ACETYLSALICYLIC ACID AS SECONDARY PREVENTION IN COLORECTAL CANCER (ASAC TRIAL)

A multi-centre, double-blinded, randomized, placebo-controlled clinical intervention trial with ASA in patients undergoing liver resection for CRC metastasis

Protocol Identification Number: ASA-CRCLM-2014

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- CONFIDENTIAL -

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Protocol revision history

Version no.	Date	Description of change
1.0	Working document	
2.0	Working document	
2.2	23 Feb 2017	First version submitted to Norwegian authorities, not approved.
2.3	Working document	
2.4	19 May 2017	Version valid for Norway.
2.5	20 July 2017	Version valid for Norway and Sweden.
3.0	20 Oct 2017	Version valid for Norway and Sweden, version submitted for start in Denmark. Main changes: Direct medical-care costs, Quality-Adjusted Life-Years (QUALYs) and life-years gained added under exploratory objective. Time for inclusion changed from 3-6 weeks postoperatively to 1-5 days. Blood samples for safety are only taken at inclusion and study termination, at all other time-points only CEA is measured and at local laboratories. Extra sample for analysis of ASA removed. Samples are not to be saved. All samples are taken and analysed locally. Patient visit to participating clinic is once per year.
4.0 (Denmark)	26 Feb 2018	Country-specific version for Denmark
5.0	06 Jun 2018	Valid for all countries. Clarifications made to adapt to all country requirements, including patient visit frequency and inclusion criteria.
5.1	21 Dec 2018	Added to Point 6.2 Molecular and genetic analysis of tumour and blood samples, which will be done at Karolinska Institute, Stockholm, Sweden. Updated site investigators.
5.2	11 Mar 2019	Revised Follow-up in Norway according to new National Guidelines for CRC liver metastases (control with CT every 6 months). Added Exclusion criteria (point 4.4): other malignancies, and decision made by the investigator.
5.3	30 Mar 2020	New PI: Sheraz Yaqub, MD PhD, is assigned as PI of the ASAC trial, replacing Dr. Bjørn Atle Bjørnbeth

SIGNATURE PAGE

Title	**Acetylsalicylic acid as secondary prevention in colorectal cancer (ASAC trial)** A multi-centre, double-blinded, randomized, placebo-controlled clinical intervention trial with ASA in patients undergoing liver resection for CRC metastasis
Protocol ID no:	ASA-CRCLM-2014
EudraCT no:	2014-003601-15
ClinicalTrials.gov:	NCT03326791

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:

Name	Title	Role	Signature	Date
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Kjetil Tasken	Professor, MD PhD	Co-PI		05.10.2020
Sheraz Yaqub	MD PhD FEBS	Coordinating investigator		05.10.2020

SIGNATURE PAGE – PRINCIPAL INVESTIGATOR

Title **Acetylsalicylic acid as secondary prevention in colorectal cancer (ASAC trial)**
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I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:

Name	Title	Role	Signature	Date
	MD	Principal investigator (PI)		

PROTOCOL SYNOPSIS

Acetylsalicylic acid as secondary prevention in colorectal cancer (ASAC trial)
A multi-centre, double-blinded, randomized, placebo-controlled clinical intervention trial with ASA in patients undergoing liver resection for CRC metastasis

Sponsor	Oslo University Hospital, Elin Henriksen
Phase and study type	Phase III, multicentre, randomized, placebo-controlled, group sequential clinical intervention trial
Investigational Medical Product (IMP) (including active comparator and placebo) :	Trombyl 160 mg (Pfizer); Active ingredient: Acetylsalicylic acid (ASA) Comparator: Placebo (by ClinStorage AB, Sweden)
Centres:	**NORWAY:** Oslo University Hospital (Oslo) Stavanger University Hospital (Stavanger) Haukeland University Hospital (Bergen) St.Olavs Hospital (Trondheim) University Hospital of Northern Norway (Tromsø) **SWEDEN:** Sahlgrenska University Hospital (Gothenburg) Karolinska University Hospital (Stockholm) Linköping University Hospital (Linköping) Lund University Hospital (Lund) Uppsala University Hospital (Uppsala) Norrlands University Hospital (Umeå) **DENMARK:** Rigshospitalet University Hospital (Copenhagen) Aarhus University Hospital (Aarhus) Odense University Hospital (Odense)
Study Period:	Estimated date of first patient enrolled: Q4 2017 Anticipated recruitment period: 5 years Estimated date of last patient completed: Q4 2025
Treatment Duration:	36 months (3 years) or until disease recurrence
Follow-up:	The patients will be followed in the outpatient clinics according to the National Guidelines for treatment of colorectal liver metastases with clinical control and CT of the chest and abdomen at 6, 12, 18, 24, 30 and 36 months to investigate for any disease recurrence and register challenges regarding the study drug. The total study duration is 36 months.
Objectives	Primary objective: To determine whether treatment with 160 mg ASA (Trombyl) once daily for 3 years can improve Disease Free Survival (DFS) in patients treated with resection for CRCLM, compared with placebo

Secondary objectives:

- To determine the effect of 160 mg ASA on Time to Recurrence (TTR) and Overall Survival (OS) compared to placebo
- To determine the effect of 160 mg ASA on Health-related Quality of Life (HRQOL) outcome measures. The related endpoints will be the eight RAND 36-Item Health Survey 1.0 (SF-36) dimension scores as well as physical and mental health summary measures, and the EQ-5D index value

Exploratory objectives:

- To determine whether 160 mg ASA can improve DFS and OS in patients with mutations in PIK3CA and KRAS
- To determine the cost-effectiveness of 160 mg ASA compared to placebo
- Direct medical-care costs, Quality-Adjusted Life-Years (QUALYs) and life-years gained

Efficacy endpoints:

Primary endpoint: Time from randomisation to disease recurrence or death by any cause (DFS)

Secondary endpoints:

- Time from randomisation to disease recurrence (TTR)
- Time from randomisation to death by any cause (OS)
- HRQOL outcome measures
    - SF36
    - EQ-5D health surveys

Study Design:

This is a multicentre, randomized, double-blinded, placebo-controlled, group-sequential trial. There will be an interim analysis when approximately half of the planned primary events (disease recurrence) have occurred. The study may be stopped for efficacy at this interim analysis.

Main Inclusion Criteria:

All patients undergoing radical liver resection for CRCLM as a part of a curative intent (macroscopic surgical free resection margin, R0 or R1) or radiofrequency or microwave ablation technique:

- First time CRCLM (synchronous or metachronous), or
- Recurrence of CRCLM (not previously included in this trial)

Main Exclusion Criteria:

- Concomitant use of ASA or other anticoagulants or platelet inhibitors such as warfarin or klopidogrel
- Inherited or acquired coagulopathy (haemophilia)
- Blood platelets (thrombocytes) < 100 x 10^9/L
- Severe heart failure

- Kidney failure
- Pregnancy
- Ongoing regular use of corticosteroids, NSAIDs
- Contraindication listed on the Summary of Product Characteristics (SmPC) of Trombyl:
    - Hypersensitivity/allergies to ASA or NSAIDs
    - Previous severe gastrointestinal haemorrhage/peptic ulcer due to ASA/NSAID
    - Active peptic ulcer
- Need to use medications contraindicated according to SmPC of Trombyl from Swedish Medicines Agency

Sample Size:	Up to 800 patients will be randomized to 2 study arms; Arm#1 receiving ASA 160 mg once daily until recurrent disease or a total period of 3 years; Arm#2 Placebo. The final sample size is conditional on the efficacy evaluation of the interim analysis.
Efficacy Assessments:	At each study visit, the patients will be assessed by CT scan or MRI for disease recurrence. Death by any cause will be recorded at time of event.
Safety Assessments:	Safety will be monitored by laboratory assessments (haematology and biochemistry), collection of AEs and physical examination. Evaluation of performance status using WHO/ECOG performance status is done at screening only.
Other Assessments:	Molecular and genetic profiling of the tumours (both primary and metastases) to assess for gene alterations like mutations in PIK3CA (exon 9 and exon 20) or KRAS will be recorded as well as immune profile of the tumours, if available. The study subjects will complete a short-form health survey SF-36 and EQ-5D on at every visit.
Statistical Methods	Patients will be randomised to 160 mg ASA or placebo in a 1:1 ratio. The null hypothesis for the comparison of DFS, and for the secondary endpoints, is that there is no difference between the treatment arms; the alternative hypothesis is that a difference exists. All tests will be two-sided. Superiority of 160 mg ASA will be demonstrated only if the nominal p-value from the appropriate two-sided test is less than the significance level of 0.01 at the interim analysis and 0.0456 at the final analysis, and the efficacy estimate is in favour of the ASA arm. The primary efficacy endpoint, time from randomisation to disease recurrence or death by any cause, as well as all time-to-event secondary endpoints will be analysed using a stratified log-rank test accounting for the stratification factor (study centre), and the treatment effect will be estimated using the Cox proportional hazards regression model stratified by study centre. A subgroup analysis will be performed to assess the interaction effect between ASA and the mutations in PIK3CA and KRAS. The analysis will be performed on primary and secondary time-to-event endpoints using a stratified Cox proportional model with an ASA/mutation in PIK3CA/KRAS mutation interaction term.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
AE	Adverse Event
ASA	Acetylsalicylic acid
CRF	Case Report Form (electronic/paper)
cAMP	Cyclic adenosine monophosphate
CEA	Carcinoembroynal antigen
COX	Cyclooxygenase
CRC	Colorectal cancer
CRCLM	Colorectal cancer liver metastases
CT	Computed tomography
DFS	Disease Free Survival
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product (includes active comparator and placebo)
NSAID	Non-steroid anti-inflammatory drug
OS	Overall Survival
OUH	Oslo University Hospital
PGE2	Prostaglandin E2
PKA	Protein kinase A
SAE	Serious Adverse Event
SD	Stable Disease
SOP	Standard Operating Procedure
Treg	Regulatory T cell
TTR	Time to Recurrence

1 INTRODUCTION

1.1 Background – Colorectal cancer and liver metastases

Norway has a high incidence of colon and rectum cancer (CRC) with more than 4,000 cases diagnosed each year (Cancer in Norway, 2011). A number of reports have shown that intake of non-steroidal anti-inflammatory drugs (NSAIDs) or acetylsalicylic acid (ASA) inhibiting cyclooxygenases (COX) reduce the risk of CRC development (primary prevention) (Baron JA et al, 2003; Ricchi et al, 2003, Din et al, 2010). Other studies have shown that selective COX-2 inhibitors also are associated with a decline in the incidence of CRC and reduced mortality rate, but undesirable cardiovascular events precluded further studies, in particular with rofecoxib (Kerr et al, 2007). ASA, on the other hand, has a favourable cardiovascular profile and better safety. Recent meta-analyses of 5 to 51 different interventional studies with ASA originally conducted for cardiovascular indications and with 5 to 20 years follow-up have shown when they are re-analysed and linked with cancer registry data, that ASA indeed reduces the risk of later CRC (and other gastrointestinal cancers) with hazard ratios of 0.45 to 0.75 for cumulative incidence and mortality and with doses as low as 75 mg per day, but with increasing benefit with duration of treatment (Rothwell et al, 2010, 2011, 2012a). Preventive effects of ASA were also seen with shorter duration of ASA treatment, but then only with higher dose than 75 mg per day (Rothwell et al, 2012a). In contrast, a large study in the US (Physicians Health Study, with 22,000 subjects) where ASA was administered every second day did not show effect on CRC development (Stürmer et al, 1998). However, a comparison between meta-analysis of randomized studies, case-control studies and observational registry studies with daily administration of ASA all showed similar and highly significant reduction in the risk of CRC (Odds Ratio 0.54-0.69), indicating a distinct and robust effect of ASA in primary prevention of CRC (Algra and Rothwell, 2012). These and other studies have led to a current and on going discussion on the use of ASA as a cost-effective primary prevention for CRC

with added benefit of the cardiovascular effects and versus its safety profile (Krauss and Arber 2008, 2011; Benamouzig and Uzzan, 2010; Jacobs, 2011; Ferrandez et al. 2012; Chan and Cook, 2012). The PI3K pathway is frequently altered in CRC e.g. through mutations of PIK3CA. Recently, acquired mutations in the PIK3CA gene were shown to predict benefit from treatment with ASA (Liao et al, NEJM 2012; Domingo, J Clin Oncol 2013). While patients whose tumours did not carry PIK3CA a mutation had no benefit from aspirin therapy, patients whose tumours carry PIK3CA mutations had an HR of 0.11-0.18 for colorectal cancer-specific death. These data are based on retrospective analyses and require confirmation in prospective randomized trials to establish treatment recommendations with ASA in patients with colorectal cancer.

1.2 Pre-Clinical & Clinical Experience with ASA

Combined with ours and other studies on mechanisms of action of prostaglandins in CRC and effects of perturbing COX by NSAIDs or ASA (reviewed in Yaqub and Taskén, 2008, Brudvik and Tasken 2012), this argues that further studies on effect of COX inhibition post-diagnosis in CRC are warranted.

COX-2 levels are elevated in as many as 85% of human CRCs and approximately 50% of colorectal adenomas (Eberhardt et al, 1994). PGE2 has been shown to be an important mediator of COX-2 associated effects (reviewed in Lone and Taskén, 2013) and PGE2 levels are elevated in CRC biopsies compared with normal mucosa and homozygous deletion of the gene for the PGE2 receptor EP2 that signals through cAMP reduced the number and size of colorectal polyps in a polyposis mouse model (reviewed in Yaqub and Taskén, 2008). Beside a well-documented pro-angiogenic effect (Tsjuii et al, 1998 a.o,), PGE2 promotes apoptosis and stimulates growth of tumour stem cells both through the EP2 and EP3 receptors by synergizing with the Wnt/β-catenin pathway, relevant in cancers where the adenomatous polyposis coli (APC) gene is activated (Taurin et al, 2008; Goessling et al., 2009, see also our report Brudvik et al, 2011). This leads to translocation of beta-catenin, which turns on a programme of genes including c-myc and COX-2. Furthermore, COX-2 over-expression also correlates with tumour recurrence and metastasis of CRC and in this context tumour immunology may be particularly important. Our novel observations in two clinical observational studies show that the PGE2 produced also inhibits anti-tumour immunity (Yaqub et al 2008, Brudvik et al, 2012, described in more detail below). Hence, the effect of COX inhibition to perturb PGE2 signalling in established CRC would be 3-fold: inhibiting angiogenesis and tumour growth and stimulating anti-tumour immunity.

In terms of anti-tumour immunity, a growing tumour with several activating mutations should normally be recognized as foreign by the immune system and eliminated. However, by cancer immuno-editing cancer cells with additional mutations that activate various tumour evasion mechanisms are selected (reviewed in Schreiber et al, 2011). These mechanisms encompass i) Downregulation of MHC-I to avoid recognition; ii) Overexpression of Indoleamine 2,3 dioxygenase (IDO) to induce tolerance; iii) Activation of inhibitory co-receptors on immune cells or triggers of apoptosis; iv) Recruitment and expansion of regulatory T cells (Tregs) and v) Secretion of immunosuppressive tumour-derived soluble factors such as prostaglandin E2 (PGE2) that inhibit immune responses (Zou, 2006 for review and references). Interestingly, several tumour evasion mechanisms such as secretion of PGE2 from tumor cells and several suppressive mechanisms by Tregs converge on cAMP immune suppression in effector T cells (our discovery; reviewed in Yaqub and Taskén, 2008).

Based on the work of the Taskén laboratory and that of other groups, the role of cAMP in the induction of Tregs and Treg-mediated suppression is increasingly getting focus (reviewed in Yaqub and Taskén, 2008; Bjørgo et al., 2011). Our data show that continuous activation of T cells leads to generation of adaptive Tregs (iTregs, Aandahl et al, 2004). Adaptive iTregs express COX-2 as a consequence of continuous exposure to antigen, leading to secretion of PGE2. PGE2 in turn stimulates FOXP3 expression in the Tregs and inhibits effector T cell function through activation of the cAMP inhibitory pathway (Mahic et al., 2006). Finally, LPS-activated monocytes also secrete high levels of PGE2, inhibiting T cell activation and inducing FOXP3 expression (Bryn et al., 2008).

Cyclic AMP (cAMP) and protein kinase A (PKA) are involved in the regulation of a broad range of body functions and involve most organ systems. A major goal of the Taskén research group has been to identify the role of cAMP in the regulation of T cell function and translate involvement in disease mechanisms into therapeutic strategies and possibly clinical practice. Cyclic AMP acts as an acute inhibitor of T cell activation that prevents T cell proliferation and cytokine production. We have previously mapped an inhibitory pathway in effector T cells that modulates immune function and involves cAMP, PKA type I and C-terminal Src kinase (Csk) (Vang, 2001, Ruppelt, 2007 and about 100 other papers on cAMP regulation of immune function) and devised a number of strategies to perturb this anchored pathway at different levels (cAMP antagonists, PKA and substrate anchoring disruptor peptides and small molecules) to get proof of concept of immune regulation in cellular and in vivo experiments. We demonstrate that Teff immune suppression can be reversed by small molecule pharmaceutical inhibitors of the cAMP signalling pathway in normal blood donors, patient samples and in vivo in CRC animal models (Mahic et al., 2006; Yaqub et al., 2008; Oberprieler et al, 2010; Brudvik et al, 2011, a.o.). Furthermore, as we have shown that iTregs formed upon chronic antigen stimulation express COX-2, secrete PGE2 and

suppress effector cells through the cAMP pathway (Mahic et al., 2006, a.o.) as discussed above, this nicely links in vivo immune regulation to molecular mechanisms and points to targeting COX with available drugs as an attractive possibility.

To examine the role of iTreg- and PGE2-mediated suppression of anti-tumour immunity in CRC we first conducted an observational study on patients referred for surgery of primary CRC. Looking at tumour specimens and peripheral blood samples from patients with CRC we found significantly elevated PGE2 levels and suppressed anti-tumour immune responses that could be reversed by removal of CD25+ iTreg or by pharmacological perturbation of the COX-2 - PGE2 - cAMP pathway at the level of COX or PKA (Yaqub et al. 2008). This was the first demonstration of Treg-mediated suppression of anti-tumour immunity in CRC and indication that pharmacological intervention with NSAIDs could block that effect.

About 50% of patients with CRC develop metastatic disease and this population has a different prognosis than the remaining patients that do not display recurrence following surgical removal of the primary tumour. The liver is the primary site for CRC metastasis and about 30-40% of the patients are available for surgery with primary liver resection or subsequent to down-staging with neoadjuvant chemotherapy, which has significantly improved the life expectancy in this group of patients. However, the population of resected patients is also heterogeneous and while some are cured, others show a rapid recurrence and progression of disease despite a resection with curative intent. Statistically, within 5 years approximately 60-80% of the liver resected patients will suffer from recurrent metastatic disease, and the majority of the recurrences will occur within the first two years after treatment. It was therefore of interest to examine whether Treg suppression of immune responses had impact on clinical fate. We examined Treg-mediated suppression in CRC patients with liver metastasis undergoing liver resection surgery at Oslo University Hospital. We found that the level of Treg-mediated suppression of anti-CEA tumour immune responses (TNFα, IFNγ) through the PGE2-cAMP pathway at the time of surgery predicts future outcome as patients with recurrent disease after 18 months had significantly more Treg-suppression of anti-tumour responses than patients that did not recur and also presented with elevated PGE2 levels as the disease recurred (Brudvik et al, Cancer Immunol. Immunother, 2012).

1.3 Rationale for the Study and Purpose

Although several studies have shown beneficial effect of ASA on primary prevention of CRC, little has been done to examine the effects of NSAIDS or ASA as secondary prevention after diagnosis of CRC where anti-tumour immune regulation would have more impact and which could have clinical benefit. However, examination of occurrence of metastatic disease in the meta-analysis of randomized ASA studies revealed lower frequency of metastasis in ASA users, which could account for the reduced mortality (Rothwell et al, Lancet 2012b). Furthermore, a recent registry study from the Tayside region in Scotland with 3000 cases looking at post-diagnosis ASA use showed reduced CRC-specific mortality (HR 0.58-0.72, depending on method of analysis and site of cancer), indicating a potentially beneficial effect post-diagnosis as secondary prophylaxis (McCowan et al, 2013). Other studies with patient numbers between 536 (Reimers et al 2012) and 4481 (Bastianet et al, 2012) have also shown effect of ASA as secondary prophylaxis on overall survival in patients with CRC (HR 0.59 and 0.77, respectively). In total, ten previous studies have assessed the survival benefit of aspirin use after the diagnosis of colorectal cancer. Seven of these studies were included in a meta-analysis conducted by Li et al. (Li et al. Gut 2014), which showed a significant overall survival benefit with a HR of 0.84 (95% CI, 0.75 – 0.94). However, in this meta-analysis, no significant benefit was found for colorectal cancer-specific survival, or for patients using aspirin prior to diagnosis. This was also the case in a meta-analysis by Ye et al. (Ye et al. Br J Cancer 2014), which found an overall survival benefit with an HR of 0.74 (95% CI, 0.62 – 0.89), but no significant advantage with regards to colorectal cancer-specific survival, with an HR of 0.74 (95% CI, 0.51 – 1.10). A recent review on the role of ASA in gastrointestinal oncology concluded that more evidence is required as to whether starting ASA after diagnosis of cancer is effective (Langley et al. 2014). However, weaknesses with these previous studies as well as the meta-analyses that summarized the observations include small sample sizes, unreliable assessment of aspirin use, highly selected study populations and recall bias.

In order to look at the effect of ASA on a large population, we have performed a register-based analysis (Norwegian Cancer Registry / Norwegian Prescription Database) from 2004 to 2011 of all patients in Norway diagnosed with CRC (n=29495 of which 23162 met criteria for inclusion in the study) and divided these in ASA (n=6109) and non-ASA (n=19535) users. Our data show a 15% reduced cancer-specific mortality in CRC patients taking ASA (> 6 months) compared to non-ASA users with CRC (Bains SJ et al., J. Clin. Oncol. 2016). Furthermore, patients also taking ASA prior to their CRC diagnosis had even further reduced risk (to 0.75).

Domingo et al. (2013) analysed retrospectively the 896 patients enrolled in a study of the COX-inhibitor rofecoxib (Vioxx). PIK3CA mutations were identified in 12% of the tumours. In the ASA-treated group, the risk of relapse correlated with PIK3CA mutations. Of the patients whose tumours did not have PIK3CA mutations, 23/90 (26%) relapsed,

compared to 0/14 ASA-treated patients with PIK3CA mutant tumours. This finding corresponds to a HR for relapse at 0.11. Liao et al. (2012) used a retrospective cohort (Nurses Health Study) to study 964 patients with CRC. They reported PIK3CA mutations in 17% of the tumours. In patients with tumours lacking the mutations there was no difference in prognosis in relation to ASA use, whereas patients whose tumours carried PIK3CA mutations showed longer cancer-specific survival (HR 0,18). Only 3/66 patients with PIK3CA mutated tumours died of the disease. Patients taking aspirin before the cancer diagnosis and those who started after the diagnosis had the benefit of treatment, if the tumour had a PIK3CA mutation. 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 signalling, which in turn up-regulates COX-2. Inhibition of COX-2 in tumour cells with mutant PIK3CA will inhibit tumour cell survival. In summary, 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 CRC who for some reason were treated with ASA, whereas patients with wild-type tumours did not benefit from the treatment to the same extent (Liao et al, NEJM 2012; Domingo, J Clin Oncol 2013). The current study will in addition to investigate the role of ASA on DFS and OS in patients treated for CRCLM also look into the subgroup of patients with mutations in the PIK3CA.

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 (Liao, X et al. NEJM 2012). Studies of the primary preventive effect from ASA have demonstrated an effect from daily doses of 300 mg (Rothwell, PM et al. Lancet 2012b; Flossman E et al. Int J Epidemiol 2007). There is thus evidence for an effective dose between 100-300 mg/day, which led us to choose the 160 mg daily regimen.

Based on the in-vitro and in-vivo animal work and the two clinical observational studies described above as well as the recently completed project on register-based data, we now have the basis for asking the question of how a clinical intervention with an NSAID or ASA to block PGE2 production will affect secondary prevention after primary cancer (not examined earlier despite many studies on primary prevention). In conclusion, these findings strongly support initiation of a placebo-controlled trial that investigates the role of ASA as adjuvant treatment in CRC patients.

2 STUDY OBJECTIVES AND RELATED ENDPOINTS

The working hypothesis of the present trial is that the use of a COX inhibitor (ASA, Trombyl) to boost anti-tumour immune responses after surgery for primary CRC or CRCLM may have a beneficial effect on disease recurrence. Postponing or avoiding disease recurrence could next potentially have effect on HRQOL and cost-effectiveness, as the pharmacological intervention would be cheap whereas liver reductive surgery is costly.

2.1 Primary Objective and Endpoint

The primary objective is to determine whether treatment with 160 mg ASA (Trombyl) once daily for 3 years can improve Disease Free Survival (DFS) in patients treated with resection for CRCLM, compared with placebo. The primary endpoint is time from randomisation to disease recurrence or death by any cause.

2.2 Secondary Objectives and Endpoints

- To determine the effect of 160 mg ASA on Time to recurrence (TTR) and Overall Survival (OS) compared to placebo. Related endpoints will be time from randomisation to disease recurrence and death of any cause.

- To determine the effect of 160 mg ASA on Health-related Quality of Life outcome measures. The related endpoints will be the eight RAND 36-Item Health Survey 1.0 (SF-36) dimension scores as well as physical and mental health summary measures, and the EQ-5D index value.

2.3 Exploratory Objectives and Endpoints

- To determine whether 160 mg ASA can improve DFS and OS in patients with mutations in PIK3CA or KRAS, in patients where this data is available. Related endpoints are time from randomisation to disease recurrence and death of any cause.

- To determine the cost-effectiveness of 160 mg ASA compared to placebo. Related endpoints are direct medical-care costs, Quality-Adjusted Life-Years (QUALYs) and life-years gained.

2.4 Safety Objectives

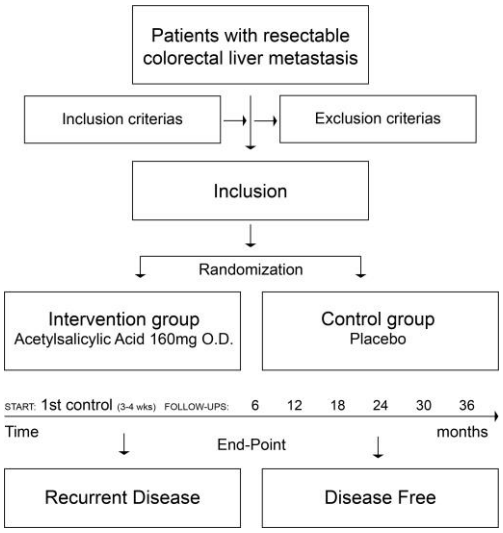
- To assess the safety of 160 mg ASA as adjuvant treatment in CRCLM compared to placebo. Related endpoints include:

- Incidence and severity of adverse events and laboratory abnormalities
- Incidence of Serious Adverse events (SAEs)
- Incidence of treatment discontinuation due to adverse events

3 OVERALL STUDY DESIGN

The study is a phase III, multicentre, randomized, placebo-controlled group-sequential clinical intervention trial to investigate whether ASA treatment is associated with a significantly increased disease free survival. The design is aimed to be robust: well aligned with normal clinical procedures and practice, with mainly hard end-points and with a minimum of sample logistics.

Before surgery, all patients are discussed in a multidisciplinary team with respect to surgical strategy and indication of neo-adjuvant treatment. Patients selected for liver resection, will be approached for inclusion in the study. All patients will give an informed consent in line with the rules and regulations of the Regional Ethical Committee (EC) and can at any time decide to step out of the study. Inclusion in the study will not affect the choice of operative approach or other clinical decisions (see flow chart).



Study Period: Estimated date of first patient enrolled: Q4 2017

Anticipated recruitment period: Five years

Estimated date of last patient completed: Q4 2025

Treatment Duration: 36 months or until disease recurrence

Follow-up: The patients will be followed in the outpatient clinics at each centre according to the National Guidelines for treatment of colorectal liver metastases with clinical control and CT of the chest and abdomen at 6, 12, 18, 24, 30 and 36 months to investigate for any disease recurrence and register challenges regarding the study drug. In Sweden and Denmark, the patients are also followed every 6 months. The total study duration is 36 months per patient.

4 STUDY POPULATION

4.1 Selection of Study Population

Patients referred for CRC liver metastases to participating university hospitals in Norway, Sweden, and Denmark (see separate site list) will be discussed in a multidisciplinary team with respect to surgical strategy and indication of neo-adjuvant treatment. Patients selected for liver resection, will be approached for inclusion in the study. The time point of inclusion will be postoperatively (1-5 days) when the patient is radically operated. Patients with surgical (macroscopic) free resection margins (R0/R1) or in combination with radiofrequency/microwave ablation will be regarded as eligible for study inclusion.

4.2 Number of Patients

A total of 800 patients will be included in this trial and randomized into two arms, with 400 patients in each arm.

4.3 Inclusion Criteria

All subjects undergoing liver resection or ablation (RF or MW) for CRCLM as a part of a curative intent may be included in the study if

- They meet one of the following criteria:
    - First time CRCLM (synchronous or metachronous).
        - For patients with synchronous CRCLM:
            1. Primary tumour resected before hepatic metastasis
            2. Synchronous resection of primary CRC and hepatic metastasis
            3. Hepatic metastasis resected before primary CRC (“liver-first approach”) and the primary tumour operated within 6 weeks after the liver surgery
    - Recurrence of CRCLM (not previously included in this trial)
- Primary tumour radically operated / radiated (rectum)
- No extra-hepatic metastases
- Hepatic metastasis resected with a macroscopic (surgical) free resection margin (R0/R1)
- Ambulatory with a performance status ECOG 0-2
- Age 18 years or above
- Signed informed consent and expected cooperation of the patients for the treatment and follow-up must be obtained and documented according to ICH GCP, and national/local regulations.

4.4 Exclusion Criteria

Patients will be excluded from the study if they meet any of the following criteria:

- For patients operated with “liver-first” approach if the primary is not removed within 6 weeks after the liver surgery.
- Concomitant use of ASA or other anticoagulants or platelet inhibitors such as warfarin or klopidogrel
- On-going regular use of systemic corticosteroids or NSAIDs
- Inherited or acquired coagulopathy (haemophilia)

- Blood platelets (thrombocytes) < 100 x 10^9/L
- Severe heart failure (classified as NYHA class >III)
- Severe kidney failure > grade III
- Pregnancy or breastfeeding. For women in childbearing age there will be pregnancy test at monthly intervals (urine HCG pregnancy tests (for home testing) will be given to the patients for monthly tests and the patient will self-report the results at each control). Furthermore, highly effective contraceptives will be required.
- Childbearing potential without proper contraceptive measures such as oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device to avoid pregnancy for the entire study period.
- Liver cirrhosis with a Child-Pugh score > B7
- Known alcoholism
- Previous malignancies within past 5 years, except non-melanomatous skin or non-invasive cervical cancers.
- Contraindication listed on the Summary of Product Characteristics (SmPC) of Trombyl:
    - Hypersensitivity/allergies to ASA
    - Thrombocytopenia
    - Previous severe gastrointestinal haemorrhage/peptic ulcer due to ASA/NSAIDs
    - Active peptic ulcer
    - Haemophilia
    - Liver cirrhosis
    - Severe congestive heart failure.
- Need to use concomitant medications contraindicated according to SmPC of Trombyl (see point 5.4)

5 TREATMENT

For this study Trombyl 160 mg tablets is defined as Investigational Medicinal Product (IMP). IMP includes also placebo.

5.1 Drug Identity, Supply and Storage

Trombyl and placebo-drug will be packed by Recipharm AB, Sweden (100 tablets active or placebo in each package/bottle). Recipharm AB will send all tablets packaged and labelled and ready to use for this clinical study to Clinstorage AB, Sweden.
Clinstorage AB will distribute the packages to the participating clinical sites in Norway, Sweden and Denmark.

The drug and placebo will be stored at the clinical sites according to the storage instructions on the package labelling.

5.2 Dosage and Drug Administration

Trombyl tablets, 160 mg, or placebo will be administered per orally in one daily dose. Dose justification: meta-analysis of studies of ASA in primary prevention of CRC show effect only at doses higher than 75 mg/day if duration shorter than 5 years (Rothwell et al., 2012a). The treatment will be continued for 36 months or until verification of disease recurrence.

5.3 Duration of Therapy

The treatment will be continued for a maximum of 36 months or until verification of disease recurrence (primary end-point), measured by CT or other relevant radiography.

5.4 Concomitant Medication

The following medication is not allowed while the patient is included in the study after start of the study treatment, neither for chronic or prophylactic use (based on SmPC of Trombyl):

- NSAIDs
- ASA
- Clopidogrel
- Per-oral anti-coagulants like warfarin, rivaroksaban or apixaban
- Pharmacodynamics: other platelet inhibitors, probenicide, cyclosporine, tacrolymus, steroids
- Pharmacokinetics: methotrexate, digoxin, litium, valproate, phenytoin, Sulfonureids
- Systemic corticosteroids

All concomitant medication (and "over-the-counter" drugs) used by the patient will be recorded in the CRF.

Patients will receive thrombosis prophylaxis with low molecular weight heparin (LMWH) for about 4 weeks after the liver surgery, as a part of the post-operative standard of care. The start of study treatment is about 4 weeks after the liver resection surgery, when the LMWH treatment has been ceased. However, for the "liver-first" patients with synchronous metastases the start of study treatment will be 4 weeks after surgery for primary CRC, when the LMWH treatment has been ceased.

5.5 Drug Accountability

The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return, and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed and specified procedures.

All investigational product containers (opened, unopened, or empty) must be returned to the sponsor after the study. A drug dispensing log must be prepared for each subject. At the drug dispensing occasions at 0, 12, 24, and 36 months and at any other relevant time-point the following information must be entered into the drug-dispensing log:

- Date of visit
- Number of containers given to the patient, including individual container number(s)
- Number of containers returned from the patient, including individual container number(s)
- The number of tablets left in the containers will be counted
- Explanation of any discrepancies
- Signature of the person distributing/collecting the container(s)

After completion of the study, the completed drug dispensing logs must be signed by the investigator.

5.6 Drug Labelling

The investigational product will have a label permanently affixed to the outside and will be labelled according with ICH GCP and national regulations, stating that the material is for clinical trial / investigational use only and should be kept out of reach of children.

Labelling of IMP will be done by Recipharm AB Sweden. The labelling will be done according to national regulatory requirements in the participating countries.

In Norway the labelling will be done according to chapter 4.4."Merking av utprøvingspreparatet" in "Forskrift om klinisk utprøving av legemidler til mennesker" (http://www.lovdata.no/for/sf/ho/xo-20091030-1321.html) and "Veiledning til forskrift av 30. oktober 2009 om klinisk utprøving av legemidler til mennesker" (http://www.legemiddelverket.no/Godkjenning_og_regelverk/Klinisk-utproving/Regelverk%20og%20veiledninger/Documents/Veiledning%20-%20revidert%20versjon%202.2%2006.11.2012.pdf) as:

ASAC-studiemedisin, tabletter, no 100

- TIL KLINISK UTPRØVING
- Patient's name
- 1 tablett 1 gang daglig
- ASA-CRCLM Pasientnr *(to be filled in)*
- Kit number:
- Batch number:
- Date dispensed
- Name of prescribing doctor
- Studieansvarlig: Dr. Sheraz Yaqub, Gastrokirurgisk avdeling, Oslo universitetssykehus HF.
  Telefon +47-23070000 Ta kontakt i nødstilfelle for informasjon om ASAC-studien.
- TA MED PAKNINGEN VED NESTE KONTROLL
- Ikke kast tomme pakninger
- Oppbevares utilgjengelig for barn
- Utløpsdato:
- Oppbevares tørt i romtemperatur

5.7 Subject Numbering

Each study participant is identified by a unique subject number that is assigned when the subject is randomized and is entered into the eCRF. Once assigned the subject number cannot be reused for any other subject. The same primary identifier will be used throughout the study.

6 STUDY PROCEDURES

6.1 Flow Chart

Table 1. Trial flow chart

Visits	1	2 *Drug start**	3	4	5	6	7	8 *Drug stop*	Withdrawal visit
Timeline (months) (+/- 14 days), Norway, Sweden and Denmark	**0**	**0**	**6**	**12**	**18**	**24**	**30**	**36**	
Informed consent	X								
Subject screening	X								
Demographics	X								
Medical history	X								
Surgery	X								
Concomitant medicines	X	X	X	X	X	X	X	X	X
Vital signs	X								
Physical exam	X								
Tumour markers (CEA)	X		X	X	X	X	X	X	X
Blood samples	X								X
Study drug dispensing		X		X		X			
Study drug collecting				X		X		X	X
CT-scan (chest & abdomen) or MRI of the liver	X (pre-op.)		X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X
Questionnaires (SF-36 and EQ-5D)	X		X	X	X	X	X	X	X
Recurrence assessment			X	X	X	X	X	X	X
Survival assessment			X	X	X	X	X	X	X

***** Visit 2: This is right after liver surgery, before discharge from the hospital. The patient will be randomized and IMP delivered. The start of study medicine will be 4 weeks after surgery, when LMWH is ceased. For patients with "liver-first" approach this visit will be after the surgery for primary CRC, which is within 6 weeks after liver resection (see point 5.4 last paragraph).

6.2 By Visit

Informed consent

Informed consent must have been given voluntarily by each subject and signed by the patient and an investigator, before any study specific procedures are initiated. The following tests will be done at screening:

Clinical status

Medical history (including disease history and corresponding treatment details), time of primary cancer and time of metastases, previous oncological treatment, on-going oncological treatment, other malignancies, physical examination (cor/pulm/abdomen and peripheral lymph node status), vital signs (weight, blood pressure, and pulse) and ECOG performance status.

Concomitant medication

All concomitant medication (incl. "over-the-counter" drugs) used by the subject within 28 days of study treatment start must be recorded in the CRF. Only the starting dose is to be recorded in the eCRF.

Tumour evaluation

CT scan of chest and abdomen no more than 8 weeks prior to the screening visit.

Molecular profiling of the tumours

In all patients operated for CRC liver metastases, the specimen with tumour is routinely fixated in formalin at the local pathology department. The formalin-fixated tumour is sectioned, stained and the tumour content in the selected piece is estimated. From patients included in the ASAC trial, there will be selected six (6) slices á 10 µm of tumour that will be sent to the National reference laboratory at Science for Life Laboratory in Stockholm, Sweden, in collaboration with Karolinska Institute, Stockholm, Sweden.

In addition to a panel of gene analysis of the tumour, mutation analysis of the BRAF, KRAS and NRAS genes and Mismatch Repair (MMR) status (Microsatellite instability, MSI, Immunohistochemistry, IHC, for MMR genes) will be performed on the selected tumour sections and forwarded back to the treating doctor as these genomic alterations are already used in clinical routine today and will add value to the clinic.

All samples will be stored for 15 years in the biobank in the National reference laboratory at Science for Life Laboratory in Stockholm, Sweden before they will be destructed.

Laboratory analysis

Blood samples will be taken at time of inclusion and at withdrawal visit to determine Haemoglobin, Leucocytes, Thrombocytes, Creatinine, ASAT, ALAT, Bilirubin, CRP, CEA. At other visits the local laboratories will analyse blood samples for CEA.

A 2x4 ml (EDTA) blood sample will be taken at time of surgery and sent to Karolinska Institutet Biobank, Stockholm, Sweden for extraction of germline DNA to be used for the molecular profiling.

All eligibility criteria should be assessed together with relevant baseline parameters prior to study inclusion (inclusion/exclusion criteria).

6.2.1 Before Treatment Starts

- Evaluating patient eligibility
- Assessing baseline values of parameters used as end-points
- For women in childbearing age, provide home-based urine HCG pregnancy tests (for monthly testing) and information about use of proper contraceptives (see point 4.4)

6.2.2 During Treatment

The patients will be monitored with CT scan or MRI and analysis of CEA at each visit in order to diagnose signs of disease recurrence (primary endpoint). The clinical visits and CT scans will be performed at local hospitals at time-points according to National Guidelines for surveillance after surgery for CRCLM. The local hospitals will report the results of CT scans, CEA, and any AEs back to the hospitals running the ASAC trial. Recruiting site study nurses will approach the

local hospitals by telephone to ensure all study information is being transferred to the recruiting site for entry into the study database (eCRF).

The visits shown in Table 1 will be as following for Norway; visit 1 at the time of Surgery, Visit 2 will be immediately postoperatively before discharge from the hospital and the patient will be randomized to Trombyl 160 mg or Placebo. For patients operated with "liver-first" approach, Visit 2 will be after resection of primary CRC, which has to occur within 6 weeks after liver surgery. Visit 3 at four months after surgery, Visit 4 at eight months, Visit 5 at 12 months, Visit 6 at 18 months, Visit 7 at 24 months, Visit 8 at 30 months and visit 9 at 36 months when the study drug also will be withdrawn.

The visits shown in Table 1 will be as following for Sweden and Denmark; visit 1 at the time of Surgery, Visit 2 will be immediately postoperatively before discharge from the hospital and the patient will be randomized to Trombyl 160 mg or Placebo. For patients operated with "liver-first" approach, Visit 2 will be after resection of primary CRC, which has to occur within 6 weeks after liver surgery. Visit 3 at six months after surgery, Visit 4 at 12 months, Visit 5 at 18 months, Visit 6 at 24 months, Visit 7 at 30 months and visit 8 at 36 months when the study drug also will be withdrawn.

The patient will fill out questionnaires regarding health-related Quality of Life (SF-36 and EQ-5D) at the time of inclusion and further at each visit until study completion at 36 months. Study nurses will approach the patient by phone at each time of visit in order to register any AEs and give technical support in completing QoL surveys. Every 12 months during the study period, the included patients will be followed-up at the recruiting sites for a control and study drugs will be accounted and drug supply for the next 12 months will be dispensed.

6.2.3 End of Treatment Visit

The patients will be monitored with respect to the primary and secondary endpoints.

6.2.4 Withdrawal Visit

In case of withdrawal due to withdrawal of consent, data will be registered as shown in the flow chart.

6.2.5 After End of Treatment (Follow-up)

After the protocol treatment is discontinued, the patients will be followed up according to National Guidelines for controls after CRCLM.

6.3 Criteria for Patient Discontinuation

Patients may be discontinued from the study at any time 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. Discontinued patients must also discontinue treatment. Specific reasons for discontinuing a patient for this study are:

- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
- If further study participation (even with treatment discontinuation) is regarded as a liability to the patient with respect to safety and well-being.
- Incorrect enrolment i.e., the patient does not meet the required inclusion/exclusion criteria for the study, prior to randomisation. Incorrectly enrolled randomised patients should be kept in the study to comply with the intention to treat principle.
- Patient lost to follow-up

6.4 Procedures for Treatment Discontinuation

Patients may be discontinued from study treatment at any time, but should still continue in the study. Any study treatment interruptions are not criteria for discontinuation of the study treatment. The interruption is recorded in the eCRF, and the patient may continue study treatment and study participation.

Specific reasons for discontinuing treatment only are:

- A female patient becoming pregnant
- Disease progression – please refer to section 2
- Deterioration in the patient's condition which in the opinion of the Principal Investigator warrants study medication discontinuation (to be records as an AE or under Investigator Discretion)
- Patients non-compliance to procedures or study protocol
- Unacceptable AE, including laboratory values
- Patient refuses to cooperate
- Unblinding of treatment
- Patient that, during the trial, need ASA for other indications, e.g. CVD will be withdrawn from treatment but will remain on study
- Patient that, during the trial, need anti-coagulation therapy for any medical reason, e.g. pulmonary embolism or venous thrombosis, will be withdrawn from treatment but will remain on study
- Other medical reasons

6.5 Procedures for Discontinuation

6.5.1 Patient Discontinuation

Patients who withdraw or are withdrawn from the study, will stop further treatment. However, the patients will be followed up according to the National Guidelines for controls following treatment of CRCLM.

If possible, a final assessment will be made (end of study visit). The reason for discontinuation shall be recorded.

6.5.2 Treatment discontinuation

Patients who withdraw from treatment should follow all study procedures except those regarding treatment, CT/MR scan, CEA, safety reporting. The patients will be encouraged to continue to complete the SF-36/EQ-5D-5L questionnaires. The reason for study treatment discontinuation will be recorded.

6.5.3 Trial Discontinuation

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial
- Difficulties in the recruitment of patients

The sponsor and principal investigator will inform all investigators, the relevant Competent Authorities and Ethics Committees of the termination of the trial along with the reasons for such action. If the study is terminated early on grounds of safety, the Competent Authorities and Ethics Committees will be informed within 15 days.

6.6 Laboratory Tests

Blood samples will be collected at time of inclusion and at each study visit, in the local laboratory. The samples are part of a routine control and will be collected, handled in accordance with hospital/laboratory standard procedures. The level of CEA will be recorded in the eCRF at each visit.

7 ASSESSMENTS

7.1 Assessment of Efficacy

All included patients will be monitored with CT-scan of the chest and abdomen every 6 months till end of study period (three years). This is in-line with the National guidelines for follow-up of patients operated for CRC liver metastases. New metastases or disease recurrence found on CT scans will be registered in the CRF and the patient will be discussed on MDT liver meeting for further treatment.

7.1.1 Assessment of Disease recurrence

Disease recurrence is defined as a new metastatic lesion regardless of location, observed on CT scan of chest and abdomen or MRI of the liver (Punt et al. 2007). The date of disease recurrence will be registered, which is the date of CT/MR scan taken. The assessment of disease recurrence will be done until study end (36 months) or until withdrawal of consent. The observation will be censored at this time point.

7.1.2 Assessment of Overall Survival

Overall survival time is the time from randomisation to death of any cause. Patients will be followed for 3 years. Patients who are still alive at end of follow-up or are lost to follow-up (withdrawn) will be censored at the last available date on which they were known to be alive. The cause of death will be recorded.

7.1.3 Assessment of Disease Free Survival

Disease free survival is defined as the time from randomization until disease recurrence/new metastases or death from any cause (Punt et al. 2007).

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit. Significant findings that are present prior to the signing of informed consent must be included in the relevant medical history/ current medical condition page of the eCRF. For details on AE collection and reporting, refer to Section 8.

For the assessment schedule refer to Flow chart in Section 6.

The procedures for determining the safety and tolerability parameters include physical examination, reporting of adverse events and laboratory tests.

Laboratory evaluation:

Local laboratory will be used for the analysis of haematology and biochemistry specimens collected (routine analysis). In particular serum level of CEA will be measured in order to assess tumour recurrence.

7.2 Other Assessments

Demographic and baseline characteristics will be recorded for all included patients. All relevant medical oncological history will be recorded in the eCRF. Molecular profiling of the tumours (both primary and metastases) to assess for gene alterations like mutations in PIK3CA (exon 9 and exon 20) will be recorded as well as immune profile of the tumours, if available. The study subjects will complete a short-form health survey SF-36 and EQ-5D on every study visit according to Flow chart in Section 6.

8 SAFETY MONITORING AND REPORTING

The investigator is responsible for the detection and documentation of any untoward medical events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each patient will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

The methods for collection of safety data are described below.

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The term AE is used to include both serious and non-serious AEs.

An AE is defined as any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the investigational product. Only symptoms/signs that begin or worsen in severity after the start of investigational product administration will be recorded as AEs in the case report form.

The patients will be closely observed and questioned for any kind of AE during the study. The patients will be instructed to immediately report any symptoms and signs to the study staff, also between the formal controls.

If an AE has already been reported it is not necessary to report each individual sign and symptom of that AE as a separate AE. For example, if myocardial Infarction is reported as an AE there is no need to report an elevated CK, TnT, abnormal ECG or other related signs, symptoms or laboratory values as separate AEs.

All known postoperative complications (such as wound dehiscence, surgical site infections, bile leakage, deep venous thrombus) are known complication after liver resection and will not be registered as AEs. Patients on chemotherapy having nausea or leukopenia (known side-effects of chemotherapy) will not be registered as AEs.

8.1.2 Serious Adverse Event (SAE)

Any untoward medical occurrence that at any dose:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Hospitalization for administrative reason (for observation or social

reasons) is allowed at the investigator's discretion and will not qualify as serious unless there is an associated adverse event warranting hospitalization.

8.1.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse Reaction: all untoward and unintended responses to an investigational medicinal product considered by the investigator to be related to any dose of the study treatment administered.

Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Suspected Unexpected Serious Adverse Reaction: SAE (see section 8.1.2) that is unexpected as defined in section 8.2 and possibly related to the investigational medicinal product(s).

8.2 Expected Adverse Events

The adverse drug reaction (ADR) of Trombyl gives reason to expect gastrointestinal ADRs as the most commonly occurring. Expectedness of SAEs will be evaluated based on the SmPC for Trombyl.

8.3 Disease Progression/Recurrence

Expected progression of the disease under the study and/or expected progression of signs and symptoms of the disease under study, unless more severe in intensity or more frequent than expected for the patient's condition should not be reported as an AE. Events which are definitely due to disease progression will not be reported as an AE/SAE. However, if the investigator considers that there was a causal relationship between treatment with IP and the disease progression/recurrence, then this must be reported as an SAE.

Death due to progressive disease is to be recorded on a specific from in the CRF but not as an SAE.

Any new primary cancer (non-related to the cancer under study) will be reported as an SAE.

8.4 Time Period for Reporting AE and SAE

The standard time period for recording AE and SAEs will begin from the time point informed consent is given by the patient.

During the course of the study all AEs and SAEs will be proactively followed up for each patient; events should be followed up to resolution, unless the event is considered by the investigator to be unlikely to resolve due to the underlying disease. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

8.5 Recording of Adverse Events

If the patient has experienced adverse event(s), the investigator will record the following information in the CRF:

- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient).
- The duration of the event will be described in terms of event onset date and event ended data.
- The intensity of the adverse event will be graded as mild, moderate, or severe using the following definitions:
  - Mild: Tolerable
  - Moderate: Interferes with normal activity
  - Severe: Incapacitating (causes inability to perform usual activity or work)

- The Causal relationship of the event to the study medication will be assessed as one of the following:

  Unrelated:

  There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

  Unlikely:

  There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.

  Possible:

  There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.

  Probable:

  There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.

  Definite:

  There is a reasonable causal relationship between the investigational product and the AE.

- Action taken

- The outcome of the adverse event – whether the event is resolved or still on-going.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but is not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.6 Reporting Procedure

8.6.1 AEs and SAEs

All adverse events and serious adverse events that should be reported as defined in section 8.1.1 will be recorded in the patient's CRF.

SAEs must be reported by the investigator to the sponsor within 24 hours after the site has gained knowledge of the SAE. Every SAE must be documented by the investigator on the SAE pages (to be found in the CRF). The Serious Adverse Event Report Form will be reviewed by the sponsor designee, the Head of Surgical Clinic Morten Tandberg Eriksen, OUH who will evaluate expectedness and relation to IMP. In case of SUSARs the report will be sent to Martha Colban, OUH Clinical Trial Unit, for further reporting to competent authorities, if applicable. The initial report shall promptly be followed by detailed, written follow-up reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter.

The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to seriousness, causality and expectedness. The sponsor will inform all participating sites and principal investigators about any occurred SAEs related to the investigational product.

8.6.2 SUSARs

The following timelines should be followed:

The sponsor will ensure that all relevant information about suspected unexpected serious adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the Competent Authority in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days.

All other suspected unexpected serious adverse reactions will be reported to the Competent Authority concerned as soon as possible but within a maximum of fifteen (15) days of first knowledge by the sponsor.

8.6.3 Annual Safety Report

Once a year throughout the clinical trial, the sponsor will provide the Competent Authority with an annual safety report. The format will comply with national requirements.

8.6.4 Clinical Study Report

The adverse events and serious adverse events occurring during the study will be discussed in the safety evaluation part of the Clinical Study Report.

8.7 Procedures in Case of Emergency

The investigator is responsible for assuring that there are procedures and expertise available to cope with emergencies during the study.

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 should preferably contact the **HPB-surgeon on-call at Oslo University Hospital Rikshospitalet (Norway) (Tel : 02770 / +47-915 02770) who will be available 24/7 during the trial period**. Alternatively during office hours, the study nurses at Oslo University Hospital (Victoria Bringsjord, Gyda Christiansen, and Kornelia Borgen; cell phone: +47-47908528 / Office: +47-2307117), or Principal investigator Sheraz Yaqub (cell phone: +47-90953996) at Oslo University Hospital The unblinding process will be done online in Viedoc™ for the trial and can only be performed by the study nurses at OUH, coordinator investigator, PI at OUH, or on-call HPB Surgeon at OUH Rikshospitalet by using a specific login and password. The information about reason for unblinding will be registered in the eCRF and the patient will discontinue the trial drug, but however be followed up according to the trial protocol with respect to intention-to-treat analysis.

8.8 Data Monitoring Committee (DMC)

A DMC will be appointed consisting of clinicians and a biostatistician that are independent of the trial. The DMC will perform the interim analysis where they will analyse safety of the trial as well as efficacy of ASA compared to placebo (see point 9.4 below).

9 DATA MANAGEMENT AND MONITORING

9.1 Electronic Case Report Forms (eCRFs)

The designated site staff will enter the data required by the protocol into the e-case report forms (eCRF). The Prinicipal Investigator at each participating site is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections, will also be recorded/tracked in the eCRF.

The Clinical Data Management System (CDMS) used for the eCRF in this study is Viedoc™. The setup of the study specific eCRF in the CDMS will be performed by department of clinical research support, Oslo University Hospital. After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

9.2 Source Data

The medical records for each patient should contain information which is important for the patient's safety and continued care, and to fulfil the requirement that critical study data should be verifiable.

To achieve this, the medical records of each patient should clearly describe at least:

- That the patient is participating in the study, e.g. by including the enrolment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- WHO performance status assessments conducted as part of the study, if applicable;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;
- Additional information according to local regulations and practice.

9.3 Study Monitoring

The investigator will be visited on a regular basis by the Clinical Study Monitor, who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required.

When the responsible study monitor has checked and verified the CRFs, the data will be managed at the Oslo University Hospital scientific server for further handling and statistical evaluation.

Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

9.4 Interim analysis

An interim analysis will take place when approximately 135 of the primary study events (disease recurrence or death by any cause) have occurred. Data on the primary variable will be entered, verified and validated and then handed over to the DMC to assess efficacy of ASA compared to placebo. Further procedures are described in section 10.5.

9.5 Confidentiality

The investigator shall arrange for the secure retention of the patient identification and the code list. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (eCRFs, Site File etc) shall be retained and stored during the study and for 15 years after study closure. All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

9.6 Database management

Data management will be performed by the department of clinical research support, Oslo University Hospital. The Data management procedures will be performed in accordance with the departments SOPs and ICH guidelines.

The data management process will be described in the study specific data handling plan and the study specific data handling report after database closure.

After database closure the data will be stored in a dedicated and secured area at OUS. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be archived for the time period requested by the competent authorities.

10 STATISTICAL METHODS AND DATA ANALYSIS

10.1 Statistical Hypotheses and Tests

The primary objective of this trial is to determine whether treatment with 160 mg ASA once daily can improve DFS in patients treated with resection for CRCLM, compared with placebo.

The null hypothesis for the comparison of DFS, and for the secondary endpoints, is that there is no difference between the treatment arms; the alternative hypothesis is that a difference exists. All tests will be two-sided. Superiority of 160 mg ASA will be demonstrated only if the nominal p-value from the appropriate two-sided test (see section 10.6.2) is less than the significance level of 0.01 at the interim analysis and 0.0456 at the final analysis, and the efficacy estimate is in favour of the ASA arm. The significance levels follow from the applied group sequential clinical trial design with one interim analysis and with an asymmetric fixed boundary shape O' Brien-Flemming-like method.

10.2 Determination of Sample Size

The sample size estimation for this study is based on the following assumptions:

- Two-sided test with a 5% significance level
- Power: 80%
- Treatment allocation ratio: 1:1
- Follow-up time: 36 months
- One interim efficacy analysis with an O' Brien-Flemming-like alpha-spending function
- Disease recurrence rate in the placebo group after 36 months: 40%
- Hazard ratio (HR) between the treatment groups: 0.7
    - Corresponding to a Disease recurrence rate in the ASA group after 36 months of 30%

Assuming a hazard ratio of 0.7, a total of 254 events (disease recurrences) are required to be 80% sure to reach a statistical significant difference between the treatment groups at the 5% level using a fixed sample size calculation. Adjusting for an inflation rate of 1.06 due to the planned interim analysis, we need 270 events during the study if the study is not stopped at the interim analysis for efficacy. With an approximate overall recurrence rate of 35% and adjusting for loss to follow-up, a total of 800 patients (400 in each arm) will be randomised.

10.3 Randomization

10.3.1 Allocation- sequence generation

Eligible patients will be allocated in a 1:1 ratio between Trombyl 160 mg and placebo, using a computer randomization procedure stratified by centre. The randomization will be blocked within each stratum.

Details of block size and allocation sequence generation will be provided in a separate document that is unavailable to those who enrol patients or assign treatment.

The time of randomization will be slight different for patients with “liver-first” approach. Patients who are already operated for primary CRC or are synchronously operated for primary and hepatic metastases will be randomized postoperatively after the liver surgery. The “liver first” patients will be randomized after the surgery for the primary CRC.

10.3.2 Allocation- procedure to randomize a patient

Each centre will be provided with batches of treatment kits on a regular basis. Each container will be identified by a kit number and contain 100 tablets. Four containers is sufficient for one year use, and will have the same kit number. When a patient is deemed eligible and ready for randomisation, the investigator will receive the patient kit number through the eCRF system upon randomisation. Four containers with the corresponding kit number are then given to the patient. During the study, patients will be given new containers with the same kit number, but with adequate expiry date. Details of given kit numbers are recorded in the eCRF.

10.3.3 Blinding and emergency unblinding

Following the randomization, the patient, investigator, and clinical physician will be blinded.

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 should contact the Principal investigator in Norway Sheraz Yaqub (cell-phone: +47-90953996), or on-call HPB Surgeon at OUH Rikshospitalet (Tel : 02770 / +47-915 02770) who will be available 24/7 during the trial period.

10.4 Population for Analysis

The following populations will be considered for the analyses:

- Intention to treat (ITT) population: All randomised participants, regardless of protocol adherence
- Full Analysis Set (FAS): All randomised patients who have taken at least one dose of study medication
- Per Protocol (PP) set: All randomised patients who sufficiently comply with the protocol. Criteria for inclusion in the PP population will be specified in the statistical analysis plan, and the final criteria will be defined prior to database lock.
- Safety population: All randomised patients who have taken at least one dose of study medication, i. e. identical to the FAS.
- Sub-group analysis on patients in both groups with mutations in PIK3CA, KRAS and others.

The primary analysis will be done on the Full Analysis Set.

10.5 Planned analyses

There will be one interim analysis and one main analysis planned in this study.

- The interim analysis will be performed when approximately half of the planned primary events (135) have occurred and the primary endpoint has been entered, verified and validated according to the data management plan. A separate document (the Data Monitoring Committee (DMC) charter) will detail the procedures for the interim analysis. A report will be written following the interim analysis, describing any deviations from the planned analysis, and a recommendation to either continue or stop the study for efficacy. No efficacy information such as hazard ratios, confidence intervals or p-values will be presented in the report.
- If the DMC recommends stopping the study for efficacy, the sites will be given notice and no more patients will be randomised. All remaining data must be entered, verified and validated according to the data management plan. The database will then be locked for further entering or altering of data. The allocation list will be opened, and final analyses will be performed. Patients will be informed of their allocation, and patients having received placebo will be offered ASA treatment.
- If the DMC recommends continuing the study, the final analysis will be performed when the planned primary events (270) have occurred and all data have been entered, verified and validated according to the data management plan.

A separate statistical analysis plan (SAP) will provide further details on the planned statistical analyses. The SAP will be finalized, signed and dated prior to database lock. The treatment allocation will be revealed after the database lock and used in the statistical analysis.

Deviation from the original statistical plan will be described and justified in the Clinical Study Report. Amendments to plan can be done until day of database (DB) lock.

10.6 Statistical Analysis

This randomized clinical trial aims primarily to describe and estimate efficacy parameters and test pre-specified statistical hypotheses.

10.6.1 Description of data

Continuous variables will be summarized using standard summary statistics such as number of observations (n), mean value, standard deviation (SD), minimum and maximum value, median value, and 1st and 3rd quartiles. Time-to-event variables will also be summarized using the Kaplan-Meier method to estimate the median, 25th and 75th percentiles, minimum and maximum and the 95% confidence interval for the median time to event. Kaplan-Meier plots will also be generated. Categorical variables will be summarized in frequency tables as counts and percentages.

Demographic data and other baseline characteristics will be summarized using descriptive statistics.

10.6.2 Analysis of Efficacy Data

The efficacy endpoints to be analysed are described in Section 9 and will be summarized and analysed both for the FAS and PPS. The FAS will be regarded as the primary analysis set.

The primary efficacy endpoint, time from randomisation to disease recurrence or death by any cause, as well as all time-to-event secondary endpoints will be analysed using a stratified log-rank test accounting for the stratification factor (study centre), and the treatment effect will be estimated using the Cox proportional hazards regression model stratified by study centre. Type of patient (“liver first” or “primary CRC first / synchronously primary CRC and CRCLM”) will be adjusted for in the analysis.

As exploratory analyses, these endpoints will also be analysed using stratified Cox proportional hazards models adjusted for other baseline covariates considered to be of potential prognostic value such as clinical classification, type of chemotherapy, number and size of metastases and tumour biomarker analysis.

A subgroup analysis will be performed to assess the interaction effect between ASA and the mutations in PIK3CA and KRAS. The analysis will be performed on primary and secondary time-to-event endpoints using a stratified Cox proportional model with an ASA/mutation in PIK3CA/KRAS mutation interaction term.

All time-to-event endpoints will be summarized using Kaplan-Meier/Nelson-Aalen plots, estimates of median, 25th and 75th percentiles and hazard ratios.

10.6.3 Analyses of Safety Data

All safety summaries will be presented for the safety population. No formal statistical analysis of safety endpoints will be performed.

An overall summary of AEs during the trial will be presented by treatment arm. This summary will include the number and percentage of patients with any AE, a TEAE, a serious TEAE, a related TEAE, a serious related TEAE, a TEAE leading to discontinuation and a TEAE leading to death.

Frequency tables of TEAEs will be presented by treatment arm, MedDRA system organ class and preferred term and will include the number and percentage of patients reporting the event. The same summary tables will also be presented for serious TEAEs, related TEAEs, serious related TEAEs, TEAEs leading to discontinuation and TEAEs leading to death.

Descriptive statistics (number of observations, mean, standard deviation, minimum, median and maximum values) will be presented for clinical laboratory tests (haematology, biochemistry, urinalysis) and vital signs by treatment at applicable visits.

10.6.4 Handling of Loss to Follow-up and/or Missing Data

There will be no handling of missing data for the time-to-event endpoints. Patients without event will be censored at the last point of contact.

11 STUDY MANAGEMENT

11.1 Investigator Delegation Procedure

The principal investigator is responsible for making and updating a “delegation of tasks” listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

11.2 Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations.
All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

11.3 Study Amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

11.4 Audit and Inspections

Authorized representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise, representatives from the sponsor may visit the centre to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The principal investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

12 ETHICAL AND REGULATORY REQUIREMENTS

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

12.1 Ethics Committee Approval

The study protocol, including the patient information and informed consent form to be used, is approved by the Regional Ethics Committee in each country.

The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national regulatory requirements.

12.2 Other Regulatory Approvals

The protocol will be approved by competent authorities in each country before commencement of the study.

The protocol will also be registered in www.clinicaltrials.gov before inclusion of the first patient.

12.3 Informed Consent Procedure

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements. The investigator is responsible for obtaining signed informed consent.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed in the Investigator Site File binder.

12.4 Subject Identification

The investigator is responsible for keeping a list of all patients (who have received study treatment or undergone any study specific procedure) including patient's date of birth and personal number, full names and last known addresses.

The patients will be identified in the CRFs by patient number, initials and date of birth.

13 TRIAL SPONSORSHIP AND FINANCING

The study is funded by the Research Council of Norway, the Norwegian Cancer Society and The national programme for clinical therapy research, KLINBEFORSK.

14 TRIAL INSURANCE

The Principal investigator has insurance coverage for this study through membership of the Drug Liability Association (see http://www.laf.no for more details) in Norway (ref: #6996401/1). Likewise, there will also be insurance for the trial in Sweden and Denmark.

15 PUBLICATION POLICY

Upon study completion and finalization of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations.

All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors.

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17 LIST OF APPENDICES