INAS-FOCUS: *Study Protocol*

International Active Surveillance Study - Folate in Oral Contraceptives Utilization Study

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PRINCIPAL INVESTIGATOR:	Jürgen Dinger Invalidenstrasse 115 10115 Berlin Germany
STUDY CONDUCT:	ZEG Berlin, Center for Epidemiology and Health Research Invalidenstrasse 115 10115 Berlin Germany
SAFETY MONITORING AND ADVISORY COUNCIL:	Prof. Samuel Shapiro (South Africa, Chair 2010-2016)[1] Dr. David Grimes (US, Chair 2016 -) Dr. Edward Pritchett (US) Dr. Andrea Rapkin (US) Dr. Carolyn Westhoff (US) Dr. Anne Szarewski (UK)[2] Dr Peter Collins (UK)[3]
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[1] Prof. Samuel Shapiro (SMAC Member and Chair 2010 – 2016; RIP 2016)
[2] Dr. Anne Szarewki (SMAC Member 2010 – 2013; RIP 2013)
[3] Dr Peter Collins (SMAC Member 2014 -)

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1. ABBREVIATIONS

Abbreviation	Definition
AMI	Acute Myocardial Infarction
AT	As Treated
ATE	Arterial Thromboembolism
BMI	Body Mass Index
COC	Combined Oral Contraceptive
COC+	COC plus metafolin
CRC	Colorectal Cancer
CVA	Cerebrovascular Accident
DRSP	Drospirenone
DVT	Deep Venous Thrombosis
EE	Ethinylestradiol
EURAS	EURopean Active Surveillance (study)
FFQ	Food Frequency Questionnaire
FU	Follow-Up
HR	Hazard Ratio
INAS	INternational Active Surveillance (study)
IRB	Institutional Review Board
ITT	Intention To Treat
LNG	Levonorgestrel
OC	Oral Contraceptive
PASS	Post-Authorization Safety Study
PE	Pulmonary Embolism
SAE	Serious Adverse Event
SMAC	Safety Monitoring and Advisory Council
VTE	Venous Thromboembolism
WY	Women-years
ZEG	Berlin Center for Epidemiology & Health Research (acronym for the German term 'Zentrum für Epidemiologie & Gesundheits-forschung Berlin')

2. EXPLANATORY NOTE

INAS-FOCUS protocol was conceived in June 2010 as a post-authorization safety study (PASS) for newly marketed combined oral contraceptives containing metafolin. At the time of writing, this included combined oral contraceptives containing both Drosperinone (DRSP) and Dinogest (DNG). During study recruitment, only one combined oral contraceptive was launched containing metafolin (DRSP/EE+) and the planned third cohort arm (users of DNG/EE+) was rendered obsolete.

In addition, early recruitment rates and exposure figures in INAS-FOCUS suggested that the study would be inadequately powered to accurately assess the risk of colorectal cancer at 15 years. Interim reports were presented to the Safety Monitoring and Advisory Council (SMAC) and in consultation with the funder, a decision made to discontinue INAS-FOCUS following analysis of cardiovascular events (Part 1 of study).

The current protocol reflects the developments discussed above. The original three cohorts have been reduced to two (DRSP/EE+ and other OCs containing estrogen/progestogen) and Part II (long-term assessment of risk of colorectal cancer) has been removed from the protocol. Colorectal cancer outcomes will be analyzed as a secondary outcome for signal detection purposes only.

For historical accuracy and context, the introduction and background sections of the protocol have been left unchanged.

3. INTRODUCTION

In the forthcoming months new oral contraceptives (OCs) containing drospirenone (DRSP) as well as ethinylestradiol (EE), and metafolin (INN: levomefolate calcium) will be introduced. Oral contraceptives are an effective and popular method of birth control, with the majority of women in Europe and the US using OCs at least once in their lifetime. Over the past 50 years their safety has been improved with reductions in the estrogen and progesterone dose. Overall, the risk/benefit ratio is positive for the majority of women who need reversible and reliable contraception. However, special attention regarding oral contraceptive safety amongst women with risk factors for venous and arterial thromboembolism, as well as cancer, is necessary.

OCs containing DRSP - a progestogen that is also an aldosterone antagonist - have been available since the early 2000s. Results from the EURAS-OC [1] and the Ingenix study [2] showed that for all clinical outcomes studied - and in particular cardiovascular outcomes – there was no increased risk for users of DRSP/EE compared to users of other OCs (including OCs containing levonorgestrel (LNG)). The study results were robust enough to show non-inferiority of DRSP/EE regarding the cardiovascular outcomes of interest. In contrast, two recently published studies

[3, 4] suggested that the risk of venous thromboembolism (VTE) might be slightly higher for DRSP/EE compared to LNG/EE. However, these two studies had several methodological shortcomings that resulted in an overestimation of the DRSP cohort risk [5]. Overall, the scientific discussion concerning the impact of bias and confounding on the estimates for the VTE risk associated with OC use has not been fully resolved [1-18]. The most parsimonious interpretation of the evidence that exists to date suggests that the VTE risk associated with the use of DRSP/EE and LNG/EE are similar. The addition of folate to progestogen/estrogen combinations has probably no impact on the risk of VTE. However, robust clinical data is not available and therefore an investigation of the VTE risk associated with the combination of DRSP, EE and folate are required.

In 1995, a monophasic, low-dose combined oral contraceptive (COC) containing 2mg of dienogest and 30µg of ethinylestradiol (DNG/EE) was introduced to the German market. For the past decade, this combined oral contraceptive has been the most widely used OC brand in Germany. A recently conducted case-control study showed that the VTE risk is similar to another low-dose COCs (including LNG/EE). The impact of folate fortification on the VTE risk of this progestogen/estrogen combination needs further elucidation.

In addition to concerns raised regarding the VTE risk profile of OCs, there has been on-going debate regarding the potential impact of OCs on the development of several cancers. Until recently, research has focused on gynecological cancers. Inconsistent results surrounding OCs and breast cancer reveal the need for on-going surveillance [19-21]. In contrast, OCs and non-gynecological malignancies have received less attention. However, many studies suggest that progestogen/estrogen combinations - including OCs - reduce the risk of colorectal cancer (CRC) [22-24]. To date, published studies have generally had short follow-up periods and insufficient power to determine if ‘duration of use’ is an influential factor in the development of CRC. A recent meta-analysis on the association between oral contraceptives and CRC showed a decreased risk with ‘ever use’ of OCs although the analysis found no association with duration of use [25]. In contrast, Lin’s analysis reported a trend towards increasing protection from CRC with increased duration of OC use (multivariate trend p=0.09) [26].

Folic acid supplementation has also been implicated in both the prevention and the promotion of several cancers, including colorectal cancer (CRC). However, results from different studies are conflicting. Until recently, the majority of large observational trials and randomized controlled trials assessing dietary folate and CRC seemed to suggest a moderate inverse, or no association between dietary folate and CRC [27 - 30]. However, some trials (The Netherlands Cohort Study (2002) [31], Cole (2007) [32]), and two meta-analyses by Fife et al (2009) [33] and Ebbing [34] point to a more complex picture, with a suggestion that folate supplementation may have a tumor-promoting effect in already established neoplasm. These studies have tended to

examine older, predominantly male populations and extrapolating data to a young, female population is problematic. Additionally, there is some discussion as to whether the potential accelerating effect of folic acid on tumor growth is related to unmetabolized folic acid in the bloodstream. There is currently no clear evidence that folate supplementation either increases or decreases the risk of colorectal cancer. A study investigating the long-term effects of folate supplementation in a population of reproductive aged women is missing from the analysis.

Conversely, epidemiological evidence collectively suggests that low-folate diets are associated with an increased risk of malignancy, including colorectal cancer. It is possible that there is a complex interaction between folate and the cell-cycle, with folate exerting a differential effect depending on the cell status. Chronic folate deficiency seems to be associated with colorectal carcinogenesis, while high folic acid levels may have a tumor-promoting effect. On balance an oral contraceptive containing folate may be advantageous for several reasons. It may increase baseline folate levels with potential for protecting against some malignancies and concurrently decrease the risk of neural tube defects in women who become pregnant due to OC-failure, incorrect OC-use or after stopping the OC for a planned pregnancy.

Although unexpected, it is unclear whether combined oral contraceptives plus metafolin, (COC+) in general, and specifically DRSP/EE plus metafolin (DRSP/EE+), will alter the risk profile of established oral contraceptives. A non-interventional post-authorization safety study (PASS) is planned to investigate the safety of this new combination of oral contraceptive with regard to cardiovascular outcomes and that of cancer.

The EURAS study has demonstrated that a large, prospective, controlled, non-interventional, long-term cohort study is suitable for

1. Safety monitoring of an oral contraceptive
2. Reliable identification of relevant clinical outcomes and
3. Providing robust estimates of their incidence.

This study has a similar study design, however, the procedures for recruitment, informed consent and follow-up were modified to comply both with European and US regulations, and to ensure good recruitment rates and low loss to follow-up in a transatlantic environment. This study design has already proven to be successful in ongoing INAS studies (INAS-OC, INAS-SCORE).

The study should provide early information and regular updates on relevant clinical outcomes which will contribute to a continuous risk - benefit assessment during long-term follow up (up to 8 years).

4. STUDY OBJECTIVES

The primary objective of the study is to assess the risks of short and long-term use of DRSP/EE plus 451 µg of metafolin *(L-5-methyltetrahydrofolate)* and of established OCs in a study population that is representative for the actual users of the individual preparations. This includes an estimate of the absolute risk of rare serious adverse outcomes.

The main clinical outcomes of interest for the short and long-term follow-up are:

- Venous Thromboembolic Events (VTE[4]; primary variable (cf. section 11))
- Acute Myocardial Infarction (AMI)
- Cerebrovascular Accidents (CVA)

Secondary objectives are:

- To analyze the drug utilization pattern of COC+ and established OCs in a study population that is representative for typical use of the individual preparations under routine medical conditions. Interference of study-specific requirements and measures with the normal drug utilization pattern should be minimized by using a non-experimental study design.
- To characterize the baseline risk of users of the individual formulations (lifetime history of co-morbidity, risk markers, co-medication, socio-demographic and lifestyle data).
- To investigate risks of short and long-term use of COC+ and of established OCs in adolescents below the age of 18 years.
- To investigate pregnancy related data on discontinuation of COC+ and established OCs, i.e. return to fertility, congenital anomalies and in particular neural tube defects in abortion, stillbirths and live births.
- To characterize folate intake with respect to diet, COC+ use, vitamin use (information on preparation: trade name, vitamin ingredients and folate dose), and food fortification status of participating countries.
- Colorectal cancer (CRC)
- Other cancer entities

5. STUDY DESIGN

This is a large, transatlantic, prospective, controlled, non-interventional, long-term cohort study which follows two cohorts, users of DRSP/EE+[5], and users of OCs containing other estrogen/progestogen combinations. The users will be grouped to ‘starters’ (first-ever users) ‘recurrent users with a break’ (re-starters and switchers with a pill intake break) and ‘recurrent users without a break’. ‘A break’ is defined as

[4] includes deep venous thrombosis (DVT) and pulmonary embolism (PE)

[5] e.g., 21-day regimen of 3 mg DRSP/30 µg EE and 24-day regimen of 3 mg DRSP/ 20 µg EE containing metafolin

cessation of OC intake of at least one treatment cycle. A "non-interference" approach[6] will be used to provide standardized, comprehensive, reliable information on these groups of OCs under routine medical conditions. In this study, regular, active contacts with the cohort members (active surveillance) should provide all necessary information on health-related events or changes in health status during new OC use.

There will be active contacts with all study participants at baseline, every six months for the first two years, annually from years 3-5, making a total of 7 FUs (6,12, 18, 24, 36, 48, and 60 months)[7]. By means of these contacts, almost all relevant clinical outcomes will be captured. However, laypersons often misclassify adverse events (e.g., pain in the legs after standing a long period of time as "thrombosis" or migraine attacks as "stroke" even if modern imaging procedures do not provide any indication of the perceived event). This type of inaccuracy in patient reports requires careful validation of the reported events. This will be accomplished by contacting the relevant physicians and by reviewing relevant source documents. Under routine medical conditions, diagnosis of a VTE is not always confirmed by medical imaging. A standard algorithm will be used to classify VTE cases as "confirmed" or "not confirmed". For cancer endpoints, a disease-specific algorithm incorporating histological diagnosis will be used (cf. section 8 and Appendix 1). At the end of the study this classification will be verified by blinded independent adjudication (cf. Appendix 2).

6. STUDY CENTERS

Recruitment of the cohort members will be conducted via a network of approximately 2,500 OC prescribing physicians (study centers) in Europe and the United States.

The combined cohort will include 80,000 women, of which about 50,000 are recruited in the United States and 30,000 in Europe. The study will be implemented in two European countries (Russia and Ukraine) and the US (USA and Canada). The sequence for starting the study in individual countries will depend upon the sequence of COC+ launches. Study participants should undergo follow-up for 5 to 8 years.

Study measures should not interfere with the prescribing behavior of physicians or with the individual needs of the participating women. Influence on the preference for specific OCs is to be avoided but significant efforts are to be undertaken to ensure

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[6] i.e., 1) all patients who are new users of an OC - regardless of the type of estrogen or progestogen - are eligible for enrollment if they give their informed consent and 2) the recruitment of patients should not (significantly) influence the physician's prescribing behavior.

[7] planned follow-up for an individual patient at least 5 years but can be longer based on time point of study entry. (cf. section 7)

standardized, comprehensive and reliable documentation of all baseline characteristics and adverse events during the follow-up period.

7. STUDY PARTICIPANTS

The study participants are women who

- are new users of an OC (first ever use, recurrent use with and without pill break)
- are willing to participate in this long-term follow-up study

Women will be categorized into three different groups depending on OC-user characteristics. These groups have been previously found to be important in assessing cardiovascular risk [31]. The groups are defined as 1) OC starters (first-time users), 2) recurrent users with a break (re-starters and switchers with a break) and 3) recurrent users without a pill-intake break.

There are no specific medical inclusion or exclusion criteria. However, women

- who are not cooperative/available for follow-up may be excluded from study participation
- with a language barrier will not be eligible for study inclusion

At the participating centers, all women seeking a prescription for a new OC are to be asked by their physician if they are willing to participate. The physician is to explain the nature of the study, its purpose and associated procedures, and the expected duration of follow-up for each woman prior to her study entry. Each woman is to have ample opportunity to ask questions and must be informed about her right to withdraw from the study at any time without disadvantage and without having to provide reasons for her decision. This information will be provided on an informed consent and data privacy form which must be signed by all study participants. These documents are to be approved by the relevant local Ethics Committees/ Institutional Review Boards (IRB) and the relevant Data Privacy Office, if applicable. Local regulations and/or local ethical approval might require a parent's or guardian's signature for the recruitment of adolescents (e.g., United States); the informed consent form will be modified accordingly.

Once enrolled, a subject may discontinue use of OCs at any time. However, subjects will continue to be followed whether or not they remain on OCs, provided that they do not withdraw their consent. During the follow-up phase, subjects will be asked whether they have discontinued OC use or whether they have switched to another OC preparation. Information on the date and reason for discontinuation or switching during the follow-up phase will also be collected.

8. BASELINE SURVEY

Each physician's office will be provided with simple case report forms (questionnaires) for collecting data at baseline. The baseline visit will take place at the participating physician's office. All women who receive a new prescription for an oral contraceptive are to be asked if they are willing to participate. Only after the OC has been prescribed will the physician discuss the study with the subject. This ensures that participation in the study is not considered a requirement for treatment. After discussing the study details (including follow-up procedures and intervals, content and duration of follow-up contacts, use of data collected, etc.), each subject will be asked to provide written informed consent to participate in the study. If the subject needs time to consider participation, she will be permitted to leave the physician's office with her prescription and take an appropriate period to decide whether to participate.

The informed consent will include permission for study data to be collected and analyzed and for contacts to be made by the study team at intervals during the follow-up phase for collection of study information. Each subject will also be asked to provide information regarding alternative contacts (a close relative or friend, or primary care physician) if the study team cannot reach the subject after several attempts. Permission for the study team to contact a subject's primary care physician/ attending physician(s) and to review relevant source data (e.g., medical reports for validation of reported serious clinical outcomes) will also be sought. Follow-up frequency by the study team will be explained, and the content of follow-up contacts will be described.

Confidentiality will be maintained throughout the study and no personal information will be shared with alternative contacts. The funder - Bayer AG - will not have access to names, addresses, or alternative contact information for the subjects and all individual subject data will remain anonymous. Personal and medical information will be recorded on separate documents. ZEG will ensure that access to personal information is restricted in accordance with data privacy rules.

Baseline data will be recorded on a self-administered questionnaire containing queries relating to the participant's state of health and potential risk factors. Demographic data, medical and gynecological history, medication history (incl. vitamins) and history of OC use, family history of cardiovascular, colorectal and breast cancer related outcomes, reasons for OC use, as well as the addresses, e-mail addresses and phone numbers of the patient, relatives or friends, and the primary care physician are to be provided. In compliance with data protection regulations names, addresses and phone numbers are to be documented on a separate sheet.

9. FOLLOW-UP

A follow-up assessment for each woman is scheduled 6, 12, 18, 24 months after baseline, and then 3, 4, 5 years after recruitment. Women will be followed-up for at least 3 years. Women recruited in the early phase of the study will be followed-up until study endpoint [max. 8 years].

Follow-up questionnaires will be mailed to the participating women, who often know more about their own personal health related issues than the physician who prescribes their OC. This is especially true for potential adverse events treated by other physicians. Occasionally, events may be reported by the participant or by a participant's relatives, friends or attending physicians between the regular follow-ups. These reports will be documented and validated in the same way as regular reports (see section 8).

In addition to the follow-up questionnaires, at the 18-month follow-up, a validated food-frequency questionnaire (Dietary Folate Equivalents (DFE) screener) specific for folate intake will be sent to study participants. Folate intake has a relatively stable intra-person variability, particularly in individuals consuming a western diet. In contrast, inter-person variability tends to vary significantly. A single DFE screener during the follow-up period will rank individuals in regard to their average dietary folate intake (inter-person variability) and provide sufficient information for analysis purposes. The DFE is a validated and widely used methodology for capturing micronutrient data in large populations and can be tailored for individual countries. It is expected that women use OCs for an average of approx. 3 to 4 years. Follow-up at 18 months would therefore be a good predictor of folate intake during OC use.

A low "lost to follow-up rate" will be essential for the validity of the study. In order to minimize loss to follow-up a multi-faceted, four-level follow-up process will be established. Level 1 activities include mailing of the follow-up questionnaire and – in case of no response – reminder letter(s). If level 1 activities do not lead to a response, multiple attempts are to be made to contact the woman, friends, relatives and the gynecologist/primary care physician per phone. In parallel to these level 2 activities searches in national and international telephone and e-mail address directories are started (level 3 activities). If this is not successful, an official address search via the respective governmental administration will be conducted. This level 4 activity can provide information on new addresses (or emigration or death). If necessary, a search in the national death registers could be started at the end of the study to clarify the vital status of patients who are lost to follow-up after level 4 activities. Specific follow-up procedures will be governed by local peculiarities. Overall, the loss to follow-up of the combined cohort should be kept at less than 5% and 10% of the recruited population for Europe and the US, respectively.

The follow-up questionnaires will address the occurrence of adverse events. Reasons for switching to another OC or discontinuation will be requested if applicable. In case the use of COC+ or other OCs was discontinued due to the wish to conceive, or due to intended or unintended pregnancy, a follow-up questionnaire requesting data on pregnancy related outcomes (abortions, stillbirths and congenital abnormalities) will be sent to the study participant.

10. VALIDATION OF SELF-REPORTED EVENTS

A self-administered questionnaire used by study participants is a very sensitive tool which captures almost all serious clinical outcomes. From a methodological point of view, it captures a much higher proportion of these outcomes than methods relying only on the prescribing gynecologist who often is not involved in the diagnosis and treatment of these outcomes. However, it must be considered that there is a significant difference between the rates of reported and validated events, because laypersons often misclassify adverse events. Therefore, validation of the self-reported events is of utmost importance.

Validation of self-reported events begins at the level of the national field organization with a review of all subjective “events.” Potential serious outcomes are reported to ZEG on a daily basis and validated by ZEG.

If an event is reported by a participant, the subjectively perceived symptoms, signs of a disease and if possible, the diagnosis as understood by the patient has to be recorded. The name and address of the relevant physician (attending physician, physician responsible for the follow-up treatment after discharge from hospital, or primary care physician) should be provided by the participant.

Follow-up questionnaires containing information on such an event are to be immediately passed on to the medical reviewer group at ZEG. If the information is unclear or missing, the woman will be contacted by phone, e-mail or other means. For many serious outcomes it will be necessary to contact the diagnosing and/or treating physician for clarification and validation of the information received from the patient. This procedure is mandatory for all serious clinical outcomes (incl. VTE, ATE and cancer).

Under routine medical conditions, diagnosis of an SAE is not always confirmed by a diagnostic method with high specificity. Therefore, SAEs are classified by the investigators as “confirmed” or “not confirmed” according to a predefined algorithm (cf. Appendix 1).

In order to minimize classification bias - particularly if selectively affecting an individual exposure cohort - classification of self-reported serious events into confirmed and not confirmed cases will be adjudicated by two blinded medical

boards. The 'Cardiovascular Board' consists of three independent medical experts specialized in radiology/nuclear medicine, cardiology, and internal medicine/phlebology. The meetings of this board should take place 5 years after study start. The Safety Monitoring and Advisory Council (see section 13) will appoint these experts who will review all available information on the reported VTE/ATE. However, brand names, dose regimen and composition of the OC(s) used by the reporting woman will be rendered anonymous. The adjudicators will perform the review independently of each other and without knowing the judgement of the other adjudicators. If at least one adjudicator classifies a report as confirmed VTE or colorectal cancer, the reported event will be considered 'confirmed'. More details on the blinded adjudication procedure are given in Appendix 2.

11. REPORTING OF SERIOUS AND/OR UNEXPECTED ADVERSE EVENTS

ZEG will report all serious[8] and/or unexpected events that are possibly related to the use of any OC to the relevant pharmaceutical companies. A physician on the ZEG study team will assess the likelihood of a causal relationship to OC use for each serious adverse event in accordance with a predefined algorithm (cf. Appendix 3). Overall, the handling of adverse events will follow Volume 9A of 'The Rules Governing Medicinal Products in the European Union (part I, section 7).

ZEG will not monitor whether these companies meet their obligation to report these events to the Health Authorities according to (inter)national rules.

12. DATA MANAGEMENT

When questionnaires are received from study participants, all pages are counted, and the questionnaire is date-stamped. Questionnaires are to be checked for correct subject identification number, missing pages, legibility, and incomplete information on the questionnaires (cf. section 7 'Follow-Up'). Missing pages, illegible or missing information are requested from the study participants prior to data entry of the respective questionnaire.

Data are entered by double data entry via formatted entry screens designed to reflect the appearance of the questionnaire. Discrepancies between first and second data entry are identified by comparison of the two entry files within the statistical software SAS. The decision on the true entry is done by the responsible data manager at ZEG. This may require direct contact with the study participant who filled in the questionnaire. Corrections will be made to the questionnaire only after contact with

[8] Serious adverse event means any adverse event that results in death, a life-threatening experience, inpatient hospitalization, persistent or significant disability/incapacity, or requires medical/surgical intervention to prevent one of said outcomes.

the study participant or her treating physician (cf. section 8 'Validation of Self-Reported Events'). All corrections are dated and initialled by the data manager who received the relevant new information (e.g., via direct contact or by a copy of medical reports/documents). The incorrect CRF entry will be crossed out; however, it must remain legible, and the correct entry will be placed next to it. The reason for any correction of medical data on the questionnaire must be documented.

Quality control of entered data will be supported by SAS plausibility programs which include range, coding, missing and date checks as well as cross-reference (consistency) checks between variables.

13. DATA ANALYSES

The primary variable for inferential statistics is VTE. Based on available data and pharmacological/pharmacokinetic considerations the *a priori* assumption is that use of DRSP/EE+ is not associated with an increased risk of VTE compared to established OCs. That is, a statistical comparison of DRSP/EE+ and established OCs is not expected to show a difference. Therefore, a non-inferiority design to investigate the VTE risk of DRSP/EE+ was chosen. The primary analysis will be based on the comparison of the upper confidence limit for the point estimate of the VTE hazard ratio with the predefined non-inferiority limit (cf. section 12).

The null hypothesis to be tested is: $\mathbf{HR_{VTE} \geq 2}$ (i.e., the VTE hazard ratio for DRSP/EE+ vs. established OCs is higher or equal to 2). The alternative hypothesis is: $\mathbf{HR_{VTE} < 2}$.

Sub-analyses will include COC+, specific regimens of DRSP/EE+ (e.g., the 24-day regimen of 3 mg DRSP/20 µg EE and users of other COCs) as well as starters and recurrent user with and without pill-intake break.

Safety monitoring during study conduct will be based primarily on the ITT analysis of crude data. The final analyses will include both an "as treated" (AT) and an intention-to-treat (ITT) analysis using Cox regression models. The safety conclusions of the study, however, will be based on the AT analyses because the ITT approach potentially dilutes differences between treatments.

Crude as well as adjusted hazard ratios will be calculated. The appropriate confounding variables will be built into the statistical model. Based on the expectation of a small absolute number of serious outcomes of interest the number of confounding variables will be limited to well established risk factors for these outcomes. For VTE/ATE these will include age, body mass index (BMI), duration of current use, and VTE history. For Colorectal cancer age, BMI, alcohol intake and

family history of CRC are planned. The final decision on the confounding variables will be made by the Safety Monitoring and Advisory Council before the first interim analysis of follow-up data. In addition, alternative analysis will be performed with other potential baseline risks to check the appropriateness of this decision. Similar analyses will be performed for arterial thromboembolism (e.g., acute myocardial infarction and stroke), colorectal cancer and other serious adverse events.

A detailed statistical analysis plan will be developed by the Principal Investigator during the first year after study start. The final analysis plan will be approved by the Safety Monitoring and Advisory Council before the first interim analysis of follow-up data.

Within the first 5 years biannual interim reports will be provided to the funder following release of the interim analyses results by the independent Safety Monitoring and Advisory Council (cf. section 13). In the following years annual reports will be provided.

14. SIZE OF THE STUDY AND EVALUTION

The 5 to 8-year follow-up of 80,000 women should result in about 620,000 documented women-years. This estimate is based on the assumptions that (1) ZEG's physicians' network could recruit 80,000 women within three years, and (2) the annual drop-out rate is less than 10% (based on the EURAS-OC [1] and LASS studies: 10% for FU years 1-5, 7% for FU years 6-10, and 5% for FU years 11+). Details for 36-months recruitment scenario are given in Table 1a and 1b: Table 1a based on the assumption that the follow-up period is max. 8 years, Table 1b calculates the observation time with extending the follow-up period by one year (and therefore increasing the number of women-years).

Table 1a: Expected observation time (max. 8 years follow-up): Patient recruitment within 3 years (annual recruitment rate = 26,667 women)

	Sub-cohorts recruited during the					
Time (y)*	**1st study year**		**2nd study year**		**3rd study year**	
	No. of women	Time of observation	No. of women	Time of observation	No. of women	Time of observation
1	25,310	12,913				
2	22,779	24,045	25,310	12,913		
3	20,501	21,640	22,779	24,045	25,310	12,913
4	18,451	19,476	20,501	21,640	22,779	24,045
5	16,606	17,529	18,451	19,476	20,501	21,640
6	15,444	16,025	16,606	17,529	18,451	19,476
7	14,363	14,903	15,444	16,025	16,606	17,529
WY (total)		126,531		111,628		95,603
WY (grand total)	333,762					

* Time after start of recruitment

** The number of recruited women equals 26,667. However, the number of women at the end of the first year is lower because some women will drop out during the first year (daily drop-out rate for follow-up years 1-5: ~ 0.029%).

Table 1b: Expected observation time (alternative of extending the follow-up by one year): Patient recruitment within 3 years (annual recruitment rate = 26,667 women)

	Sub-cohorts recruited during the					
Time (y)*	**1st study year**		**2nd study year**		**3rd study year**	
	No. of women	Time of observation	No. of women	Time of observation	No. of women	Time of observation
1	25,310	12,913				
2	22,779	24,045	25,310	12,913		
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5	16,606	17,529	18,451	19,476	20,501	21,640
6	15,444	16,025	16,606	17,529	18,451	19,476
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* Time after start of recruitment

** The number of recruited women equals 26,667. However, the number of women at the end of the first year is lower because some women will drop out during the first year (daily drop-out rate for follow-up years 1-5: ~ 0.029%).

An evaluation of the cardiovascular events will take place 5 years after study start. In Table 2 are the total women-years shown which will be observed at that point in time. Based on the results of EURAS-OC [1], it is assumed that at that point in time about 4/5 of the observed time represents exposure time. Therefore, 232,000 WY will result in approx. 185,000 WY of OC exposure 5 years after study start.

Table 2: Expected observation time for cardiovascular risk (5 years): Patient recruitment within 3 years (annual recruitment rate = 26,667 women)

	Sub-cohorts recruited during the					
Time (y)*	**1st study year**		**2nd study year**		**3rd study year**	
	No. of women	Time of observation	No. of women	Time of observation	No. of women	Time of observation
1	25,310	12,912				
2	22,779	24,044	25,310	12,913		
3	20,501	21,640	22,779	24,045	25.310	12,913
4	18,451	19,476	20,501	21,640	22.779	24,045
5	16,606	17,528	18,451	19,476	20.501	21,640
WY (total)		95,600		78,074		58,598
WY (grand total)	232,272					

* Time after start of recruitment

** The number of recruited women equals 26,667. However, the number of women at the end of the first year is lower because some women will drop out during the first year (daily drop-out rate ~ 0.029%).

The study was designed to analyze rare and very rare events (according to the CIOMS classification 1 – 10 and less than one event(s) per 10,000 women-years, respectively). The adverse events of particular interest for the sample size calculation are VTE, ATE and colorectal cancer. Based on the EURAS-OC results (see section 1) the estimated VTE and ATE incidence rates in the young study population are ~9/10,000 WY for VTE and ~2/10,000 WY for ATE. However, in the DRSP/EE cohort, a reduced incidence for ATE (<1/10,000 WY) was observed. Since the EURAS-OC results are the most reliable and comparable data available, further calculations are based on these results. For colorectal cancer the expected incidence is 0.65/10,000 WY based on the CancerMPact database, which is the best source to derive incidence rates for colorectal cancer in certain sub-populations. Based on this incidence, the following sample size was calculated; in case the study shows other incidence rates at a later point in time, a re-calculation might be necessary and has to be discussed with the Safety Monitoring and Advisory Council.

The COC+ cohort consists of several newly marketed products and for this combined cohort a proportion of 25-35% of the total study population seems to be realistic. Furthermore, it is assumed that the proportion for the individual products will be in the range of 7-12%. Power calculations based on the incidences given above showed that approximately 110,000 to 180,000 WY would be needed to show non-inferiority of individual COC+ products versus established OCs for VTE. The calculations for VTE are based on the assumptions given in Table 3. In essence, the study is powered to exclude a twofold risk of VTE for the DRSP/EE+ cohort (primary variable)

as well as for folate-containing products with at least 7% of the total exposure – if the true risk of VTE is not different for the individual (sub)-cohorts.

Table 3: Power calculation[32] for VTE based on the assumption that the true incidence in the relevant COC+ (sub)-cohort is not different from the reference cohort.

Test significance level, α (one-sided)	0.025 (= 0.05 two-sided)	
VTE Incidence for reference cohort	9/10,000 WY	
Non-inferiority margin	9/10,000 WY (equal to the VTE incidence for the reference cohort)	
Expected VTE incidence for COC+ cohort	9/10,000 WY	
Power (%)	90	
Proportion of COC+ users (% of study population)	7	12
Required women years in COC+ cohort	12,543	13,256
Required women years in reference cohort	166,635	97,204
Total women years	179,178	110,460

Furthermore, 124,000 to 172,000 WY of exposure would be sufficient to also exclude a twofold risk for ATE (cf. Table 4), assuming that 1) the incidence for DRSP/EE+ is comparable to the EURAS-OC results (~ 1/10,000) and 2) DRSP/EE+ accounts for at least 8% of the total exposure.

Table 4: Power calculation[32] for ATE based on the assumption that the true incidence in the DRSP/EE+ cohort is ~ 1/10,000 compared to ~ 2/10,000 in the reference cohort.

Test significance level, α (one-sided)	0.025 (= 0.05 two-sided)	
ATE Incidence for reference cohort	2/10,000 WY	
Non-inferiority margin	2/10,000 WY (equal to twice the ATE incidence for the reference cohort)	
Expected ATE incidence for DRSP/EE+ cohort	1/10,000 WY	
Power (%)	90	
Proportion of DRSP/EE+ users (% of study population)	8	12
Required women years in DRSP/EE+ cohort	13,704	14,858
Required women years in reference cohort	157,594	108,953
Total women years	171,298	123,811

These power calculations suggest that this study is sufficiently powered to show non-inferiority of COC+ compared to established OCs. However, exact power calculations based on actual incidences and drop-out rates should be done on the basis of two years follow-up data. If these calculations do not confirm the assumed incidences and drop-out rates the independent Safety Monitoring and Advisory Council (SMAC) may discuss the need for adapting patient numbers and follow-up times.

15. SAFETY MONITORING AND ADVISORY COUNCIL

This study will maintain scientific independence and will be governed by an independent Safety Monitoring and Advisory Council (SMAC). Bayer AG will provide an unconditional grant. The Berlin Center for Epidemiology and Health Research (ZEG), Germany and its research team will be accountable to SMAC in all scientific matters.

The SMAC members will be international experts in relevant scientific fields (e.g., epidemiology, gynecology, cardiology and oncology). The members will receive remuneration of expenses and an honorarium to compensate for loss of potential earnings during their work for SMAC. The members will not be involved in or paid for the operational conduct of the study.

16. STUDY MANAGEMENT

This study will be conducted in accordance with

- 'Guidelines for Good Pharmacoepidemiology Practices (GPP)' issued by the International Society for Pharmacoepidemiology in 2007
- 'Good Epidemiological Practice (GEP) – Proper Conduct in Epidemiologic Research' issued by the International Epidemiological Association (IEA) European Federation in 2007
- The ethical principles that have their origin in the Declaration of Helsinki.

17. ETHICS AND PRIVACY

The study will only start after all relevant legal, administrative and ethical requirements (including all requirements regarding the enrollment of minors) have been fulfilled. Information on the identity of the patients and treating physicians will be kept separated from the clinical information throughout the study. All relevant national data protection laws will be followed. The study protocol will be submitted to the relevant Ethics Committees, Institutional Review Boards and regulatory authorities for comments and approval.

Subjects will sign informed consent forms at baseline after reading a subject information sheet and discussing the study with the participating physician. The physician will describe the purpose of the study, the non-interventional character of the study, timing and expected content of follow-up phase contacts, and collection of alternative contact information. Consent will include permission to contact any treating physician to follow up on specific safety outcomes. Subjects will be informed that ZEG's study team will contact them during the follow-up phase to ask a predefined set of safety related questions or to update alternative contact information. Answers to these questions will remain anonymous when forwarded to Bayer AG or the Safety Monitoring and Advisory Council.

Subjects will be asked to provide personal contact information (e.g., telephone number, home and e-mail address) and information regarding alternative contacts (e.g., relative, friend, general practitioner) in case they cannot be reached. In the event that a subject cannot be reached during the follow-up phase, local organizations will attempt to reach an alternative contact to re-establish contact with the subject. Subjects may be contacted between two follow-up points to confirm that their personal contact information is correct.

Subjects retain the right to withdraw their consent at any time during the study.

18. STUDY FEASIBILITY

ZEG has performed large, multi-national (including transatlantic), observational studies on pharmacoepidemiological issues for the past 20 years – in particular in the area of women's health care. Many of these studies have prospectively followed-up

patients for 5-10 years. In addition, ZEG has played a pivotal role in developing the EURAS/INAS study design. ZEG has established a broad network of several thousands gynecologists and OC prescribing physicians in the US and Europe who are currently recruiting women for INAS-like studies. With this established international network, no major problems are expected for the recruitment of study participants. Furthermore, the drop-out and follow-up rates presented in this study protocol are based on comprehensive experience from similar studies. Therefore, the conduct of the INAS–FOCUS study according to this study protocol has a high probability of success.

19. MILESTONES

Precise recruitment and follow-up milestones will depend on the specific launch dates of folate-fortified OCs in Europe and the USA. However, we anticipate the first patient to be recruited in November 2010 in the USA and 2011 in Europe. The first Safety and Medical Advisory Council (SMAC) will take place in the second quarter of 2011, and 6-monthly during Part I of the study. Unless otherwise agreed upon by SMAC members, SMAC meetings will take place annually (years 6 – 8). Written reports up-dating regulatory authorities of the study progress and important SMAC decisions will follow these meetings. The evaluation and analysis of cardiovascular end-points (VTE, ATE) will take place 5 years after the recruitment of the first study participant. After 8 years of follow-up, the final evaluation and analysis of colorectal and other cancer end-points will take place. A comprehensive study report will be made available at the completion of the 5 and 8-year evaluation.

20. PUBLICATIONS

The results of this study will be published. In accordance with the International Committee of Medical Journal Editors (ICMJE) initiative requiring prior entry of clinical studies in a public registry as a condition for publication, the study will be registered in the U.S. National Institutes of Health's protocol registration database (http://ClinicalTrials.gov).

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APPENDIX 1: VALIDATION OF SELF-REPORTED EVENTS

- Definite Event:

  Confirmed by diagnostic measures with high specificity (e.g., phlebography for DVT, spiral CT for pulmonary embolism, cerebral MRT for cerebrovascular accidents, ECG with typical ST segment elevation for acute myocardial infarction, histology for cancers, two-sided blood pressure measurement with diastolic blood pressure of more than 120 -mmHG for hypertensive crisis)

- Probable Event:

  Absence of confirmation by a diagnostic measure with high specificity, but clinical diagnosis confirmed by a health professional or supported by diagnostic tests with low specificity (such as D-dimer for VTE, typical ECG/blood gas tests for PE or confirmation of diagnosis by the treating physician for colorectal cancer). These cases are usually characterized by a subsequent specific therapy (such as fibrinolysis, long-term anticoagulant therapy or chemotherapy/radiotherapy). However, if the attending physician confirms that the diagnosis is correct, the event will be classified as a probable event even if specific treatment was not given.

- Event not confirmed:
  - Diagnosis reported by the patient is excluded by diagnostic procedures
  - A different medical condition is diagnosed by the attending physician
  - The woman did not contact a health professional to clarify her symptoms and no diagnostic measures were performed that could have clarified the diagnosis

Definite and probable events will be classified as ‘confirmed events’.

APPENDIX 2: BLINDED ADJUDICATION

The following adjudication procedure will be established:

1) Independent adjudication by the individual specialists
2) Documentation of the individual assessments
3) Comparison of the individual assessments
4) Discussion of "split decisions" among the adjudicators without enforcement of a unanimous decision
5) Independent re-adjudication of the discussed cases by the individual adjudicators
6) Documentation of the individual assessments

Based on this procedure four different classification strategies will be possible

I. Classification of the reported event according to the assessment of the majority of adjudicators before the discussion of "split decisions" takes place (i.e., "majority vote" based on step 2 of the six-step procedure described above)
II. Classification of the reported event according to the assessment of the majority of adjudicators after discussion of "split decisions" takes place (i.e., majority classification based on step 6 of the six-step procedure described above)
III. Classification of the reported event as confirmed if at least one adjudicator had classified the event as confirmed before the discussion of split decisions took place (i.e., "worst case decision" based on step 2 of the six-step procedure described above)
IV. Classification of the reported event as confirmed if at least one adjudicator had classified the event as confirmed after the discussion of split decisions took place (i.e., "worst case decision" based on step 6 of the six-step procedure described above)

The final analysis will be based on strategy III (worst case decision without discussion of split decisions) because it represents the most conservative approach. Alternative analyses will be possible on request of the Safety Monitoring and Advisory Council or regulatory authorities.

APPENDIX 3: CAUSALITY ASSESSMENT

Categories (Code)	Definition
no (1)	The time course between administration of the study drug and occurrence or worsening of the adverse event rules out a causal relationship and/or another cause is confirmed and no indication of involvement of the study drug in the occurrence/worsening of the adverse event exists.
unlikely (2)	The time course between administration of the study drug and occurrence or worsening of the adverse event makes a causal relationship unlikely and/or the known effects of the study drug or of the substance class provide no indication of involvement in occurrence/worsening of the adverse event and another cause adequately explaining the adverse event is known and/or regarding the occurrence/worsening of the adverse event a plausible causal chain may be deduced from the known effects of the study drug or the substance class, but another cause is much more probable and/or another cause is confirmed and involvement of the study drug in the occurrence/worsening of the adverse event is unlikely.
possible (3)	Regarding the occurrence/worsening of the adverse event, a plausible causal chain may be deduced from the pharmacological properties of the study drug or the substance class, but another cause just as likely to be involved is also known or although the pharmacological properties of the study drug or the substance class provide no indication of involvement in the occurrence/worsening of the adverse event, no other cause gives adequate explanation.
probable (4)	The pharmacological properties of the study drug or of the substance class and/or the course of the adverse event after dechallenge and, if applicable, after rechallenge and/or specific tests (e.g. positive allergy test, antibodies against study drug/metabolites) suggest involvement of the study drug in the occurrence/worsening of the adverse event, although another cause cannot be ruled out.
definite (5)	The pharmacological properties of the study drug or of the substance class and the course of the adverse event after dechallenge and, if applicable, after rechallenge and specific tests (e.g. positive allergy test, antibodies against study drug/metabolites) indicate involvement of the study drug in the occurrence/worsening of the adverse event and no indication of other causes exists.