table template for Table 2: Results from analysis of primary endpoint, and secondary endpoint 1-3. If the PHA is violated, one HR per time-interval (in addition to the overall HR).

Outcome	**N (n events) in ASA arm**	**N (n events) in Placebo arm**	**HR ASA vs Placebo (95% CI; p)**
TTR in PIK3CA exons 9/20			
DFS in PIK3CA exons 9/20			
TTR in PIK3CA (other exons), PIK3R1, PTEN			
DFS in PIK3CA (other exons), PIK3R1, PTEN			

Table 16. Table template for Table 3: Results from analysis of secondary endpoints 4-5.

Outcome	**N (n events) in ASA arm**	**N (n events) in Placebo arm**	**HR ASA vs Placebo (95% CI; p)**
OS in PIK3CA exons 9/20			
OS in PIK3CA (other exons), PIK3R1, PTEN			

Table 17. Table template for Table 4: Results from analysis of exploratory endpoints.

Outcome	**Subgroup**	**N (n events) in ASA arm**	**N (n events) in Placebo arm**	**HR ASA vs Placebo (95% CI)**
TTR	PIK3CA exons 9/20 (PP3 population)			
TTR	PIK3CA (other exons), PIK3R1, PTEN (PP3 population)			
…				

Table 18. Table template for Table 5: Number of SAEs per randomized arm.

		Randomized to ASA		**Randomized to Placebo**	
		Number of SAEs (%)	**N subjects in SP**	**Number of SAEs (%)**	**N subjects in SP**
Total					
Time from randomization (months)	Median (IQR, min-max)				
Type of SAE	Hematological				
	Cancer				
	Cardiovascular				
	CNS/Neurological				
	Dermatological				
	Endocrine				

		Randomized to ASA		Randomized to Placebo	
		Number of SAEs (%)	**N subjects in SP**	**Number of SAEs (%)**	**N subjects in SP**
	Eyes, ears, nose, throat				
	Gastrointestinal				
	Urogenital				
	Immunological				
	Musculoskeletal				
	Respiratory				
	Other				
SAE description	Leads to death				
	Is life threatening				
	Other important medical event				
	Requires hospitalization for more than 24 hours or extended medical care				
	Causes permanent or significant invalidity/functional impairment				
Intensity	Mild				
	Moderate				
	Severe				
Treatment modification	Unchanged				
	Stopped				
	Other, e.g. treatment pause				
Result	Restored				
	Under improvement				
	Not Restored, with sequels				
	Death				

Table 19. Table template for Table 6: Number of AEs per randomized arm.

		Randomized to ASA		Randomized to Placebo	
		Number of AEs (%)	**N subjects in SP**	**Number of AEs (%)**	**N subjects in SP**
Total					
Time from randomization (months)	Median (IQR, min-max)				
Time from start of AE until end of AE (days)	Median (IQR, min-max)				
Type of SAE	Hematological				
	Cancer				
	Cardiovascular				
	CNS/Neurological				
	Dermatological				
	Endocrine				

	Eyes, ears, nose, throat				
	Gastrointestinal				
	Urogenital				
	Hormonal				
	Immunological				
	Musculoskeletal				
	Respiratory				
	Other				
Intensity	Mild				
	Moderate				
	Severe				
Treatment modification	Unchanged				
	Stopped				
	Other, e.g. treatment pause				
Ongoing AE	Yes				
	No				
Related to the IMP	Yes				
	No				
Result	Restored				
	Under improvement				
	Not Restored, with sequels				
	Death				

10.2 Figure templates

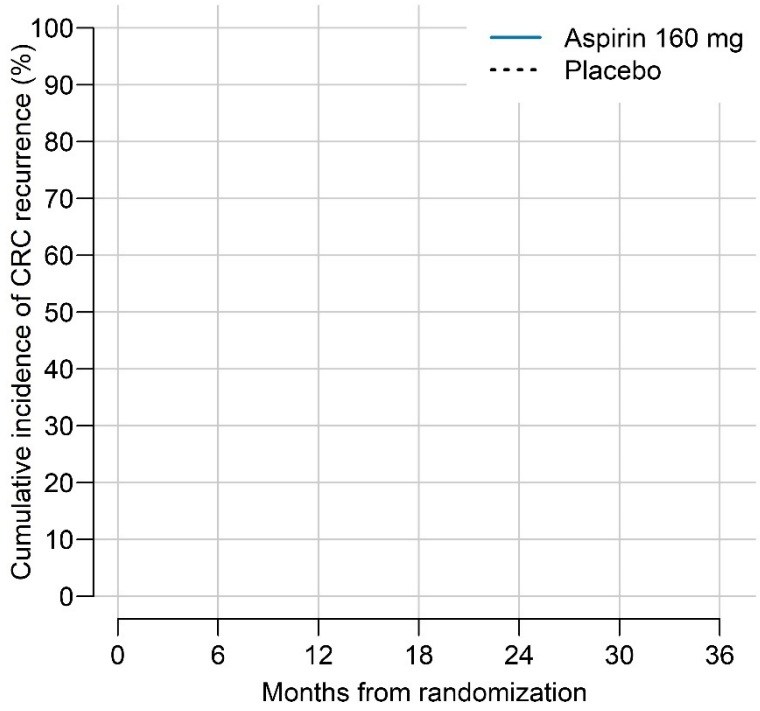

Figure 4. Template for Figures 2a, 2b, and 4.

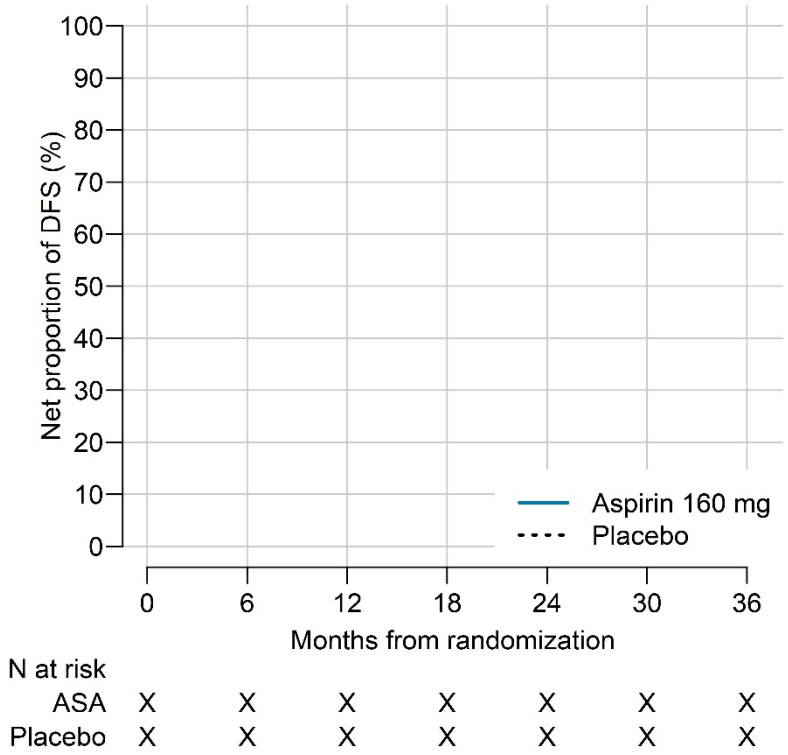

Figure 5. Template for Figure 3a and 3b.

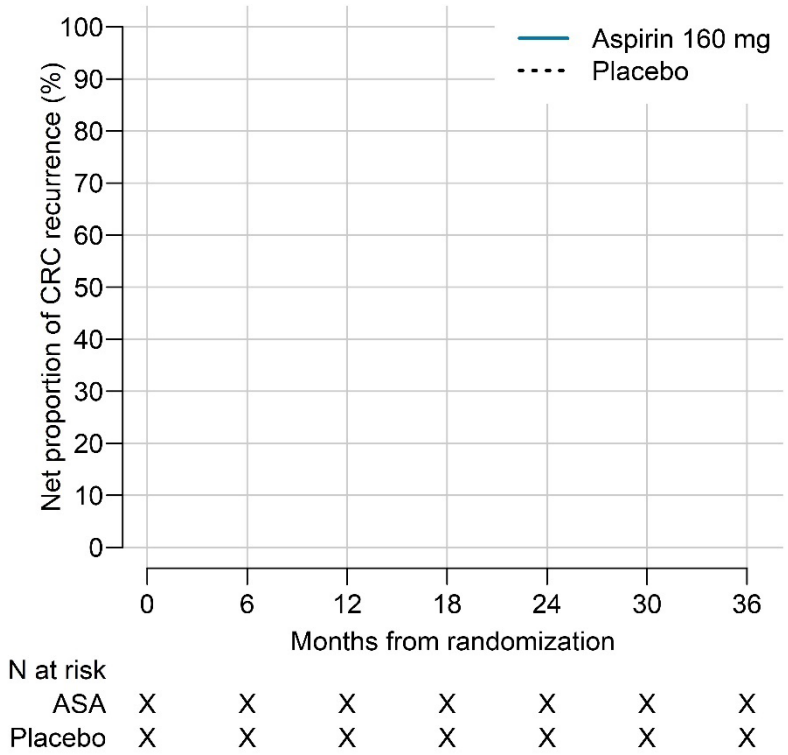

Figure 6. Template for Supplementary Figure 1.

11 References

1. Osterman, E. and B. Glimelius, *Recurrence Risk After Up-to-Date Colon Cancer Staging, Surgery, and Pathology: Analysis of the Entire Swedish Population.* Dis Colon Rectum, 2018. **61**(9): p. 1016-1025.
2. Yelland, L.N., et al., *Handling misclassified stratification variables in the analysis of randomised trials with continuous outcomes.* Stat Med, 2023. **42**(19): p. 3529-3546.
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