A Phase III double-blind placebo-controlled Randomised Trial of Aspirin on Recurrence and Survival in Colon Cancer Patients

NL 38132.058.14

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PROTOCOL SIGNATURE SHEET

I declare that I have read and familiarized myself with the following protocol:

A Phase III double-blind placebo-controlled Randomised Trial of Aspirin on Recurrence and Survival in Colon Cancer Patients

ASPIRIN trial

NL 38132.058.14

Version: v2.4 – The Netherlands

I agree to conduct the study as described in the protocol.

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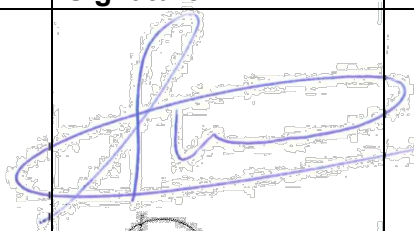
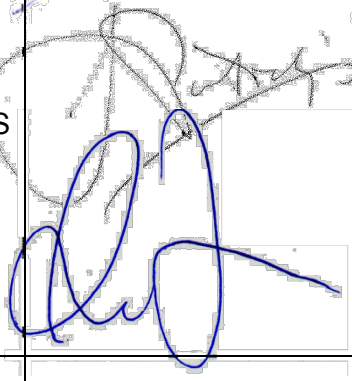
Dr. G.J Liefers	LUMC		
Dr. J.E.A. Portielje	HAGAZIEKENHUIS		
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1. LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AFAP	Attenuated Familial Adenomatous Polyposis
AR	Adverse Reaction
ASA	American Society of Anesthesiologists
BCC	Basal-cell carcinoma
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CIN	Cervical intraepithelial neoplasia
CRF	Case Report Form
CEA	Carcinoembryonic antigen, tumour marker
CV	Curriculum Vitae
CAPOX	Chemotherapy regimen consisting of capecitabine combined with oxaliplatin CAP- Capecitabine (Xeloda) OX- Oxaliplatin (Eloxatin)
DSCA	Dutch Surgical Colorectal Audit
DSMB	Data Safety Monitoring Board
EORTC	European Organisation for Research and Treatment of Cancer
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FAP	Familial Adenomatous Polyposis
FOLFOX	a chemotherapy regimen for treatment of colorectal cancer, made up of the drugs FOL- Folinic acid (leucovorin) F- Fluorouracil (5-FU) OX- Oxaliplatin (Eloxatin)
GCP	Good Clinical Practice
(I)ADL	(Instrumental) Activity of Daily Living
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LMWH	Low molecular weight heparin
LUMC	Leiden University Medical Center
MAP	MYH-Associated Polyposis
METC	Medical research ethics committee (MREC); in Dutch: Medisch Ethische Toetsingcommissie (METC)
NFU	Netherlands Federation of University Medical Centres; in Dutch: Nederlandse Federatie van Universitair Medische Centra (NFU)

Po	Per os
PPI	Proton-pump inhibitor, medication for reduction of gastric acid production
QLQ	Quality-of-Life-Recorder
(S)AE	(Serious) Adverse Event
SCC	Squamous Cell Carcinoma
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
UICC	International Union Against Cancer
UZA	Antwerp University Hospital
WBP	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

2. SUMMARY

Rationale: Preclinical, epidemiologic and clinical evidence suggest that acetylsalicylic acid use may reduce overall cancer risk and mortality in colon cancer patients.

Objective: To investigate acetylsalicylic acid 80 mg po daily for 5 years as an adjuvant therapy in curatively operated, stage II and III colon cancer patients.

Study design: A phase III double-blind placebo-controlled randomised trial of adjuvant low-dose acetylsalicylic acid in colon cancer patients. The question will be addressed by means of two parallel studies in patients treated with or without adjuvant chemotherapy.

Study population:

- Patients 45 years and older with histologically confirmed adenocarcinoma of the colon
- Patients must have TNM stage that is one of the following: pT3-4; N0-2 and M0, or pT1-2 and N1-2 (UICC stage II and III) (in case of >1 tumour: more advanced tumour is stage II or III)
- Patients must have completed surgical resection (R0) (both laparoscopic and open surgery) within 12 weeks of randomisation

Exclusion:

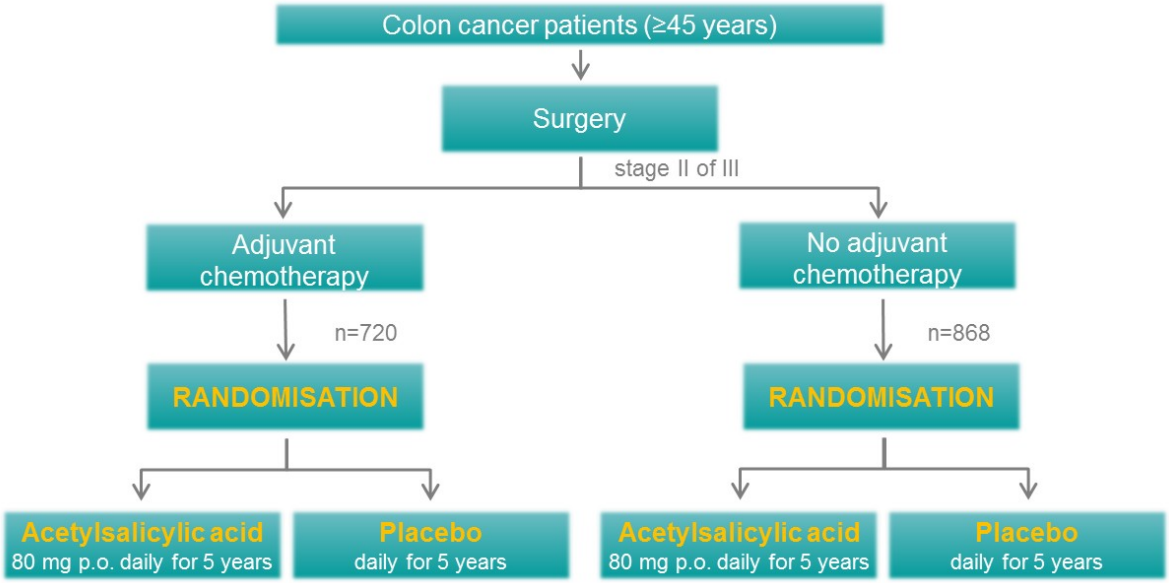
- Patients with rectal cancer (defined as tumour within 15 cm from the anal verge)
- Patients currently taking oral anti-coagulants or use of LMWH
- Patients currently taking acetylsalicylic acid for any reason
- Patients with a history of bleeding disorders or active gastric or duodenal ulcers
- Patients currently taking high dose systemic glucocorticoids (≥ 30 mg predniso(lo)n or equivalent)
- Patients with (suspected) (non-) polyposis syndrome (FAP/AFAP, MAP, Lynch syndrome)
- Patients with >100 polyps of the colon **or** a known hereditary syndrome of the colon in a first degree family member
- Allergy or intolerance to salicylates
- Patients with local or distant recurrent disease
- Previous malignancies other than CIN, BCC or SCC with a disease free survival *less than* 5 years
- Presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial

Intervention: Patients will be randomised for acetylsalicylic acid 80 mg po daily for 5 years versus placebo.

Main study parameters/endpoints: The primary endpoint of the trial is 5 year Overall Survival (5-yr OS). Secondary endpoints are Time to Treatment Failure (TTF; time elapsed between randomisation until treatment discontinuation due to disease recurrence, unacceptable toxicity, death or any other event of interest) and 3-year Disease Free Survival (DFS); time to recurrence or death due to any cause. The trial is powered to identify a hazard ratio of 0.75 in the acetylsalicylic acid arm, with 80 percent power and a type 1 error of 0.05 (2-sided). In order for the trial to be successful, 1588 patients need to be randomised.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Unexpected adverse events may occur. Given the widespread use of low dose acetylsalicylic acid in cardiovascular risk management, it is unlikely that new toxicities will be identified. However, adverse anti-cancer effects may be identified. An independent data safety monitoring board will oversee trial conduct. A planned interim futility analysis allows early termination of the study if it appears that acetylsalicylic acid will not favourably impact colon cancer outcome.

3. FLOW CHART ASPIRIN TRIAL



Primary endpoint: 5 year OS, assuming HR 0.75 with 80% power and type 1 error of 0.05 (2-sided), 1588 patients need to be randomised.

4. INTRODUCTION AND RATIONALE

Epidemiology

Colorectal cancer is one of the most common cancers in developed countries, with about one million new cases each year and a mortality rate of nearly 33%(1). For both men and women, in Europe colorectal cancer is the second cause of cancer death. In Europe, in 2012 there were 447.000 new colorectal cancer patients and 215.000 patients died of colorectal cancer(2). In the Netherlands, in 2013 there were 9.411 new patients diagnosed with colon cancer. The number of patients with colorectal cancer in the Netherlands has doubled between 1990 and 2014(3).

Initial therapy after diagnosis usually comprises primary surgical resection. When there is evidence of nodal involvement patients often receive adjuvant chemotherapy following initial surgery in an attempt to prevent metastatic spread(1). 50-60% of patients diagnosed with colorectal cancer eventually develop metastasis(4). Approximately 35% of patients presenting with stage III, 20% of patients with stage II, and 5 to 10% of patients with stage I cancer eventually relapse and subsequently die from metastatic disease(5).

Adjuvant therapy in colon cancer

Surgical resection remains the corner stone of colon cancer treatment. For patients with an indication for adjuvant treatment, the combination of 5-fluorouracil and leucovorin or capecitabine and oxaliplatin (FOLFOX or CAPOX) is the current standard of care(5;6). The MOSAIC trial randomised 2.246 patients with stage II or III colon carcinoma between 5FU/LV or 5-FU/LV+oxaliplatin. Results of this trial showed an increase in disease-free survival at 3 years from 72.9 to 78.2% ($p = 0.002$) for the addition of oxaliplatin to FU/LV. Five-year disease-free survival remained significant (HR: 0.80; $p = 0.003$) and at 6 years there was an overall survival benefit for stage III patients (68.3% versus 72.9%). The addition of oxaliplatin to LV5FU2 was associated with an increase in neutropenia, febrile neutropenia and neuropathy(7).

In the current guidelines in the Netherlands, there is no room for adjuvant treatment with irinotecan or any targeted therapy, e.g. cetuximab or bevacizumab (3;8-10).

In January 2014 the screening program for colorectal cancer in the Netherlands started. This program is for both men and women in the age group 55-75. This program will be introduced in phases. In the first 6 months of the screening, 763 new cases of colorectal cancer were diagnosed. Because the screening is introduced in phases, this was only 21% of the initial target audience(11).

In addition to the increasing incidence worldwide, with the recently started screening it is expected that the number of new patients with colorectal cancer in the Netherlands will grow exponentially the coming years.

Acetylsalicylic acid and cancer

Acetylsalicylic acid and other nonsteroidal anti-inflammatory drugs (NSAIDs) have shown to be effective in the prevention of colorectal cancer. Evidence for the role of acetylsalicylic acid

as an anti-cancer agent comes from large trials primarily assessing the cardiovascular benefits of daily acetylsalicylic acid. In a pooled analysis of 5 large trials it was recently shown that use of acetylsalicylic acid taken for several years at doses of at least 75 mg daily reduced long-term incidence and mortality due to colorectal cancer(12).
In another meta-analysis acetylsalicylic acid was shown to significantly reduce adenoma formation in patients with a history of colorectal cancer(13).

Indirect evidence for acetylsalicylic acid as an adjuvant therapy comes from a report from Chan *et al*. In a non-randomised study they found that regular users of acetylsalicylic acid after the diagnosis of colorectal cancer had a reduced colorectal cancer-specific mortality (HR 0.71, 95% CI: 0.53-0.95). (14)
These data are very similar with data from our own study. In a large retrospective series of 4,481 patients with colon cancer we found that acetylsalicylic acid use initiated after the diagnosis colon cancer was associated with a significantly increased overall survival (adjusted RR 0.61 (95%CI 0.46-0.81; p=0.001)(15).
In a third population based study from Scotland, the same conclusion was reached as well. In 2.990 patients, acetylsalicylic acid use post-diagnosis was associated with a lower risk of all-cause mortality (HR 0.67, 95% CI: 0.57-0.79) (16). Increasing age at diagnosis was also associated with increased risk reduction.
In contrast a fourth study by Walker *et al*. showed a more modest risk reduction of 9 percent (HR 0.91. 95% CI: 0.84-0.98). Subgroup analysis showed that the benefit was confined to those patients who also used acetylsalicylic acid before the diagnosis(17). Again, in the report by Walker *et al*. the effect of acetylsalicylic acid seemed stronger in older patients and in colon cancer patients.
In recent publications from Chan *et al.* and Liao and co-workers, using the same cohort, acetylsalicylic acid use was associated with longer survival in PTGS2 expressing tumours and*PIK3CA* mutated tumours only. In that study, the hazard ratio for cancer related death was even 0.18 (95% CI 0.06-0.61), but only in tumours harboring a *PIK3CA* mutation(18).
These results were validated by Domingo *et al*. where *PIK3CA* mutations were predictive for acetylsalicylic acid benefit (19). Interestingly, this study was not able to validate the predictive value of PTGS2 expression.
The magnitude of the clinical benefits seen with acetylsalicylic acid in colorectal cancer seem to be larger, for example in Peter Rothwell's data, than could be explained by an effect on the approximately 15% of colorectal tumours with mutated *PIK3CA*. It is therefore unlikely that all the effects of acetylsalicylic acid will be explained by this one mutation.
Acetylsalicylic acid use has also been associated with a decreased risk of developing a colorectal tumour with an intact *BRAF* gene but no association between post-diagnosis acetylsalicylic acid use, *BRAF* mutation status and clinical outcome has been found (20). Recently, our group was not able to validate the predictive value of *PIK3CA* mutations and PTGS2 expression with low dose acetylsalicylic acid, but did find a survival benefit with acetylsalicylic acid in tumours expressing HLA class I antigen only(21).

Altogether, these data indicate that in subgroups of patients the effect may be larger than in others, however, the findings remain inconsistent. A trend towards multifactorial approach of the etiology and treatment of colorectal cancer is ongoing. Not just isolated biomarkers seem to determine prognosis but molecular subtyping of tumours will be the future. This can also be helpful in developing new agents for the treatment of colorectal cancer(22).

Currently three other trials are studying the effect of acetylsalicylic acid as adjuvant treatment in colorectal cancer. The ASCOLT trial in Singapore is a randomised, placebo-controlled trial studying the effect of acetylsalicylic acid 200 mg in patients with Dukes B and C colorectal tumour. The ALASCCA trial, currently under development, will be carried out in Sweden, and studies the effect of 80 mg acetylsalicylic acid in patients with PIK3CA mutated colorectal tumours.
In the near future the Add-Aspirin trial, carried out in India and the United Kingdom, will start including patients. This trial will randomise patients with different types of cancer for acetylsalicylic acid vs placebo treatment. Patients with upper gastro intestinal tumours, breast cancer, prostate cancer and colorectal cancer are eligible for inclusion.
Very recent new retrospective data again report that in parallel to the chemo preventive action of acetylsalicylic acid, this cheap and well tolerated drug is also implicated in prevention of distant metastases(23-25). These data from Rothwell indicate that acetylsalicylic acid might help in treatment of some cancers and provides proof of principle for pharmacological intervention specifically to prevent distant metastasis. Nevertheless, there is an urgent need for more randomised trials to prove the role of acetylsalicylic acid in the *adjuvant treatment* of cancer.
The mechanism by which acetylsalicylic acid exerts its activity is not completely understood. The protective activity of acetylsalicylic acid has been attributed to direct inhibition of the prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase (COX), family of enzymes involved in prostaglandin synthesis. The PTGS2 enzyme is strongly and rapidly induced in response to mediators of inflammation, growth factors, cytokines, and endotoxins; and its expression correlates with increased cell proliferation and tumour promotion(26).
Acetylsalicylic acid can decrease the production of potentially neoplastic prostaglandins arising from PTGS2-mediated catalysis of arachidonic acid(27). However, acetylsalicylic acid has a much broader range of downstream effectors, such as NF-KB, insulin-like growth factor I (IGF-I) and many others, as well as the inhibition of Wnt signalling and stem cell growth possibly as the result of enhanced beta-catenin phosphorylation(28-30).
Cancer is a heterogeneous disease encompassing differentiated cell types but also less committed stem-like cells. Recent evidence suggests that a subpopulation of tumour cells with distinct features such as self-renewal and the ability to differentiate into multiple lineages is responsible for tumour initiation, invasive growth and possibly dissemination to distant organ sites. The acquired ability of a cell to resist to apoptosis is a hallmark of almost all types of cancer. Recently, it has been shown that IL-4 expression is essential for the resistance to DNA damage-induced apoptosis of colon cancer stem cells (CSCs)(31). CSCs are also resistant to the cytotoxic effect of chemotherapy. It has been shown that IL-4 confers colon CSCs with resistance to apoptosis.(31) Consistently, treatment with IL-4Ra

antagonist or anti-IL-4 neutralizing antibody strongly enhances the antitumour efficacy of standard chemotherapeutic drugs through selective CSCs sensitization. Notably, acetylsalicylic acid inhibits IL-4 gene expression(32).
Based on the above observations, it is plausible that acetylsalicylic acid may both act as a preventive agent in CRC onset by modulating the Wnt pathway in CSCs, but also as adjuvant treatment by increasing CSCs' sensitivity to conventional chemotherapy regimens.

Acetylsalicylic acid and (non-) polyposis syndrome
Patients with (non-) polyposis syndrome are not eligible for inclusion in this trial. These patients have a different prognosis and different tumour characteristics(33-35).
Additionally, the CAPP2 trial has examined the effect of 600 mg acetylsalicylic acid vs placebo in patients with Lynch syndrome plus previously affected patients within families meeting the Amsterdam criteria. This is the first large scale genetically targeted chemoprevention trial, which randomly assigned 861 participants to acetylsalicylic acid or placebo. Results showed when acetylsalicylic acid is used for at least two years, cancer incidence is reduced by 63% (IRR 0.37, 95% CI 0·18–0·78, p=0·008)(36). At this moment the CAPP3 trial is investigating the optimum low-dose acetylsalicylic acid to administer in at least 3.000 gene carriers(37).
By excluding patients younger than 45 years, patients with a high a priori chance of (non-) polyposis syndrome are excluded.

Risk-benefit
Acetylsalicylic acid, even at low doses appropriate for cardiovascular risk management, roughly doubles the incidence of gastric bleeding and one or two people in every thousand are likely to have a bleeding every year. This risk rises with age and in people older than 80 years, it might be as high as seven per 1000 people every year (38).
Age specific changes in the risk-benefit ratio of the preventive role of acetylsalicylic acid remain unclear. Data on the risk-benefit ratio for cancer prevention are insufficient and no definitive recommendations by a recent international consensus statement could be made regarding the preventive use of acetylsalicylic acid(39).
However, the demonstration of a significant therapeutic effect of acetylsalicylic acid as adjuvant treatment will alter the balance of risk and benefit importantly. The data from Chan et al., our own data and many more observational studies show a reduction of all-cause mortality (thereby including any fatal bleeds) up to 30 percent(14;15;21;40).. This potential benefit of acetylsalicylic acid is therefore additional to the risk-benefit equation in which the risk of bleeding is already offset by other benefits of acetylsalicylic acid i.e. prevention of ischaemic stroke, myocardial infarction.

Relevance
The current limiting testing of acetylsalicylic acid as a therapeutic agent in the adjuvant setting, is in marked contrast to the numerous studies that were initiated using selective PTGS2 inhibitors before 2004.

And, with recent high profile failures of adjuvant bevacizumab and cetuximab in resected CRC, findings of a potential adjuvant benefit with acetylsalicylic acid, is not only timely, but highly important. Acetylsalicylic acid is an unlikely anti-cancer drug, however if proven beneficial, has the potential to change treatment paradigms, with significant global health and socio-economic impact. Because acetylsalicylic acid is off-patent and costs a-penny, development of acetylsalicylic acid as an anti-cancer therapy will be critically dependent on public funding and collaborative support from academia. In an era of escalating cancer treatment costs, providing the evidence for cost-competitive solutions must also be a necessary strategy and imperative. In this process, acetylsalicylic acid should not be over looked because it is neither new nor expensive.

Key points

- *The number of colon cancer patients is increasing and there is a strong need for therapeutic improvement*
- *The colorectal cancer screening in the Netherlands has raised the incidence*
- *Pre-clinical, epidemiological and clinical data demonstrate that acetylsalicylic acid use is associated with improved colon cancer outcome.*
- *Demonstration of a significant therapeutic effect of a well-tolerated drug that costs mere pennies per day would be a major clinical advance.*

5. OBJECTIVES

Primary Objective:

- To study effect of 80mg acetylsalicylic acid (given orally once daily for 5 years) on 5 year overall survival (OS) for stage II and III colon cancer patients.

Secondary Objective(s):

- To study the effect of acetylsalicylic acid on 3 year disease free survival (DFS)
- To study the effect of acetylsalicylic acid on time to treatment failure (TTF)
- To study the effect of acetylsalicylic acid on toxicity, for example the interaction of acetylsalicylic acid with chemotherapy.

Tertiary Objective (pending additional funding):

- *PIK3CA, BRAF* and *KRAS* mutation analysis, PTGS2 and HLA class I expression analysis in tumour samples from all patients.

6. STUDY DESIGN

A phase III double-blind placebo-controlled randomised trial is proposed. Stage II and III colon cancer patients with or without adjuvant chemotherapy will be randomised between low-dose acetylsalicylic acid (80 mg) or placebo, within 12 weeks after radical resection. Treatment will start at least 10 days after surgery. Study medication will be continued for 5 years. The research question will be addressed in patients with and without adjuvant chemotherapy by means of two parallel studies.

Stratification factors:

- Stage in the 'no adjuvant chemotherapy' arm
- Age <70 years and ≥70 years

7. STUDY POPULATION

7.1 Population (base)

Patients with stage II or III colon cancer 45 years of age and older after radical (R0) resection will be randomised. From these patients 24% received any kind of adjuvant chemotherapy, but these percentages are increasing over time.

	All patients* Stage II & III Older than 45yrs		Patients not using aspirin at diagnosis**		Inclusion	
	No CT	CT	No CT	CT	No CT	CT
2010	3498	1229	1539	664		
2011	3579	1257	1575	679		
2012	3649	1282	1606	692		
2013	3614	1270	1590	686		
2014	4201	1476	1848	797		
2015	4450	1564	1958	844		
	Increase of app. 172 pt/yr	Increase of app. 60 pt/yr				
2016	4622	1624	2034	877		
2017	4794	1684	2109	910		
2018	4966	1745	2185	942		
2019	5138	1805	2261	975		
2020	5310	1866	2336	1007		
2021	5482	1926	2412	1040		
Total 2015-2021			**15296**	**6596**	868 6% participation needed	720 11% participation needed

*Under the assumption that 24% of the colon cancer patients will receive chemotherapy in the period 2015-2017. **Assumption of aspirin and VitK antagonist use in patients who received no chemotherapy 56%, in patients who did receive chemotherapy 46%.

CT=Chemotherapy

Numbers based on incidence in the Netherlands Cancer Registry and data from ECR-Pharmo cohort

7.2 Inclusion criteria

- Patients 45 years and older with histologically confirmed adenocarcinoma of the colon

- Patients with histologically confirmed adenocarcinoma of the colon
- Patients must have TNM stage that is one of the following: pT3-4; N0-2 and M0, or pT1-2 and N1-2 (UICC stage II and III) (in case of >1 tumour: more advanced tumour is stage II or III)
- Patients who have undergone curative radical resection (R0 resection) within 12 weeks prior to study entry
- Written informed consent according to national and local regulation

7.3 Exclusion criteria

- Patients with rectal cancer (defined as tumour within 15 cm from the anal verge).
- Patients currently taking (low-dose) acetylsalicylic acid for any reason.
- Patients currently taking oral anti-coagulants or use of LMWH
- Patients with a history of bleeding disorders or active gastric or duodenal ulcers.
- Patients currently taking high dose systemic glucocorticoids.(≥ 30 mg predniso(lo)n or equivalent)
- Patients with (suspected) (non-) polyposis syndrome (FAP/AFAP, MAP, Lynch syndrome)
- Patients with >100 polyps of the colon **or** a known hereditary syndrome of the colon in a first degree family member (father/mother/brother/sister/son/daughter)
- Allergy or intolerance to salicylates
- Patients with local or distant recurrent disease
- Previous malignancies other than CIN, BCC or SCC with a disease free survival *less than* 5 years
- Presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial

7.4 Sample size calculation

Overall Survival

The sample size calculation was performed on Overall Survival with the assumption that acetylsalicylic acid will increase Overall Survival with a hazard ratio of 0.75 in the acetylsalicylic acid group versus placebo (in our retrospective study the *upper* limit of the confidence interval in older patients was 0.78(15)) The study will consist of two parallel studies in patients who do and do not receive adjuvant chemotherapy (see flow diagram on page 10). These two arms will be powered separately as we aim to achieve significant results in both trials.

- Sample size in patients who receive no chemotherapy.

The number of events needed in this population was calculated used the next formula:
$d = (1.96 + z_{\beta})^2 (\delta+1 / \delta-1)^2$ where β=0.8 and δ=0.75.
Overall, the calculation showed that 384 events are needed; so in the control group 219 events are needed. Patients will be included in 3 years and follow-up will be for at least 5 years. In the life tables of patients who received no chemotherapy 912 events were observed in 1809 patients in 6.5 years. To observe the 219 events in 6.5 years we need to include 219/(912/1809) = 434 patients per arm with a total of **868 patients**.

- Sample size in patients who do receive chemotherapy

In the life tables of the patients who received chemotherapy 204 events were observed in 335 patients in 6.5 years. To observe the 219 events in 6.5 years in this trial we need to include 219/(204/335) = 360 patients per arm with a total of **720 patients**.

In conclusion, sample size calculation showed that an inclusion of in total **1588 patients** will be sufficient to detect a hazard ratio of 0.75.

7.5 International Collaborations

7.5.1 Collaboration between LUMC and UZA

Several hospitals in the surroundings of Antwerp have declared their interest to participate in the Aspirin trial, with funding from the Anticancer Fund. By the end of 2015 the first meeting between Leiden University Medical Center, the Anticancer Fund and Antwerp University Hospital was organised to discuss the expansion of the ASPIRIN trial to Belgian hospitals. The rationale for this collaboration is to accelerate accrual, because finalising the trial within the timeframe is highly important. For both countries, separate protocols are in place, since the Belgian trial has several additional side studies implemented that will not take place in the Netherlands. Nonetheless, both protocols are similar in a way that allows the data to be pooled for analysis. One database will be used to collect all data for both countries, central data management will be performed by the Datacenter Heelkunde in Leiden, The Netherlands. The total number of required patients will remain the same.

7.5.2 Collaboration with the Add-Aspirin trial

The MRC clinical trial unit in the United Kingdom is ongoing in the development of the 'Add-Aspirin' trial. This trial is expected to start recruiting patients in September 2015. In collaboration with this group we agreed in principle that patients in both trials will be shared after both trials are analysed and published separately. The expected accrual in the UK is 2300 colorectal cancer patients older than 18 years. These patients will be randomised for high-dose acetylsalicylic acid, low-dose acetylsalicylic acid or placebo for 5 years. Assuming comparable demographics and incidence of colon cancer, data from approximately 400 patients can be shared.

8. TREATMENT OF SUBJECTS

8.1 Investigational product/treatment

Patients will be randomised either to receive orally acetylsalicylic acid 80 mg once daily or placebo.

8.2 Concomitant medication

Patients are allowed to use their concomitant medication in consultation with their treating physician. Concomitant medication use will be documented with every follow-up visit. Patients who need to undergo elective surgery or other interventional procedures do not have to stop taking the study drug. Evidence suggests that acetylsalicylic acid use prior to and after surgery does not increase (post) operative bleeding risk(41). In case a specific situation (e.g. a procedure at the dentist) requires stopping study medication, the patient may stop 7 days prior to the procedure. Recommence of study medication is directly after recovery, if clinical condition allows.

8.2.1 Gastroprotection

The Dutch College of General Practitioners advises gastroprotection concomitantly with acetylsalicylic acid only in patients above 70 years of age. However, the ASPREE study (ASPirin in Reducing Events in The Elderly) safely enrolled 19,000 patients above 70 years of age in Australia. In this study where patients were randomized for low-dose acetylsalicylic acid (100 mg) or placebo, proton pump inhibitors (PPI) were not routinely advised as concomitant medication (Prof. Dr. M. Nelson, *personal communication*) Also in the before mentioned ASCOLT-study and Add-Aspirin study standard PPI use is not recommended.
If dyspeptic symptoms do occur we recommend to cease the study medication for a period of 14 days, and then re-challenge. If symptoms recur then the treating physician may add other medication (e.g. a PPI). Gastroprotection is advised in patients who chronically use NSAID's, systemic glucocorticoids, SSRI, venlafaxine, duloxetine, trazodon or spironolactone. Also in case of severe incapacitating rheumatoid arthritis, heart failure or diabetes, standard PPI's may be considered.

8.2.2 Chemotherapy

Concomitant use of chemotherapy and acetylsalicylic acid is allowed. When patients are treated with chemotherapy and thrombocytopenia is observed, study treatment may be discontinued until thrombocytes reach an acceptable (according to the treating oncologist) level for the readministration of the study drug. The study drug may be restarted after the completion of chemotherapy, even if chemotherapy duration exceeds the maximum period of study drug interruption (see 10.4.1 Specific criteria for withdrawal)

9. INVESTIGATIONAL MEDICINAL PRODUCT

9.1 Aspirin

Aspirin, also known as acetylsalicylic acid is a salicylate often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Salicylic acid, the main metabolite of acetylsalicylic acid, is an integral part of human and animal metabolism. While much of it is attributable to diet, a substantial part is synthesized endogenously. Acetylsalicylic acid also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, acetylsalicylic acid is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk for developing blood clots(42). It has also been established that low doses of acetylsalicylic acid may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue. Acetylsalicylic acid is part of a group of medication called non-steroidal anti-inflammatory drugs (NSAIDs). Though acetylsalicylic acid, and other salicylates have similar effects (antipyretic, anti-inflammatory, analgesic) and inhibit the same enzyme cyclooxygenases their mechanism of action, acetylsalicylic acid does it in an irreversible manner. For example, NSAIDs antiplatelet effects last in the order of hours, whereas acetylsalicylic acid's effect lasts for days (until the body replaces the suppressed platelets).

Kinetics:

Resorption: orally 80–80%. T_{max} = ½–2 hours. Metabolism: in the liver, but also in plasma, Elimination: With the urine as metabolites and unchanged acetylsalicylic acid (1%).

9.2 Summary of findings from non-clinical studies

Acetylsalicylic acid inhibits prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2)(14). PTGS2 is an inducible enzyme whose expression and activity are up-regulated in response to a variety of cytokines, growth factors, and tumour promotors. (43)PTGS2 was shown to be up-regulated in colorectal adenomas and adenocarcinomas. Approximately, 70% of colorectal tumours express PTGS2, which increases with stage(44). PTGS2 plays an important role in colorectal carcinogenesis, invasion, angiogenesis and metastasis. The importance of PTGS in tumourigenesis was established by knockout of the PTGS2 gene in mice containing a truncating mutation in the adenomatous polyposis coli (APC) gene. These mice developed markedly fewer intestinal adenomas than did APC-mutated mice with intact PTGS2 (45). Several studies showed that this PTGS2 effect can be reversed by selective PTGS2 inhibitors(46).

9.3 Summary of findings from clinical studies

Acetylsalicylic acid and other nonsteroidal anti-inflammatory drugs (NSAIDs) have shown to be effective in the prevention of colorectal cancer. However, results from the Victor trial, a phase III randomised trial assessing rofecoxib in the adjuvant setting of colorectal cancer, demonstrated no difference in overall survival(44).

Previous studies showed that regular use of acetylsalicylic acid was associated with a lower risk of colorectal cancer(14;47). A recent study in patients with stage I-III colorectal cancer selected from two nationwide health professional cohorts in the U.S. showed that regular acetylsalicylic acid use after the diagnosis of colorectal cancer compared with non-users is associated with a lower risk of colorectal cancer-specific and overall mortality (adjusted Hazard Ratio (HR) 0.71), especially among individuals with tumours that over express PTGS2 (adjusted HR 0.39)(11). Another study where prediagnosis non-steroidal anti-inflammatory drug use and survival after diagnosis of colorectal cancer was evaluated, showed a higher reduction in colorectal cancer mortality risk after diagnosis by acetylsalicylic acid use than overall NSAID use(48). The magnitude of effect of acetylsalicylic acid in a large Scottish cohort (14) (N=2.990) on all-cause mortality (adjusted HR 0.67, 95% CI 0.57–0.79) was more marked than that seen in the Dutch study by Bastiaannet *et al*. (12) (HR 0.77, 95% CI 0.63–0.95) and for colorectal cancer mortality (adjusted HR 0.58, 95% CI 0.45–0.75) than Chan *et al.* (HR 0.79, 95% CI 0.53–0.95)(11). Recently, subgroup analysis in a Dutch cohort of elderly patients (75 and older) who received no chemotherapy the survival gain was especially large (adjusted RR 0.52 (95%CI 0.35-0.78), p=0.001)(15).

An important addition to our current understanding of the anticancer effect of acetylsalicylic acid is a meta-analysis involving 17,285 persons from 5 randomised trials assessing the effect of acetylsalicylic acid to prevent cardiovascular events (18). Allocation to acetylsalicylic acid reduced the risk of death due to cancer in patients who developed adenocarcinoma, particularly in those without metastasis at diagnosis (HR 0.50 95% CI: 0.34-0.74).

Since all data on the use of acetylsalicylic acid as an adjuvant treatment are retrospective cohort studies or from trial designed for cardiovascular risk management, the magnitude of effect on all-cause and cancer specific mortality remains an open question. But, combining all available data, an assumption of 25% risk reduction for all-cause mortality, in patients with colon cancer only seems a conservative estimation.

9.4 Summary of known and potential risks and benefits

9.4.1 Gastrointestinal

Acetylsalicylic acid use has been shown to increase the risk of gastrointestinal bleeding. Combining acetylsalicylic acid with other NSAIDs has also been shown to further increase this risk. Using acetylsalicylic acid in combination with clopidogrel or warfarin also increases the risk of upper gastrointestinal bleeding. In addition to enteric coating, "buffering" is the other main method companies have used to try to mitigate the problem of gastrointestinal bleeding. Buffering agents are intended to work by preventing the acetylsalicylic acid from

concentrating in the walls of the stomach, although the benefits of buffered acetylsalicylic acid are disputed.

9.4.2 Central effects

Large doses of salicylate, a metabolite of acetylsalicylic acid, have been hypothesized to cause tinnitus based on experiments in rats, via the action on arachidonic acid and N-methyl D-aspartate receptors cascade.

9.4.3 Hives and swelling

For a small number of people, taking acetylsalicylic acid can result in symptoms that resemble an allergic reaction, including hives, swelling and headache. The reaction is caused by salicylate intolerance and is not a true allergy, but rather an inability to metabolize even small amounts of acetylsalicylic acid, resulting in an overdose.

9.4.4 Other effects

Acetylsalicylic acid can induce angioedema in some people. Acetylsalicylic acid causes an increased risk of cerebral microbleeds. Such cerebral microbleeds are important since they often occur prior to ischemic stroke or intracerebral haemorrhage, Binswanger disease and Alzheimer's disease. Acetylsalicylic acid can cause prolonged bleeding after operations for up to 10 days.

9.4.5 Precautions

Acetylsalicylic acid should not be taken by people who are allergic to ibuprofen or naproxen, or who have salicylate intolerance or a more generalized drug intolerance to NSAIDs, and caution should be exercised in those with asthma or NSAID-precipitated bronchospasm. Owing to its effect on the stomach lining, manufacturers recommend people with mild diabetes seek medical advice before using acetylsalicylic acid. Even if none of these conditions is present, there is still an increased risk of stomach bleeding when acetylsalicylic acid is taken with alcohol. Acetylsalicylic acid is known to cause haemolytic anaemia in people who have a genetic disease glucose-6-phosphate dehydrogenase deficiency (G6PD), particularly in large doses and depending on the severity of the disease. Use of acetylsalicylic acid during dengue fever is not recommended owing to increased bleeding tendency. In people with kidney disease, hyperuricemia, or gout caution must be taken because acetylsalicylic acid inhibits the kidneys' ability to excrete uric acid, and thus may exacerbate these conditions. Acetylsalicylic acid can increase of the activity of several drugs, such as sulfonylureumderivates, insulin, and acetazolamide, and the side effects of methotrexate.

9.4.6 Pregnancy

Regular use of aspirin may adversely affect a pregnancy and/or foetal development. Regular aspirin use whilst breast feeding can also cause complications in the neonate/infant and should be avoided. Therefore, participants joining the Aspirin trial should not be pregnant or breast feeding at registration and will be advised against becoming pregnant during the

treatment period of the trial. If a participant becomes pregnant during the trial, the study drug should be stopped and the pregnancy should be reported on the 'Comment form'. This must also be discussed directly with the trial coordinator. Follow-up within the trial will continue.

9.4.7 Overdose

Acetylsalicylic acid overdose can be acute or chronic. In acute poisoning, a single large dose is taken; in chronic poisoning, higher than normal doses are taken over a period of time. Acute overdose has a mortality rate of 2%. Chronic overdose is more commonly lethal, with a mortality rate of 25%. Chronic overdose may be especially severe in children. Toxicity is managed with a number of potential treatments, including activated charcoal, intravenous dextrose and normal saline, sodium bicarbonate, and dialysis. The diagnosis of poisoning usually involves measurement of plasma salicylate, the active metabolite of acetylsalicylic acid, by automated spectrophotometric methods. Plasma salicylate levels generally range from 30–80 mg/L after usual therapeutic doses, 50–300 mg/L in patients taking high doses and 700–1400 mg/L following acute overdose.

9.5 Description and justification of route of administration and dosage

Following post-operative randomisation, patients will receive acetylsalicylic acid 80 mg or placebo orally for 5 years. Adequate instruction regarding dose and frequency of the study medication will be given to the patient.

9.6 Dosages, dosage modifications and method of administration

Study medication can be taken during any moment of the day. However, to avoid gastric complaints, it is advised to take study medication during or just after a meal. There will be no dose reduction for adverse events related to acetylsalicylic acid. Acetylsalicylic acid should be stopped if the patient develops an adverse event that is related to the study drug (toxicity). The drug will not be reintroduced in case of anaphylaxis, angioedema or gastrointestinal bleeding. Otherwise, re-introduction of the drug will be done after complete resolution of the adverse event following a rest period without study drugs (maximal duration 6 weeks). If the adverse event subsequently recurs, the patient will be taken off treatment with study drug.
In the case of observed thrombocytopenia when patients receive chemotherapy, it is allowed to interrupt study treatment according to the treating medical oncologist. Patients can restart the study drug after completion of chemotherapy or when thrombocytes reach an acceptable level according to the treating medical oncologist. The study drug may be restarted even if chemotherapy duration exceeds the 6 weeks (maximum period of study drug interruption) (see 10.4.1 Specific criteria for withdrawal)

9.7 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling will be done according to relevant GMP guidelines. The acetylsalicylic acid 80mg tablets and matching placebo tablets are manufactured by Tiofarma Beheer B.V. (Oud-Beijerland, the Netherlands). The IMPD of the acetylsalicylic acid and Placebo are provided by Tiofarma Beheer B.V..
The medication is released by the Qualified Person of the LUMC Pharmacy.

9.8 Drug accountability

Tiofarma is responsible for the manufacturing, packaging, labelling and shipment of the study medication, conform GDP. The local (hospital) pharmacy is responsible for accountability and distribution of the investigational product on patient's level.
Randomisation codes are kept strictly confidential and are accessible to authorised persons only.
At the end of the study, all used and unused containers/products must be destroyed on site as dictated by the appropriate standard procedures. Under no circumstances should the investigator or site personnel supply product to other investigators or clinics or allow the study supplies to be used otherwise than as directed by this protocol.

10. METHODS

10.1 Study parameters/endpoints

10.1.1 Main study parameter/endpoint

The primary endpoint is 5 year Overall Survival (5y-OS). The time to an event for OS is defined as the time interval between the date of randomisation and the date of death. A patient that is still alive at the last date of follow-up patient is censored for analysis and the time at risk corresponds to the time interval between the date of randomisation and the date of last follow up.

10.1.2 Secondary study parameters/endpoints

Two secondary endpoints:

- Disease Free Survival (DFS). The time to an event for DFS is defined as the time interval between the date of randomisation and the date of disease recurrence or death, whichever comes first. Recurrence of a disease can be a loco-regional recurrence, a distant recurrence or a new primary colon cancer. The evidence for recurrence must be documented in the patients' file. The date of radiological evidence, e.g. CT, MRI, etc., or colonoscopy will be considered as date of recurrence. An elevated CEA level, as solitary finding, will not be considered acceptable evidence of colon cancer recurrence.
- Time to Treatment Failure (TTF). The time elapsed between randomisation until treatment discontinuation due to disease progression, unacceptable toxicity, death or any other event of interest.

10.1.3 Other study parameters

Patient and tumour characteristics will be documented consisting of:

1. Identification of the patient: unique ASPIRIN study number, DSCA registration (see paragraph 14.3) number, sex and date of birth
2. Comorbidities: cardiac, vascular, diabetes, pulmonary, neurological, gastrointestinal, urogenital, thrombosis, muscle and joints, endocrine diseases, infectious diseases and previous malignancies (also registered by the DSCA, see paragraph 14.3) Comorbidities will also be registered by using the Adult Comorbidity Evaluation-27 (ACE-27)
3. Previous abdominal surgery (registered by DSCA)
4. Previous and current medication (registered by CRF)
5. Diagnostics performed preoperative
6. Treatment: pre-operative treatment, date of surgery, ASA-score, urgency of surgery, surgical procedure.
7. Histopathology: Histology, number of resected lymph nodes, number of positive lymph nodes, pathological TNM classification.
8. Adjuvant treatment: treatment regimen, dose, no. of cycles, reason no chemotherapy.

9. Geriatric Assessment (see appendix 1-5)
   - G8 geriatric assessment screening tool;
   - ACE-27
   - Social situation

   Patient questionnaires:
   1. From the IADL and ADL we used a modified version in Dutch, which is commonly used in Dutch hospitals.
   2. EORTC QLQ-C30

   The modified IADL and ADL and EORTC QLQ C30 will be repeated after 6 months, 3 years and 5 years.
10. According to the code good medical practice (obligated in the Netherlands) tissues after surgery will be stored at the pathology department. After the trial these tissues can be used for further studies, only by using the code good medical practice. The tissues will be used for biomarker investigation: *PIK3CA*, *KRAS* and *BRAF* mutation analyses. Furthermore, HLA class I and PTGS2 immunohistochemical stainings will be performed to investigate which patients will benefit most from acetylsalicylic acid treatment after diagnosis.

10.2 Randomisation and treatment allocation

10.2.1 Patient randomisation procedure

After having properly checked all eligibility criteria and having obtained patients informed consent, patients will be randomised online through the ProMISe program or by phone, fax or email at the LUMC Datacenter.

Online randomisation

Randomisation can also be performed online 24 hours per day through the ProMISe randomisation program. Go to:

https://www.clinicalresearch.nl/PROMISE/S/HEIT/S_O_LUMC_C_HEELK_ASPIRIN_/LOGON/INDEX.HEI?MODE=1&Simple=1

Investigators, datamanagers or research nurses can apply to the Central Datacenter for a username and password. E-mails with the answered questions and the patient number will be automatically sent to the investigator and the person responsible for CRF completion.
The local pharmacist will be notified about the allocated treatment.

Randomisation by email, phone or fax

Randomisation can be done by email datacenter@lumc.nl, telephone +31 71 526 3500; Monday-Friday; 9:00-17:00 or fax +31 71 526 6744. During the randomisation procedure eligibility criteria will be checked. After randomisation, a sequential identification number will be applied. This number has to be recorded on the randomisation form, along with the randomisation date. The randomisation form must be signed by the investigator (in case of faxed randomisation, the confirmation of the data manager/research nurse also has to be

signed by the investigator) and filed with the CRFs. An automatic e-mail will be sent to the investigator (see online randomisation).

10.2.2 Unblinding

The treatment will be un-blinded in the following cases:

- End of the study, after the final analysis, prior to writing the publication
- In case of emergency (SAE to the patient possibly attributed to the study medication)
- In case of another indication that warrants acetylsalicylic acid treatment (e.g. cardiovascular incidents or a (non) polyposis syndrome that was diagnosed after inclusion in the study)

Because acetylsalicylic acid is prescribed in low dosage and a drug with only a slight risk of causing immediate life threatening events, no 24-hour service is provided for deblinding. When deblinding is necessary, local participating centre can provide deblinding the next work day.

10.3 Study procedures

10.3.1 Before treatment starts

See www.oncoline.nl for the national regulations and protocols. After surgery, when the histology of the cancer and the disease stage is known, patients will be randomised for acetylsalicylic acid use or placebo within 12 weeks after surgery. Within a week after randomisation study medication must be started. Acetylsalicylic acid or placebo will start at least 10 days after surgery. In case of treatment with chemotherapy, study medication can be used concomitantly with chemotherapy.
Local investigator fills out baseline CRF, ACE-27, Social Situation and G8 Geriatric Assessment and asks patients to fill in the questionnaires: QLQ-C30 and modified ADL and IADL (see appendix 1-5).

10.3.2 During adjuvant therapy (years 1-5)

The follow-up period will be according to the national guidelines, but with a minimum of follow-up visits every 6 months the first 2 years, and every year in the third to fifth year, according to Dutch guidelines www.oncoline.nl
In case the patient has one of the following events, special forms have to be completed:

- Recurrence/new primary Form
- Concomitant medication form
- End of study treatment form
- Off study form
- Death form
- AE form
- SAE form

After 6 months, 36 months and 60 months modified IADL and ADL questionnaires will be repeated.

Study events table: see appendix 6

10.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

10.4.1 Specific criteria for withdrawal

- Patients who develop anaphylaxis, angioedema or gastrointestinal bleeding
- Recurrent disease
- Patients with diagnosed (non-) polyposis syndrome
- Intercurrent, cardiovascular disease that warrants acetylsalicylic acid treatment
- Patients that warrant treatment with oral anti-coagulants or use of LMWH
- Greater than 6-week interruption in study drug administration due to toxicities (In case of thrombocytopenia when patients are treated with chemotherapy, study drug interruption may exceed 6 weeks)
- Unacceptable study drug toxicity
- Patient withdrawal of consent to continue treatment
- Intercurrent, non-cancer related illness that prevents continuation of therapy or regular follow-up
- Changes in a patient's condition that renders the patient not suitable for further treatment in the judgement of the investigator
- Major protocol violation or discovery of information that, if previously known, would have rendered the patient ineligible for study

10.4.2 Replacement of individual subjects after withdrawal

After withdrawal subjects will not be replaced.

10.4.3 Follow-up of subjects withdrawn from treatment

Subjects withdrawn from treatment will still have follow-up and will be corrected later in the statistical analysis for the time they used the study drug.

10.5 Premature termination of the study

The study will be terminated when there is one of the following criteria:

- Serious adverse side effects related only to acetylsalicylic acid use in >5 % of the subjects
- If the results of the interim analysis do not show survival benefit for acetylsalicylic acid use or if these results show a survival gain for control group.

11. SAFETY REPORTING

11.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

11.2 Adverse and serious adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to acetylsalicylic acid. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death
- is life threatening (at the time of the event)
- requires hospitalisation or prolongation of existing patients' hospitalisation
- results in persistent or significant disability or incapacity
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs will be reported through the web portal *ToetsingOnline* of the CCMO to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.

SAEs that result in unexpected admissions, death, or are life threatening should be reported expedited. The expedited reporting will occur no later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

All SAEs, irrespective of relationship to the study treatment must be reported to the Datacenter by fax: +31-71-5266744 or email: datacenter@lumc.nl as soon as possible but no later than one working day from the time the local investigator has first knowledge of the SAE. The Datacenter will inform the trial coordinator and the principal investigator. The Datacenter will also inform the Medical Ethics Committee(s) and the Competent Authority as described in the previous section.

NOTE In this study, the following events are not considered as an AE or SAE:

- Chemotherapy toxicity not resulting in admission
- Admission for diagnosis or treatment of recurrences. For recurrences, the CRF “new primary / recurrences” has to be filled in
- Death due to progression of disease
- Planned admissions. E.g. a knee operation (prosthesis for the knee) due to gonarthrosis. However, gonarthrosis is an AE

Relevant AE's of any kind and any grade (*e.g.* bruises, gingival bleedings, epistaxis and thrombocytopenia) should be reported.
SAE’s occurring 30 days after patients have been withdrawn from study medication do not have to be reported, unless there is a reasonable probability that there is a causal relationship between the protocol treatment and the SAE.

11.2.1 Suspected unexpected serious adverse reactions (SUSAR)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.
Suspected unexpected serious side effects. This is the case in the following:

1. Serious event
2. Suspected is that the administered drug is related to the event
3. The side effect has not been written in existing product information e.g. Investigator’s Brochure or SPC.

The investigator will report expedited SUSARs through the web portal *ToetsingOnline* of the CCMO to the METC.
The expedited reporting will occur not later than 7 days (in case life threatening) 15 days (in case not life threatening) after the investigator has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

11.2.2 Recording of adverse events

All AEs grade 3 or more, reported spontaneously by the subject or observed by the investigator or his staff will be recorded on the AEs form of the CRF with the following information:

1. The severity grade according to the NCI-CTCAE version 4.0, published May 28, 2009 (appendix 7) (1=mild, 2=moderate, 3=severe, 4=life threatening)
2. Whether it constitutes a SAE

All AEs should be treated appropriately. Treatment may include one or more of the following: no action taken (i.e. further observation only); study drug dosage temporarily interrupted; study drug permanently discontinued due to this AE; concomitant medication given; non-drug therapy given; patient hospitalized/patient’s hospitalization prolonged. The action taken to treat the AE should be recorded on the AE CRF.
Once an AE is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changed in severity, the suspected relationship to the study drug, the interventions

required to treat it, and the outcome. Information about common side effects already known about the investigational drug can be found in SPC.

11.2.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority and the Medicine Evaluation Board.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

11.3 Follow-up of adverse events

All adverse events will be followed up until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

11.4 Data Safety Monitoring Board (DSMB)

A DSMB is established to perform ongoing safety surveillance and to evaluate interim analyses on the safety data. This committee is independent of the conducted trial. The DSMB is composed of three independent members, of whom at least one is a statistician. The members of the DSMB are independent and have no conflicts of interest with the conducted trial, principal investigator or sponsor of the study. Interim analyses are performed according to chapter 12.4. Accumulating data is reviewed, including updated figures on recruitment, data quality, primary outcome and safety data. The interim analysis will be performed deblinded by an independent statistician. The statistician will report to the independent DSMB. The DSMB will discuss the results of the interim-analysis and advice the steering committee. Discontinuation of the trial is advised by the DSMB according to the predefined stopping guidelines stated in paragraph 10.4.

11.5 Monitoring

Monitoring in the Netherlands will be done according to table 2 of the NFU guideline 'Kwaliteitsborging mensgebonden onderzoek 2.0' chapter 6. The ASPIRIN Trial is classified as a low-risk trial and therefore minimal monitoring is required (appendix 9).

Objective of on-site monitoring:

- To identify procedural errors in the execution of the research

Central monitoring purpose:

- Detection of missing, late and inaccurate research

Source Data Verification:

According to the guideline 1 – 10% of the source data has to be monitored based on a predefined list of variables, including the primary endpoint.

- Randomisation form (F01) all items, including the
    - General information
    - Inclusion criteria
    - Exclusion criteria
    - Informed consent date
    - Specific questions
- Baseline form (F02)
    - Chemotherapy given according to stratification at randomisation
- Follow-up forms (F03)
    - Therapy compliance
    - Disease status
- Recurrence form (F04) (if applicable) all items, including the
    - Type of event
    - Locations
    - Investigations and results
    - Completeness/missed events
- End of study treatment form (F06) (if applicable)
    - Date of last study medication intake
    - Reason for end of study treatment
    - Study treatment deblinded
- Off study form (F07) (if applicable)
    - Last date in study
    - Reason off study
- Death form (F20) (if applicable)
    - Date of death
    - Cause of death
- Adverse Event forms (F30) (if applicable)
    - Completeness/missed AEs

- SAE forms (F40) (if applicable)
    - Completeness/missed SAEs

12. STATISTICAL ANALYSIS

12.1 Descriptive statistics

In both of the parallel trials, the number of patients will be described for acetylsalicylic acid and placebo. Rates and reasons for study discontinuation will be presented in contingency table(s). Patient characteristics at study entry will be summarised in frequency tables and descriptive statistics will be provided for patient and tumour variables.
The following information about the study treatment administration will be provided:

- The number of patients who have started on treatment, with details regarding the identification of the patients who should have started, and the reasons for the non-adherence to the protocol
- The number of patients who stopped the protocol treatment with the reasons for stopping treatment, complemented with a description of the cases who stopped for toxicity (detailing the patient identification and the type of toxicity)
- A summary of the duration of treatment. If all patients have finished treatment, the duration will be summarized using median and ranges of the actual treatment duration. If the treatment is still ongoing for some patients, the median treatment duration will be estimated using Kaplan-Meier curves.

The “intention to treat population” will be used for analysis. This population will include all patients randomised, regardless of whether patients received the acetylsalicylic acid or not or discontinued treatment. This population will be the primary population to evaluate survival. Besides, a “per-protocol” analysis will be made where patients will be analysed according to the study medication they actually used.

12.2 Univariate analysis

The two arms will be compared using a 2-sided non-stratified log-rank test at the 0.05 level of significance. Survival curves will be estimated using the Kaplan-Meier technique. Analysis will be performed for both parallel studies separately, so for patients who do receive chemotherapy and patients who did not receive chemotherapy. Univariate Cox proportional hazard models will be used to estimate the Hazard Ratio and confidence interval for OS and DFS in the acetylsalicylic acid arm versus placebo.

12.3 Multivariate analysis

To adjust for potential confounders, multivariable Cox proportional hazards model will be used to estimate an adjusted HR and 95%CI for acetylsalicylic acid versus placebo in which we include all factors that were associated with survival (OS, DFS) in univariate analysis.

12.4 Interim analysis

- An interim analysis will be performed when half of the required events have been observed, in both arms 219/2 (=109)
- An alpha-spending function with an O'Brien-Fleming boundary will be used in order to be flexible with respect to the timing of the analysis.
- A futility analysis will take place at the same time with two stopping rules: 1. when >5% of patients experience serious side effects and 2. when results indicate that it is unlikely that results at the scheduled end of the trial will demonstrate a significant effect (differences between acetylsalicylic acid and control group are that small that any prospect of a positive result with the planned sample size is very unlikely). Probability and conditional power will be calculated; if this probability is too small to meet the pre-specified criteria, we would stop the trial for futility.
- The final analysis of the trial will be done when the required number of events has been recorded at all patients evaluated by the Study Coordinator.

13. ETHICAL CONSIDERATIONS

13.1 Regulation statement

The responsible investigator will ensure that this study is conducted in agreement with either the Declaration of Helsinki or the laws and regulations of the country, whichever provides the greatest protection of the patient. The protocol will be approved by the local, regional or national ethics committees.

13.2 Recruitment and consent

A sequential identification number will be automatically attributed to each patient registered in the trial. This number will identify the patient and must be included on all case report forms. In order to avoid identification errors, patients date or birth will be reported on the case report forms as well.
All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. 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 he/she 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 or randomised. This must be done in accordance with the national and local regulatory requirements. The informed consent must be kept as hardcopy and preferably also digitally in the electronic patient file.
The informed consent procedure must conform to the ICH guidelines of Good Clinical Practice. This implies that the written informed form should be signed and personally dated by the patient.

13.3 Objection by minors or incapacitated subjects

Patients that are not competent to make decisions on their own are not eligible for this trial.

13.4 Benefits and risks assessment, group relatedness

The potential of the adjuvant use of acetylsalicylic acid is enormous. It involves a common, very cheap drug, with a well-known safety and side effect profile utilised in a conventional (cardiovascular) and non-conventional (adenocarcinoma, COX-2 inhibition) manner. In addition acetylsalicylic acid is not feared by patients in the same way that conventional chemotherapy is.

13.5 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.
The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003) with HDI Gerling. This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

14. ADMINISTRATIVE ASPECTS AND PUBLICATION

14.1 Paper Case Report Forms

All forms must be dated and signed by the responsible investigator or one of his/her authorized staff members (see signature log) as soon as the requested information is available. The list of staff members authorized to sign case report forms (with a sample of their signature) must be sent to the Datacenter by the responsible investigators before the start or the study.
In all cases, it remains the responsibility of the investigator to check that original case report forms are sent to the Datacenter and that they are completely and correctly filled out. The original copy must be immediately returned to the Datacenter and the investigator must keep a copy.
If an investigator needs to modify a case report form after the original copy has been returned to the Datacenter, he/she should notify the Datacenter in writing (and sign the notification) and append a copy of the notification to his own copy of the case report forms.

All data will be reported on the ASPIRIN Case Report Forms. These can be downloaded from the DCCG website or asked for at the Central Datacenter (datacenter@lumc.nl or 071-5263500). The completed forms should be sent to:

Leids Universitair Medisch Centrum (LUMC), Datacenter Heelkunde, K6-R
Postbus 9600, 2300 RC LEIDEN

The Central Datacenter will perform extensive consistency checks on the CRFs and issue Query Forms in case of inconsistent data that will be sent to the investigator. Those Query Forms must be answered and signed by the investigator (or an authorized staff member).

14.2 Electronic Case Report Forms

Data can also directly be entered on site into the web based ASPIRIN database. Investigators and/or authorized staff members should read the manual carefully and apply for a username and password at the Datacenter of LUMC.

14.3 Data retrieval from the Dutch Surgical Colorectal Audit (DSCA)

The DSCA registers every operated colorectal cancer patient. Data on co morbidity, tumour characteristics and treatment are collected. One question in the DSCA is about trial participation. If answered with yes, into ASPIRIN trial, data without any patient identifier, will be automatically transferred to the ASPIRIN database in Leiden on the basis of the unique DSCA patient number. This number will also be registered on the baseline CRF to assure a correct merge of data.

Explicit permission for the LUMC to import data from the DSCA into the ASPIRIN database has been inserted in the Clinical Trial Agreement.

14.4 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.
A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

14.5 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

14.6 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.
In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

14.7 Public disclosure and publication policy

The final publication of the trial results will be written by the Study Chairman on the basis of the final analysis performed at the Datacenter. A draft manuscript will be submitted by the study coordinator to the Datacenter for review no later than six months after receiving the Datacenter Report. After revision by the Datacenter and other co-authors the manuscript will be sent to a major scientific journal. Authors of the manuscript will include at least all Study Coordinators, the Project Manager and the member of the Datacenter team who have contributed to the trial. Due to the high number of participating centers only Local Investigators from centers who have entered the top 8 of included patients qualify for co-authorship. The other Local Investigators qualify for acknowledgements. All publications, abstracts or presentations including data from the present trial will be submitted for review to the Datacenter prior to submission.

All manuscripts will include an appropriate acknowledgement section, mentioning all investigators who have contributed to the trial, as well as supporting bodies. The Group Chairman, the Study Coordinators and the Datacenter must approve all publications, abstracts and presentations based on patients included in this study. This is applicable to any individual patient registered/randomised in the trial, or any subgroup of the trial patients.

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APPENDIX 1 ACE-27

Adult Comorbidity Evaluation-27

Identify the important medical comorbidities and grade severity using the index.
Overall Comorbidity Score is defined according to the highest ranked single ailment, except in the case where two or more Grade 2 ailments occur in different organ systems. In this situation, the overall comorbidity score should be designated Grade 3.

Cogent comorbid ailment	Grade 3 Severe Decompensation	Grade 2 Moderate Decompensation	Grade 1 Mild Decompensation
Cardiovascular System			
Myocardial Infarct	☐ MI ≤ 6 months	☐ MI > 6 months ago	☐ MI by ECG only, age undetermined
Angina / Coronary Artery Disease	☐ Unstable angina	☐ Chronic exertional angina ☐ Recent (≤ 6 months) Coronary Artery Bypass Graft (CABG) or Percutaneous Transluminal Coronary Angioplasty (PTCA) ☐ Recent (≤ 6 months) coronary stent	☐ ECG or stress test evidence or catheterization evidence of coronary disease without symptoms ☐ Angina pectoris not requiring hospitalization ☐ CABG or PTCA (>6 mos.) ☐ Coronary stent (>6 mos.)
Congestive Heart Failure (CHF)	☐ Hospitalized for CHF within past 6 months ☐ Ejection fraction < 20%	☐ Hospitalized for CHF >6 months prior ☐ CHF with dyspnea which limits activities	☐ CHF with dyspnea which has responded to treatment ☐ Exertional dyspnea ☐ Paroxysmal Nocturnal Dyspnea (PND)
Arrhythmias	☐ Ventricular arrhythmia ≤ 6 months	☐ Ventricular arrhythmia > 6 months ☐ Chronic atrial fibrillation or flutter ☐ Pacemaker	☐ Sick Sinus Syndrome ☐ Supraventricular tachycardia
Hypertension	☐ DBP≥130 mm Hg ☐ Severe malignant papilledema or other eye changes ☐ Encephalopathy	☐ DBP 115-129 mm Hg ☐ DBP 90-114 mm Hg while taking antihypertensive medications ☐ Secondary cardiovascular symptoms: vertigo, epistaxis, headaches	☐ DBP 90-114 mm Hg while not taking antihypertensive medications ☐ DBP <90 mm Hg while taking antihypertensive medications ☐ Hypertension, not otherwise specified
Venous Disease	☐ Recent PE (≤ 6 mos.) ☐ Use of venous filter for PE's	☐ DVT controlled with Coumadin or heparin ☐ Old PE > 6 months	☐ Old DVT no longer treated with Coumadin or Heparin
Peripheral Arterial Disease	☐ Bypass or amputation for gangrene or arterial insufficiency < 6 months ago ☐ Untreated thoracic or abdominal aneurysm (≥6 cm)	☐ Bypass or amputation for gangrene or arterial insufficiency > 6 months ago ☐ Chronic insufficiency	☐ Intermittent claudication ☐ Untreated thoracic or abdominal aneurysm (< 6 cm) ☐ s/p abdominal or thoracic aortic aneurysm repair
Respiratory System			
	☐ Marked pulmonary insufficiency ☐ Restrictive Lung Disease or COPD with dyspnea at rest despite treatment ☐ Chronic supplemental O_2 ☐ CO_2 retention (pCO_2 > 50 torr) ☐ Baseline pO_2 < 50 torr ☐ FEV1 (< 50%)	☐ Restrictive Lung Disease or COPD (chronic bronchitis, emphysema, or asthma) with dyspnea which limits activities ☐ FEV1 (51%-65%)	☐ Restrictive Lung Disease or COPD (chronic bronchitis, emphysema, or asthma) with dyspnea which has responded to treatment ☐ FEV1 (66%-80%)
Gastrointestinal System			
Hepatic	☐ Portal hypertension and/or esophageal bleeding ≤ 6 mos. (Encephalopathy, Ascites, Jaundice with Total Bilirubin > 2)	☐ Chronic hepatitis, cirrhosis, portal hypertension with moderate symptoms "compensated hepatic failure"	☐ Chronic hepatitis or cirrhosis without portal hypertension ☐ Acute hepatitis without cirrhosis ☐ Chronic liver disease manifested on biopsy or persistently elevated bilirubin (>3 mg/dl)
Stomach / Intestine	☐ Recent ulcers(≤ 6 months ago) requiring blood transfusion	☐ Ulcers requiring surgery or transfusion > 6 months ago	☐ Diagnosis of ulcers treated with meds ☐ Chronic malabsorption syndrome ☐ Inflammatory bowel disease (IBD) on meds or h/o with complications and/or surgery
Pancreas	☐ Acute or chronic pancreatitis with major complications (phlegmon, abscess, or pseudocyst)	☐ Uncomplicated acute pancreatitis ☐ Chronic pancreatitis with minor complications (malabsorption, impaired glucose tolerance, or GI bleeding)	☐ Chronic pancreatitis w/o complications

Cogent comorbid ailment	Grade 3 Severe Decompensation	Grade 2 Moderate Decompensation	Grade 1 Mild Decompensation
Renal System			
End-stage renal disease	☐ Creatinine > 3 mg% with multi-organ failure, shock, or sepsis ☐ Acute dialysis	☐ Chronic Renal Insufficiency with creatinine >3 mg% ☐ Chronic dialysis	☐ Chronic Renal Insufficiency with creatinine 2-3 mg%.
Endocrine System	**(Code the comorbid ailments with the (*) in both the Endocrine system and other organ systems if applicable)**		
Diabetes Mellitus	☐ Hospitalization ≤ 6 months for DKA ☐ Diabetes causing end-organ failure ☐ retinopathy ☐ neuropathy ☐ nephropathy* ☐ coronary disease* ☐ peripheral arterial disease*	☐ IDDM without complications ☐ Poorly controlled AODM with oral agents	☐ AODM controlled by oral agents only
Neurological System			
Stroke	☐ Acute stroke with significant neurologic deficit	☐ Old stroke with neurologic residual	☐ Stroke with no residual ☐ Past or recent TIA
Dementia	☐ Severe dementia requiring full support for activities of daily living	☐ Moderate dementia (not completely self-sufficient, needs supervising)	☐ Mild dementia (can take care of self)
Paralysis	☐ Paraplegia or hemiplegia requiring full support for activities of daily living	☐ Paraplegia or hemiplegia requiring wheelchair, able to do some self care	☐ Paraplegia or hemiplegia, ambulatory and providing most of self care
Neuromuscular	☐ MS, Parkinson's, Myasthenia Gravis, or other chronic neuromuscular disorder and requiring full support for activities of daily living	☐ MS, Parkinson's, Myasthenia Gravis, or other chronic neuromuscular disorder, but able to do some self care	☐ MS, Parkinson's, Myasthenia Gravis, or other chronic neuromuscular disorder, but ambulatory and providing most of self care
Psychiatric			
	☐ Recent suicidal attempt ☐ Active schizophrenia	☐ Depression or bipolar disorder uncontrolled ☐ Schizophrenia controlled w/ meds	☐ Depression or bipolar disorder controlled w/ medication
Rheumatologic	**(Incl. Rheumatoid Arthritis, Systemic Lupus, Mixed Connective Tissue Disorder, Polymyositis, Rheumatic Polymyositis)**		
	☐ Connective Tissue Disorder with secondary end-organ failure (renal, cardiac, CNS)	☐ Connective Tissue Disorder on steroids or immunosuppressant medications	☐ Connective Tissue Disorder on NSAIDS or no treatment
Immunological System	**(AIDS should not be considered a comorbidity for Kaposi's Sarcoma or Non-Hodgkin's Lymphoma)**		
AIDS	☐ Fulminant AIDS w/KS, MAI, PCP (AIDS defining illness)	☐ HIV+ with h/o defining illness. $CD4^+ < 200/\mu L$	☐ Asymptomatic HIV+ patient. ☐ HIV^+ w/o h/o AIDS defining illness. $CD4^+ > 200/\mu L$
Malignancy	**(Excluding Cutaneous Basal Cell Ca., Cutaneous SCCA, Carcinoma in-situ, and Intraepithelial Neoplasm)**		
Solid Tumor including melanoma	☐ Uncontrolled cancer ☐ Newly diagnosed but not yet treated ☐ Metastatic solid tumor	☐ Any controlled solid tumor without documented metastases, but initially diagnosed and treated within the last 5 years	☐ Any controlled solid tumor without documented metastases, but initially diagnosed and treated > 5 years ago
Leukemia and Myeloma	☐ Relapse ☐ Disease out of control	☐ 1st remission or new dx <1yr ☐ Chronic suppressive therapy	☐ H/o leukemia or myeloma with last Rx > 1 yr prior
Lymphoma	☐ Relapse	☐ 1st remission or new dx <1yr ☐ Chronic suppressive therapy	☐ H/o lymphoma w/ last Rx >1 yr prior
Substance Abuse	**(Must be accompanied by social, behavioral, or medical complications)**		
Alcohol	☐ Delirium tremens	☐ Active alcohol abuse with social, behavioral, or medical complications	☐ H/o alcohol abuse but not presently drinking
Illicit Drugs	☐ Acute Withdrawal Syndrome	☐ Active substance abuse with social, behavioral, or medical complications	☐ H/o substance abuse but not presently using
Body Weight			
Obesity		☐ Morbid (i.e., BMI ≥ 38)	

OVERALL COMORBIDITY SCORE (Circle one.)	**0**	**1**	**2**	**3**	**9**
	None	**Mild**	**Moderate**	**Severe**	**Unknown**

APPENDIX 2 G8 geriatric assessment screening tool

G8 Screening tool			
	Items	**Possible answers**	**Score**
A	Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?	**0**: severe reduction in food intake **1**: moderate reduction in food intake **2**: normal food intake	……...
B	Weight loss during the last 3 months?	**0**: weight loss >3kg **1**: does not know **2**: weight loss between 1 and 3 kg **3**: no weight loss	……...
C	Mobility	**0**: bed or chair bound **1**: able to get out of bed/chair but does not go out **2**: goes out	……...
E	Neuropsychological problems	**0**: severe dementia or depression **1**: mild dementia or depression **2**: no psychological problems	……...
F	Body Mass Index (weight in kg/height in m2)	**0**: BMI less than 19 **1**: BMI 19 to less than 21 **2**: BMI 21 to less than 23 **3**: BMI 23 or greater	……...
H	Takes more than 3 medications per day	**0**: yes **1**: no	……...
P	In comparison with other people of the same age, how does the patient consider his/her health status?	**0**: not as good **0,5**: does not know **1**: as good **2**: better	……...
	Age	**0**: >85 **1**: 80-85 **2**: <80	……...
	Total score (0-17)		……...

Score >14: Absence of geriatric risk profile
Score ≤14: Presence of geriatric risk profile

APPENDIX 3 Modified ADL and IADL

KATZ ADL
Dit is een lijst met bezigheden, die te maken hebben met uw functioneren in het dagelijks leven. We willen u verzoeken de vragen in te vullen voor de situatie zoals deze op dit moment is.

Naam patiënt:
Datum:

Kruis aan wat van toepassing is.
Bij twijfel altijd ja in vullen!

	DATUM:
ADL	
1. Heeft hulp nodig bij baden en douchen	O nee O ja
2. Heeft hulp nodig bij het aankleden	O nee O ja
3. Heeft hulp nodig bij kammen van de haren of scheren	O nee O ja
4. Heeft hulp nodig bij het naar het toilet gaan	O nee O ja
5. Maakt gebruik van incontinentiemateriaal	O nee O ja
6. Heeft hulp nodig bij opstaan uit stoel	O nee O ja
7. Heeft hulp nodig bij het lopen	O nee O ja
8. Heeft hulp nodig bij het eten	O nee O ja
SUBTOTAAL ADL	*/8*
IADL	
1. Heeft hulp nodig bij het gebruiken van de telefoon	O nee O ja
2. Heeft hulp nodig bij reizen (dagelijks vervoer)	O nee O ja
3. Heeft hulp nodig bij het boodschappen doen	O nee O ja
4. Heeft hulp nodig bij het bereiden van een maaltijd	O nee O ja
5. Heeft hulp nodig bij huishoudelijk werk	O nee O ja
6. Heeft hulp nodig bij het innemen van medicijnen	O nee O ja
7. Heeft hulp nodig bij het omgaan met geld	O nee O ja
SUBTOTAAL IADL	*/7*
(gemodificeerd, Weinberger et al. JAGS 1992)	
Ja = 1 punt; nee = 0 punt	
Totaal aantal ja = totaal score op KATZ = (svp optellen en invullen)	*/15*

APPENDIX 4 QLQ-C30 Questionnaire

EORTC QLQ-C30 (version 3)

Wij zijn geïnteresseerd in bepaalde dingen over u en uw gezondheid. Wilt u alle vragen zelf beantwoorden door het getal te omcirkelen dat het meest op u van toepassing is. Er zijn geen "juiste" of "onjuiste" antwoorden. De informatie die u geeft zal strikt vertrouwelijk worden behandeld.

		Helemaal niet	**Een beetje**	**Nogal**	**Heel erg**
1.	Heeft u moeite met het doen van inspannende activiteiten zoals het dragen van een zware boodschappentas of een koffer?	1	2	3	4
2.	Heeft u moeite met het maken van een lange wandeling?	1	2	3	4
3.	Heeft u moeite met het maken van een korte wandeling buitenshuis?	1	2	3	4
4.	Moet u overdag in bed of in een stoel blijven?	1	2	3	4
5.	Heeft u hulp nodig met eten, aankleden, u zelf wassen of naar het toilet gaan?	1	2	3	4

Gedurende de afgelopen week:

		Helemaal niet	**Een beetje**	**Nogal**	**Heel erg**
6.	Was u beperkt bij het doen van uw werk of andere dagelijkse bezigheden?	1	2	3	4
7.	Was u beperkt in het uitoefenen van uw hobbies of bij andere bezigheden die u in uw vrije tijd doet?	1	2	3	4
8.	Was u kortademig?	1	2	3	4
9.	Heeft u pijn gehad?	1	2	3	4
10.	Had u behoefte te rusten?	1	2	3	4
11.	Heeft u moeite met slapen gehad?	1	2	3	4
12.	Heeft u zich slap gevoeld?	1	2	3	4
13.	Heeft u gebrek aan eetlust gehad?	1	2	3	4
14.	Heeft u zich misselijk gevoeld?	1	2	3	4

Gedurende de afgelopen week:	**Helemaal niet**	**Een beetje**	**Nogal**	**Heel erg**
15. Heeft u overgegeven?	1	2	3	4
16. Had u last van obstipatie? (Was u verstopt?)	1	2	3	4
17. Had u diarree?	1	2	3	4
18. Was u moe?	1	2	3	4
19. Heeft pijn u gehinderd in uw dagelijkse bezigheden?	1	2	3	4
20. Heeft u moeite gehad met het concentreren op dingen, zoals een krant lezen of televisie kijken?	1	2	3	4
21. Voelde u zich gespannen?	1	2	3	4
22. Maakte u zich zorgen?	1	2	3	4
23. Voelde u zich prikkelbaar?	1	2	3	4
24. Voelde u zich neerslachtig?	1	2	3	4
25. Heeft u moeite gehad met het herinneren van dingen?	1	2	3	4
26. Heeft uw lichamelijke toestand of medische behandeling uw familieleven in de weg gestaan?	1	2	3	4
27. Heeft uw lichamelijke toestand of medische behandeling u belemmerd in uw sociale bezigheden?	1	2	3	4
28. Heeft uw lichamelijke toestand of medische behandeling financiële moeilijkheden met zich meegebracht?	1	2	3	4

Wilt u voor de volgende vragen het getal tussen 1 en 7 omcirkelen dat het meest op u van toepassing is

29. Hoe zou u uw algehele gezondheid gedurende de afgelopen week beoordelen?

1	2	3	4	5	6	7
Erg slecht						Uitstekend

30. Hoe zou u uw algehele "kwaliteit van leven" gedurende de afgelopen week beoordelen?

1	2	3	4	5	6	7
Erg slecht						Uitstekend

APPENDIX 5 Social Situation

To be completed by: Clinician or trained coder.

Question to the patient:
Which of the following statements best describes where you live?

- At home by myself.
- At home with someone.
- In institutional care (for example residential home or nursing home).

APPENDIX 6 Follow-up flow diagram

	Parameter	Before treatment starts	6 months	12 months	18 months	24 months	36 months	48 months	60 months
	CRF: Baseline[1]	x							
	CRF: Concomitant medication	x	x	x	x	x	x	x	x
	ACE-27	x							
	G8 Geriatric assessment screening tool	x							
	Social situation	x							
	Follow up and monitoring according to www.oncoline.nl; fill in accessory CRF		x	x	x	x	x	x	x
	(Serious) adverse events[2]		x	x	x	x	x	x	x
PATIENT	QLQ C30[3]	x	x				x		x
PATIENT	Modified ADL and IADL[3]	x	x				x		x

1 < 12 weeks after surgery
2. until < 30 days after stop of trial medication
3. Baseline questionnaire is present in the Investigator Site File. Subsequent questionnaires will be sent to the investigator/research nurse at the appropriate time points for distribution to the patient.

APPENDIX 7 NCI Common Toxicity Criteria (CTC)

The grading of adverse events and/or adverse drug reactions will be reported according to the NCI Common Terminology Criteria for Adverse Events, **CTCAE version 4.0**,
The complete document can be reviewed and downloaded from the following internet site:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

APPENDIX 8 RATIONALE FOR AMENDMENT PROTOCOL V2.0

In protocol v2.0, patients of 45 years and older are eligible for participation in the Aspirin trial. The age limit of 70 years has been removed as a result of renewed insights in previous data. Bastiaannet et al. (2012)(15) showed a survival benefit for patients who used acetylsalicylic acid after diagnosis of colon cancer with an adjusted rate ratio of 0.65 (95%CI 0.50-0.84). In the same cohort, Reimers et al. found an greater survival benefit for elderly patients with an adjusted rate ratio of 0.59 (95%CI 0.44-0.81), which suggested a larger survival benefit for elderly patients(49).

Materials and methods

For the present analyses, an extended dataset of this cohort from Netherlands Cancer was used with an inclusion of colon cancer patients between 1998 and 2011. Analyses were performed in a different manner than the previous studies. First, the prescriptions of patients were analysed per period of use or no use, taking into account stopping and re-starting acetylsalicylic acid use, instead of the “intention-to-treat” assumption where users were defined as acetylsalicylic acid user during the complete follow-up after the start of first use of acetylsalicylic acid. Only use of acetylsalicylic acid after diagnosis was considered in these analyses. Next, user status during these time periods of acetylsalicylic acid use or nonuse were analysed as a time varying covariate in a cox proportional hazards model. Nonusers were defined as patients who never used prescribed acetylsalicylic acid.

Results

In the present analyses, 3977 patients with colon cancer were included, with 545 (13.8%) patients using acetylsalicylic acid after diagnosis. Results of this dataset show corresponding results. The crude Hazard Ratio (HR) for Overall survival of users versus nonusers for patients with colon cancer was 0.60 (95% CI 0.46-0.78) and adjusted HR 0.55 (95% CI 0.42-0.71) Adjustments were made for age, stage, surgery (yes/no) chemotherapy (yes/no) and amount of comorbidity.

Table 1 shows the hazard ratios for patients in different age categories. Figure 1 shows the hazard ratios and confidence intervals plotted against age. This figure shows that the hazard ratio for acetylsalicylic acid use in patients younger than 60 is closer to 1.0 than for older patients. The difference is not statistically significant as in older patients, suggesting no or little effect of acetylsalicylic acid in younger patients.

Table 1: Crude and multivariate analysis for different age categories

	Crude analysis			Multivariate analysis*		
Age category	Hazard ratio	P-value	95% C.I**.	Hazard ratio	P-value	95% C.I. I
18-59	0,79	0.38	0,46 - 1,35	0,79	0.38	0,47 - 1,33
60-69	0,48	<0.001	0,28 - 0,83	0,54	0.029	0,32 - 0,94
70-79	0,47	<0.001	0,31 - 0,72	0,51	<0.001	0,34 - 0,77
80 and older	0,58	<0.001	0,36 - 0,94	0,51	<0.001	0,31 - 0,84

*Corrected for age at diagnosis, stage, surgery (yes/no), chemotherapy (yes/no) and amount of comorbidities

**C.I. Confidence Interval

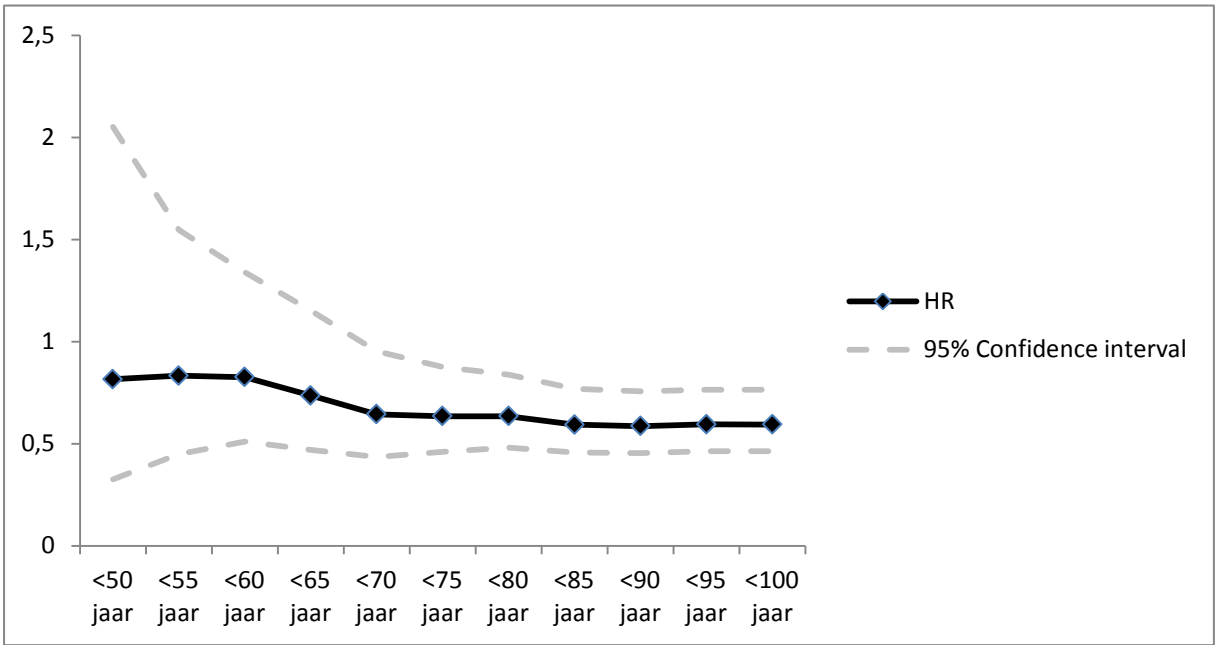

Figure 1: Adjusted rate ratio and 95%Confidence Interval according to age for colon cancer patients

Axes: overall survival in colon cancer patients (vertical) and age (horizontal)

However, when considering Dutch guidelines, the younger population of acetylsalicylic acid users reflects a specific group. In the Netherlands, acetylsalicylic acid is usually prescribed by general practitioners. The Framingham Risk Score (see Figure 2) is used as a tool for clinical decision making. This score assesses the risk of developing cardiovascular disease or dying of cardiovascular disease in the next ten years, plotted with age, blood pressure, smoking/non-smoking and ratio cholesterol/HDL cholesterol. Only patients that belong in the red boxes are eligible for drug treatment. When age rises above 70 years, nearly all patients with only one risk factor become eligible, contrary to younger patients who need to present with more risk factors before treatment is prescribed.

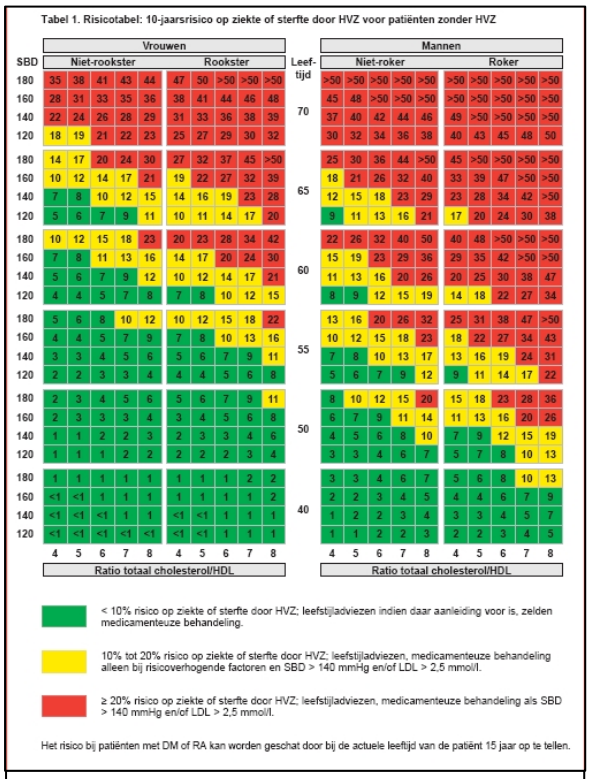

Figure 2: Framingham risk score

Source: Dutch College of General Practitioners

Our hypothesis is that younger patients who do receive acetylsalicylic acid have a significant worse health status than the general younger population due to these risk factors which are an indication to prescribe acetylsalicylic acid. The beneficial effect of acetylsalicylic acid might be attenuated due to the worse overall survival of these younger patients – contrary to older patients, as they are more likely to represent the overall older population with respect to overall survival (as acetylsalicylic acid is prescribed with less risk factors). For this reason it is not possible to assess the effect of acetylsalicylic acid on overall survival in these younger patients.

With the above observation we expect that the overall survival effect of acetylsalicylic acid in younger patients in observational studies cannot be assessed. The indication to prescribe low-dose acetylsalicylic acid to younger patients will always be strongly associated with the overall (severe worse) health status of younger patients and this will impact the survival more pronounced than in older patients. Patients above 70 years of age receive acetylsalicylic acid for a relatively mild risk factor (e.g. only mild hypertension) whilst patients below 70 will be prescribed acetylsalicylic acid if have severe hypertension and hypercholesterolemia.

As such, we do expect that the therapeutic effect of acetylsalicylic acid on cancer is not only present in older patients, but might also be present in younger patients, although this cannot be assessed with observational data. For the present protocol we assume that the beneficial

effect of acetylsalicylic acid might be present at the same rate in younger patients. To avoid the observed confounding by indication, it will only possible to assess this effect with a randomised trial.
Next to this hypothesis, we would like to emphasize that the rationale of the effect of acetylsalicylic acid on cancer (see the rationale of the protocol, chapter 4) is not age dependent.

Because of the above reasoning and the fact that the Add-Aspirin trial, ASCOLT and ALASCCA trial do not have a specific age groups, the age restriction of 70 years is removed from the inclusion criteria. Because of the high risk of patients having (non-)polyposis syndrome below 45 years, a restriction for this age is being maintained (see in- and exclusion criteria).
The original age-specific research question can still be answered when pooling the data with the Add-Aspirin trial (see paragraph 7.5).

APPENDIX 9 NFU guideline for monitoring

The monitoring will be done according to table 2 of the NFU guideline ‘Kwaliteitsborging mensgebonden onderzoek’ version 2.0, chapter 6. The complete guideline can be found at the NFU website:

http://www.nfu.nl/img/pdf/NFU-12.6053_Kwaliteitsborging_mensgebonden_onderzoek_2.0.pdf

Minimal monitoring is required because the ASPIRIN Trial is classified as a low-risk trial. In Dutch: ‘Verwaarloosbaar risico = Minimale monitoring’.