Name of Sponsor/Company: Deutsches Krebsforschungszentrum (DKFZ)		Sponsor-Code of Study: K357	*(For National Authority Use only)*
Name of (Finished) Product: ASS-ratiopharm®300 mg		Name of Active Ingredient: Acetylsalicylic acid	
EudraCT-No.: 2011-005603-32	CA Vorlage-No.: 4038264	IEC Antrags-No.: AFmu-271/2012	

End of Trial Report
SYNOPSIS

Title of Study:

Use of Acetylsalicylic Acid (ASA) for Enhanced Early Detection of Colorectal Neoplasms (ASTER-Study)

Date of Approval / Vote:
BfArM: June, 26th 2012 **Ethics Committee:** September, 17th 2012

Amendments: All substantial amendments are listed below.

Amendment No. 1:
Substantial changes include:
- Inclusion and exclusion criteria were changed with respect to pregnancy.
- Study duration was updated.
- The shipment of the colonoscopy (and histology) reports to the coordinating center was specified → "following the end of the study for each participant".
- The observation period for Adverse Events was slightly changed to "day 1 to day 9" without the inclusion of "or the day of colonoscopy" anymore.
- List of medication taken by the participant within the last 2 weeks was specified → "within the last 2 weeks before the start of the study".
- List of responsibilities of the investigators were deleted.
- Collection of laboratory values was reduced to thrombocytes.
- Direct shipment of the patient diary to the coordinating center.
- In addition to the colonoscopy and histology reports, data from the patient diary and questionnaire will not be collected in the electronic Case Report Form (eCRF).
- Check for completeness and plausibility of source documents was specified → "by the monitor at the coordinating study center"
-The causality of an Adverse Event was changed. "Unclassified" and "Unclassifiable" were replaced by "Not assessable".
- Reporting of Serious Adverse Events by investigator was specified → "within 24 hours after becoming aware of the SAE".
- Expedited reported changed to SUSARs only.

Amendment No. 2:
Substantial changes include:
- Change of LKP, because in the study center of the former LKP (University Hospital in Heidelberg) no patient recruitment was planned. New LKP in University Clinic in Ulm.
- Terminology was specified.
- Responsibilities for data management and analysis were specified → conducted by the DKFZ. The KKS Heidelberg supports the DKFZ with the eCRF.
- Time span for recording the reason for withdrawal or drop-out from the study specified to 3 weeks after enrollment.

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- Definition of drop-outs specified → "time span between taking the study drug and the colonoscopy of more than 3 months"

Amendment No. 4:
Substantial changes include:
- Change of LKP within the same study center (University Clinic in Ulm).

Amendment No. 13:
Substantial changes include:
- Study duration prolonged.
- Inclusion criteria changed from 45-75 yeas to 40-80 years.
- Inclusion and exclusion criteria changed with respect to pregnancy → pre-menopausal women are allowed participate in the study, when they have a negative pregnancy test.
- Participants are allowed to give only 2 stool samples, instead of 4 stool samples.
- Study duration for each participant changed from 9 days to 5 days, because the time interval between the administration of the study medication and the planned colonoscopy was changed from at least 9 days to at least 5 days.
-The observation period for Adverse Events was changed from "day 1 to day 9" to "day 1 to day 5".
- If colonoscopy is not performed within 3 months after start of the study, participant will not be considered as drop-outs anymore. If the colonoscopy does not take place at all, the participants will be considered as a drop-outs.
- The financial incentive for the study center was changed from 30€ to 75€ for each patient included.

Date of Approval / Vote:

Amendment No. 1:	BfArM:	16.11.2012	Ethics Committee:	26.11.2012
Amendment No. 2:		11.03.2013		22.04.2013
Amendment No. 4:		01.07.2013		02.07.2013
Amendment No. 13:		14.08.2015		02.09.2015

Investigators:

Principal Investigator (LKP according to §4 AMG): Prof. Dr. med. Thomas Seufferlein
Coordinating Investigator: Prof. Dr. med. Hermann Brenner

Study Centre(s):

The trial was a multicenter trial. 18 sites in Germany were initiated to recruit participants for this trial.

1. University Clinic in Ulm: Prof. Dr. med. Thomas Seufferlein, Albert-Einstein-Allee 23, 89081 Ulm
2. Gastroenterology private practice: Prof. Dr. med. Naktarios Dikopoulos, Prof. Dr. med. Leopold Ludwig, Zeppelinstraße 16, 89160 Dornstadt

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3. Gastroenterology private practice: Dr. med. Emil Höring, Leuschnerstraße 12, 70174 Stuttgart
4. University Clinic in Frankfurt: Universitätsklinikum Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main
5. Clinic in Memmingen: Dr. med. Thomas Luttenberger, Klinikum Memmingen, Bismarckstr. 23, 87700 Memmingen
6. Gastroenterology private practice: Internist in Regierungsbezirk Arnsberg
7. Gastroenterology private practice: Internist in Regionalverband Saarbrücken
8. Gastroenterology private practice: Prof. Dr. med. Birgit Kallinowski, Scheffelstrasse 63, 68723 Schwetzingen
9. Gastroenterology private practice: Dr. med. Jörg Mangold, Dr. med. Wolfgang Böck, Söflinger Str. 168, 89077 Ulm
10. Gastroenterology private practice: Prof. Dr. med. H. Krammer, Bismarckplatz 1, 68165 Mannheim (Not clinically active)
11. Gastroenterology private practice: Internist in Landkreis Dachau (Not clinically active)
12. Gastroenterology private practice: Internist in Regierungsbezirk Schwaben (Not clinically active)
13. Gastroenterology private practice: Internist in 81675 München (Not clinically active)
14. Gastroenterology private practice: Internist in 04103 Leipzig
15. Gastroenterology private practice: Internist in 55122 Mainz
16. Clinic in Trier: Dr. med. Heinz Kirchen, Krankenhaus der Barmherzigen Brüder Trier, Nordallee 1, 54292 Trier
17. Gastroenterology private practice: Dr. med. Thomas Eisenbach, Dr. med. Thomas Block, Franz-Kail-Straße 2, 51375 Leverkusen-Schlebusch
18. Gastroenterology private practice: Dr. med. Markus Pichler, Dr. med. Oliver Müller, Maybachstr. 50, 70469 Stuttgart

Publication (reference):

Brenner, H., et al., *Effect of a Single Aspirin Dose Prior to Fecal Immunochemical Testing on Test Sensitivity for Detecting Advanced Colorectal Neoplasms: A Randomized Clinical Trial.* JAMA, 2019. 321(17): p. 1686-1692.

Study period: (date of first enrolment) (date of last completed)	June, 18th 2013 January, 27th 2017	Phase of development:	Phase III

Objectives:

Main objective:
To evaluate diagnostic performance of 2 immunochemical Fecal Occult Blood Tests (iFOBTs) for detecting advanced colorectal neoplasms after a single dose of acetylsalicylic acid as compared to placebo.

Secondary objectives:
To study gender-specific performance of the 2 iFOBTs and the possible gain in diagnostic performance by stool sampling on multiple days.

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To study the safety of single-dose acetylsalicylic acid in the selected population. To collect blood samples for additional biomarker analyses (optional).
Methodology: Multicenter, randomized, double-blind, placebo-controlled study. Men and women aged 40 to 80 years with no recent use of aspirin or other drugs with antithrombotic effects were recruited when visiting 1 of 18 trial centers in Germany for a pre-colonoscopy appointment. Participants received trial medication (allocated in a blinded manner for both physicians and participants according to randomization list), questionnaire, and patient diary. In addition, participants received 4 stool collection kits for each of 2 iFOBTs. Participants were asked to collect a baseline stool sample, then take the trial medication and collect further stool samples 2, 3, and 4 days after trial medication intake (1 stool sample per scheduled day, 4 stool samples overall). Participants were asked to document the day of trial medication intake and the days of stool sample collection in a diary. If stool collection on these days was not possible, postponement to subsequent day(s) was allowed.
Number of Volunteers (planned and analysed): It was planned to recruit 2400 eligible participants and to include at least 2000 participants in the analysis. In total, 2422 participants have been included into the trial during the whole study period.1208 were allocated to verum and 1214 were allocated to placebo. From the verum group (n=1208), 77 participants did not receive intervention as randomized or dropped out, leaving 1131 study participants who underwent colonoscopy. From these 1131 participants, 56 were excluded from the analysis, due to no stool samples available (n=32), samples sent after colonoscopy (n=2), history of blood in stool (n=20) and Aspirin takers (n=2), leaving 1075 participants in the verum group for the analysis. From the placebo group (n=1214), 61 participants did not receive placebo as randomized or dropped out, leaving 1153 study participants who underwent colonoscopy. From these 1153 participants, 94 were excluded from the analysis, due to no stool samples available (n=56), history of blood in stool (n=30), history of colorectal cancer (n=5) and Aspirin takers (n=3), leaving 1059 participants in the placebo group for the analysis. Therefore, in total, 2134 participants were included in the final analysis.
Diagnosis and main criteria for inclusion: Men and women of 40 to 80 years of age with planned screening colonoscopy or planned diagnostic colonoscopy. Inclusion criteria: □ Age 40 to 80 years (both males and females). □ Planned screening or diagnostic colonoscopy

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☐ Able to speak and understand German sufficiently to be able to give written informed consent and comply with the study requirements

Test product (IMP being tested), trade name, MA holder, MA number, dose and mode of administration:

ASS-ratiopharm®300 mg, Ratiopharm GmbH 6367456.01.00, single oral dose of administration.

Batch numbers (Pharmacy of the University Hospital Heidelberg):

- K357/201246
- K357/201505
- K357/201624

Reference therapy (IMP used a comparator), trade name, MA holder, MA number dose and mode of administration:

NA

Duration of treatment:

Experimental intervention:
Single oral dose of acetylsalicylic acid 300 mg in the form of a tablet without enteric coating.

Control intervention:
Single oral dose of a placebo tablet.

Study duration for each participant:
5 days (in which day 1 = day on which the study medication is taken).

Criteria for evaluation: (efficacy, safety)

Efficacy:
The diagnostic performance (sensitivity and specificity) of two iFOBTs for detecting advanced colorectal neoplasms after a single dose of acetylsalicylic acid as compared to placebo. Results of the colonoscopy served as diagnostic reference standard (gold standard).

Safety:
Study participants who completed the colonoscopy (verum group, n=1131; placebo group, n=1153) were considered to have been followed up.

Statistical methods:

Diagnostic accuracy:
Sensitivity and specificity of the two immunochemical FOBTs.

These analyses were performed for the total study population for whom data on the primary endpoint and colonoscopy were available. In addition, the analyses were stratified according to gender. In addition, changes of sensitivity and specificity over the four time points of

measurement (fecal samples collected before, 2, 3 and 4 days after medication intake) in the intervention group were assessed.

Summary – Conclusions:

Efficacy Results:

Primary endpoint:
At the 10.2-µg hemoglobin/g stool cutoff, the sensitivities of the quantitative iFOBT (FOBGold®Tube Screen) for detecting advanced neoplasms, using fecal samples scheduled 2 days after tablet intake (intention-to-screen analysis), were 40.2% in the intervention group and 30.4% in the placebo group (difference, 9.8%; 95%CI, −3.1% to 22.2%; P = 0.14). At the 17-µg/g cutoff, the sensitivities were 28.6% and 22.5%, respectively (difference, 6.0%; 95%CI, −5.7% to 17.5%, P = 0.32).

Secondary endpoints:
At the 10.2-µg Hb/g stool cutoff, specificity of the quantitative iFOBT was 82.2% in the intervention group and 88.7% in the control group (difference, −6.4%, 95% CI, −9.6% to −3.2%, P < 0.001) and at 17-µg Hb/g stool cutoff was 91.7% and 94.8%, respectively (difference, −3.1%; 95% CI, −5.4% to −0.8%; P = 0.008).

For the qualitative test, sensitivity was higher by 12.7 percentage points in the intervention group (34.7% vs 22.0%) than it was in the control group (95% CI, 0.1 to 24.7, P = 0.048) but specificity was lower by 8.0 percentage points in the intervention group (83.8% vs 91.8%) than it was in the control group (95% CI, −11.0 to −4.9; P < 0.001).

Positive and negative predictive values (PPVs, NPVs) were very similar between the verum and placebo group. At the 10.2-µg hemoglobin/g stool cutoff, the PPV of the quantitative iFOBT was 22.7% in the placebo group, and increased only slightly to 23.6% in the verum group (difference, 0.9%, 95% CI, −9.3 to 10.7), whereas the NPV did not change between both groups (91.9% vs 91.9%, difference, 0.0%, 95% CI, −2.8 to 2.8). At the 17-µg/g cutoff, the PPV was 30.5% in the placebo group and decreased slightly to 30.2% in the verum group (difference, −0.3%, 95% CI, −15.3 to 14.2), and the NPV decreased slightly from 91.5% to 91.1% (difference, −0.5%, 95% CI, −3.3 to 2.3).
For the qualitative iFOBT, the PPV decreased from 23.5% (placebo) to 20.1% (verum) (difference, −3.3%, 95% CI, −14.8 to 7.7), and the NPV increased slightly from 91.3% to 91.7% (difference, 0.4%, 95% CI, −2.6 to 3.3).

Positive and negative likelihood ratios (LR+, LR-) were very similar between the verum and placebo group. At the 10.2-µg hemoglobin/g stool cutoff, the LR+ of the quantitative iFOBT was 2.7 (95% CI, 1.9-3.8) in the placebo group, and 2.3 (95% CI, 1.7-3.0) in the verum group. The LR- was 0.8 (95% CI, 0.7-0.9) in the placebo group compared to 0.7 (95% CI, 0.6-0.8) in the verum group. At the 17-µg/g cutoff, the LR+ was 4.4 (95% CI, 2.8-6.9) in the placebo group and 3.5 (95% CI, 2.4-5.0) in the verum group, and the LR- was 0.8 (95% CI, 0.7-0.9) in both groups.
For the qualitative iFOBT, the LR+ was 2.7 (95% CI, 1.7-4.1) in the control arm and 2.1 (95% CI, 1.6-2.9) in the intervention arm, and the LR- was 0.8 (95% CI, 0.8-0.9) and 0.8 (95% CI, 0.7-

0.9) in the placebo and verum group, respectively.

The area under curve (AUC) was very similar between both groups. The AUC was 64.9% (95% CI, 58.8-71.0) in the placebo arm and 63.4% (95% CI, 57.3-69.5) in the intervention arm.

In this large randomized trial, intake of a single dose of acetylsalicylic acid (300 mg) was associated with a strong increase in sensitivity of iFOBT in detecting advanced colorectal neoplasm among men but not among women. Among men, two days after pill intake, point estimates of sensitivity of the two tests were between 8.4 (95% CI, −7.5 to 23.3) and 17.6 (95% CI, 1.3 to 32.6) percent units higher in the intervention group compared to the control group. By contrast no, or only a moderate, non-significant increase (by between −0.8 (95% CI, −17.9 to 17.2) and 1.1 (95% CI, −17.7 to 20.9 percent units) was seen among women. Specificity was reduced in the intervention group by approximately 2.5 (95% CI, −6.1 to 1.1) and 6.6 (95% CI, −11.2 to −2.0) percent units among men, and between 3.7 (95% CI, −6.7 to −0.8) and 9.6 (95% CI, −13.5 to −5.5) percent units among women. P values for interaction effects between sex and intervention were all statistically non-significant and ranged from 0.08 to 0.49.
The gain in sensitivity and specificity by test application on multiple days was calculated when defining test positivity by at least 1 positive result in up to 3 fecal samples scheduled for days 2, 3, and 4. Compared with results for day-2 samples only, the sensitivities of the iFOBTs increased to estimates ranging from 38.9% to 47.7% (intervention group) and from 32.4% to 45.8% (placebo group), and the specificities decreased to estimates ranging from 72.4% to 86.3% in the intervention group and to estimates ranging from 82.8% to 91.1% in the placebo group. Differences in sensitivity and specificity between the intervention and placebo group ranged from 6.2 (95% CI, −6.4 to 18.7) to 15.3 (95% CI, 2.3 to 27.8) and from −4.9 (95% CI, −7.7 to 2.0) to −10.4 (95% CI, −14.1 to −6.6), respectively.

Safety Results:

Serious adverse events (SAE) occurred in two patients during the study. Both cases were considered as unrelated to the study drug. One patient had acute appendicitis; the other had acute suppurative cholangitis with consecutive acute kidney injury. Both patients recovered completely.

Non-serious adverse events occurred in 10 patients with mild or moderate intensity. From these, 3 patients had 4 adverse events that were associated with acetylsalicylic acid intake and had a causality of at least "possible". These adverse events were itchy rash, migraine, headache, and upper abdominal pain, which are considered to be in line with the known safety profile of acetylsalicylic acid. All patients recovered completely.

No actions regarding safety issues were taken and no change of the overall benefit-risk assessment occurred during the study.

Conclusion:

In this large randomized trial, intake of a single dose of acetylsalicylic acid (300 mg) was associated with a strong increase in sensitivity of iFOBT in detecting advanced colorectal

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neoplasm among men but not among women.
These results suggest that a single dose of acetylsalicylic acid (300 mg) taken prior to iFOBT may substantially increase iFOBT sensitivity for detecting advanced colorectal neoplasms at a very modest loss of specificity among men, but not among women.

Date of report: 06.03.2020

Principal or Coordinating Investigator(s) Signature(s)
(or Sponsor's responsible medical officer):

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study

10 March 2020	Hermann Brenner	
Date	Name	Signature

Affiliation:

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