Karisma II: A randomized, double blinded, six-armed placebo controlled study to investigate optimal dose of tamoxifen with the most favourable side effect spectra and with mammographic density reduction non-inferior to that of 20 mg tamoxifen.

Substance:	Tamoxifen
EudraCT:	2016-000882-22
Coordinating Investigator, Principal Investigator and Sponsor:	Per Hall, MD, Professor Karolinska Institutet Institution for Medical Epidemiology and Biostatistics 171 77 Stockholm Tel: +46733960590 Email: per.hall@ki.se

PROTOCOL REVISION HISTORY

Version	Date	Revised by	Description of change	Refers to chapter/page
1.0	2016-04-21	NA	Initial version.	NA
2.0	2016-06-29	NA	Revised after feedback from MPA. For details see reply to MPA dated 1 July 2016	NA
2.1	2017-01-02		Revised after inclusion in Lund 20161114-1202 and before recruitment in Sthlm	

PROTOCOL SYNOPSIS

Study Title

Karisma II: A randomized, double blinded, six armed, placebo controlled study to investigate optimal dose of tamoxifen with the most favourable side effect spectra and with mammographic density reduction non-inferior to that of 20 mg tamoxifen.

Study code KARISMA II	**EudraCT No: 2016-000882-22**

Coordinating Investigator

Per Hall

Study centre(s) –

Karma Study Centre / Breast and Mammography screening unit, Södersjukhuset, Stockholm, Sweden

Karma Study Centre / Unilabs mammography unit / Klinska prövningsenheten, Skånes Universitetssjukhus i Lund, Lund, Sweden

Study period	**Phase of development**
Estimated date of first subject enrolled, November 2016 Estimated date of last subject completed, December 2017	Phase II

Objectives and endpoints

Primary objective: Identify the minimal dose of tamoxifen non-inferior in its ability to reduce mammographic density compared to 20 mg of tamoxifen

Primary endpoint: Change in mammographic density and levels of side effects after 6 months. In particular, we will test for noninferiority in the proportion of women in the intervention arms (placebo, 1 mg, 2.5 mg, 5 mg, 10 mg) who have a density reduction as great as or greater (after 6 months) than the median density reduction in the 20 mg arm

Secondary objectives: Assess the drop-out level and the level of side effects in the intervention arms compared to the 20 mg arm.

Secondary endpoints: Drop-out rate. We will test for differences in the proportion of drop-outs after 6 months in the intervention arms compared to the 20 mg arm.

Tertiary objectives: Relate levels of tamoxifen metabolites, proteins, lipids and hormones in blood and changes in breast tissue to different tamoxifen doses. Measure genetic polymorphism in germline DNA and relate it to the other tertiary objectives. Measure mammographic density and levels of side effects 6 months after tamoxifen cessation in relation to treatment arm

Tertiary endpoints: Levels of tamoxifen metabolites, proteins, lipids and hormones in blood. Breast tissue changes. Genetic polymorphism in germline DNA. Mammographic density and levels of side effects 6 months after tamoxifen cessation

Study design

Randomized, double blinded, six-armed, placebo controlled clinical trial

Number of subjects planned

1,440

Diagnosis and main eligibility criteria

Healthy women participating in mammographic screening with a a measurable mammographic density, i.e. ≥4.5 % density (volumetric) measured by Volpara.

Investigational Medical Product

Investigational product – Tamoxifen

Dosage – 20 mg, 10 mg, 5mg, 2.5 mg, 1 mg and 0 mg (placebo) Oral administration, one tablet once daily
Duration of treatment 6 months
Duration of subjects involvement in the study 6 months for the SÖS-cohort (1400 particip), 12 months for the Lund cohort (40 paticip)
Efficacy assessments Mammographic density after 6 months Plasma levels of tamoxifen metabolites, proteins, lipids and hormones in blood Genetic polymorphism measured in germline DNA
Safety assessments Monitoring and recording of all AEs and SAEs
Statistical methods In two recent studies using change in mammographic density as a proxy for therapy response to daily intake of 20 mg tamoxifen, exactly 48% of the women responded i.e. had a significant reduction in mammographic density. This implies that approximately 50% of the women experience the beneficial effect of tamoxifen with a daily dose of 20 mg. We therefore define the response threshold to be the median decrease in absolute mammographic density of women in the 20 mg tamoxifen arm (this definition ensures that the same proportion of women exceeds the threshold as observed in previous studies). Further, we define a responder to be a woman whose mammographic absolute decrease between baseline and 6 months exceeds the response threshold. The primary efficacy endpoint is the proportion of responders. We will test for non-inferiority with respect to the primary efficacy endpoint for groups of women treated with placebo, 1, 2.5, 5, and 10 mg compared to the group of women treated with 20 mg tamoxifen. The non-inferiority margin is defined to be 16.7 percentage points; that is, we define non-inferiority to mean that the fraction of responders is not less than one third of the treated individuals. The null hypothesis is thus that the proportion of responders in women treated with placebo, 1, 2.5, 5, and 10 mg is 16.7 percentage points less than those women treated with 20 mg tamoxifen (i.e. the fraction of responders is less than one third; 50%-16.7%=33.3%). Since little is known about the threshold when absolute mammographic density is used, we will perform sensitivity analyses where the threshold is varied ±10 percentage points.

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1 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
AE	Adverse event
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
CIOMS	Council for International Organizations of Medical Sciences
CIOMS-form	Suspect Adverse Reaction Report Form
CMO	Contract Manufacturer Organization (in Pharma industry)
CRF	Case report form
CRO	Contract Research Organisation
CSA	Clinical Study Agreement
eCRF	Electronic Case report form
DCIC	Ductal Carcinoma In Situ
DDP	Data Display Plan
DMP	Data Management Plan
FAP	Full Analysis Population
GCP	Good Clinical Practice
ICF	Informed Consent Forms
ICH	International conference on harmonisation
IEC	Independent ethics committee
IMP	Investigational medicinal product
IMPD	Investigational Medicinal Product Dossier
MedDRA	Medical Dictionary for Regulatory Activities
PP	Protocol Population
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SP	Safety Population
SUSAR	Suspected Unexpected Serious Adverse Event
QC	Quality Control
QP	Qualified person. Medicinal product can not be released for sale or supply prior to certification by a QP that the batch is in accordance with the relevant requirements

2 ETHICS

2.1 Ethical and Regulatory review

Necessary approvals of the Study Protocol, the Subject Information and Informed Consent Form must be obtained before enrolment of any subject into the study. Furthermore, it is the responsibility of the Sponsor to keep the applicable Independent Ethics Committee (IEC) informed of any Suspected Unexpected Serious Adverse Reactions (SUSARs) and any substantial amendments to the protocol during the study period. The written approval from the IEC, including a study identification and the date of approval, will be filed with the Karma Group at the Karolinska Institutet and at the study site(s) together with a list of the IEC members, their titles or occupation, and their institutional affiliations.

2.2 Ethical conduct of the study

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects that were adopted in 1964 by the 18th World Medical Assembly, in Helsinki, Finland, with later revisions.

2.3 Subject information and consent

It is the responsibility of the Investigator to give each subject, adequate verbal and written information regarding the objectives and the procedures of the study as well as any risks or inconvenience involved before including the subject in the study. The subject should be informed that by signing the Informed Consent Form (ICF) she authorises monitor(s), auditor(s), the IEC and the Regulatory Authorities to have direct access to the subject's medical records for verification of clinical study procedures. The women must be informed about the right to withdraw from the study at any time and that participation is on a voluntarily basis. The subject should be allowed sufficient time for consideration of the proposal.

It is the responsibility of the Investigator to obtain signed informed consent from all subjects before including them in the study. The ICF must be signed and dated before any study-specific procedures are performed, including screening procedures. In case of possible future audits/inspections, the Investigator must file the signed ICF.

The final version of the subject information and ICF is submitted to the IEC and concerned Regulatory Authorities and must not be changed without permission from Sponsor and the local IEC.

2.4 Subject data protection

The Investigator must file a subject identification list that includes sufficient information to link records, i.e. the electronic Case Report Form (eCRF) and clinical records. This list should be preserved for possible future inspections/audits.

The subjects will be informed that the data will be stored and analysed by computer, that Swedish and local regulations for the handling of computerised data will be followed and that identification of individual subject data will only be possible for the Investigator.

Representatives of the Karma Group including a Contract Research Organisation (CRO), and/or regulatory authorities will inform the subjects about the possibility of inspections/audits of relevant parts of the clinical records. Authorisation to direct access to the subject's clinical records, as described above, is given by signing the ICF.

3 ADMINISTRATIVE STRUCTURE

Qualified investigators under the sponsorship of the Karma Group will conduct the study. The name and contact details of the responsible persons at Karma Group and the CRO will be listed in the Investigator Site File provided at the site.

Role	Name	Contact	Site
Sponsor/Principal Investigator/ Coordinating Investigator/Responsible medical officer	Per Hall	073-396 05 90 per.hall@ki.se	Karolinska Institutet /Södersjukhuset /Karma Study Centre
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SUSAR reporting	KTA, Karolinska Trial Alliance	Lotta Masousch 070-0011845	Stockholm/Lund

4 INTRODUCTION

The number of women diagnosed with breast cancer is increasing all over the world but little is done to lower these figures. The primary preventive measures that are available ranges from increased physical activity to prophylactic mastectomy. Several randomised controlled trials have shown a 40-60% decreased risk of breast cancer after anti-hormonal therapy (e.g. tamoxifen, raloxifen, aromatase inhibitors; [1]). Tamoxifen and aromatase inhibitors are normally used to treat breast cancer patients to reduce the risk of recurrence of the disease.

Despite the remarkable risk reduction in preventive randomised controlled trials, primary preventive strategies are scarcely part of clinical routine [1]. There are several possible reasons

for the reluctance, the major one being that the side effects of these therapies are not trivial. Serious side effects could only be acceptable for those that benefit from therapy, that is, there is a need of identifying women at high risk. Also, there is not a consensus on dose needed in the preventive setting. So far only full therapeutic dose has been tested and no attempts have been made to determine if lower doses prevent women from being diagnosed with breast cancer.

Karisma is an investigator-initiated study of primary prevention of breast cancer incidence. Karisma will be performed in three steps:

Karisma I, Pilot study

A previous application to Läkemedelsverket regarding the Karisma I study was accepted and the first pilot phase of Karisma was initiated in March 2015 and ended nine months later.

The exploratory pilot study including 42 women who were randomised to either 10 or 20 mg of tamoxifen. The aims of the pilot study were to identify the *time to mammographic density change, difference of density change between 10 mg and 20 mg tamoxifen and piloting procedures*.

The results from the pilot study show that density decreases within 6 months by about 50% for one quarter of the participants, about 20% for another quarter, and does not change for the remaining half of the participants.

As expected, there were more reported side effects in those groups with most density decrease. We noted no difference in the density reduction nor side effect spectra when we compared 10 and 20 mg tamoxifen. Approximately 25% of participants discontinued treatment due to adverse events and there was no dose dependant difference in discontinuation.

Karisma II (which this application refers to)

This is a dose determination study aiming to identify the optimal tamoxifen dose for reducing the risk of breast cancer. Instead of following a very large number of women, for many years, treated with different doses of tamoxifen and see if they develop breast cancer, we will include 1,440 healthy women participating in the mammographic screening program at two Mammography units (Unilabs mammography, Lund and Södersjukhuset Breast Centre, Stockholm) and measure their change in mammographic density. The change in mammographic density is a very good marker of therapy response. We will test if 1 mg, 2.5 mg, 5 mg and 10 mg reduce the mammographic density to the same extent as 20 mg. In summary Karisma II will be a randomized, double-blinded, six-armed placebo controlled study to identify the dose of tamoxifen with the most favourable side effect spectra and with a density reduction non-inferior to 20 mg tamoxifen.

The Karisma II study will be run in accordance with the ICH GCP guidelines, the principals of which have their origins in the Helsinki 2000 Declaration.

Karisma III

When the optimal tamoxifen dose has been identified in the Karisma II study, we intend to test this dose in women at high risk of breast cancer and study long-term effect of breast cancer incidence. In this phase we plan to include 8,000 women. A separate application will be sent for Karisma III.

4.1 STUDY RATIONALE

For more than 40 years 20 mg of tamoxifen has been used in breast cancer patients to reduce the risk of *recurrence*. It has also been shown that 20 mg of tamoxifen reduces the *risk* of breast cancer in perfectly healthy women but that the treatment comes with side effects. [4] The side effects are probably one reason tamoxifen is not used in the preventive setting despite the substantial risk reducing effect. The aim of the Karisma II study is to identify the lowest

tamoxifen dose that could be used for *primary preventive* purposes, with a hypothesis that a lower dose will reduce the side effects.

Mammographic density is the white part of the mammogram and consist of glandular and connective tissue. The black, non-radiolucent part is fat. The more glandular and more connective tissues, the whiter the breast, and thus, the higher the risk of breast cancer [2]. Factors that influence risk of breast cancer are known to influence density. Hormone replacement therapy increases density and risk of breast cancer, while tamoxifen has the opposite effect [3]. The molecular basis for the tamoxifen-induced changes in breast density is sparsely understood. Consequently, a secondary aim in the Karisma trial is to investigate changes in tissue markers by comparing breast tissue biopsies sampled prior to and after six months of treatment with tamoxifen or placebo.

Mammographic density has been assessed in a nested case-control study within the IBIS-1 study, a randomised prevention study of tamoxifen vs. placebo. The density decrease, over the first 18 months, in 123 women diagnosed with breast cancer, was compared to 942 women without breast cancer [4]. Forty-eight per cent of the women treated with tamoxifen experienced a 10% or greater reduction in breast density and had a 63% reduction in breast cancer risk compared to women in the placebo group. In contrast, those who took tamoxifen but experienced less than a 10% reduction in breast density had no risk reduction.

Within the Karma group a parallel study has been conducted including breast cancer patients who where treated with adjuvant tamoxifen therapy [5]. Forty-eight per cent of the women experienced a decrease in density of > 20 % over a 12 month period and had a reduced breast cancer recurrence of 50%. These findings are fully supported by a very recent finding by a US group [6].

The scientific “golden standard” for measuring mammographic density is a software supported method called Cumulus. In this semi-automated approach the breast contour is outlined and a human reader decides on what part of the breast is dense. The software then calculates the dense area. The obvious weaknesses of Cumulus are that it is time consuming, labor intensive and highly reader dependent.

In clinical practice mammographic density is measured using BI-RADS. BI-RADS categorizes breast into four categories: entirely fatty, scattered areas of fibroglandular density, and extremely dense breast. The BI-RADS measurement is done by the radiologist by looking at the mammogram. BI-RADS measurements are very crude and highly reader dependent.

Volpara is a FDA approved full automatic method for volumetric density measurement that the Karma project group in collaboration with the Mammography units at Södersjukhuset and Unilabs Lund has implemented for clinical use. The Volpara method has also been validated in the Karma cohort. We will use Volpara as a tool for assessment of eligibility (measurable density), since its implemented and the measurement can be done instantly.

In the former mentioned Karma adjuvant tamoxifen therapy study we used a newly developed fully automated density measurement, the Stratus method, which mimics how Cumulus measures mammographic density but as automated tool is not reader dependent. In the Karisma II study we will use both Volpara and Stratus to analyse the mammographic changes.

The results from the Karisma I study showed that the effect of tamoxifen on mammographic density can be determined after 6 months of treatment and indicates that the effect is equal for the doses 10 mg and 20 mg. Currently there are no studies including longitudinal measurement of mammographic density on healthy women using tamoxifen. By adding a lower dose in the Karisma I study, 10 mg, we have got an indication lower doses will be sufficient for reducing density. In Karisma II we will include six arms with 200 women in each arm; 20 mg, 10 mg, 5

mg, 2.5 mg, 1 mg and a control arm with placebo. The Subjects and Investigators will be blinded.

Side effects and quality of life will be measured as a function of tamoxifen dose. In Karisma II we will use same questionnaires as in Karisma I to evaluate general symptoms (Memorial Symptom Assesment Scale), specific symtoms of tamoxifen (FACT-ES), compliance of medicine intake (Morisky scale) and health literacy (Health Literacy Scale). The questionnaires are validated instruments and include scoring system to evaluate the symptom burden of the study participants. (References to questionnaire instruments are to be found in appendix 15.2 Questionnaires).

The Karma project forms the basis for the Karisma study. The Karma project is lead by the same group that heads Karisma. The aim of Karma is to reduce incidence and mortality in breast cancer by a preventive approach. During 2010-2013 the Karma Study Centres at Södersjukhuset and at Skånes Universitetssjukhus, Lund were used to create the Karma Cohort witch is a prospective observational study including 70,877 women. Women invited for screening mammography were invited to Karma Study Centre to sign an informed consent form, donate blood, measure blood pressure and answer a comprehensive questionnaire on lifestyle factors. The women also allowed us to access their medical information in registers and records, and to store their mammograms. In Karisma II we plan to use the same infrastructure as for the recruitment to the Karma Cohort.

5 RISK-BENEFIT ASSESMENT

5.1 Benefits

There is no obvious advantage to participate in the study on an individual level. The benefits must be seen in a larger perspective, i.e. the possibility to develop a preventive measure against breast cancer.

Every hour one Swedish woman is diagnosed with breast cancer and one in nine women will develop breast cancer during their lifetime. Tamoxifen has proven to reduce the incidence in breast cancer but treatment comes with side effects. If a reduced dose of tamoxifen could be proven to reduce the incidence in breast cancer and at the same time have less side effect it could potentially be used as a preventive measure. Such a finding has the potential to significantly reduce breast cancer incidence. We believe that a preventive approach with tamoxifen could lead to a reduction in the incidence of breast cancer by at least 10%. This corresponds to saving ≈ 800 women per year from getting the disease in Sweden alone.

Previous preventive tamoxifen studies (there are four) have exposed healthy women to tamoxifen for at least three years. In the tamoxifen study by Jack Cuzick published in 2011 it was found that women who experienced a decrease in mammographic density had a reduced risk of breast cancer [4]. It is therefore possible that the reduction in mammographic density *per see,* and not the length of treatment, is what brings the reduction in breast cancer risk. In short, it is possible that participants in the study experiencing a reduction in mammographic density will have a reduced risk of breast cancer.

5.2 Risks of side effects

Tamoxifen at a dose of 20 mg has side effects that can be divided into common side effects, that are more or less harmless and reversible, and rarer serious side effects.

The common side effects are menopause symptoms; sweating, hot flashes and insomnia. These side effects disappear shortly after stopping treatment. Women who during the study are bothered by these side effects will obviously be able to cancel participation.

The serious and uncommon side effects include cancer of the uterus. This is extremely rare and perishing of gynecological disorders. A gynecologist is involved in the study and the participants will be encouraged to contact the Karma Study Centre if they experience problems.

Another serious and uncommon side effect is blood clots. It's the same kind of problems that can arise when using oral contraceptives. It should be emphasized that the rare side effects only have been observed for tamoxifen use in doses of 20-40 mg for at least 2 years. The spectra of side effects at lower doses and shorter treatment times are unknown.

By applying strict exclusions criterias, such as a history of trombo- embolic disease, hypertension or use of hormonal contraceptive pills we will minimize the risks we expose to the participants.

Participants will report side effects after 1, 3, and 6 months (and the Lund cohort will also report after 12 months). They will also report side effects whenever they occur and there is also an alarm system when adverse events, that need to be followed up, are reported in the web questionnaire form or in the application called Karmapp installed in their smartphone or tablets. Participants will be informed to contact the Karma Study Centre immediately if they suspect side effects. The contact channels are Karmapp, phone or email and they will be manned during long-weekends and holidays seasons on a jour basis.

5.3 Study population

The study will include healthy women that participate in the mammographic screening program at Södersjukhuset, Stockholm and at Skånes Universitetssjukhus, Lund, and have a negative screening mammogram. We will not include breast cancer patients as they receive a variety of drugs that might influence mammographic density. We are excluding women with no measurable density. This is because measurable density at study start is a prerequisite for being able to see reduction of mammographic density over time. We are not selecting women based on risk of breast cancer since we currently lack a reliable risk prediction model for Swedish standards.

5.4 Study length and study period

There are three studies [4,5,6] that used mammographic density change during one year as a proxy for tamoxifen effect. Two projects studied the association between mammographic density change in patients and prognosis and one study looked at density change among perfectly healthy woman and risk of breast cancer. All three studies came to the same result, approximately half of the treated participants respond to the therapy by a significant reduction in mammographic density after one year. Those that respond with a decreased mammographic density had a lower risk of breast cancer recurrence and a lower risk of breast cancer.

The main reason we conducted the Karisma I study was to investigate time to density change. Radiologists tell us that the time to density change is most probably much shorter than one year, probably only a couple of months. However, the issue of time to density change during tamoxifen therapy has never been addressed.

The results from Karisma I show that, regardless of dose, density decreases within 6 months by about 50% for one quarter of the participants, about 20% for another quarter and not at all for the other participants. This result implicates that tamoxifen intake during 6 months gives a measurable density change of responders; therefore, the treatment period in Karisma II are set to 6 months.

From a clinical point of view and for coming study (Karisma III) we will investigate the state of density change and decline of side effects 6 months after tamoxifen cessation in relation to doses. This will be done exploratory and only for the Lund-cohort.

The study period will be at least 14 months since we plan to run Karisma II at two sites successively:

Site	Karma Study Centre Lund	Karma Study Centre Stockholm/SöS
Number of participants	Approximately 40	Approximately 1400
First enrolment	November 2016	February 2017
End of treatment	May 2017	December 2017
End of follow up	November 2017	December 2017

5.5 Risk-benefit of frequent mammograms

Eligible study participants will only be women that have negative screening mammograms before entering the study, i.e. no abnormalities have been identified. The participants will have extra mammograms taken at 6 (all participants) and 12 (only Lund cohort) months after study entry. In order to reduce radiation exposure, the mammograms consist of 2 images instead of 4, which are standard at a screening examination. The images will be used for density measurement but radiologist will also screen them for abnormalities as in clinical practise. It could be that some few cancers are diagnosed at these extra visits, which should be seen as something that benefits the study participants.
If a suspected alteration is seen, women will receive the normal routine work-up for a suspected breast cancer, that is, additional ultrasound examination and a possible fine needle biopsy. Surgical interventions are only performed if a cancer is detected.

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 Primary objective

Identify the minimal dose of tamoxifen that is non-inferior in its ability to reduce mammographic density compared to 20 mg tamoxifen.

6.2 Primary endpoint

Change in mammographic density and levels of side effects after 6 months. In particular, we will test for noninferiority in the proportion of women in the intervention arms (placebo, 1 mg, 2.5 mg, 5 mg, 10 mg) who have a density reduction as great as or greater (after 6 months) than the median density reduction in the 20 mg arm

6.3 Secondary objectives

Assess the drop-out level and the level of side effects in the intervention arms compared to the 20 mg arm.

6.4 Secondary endpoint

Drop-out rate. We will test for differences in the proportion of drop-outs after 6 months in the intervention arms compared to the 20 mg arm.

6.5 Teritary objectives

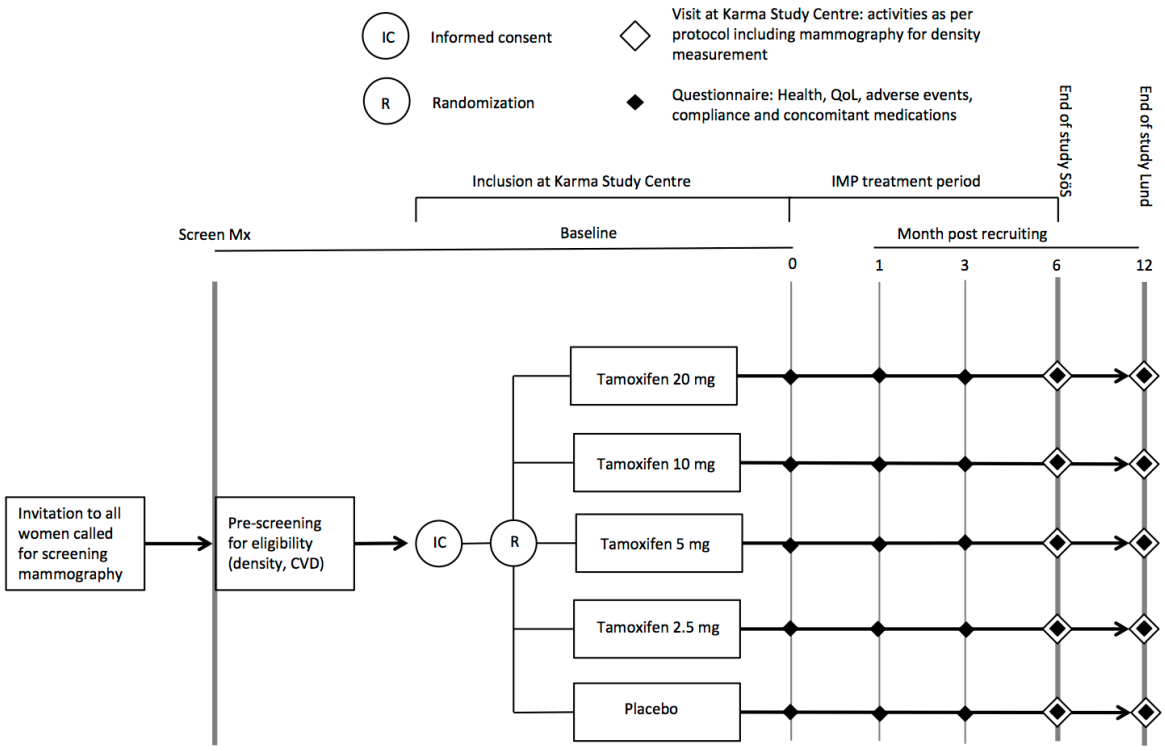
Relate levels of tamoxifen metabolites, proteins, lipids and hormones in blood and changes in breast tissue to tamoxifen doses. Measure genetic polymorphism in germline DNA and relate it to the other teritary objectives. Measure mammographic density and levels of side effects 6 months after tamoxifen cessation in relation to tamoxifen dose.

6.6 Teritary endpoints

Levels of tamoxifen metabolites, proteins, lipids and hormones in blood. Breast tissue changes. Genetic polymorphism in germline DNA. Mammographic density and levels of side effects 6 months after tamoxifen cessation

7 INVESTIGATIONAL PLAN

7.1 Overall study design



7.1.1 Invitation

Eligible women will be identified from women invited to mammography screening in Stockholm/Lund according to following:

1) A comprehensive study information about Karisma II will be sent to all women invited to mammographic screening at the collaborating sites a few days after screening invitation.
2) In connection with screening examination, interested women are invited to visit the Karma Study Centre (located within the screening facilities) to declare their interest of participation and to get further information about the study.

7.1.2 Pre-screening of eligibility of participation

At Karma Study Centre:

1) A Karma staff checks that the woman has read and understood the study information.
2) Volpara density measurement data are retrieved from the performed mammographic examination in order to see that the women meet inclusion criteria for density (≥4.5 % volumetric density)

3) Blood pressure is measured to ensure eligibility to participation.

If it at this stage turns out that the woman doesn't meet inclusion criteria or meet any of the exclusion criteria, she will have an explanation from investigator why she can't participate. A recording of non-participation is done in the Study file screening-list.

7.1.3 Baseline (Month 0)

Meeting with Investigator

- The investigator will explain the nature and aim of the study, its procedures, possible side effects, requirements and restrictions.
- The woman will sign the ICF before any further study related procedures are performed.
- A woman fulfilling the inclusion and exclusion criteria will be randomised to one of the six treatment arms; tamoxifen 20 mg, 10 mg, 5 mg, 2.5 mg, 1 mg or placebo. All treatments will be on a daily basis for 6 months
- The woman will receive a sufficient amount of IMP in order to take one tablet daily during 6 months
- Screening form, randomization number and other applicable information will be filled in by investigator in the eCRF.
- The participants will be asked if they allow to have two biopsies of the normal breast tissue taken at month 0 and 6. This is optional and complies with a separate study protocol (Protocol: Biopsy Karisma II)

Procedures at baseline

After meeting with investigator when ICF is signed and randomization is performed, the following will be obtained:

- In total 24 ml blood will be collected. The blood will be analysed for tamoxifen metabolites, plasma hormones, plasma proteins, metabolic markers. In addition, DNA will be extracted and plasma aliquots will be biobanked for later analyses.
- The participants will be asked to fill out two web-based questionnaires;
    - The Karma questionnaire including lifestyle factors that influence risk of breast cancer such as age at first birth, breast-feeding, etc., demographic, medical history, quality-of-life, and concomitant medication.
    - Karisma II baseline questionnaire including general symptoms and tamoxifen related symptoms
- Pregnancy test (urine) will be offered women of childbearing age for safety
- Measurement of weight, length and waist
- Instructions are given on how to use and report events by the Karma-app
- Biopsy of the breast. This is optional and complies with an additional translational study protocol (Protocol: Biopsy Karisma II)

7.1.4 1 month after baseline (+2/-1 weeks)

- Karisma II follow up questionnaire including general symptoms, tamoxifen related symptoms, compliance and health care contact connected to possible side effects

7.1.5 3 months after baseline (+/- 2 weeks)

- Karisma II follow up questionnaire including general symptoms, tamoxifen related symptoms, compliance and health care contact connected to possible side effects

7.1.6 6 months after baseline (+/- 2 weeks), End of treatment all participants, end of study SöS-cohort

Visit at Karma Study Centre:

- Karisma II follow up questionnaire including general symptoms, tamoxifen related symptoms, compliance and health care contact connected to possible side effects
- In total 24 ml blood will be collected. Planned analyses are tamoxifen metabolites, plasma hormones, plasma proteins, metabolic markers.
- Mammogram (one image per breast only for density measurement purposes)
- Measurement of weight, length and waist
- A 2nd biopsy will be performed if participation at baseline

End of study SöS cohort. The women will be informed that the study period now has ended and she will be called to mammography within the national screening program.

7.1.7 12 months after baseline (+/- 2 weeks), End of study Lund cohort

Visit at Karma Study Centre:

- Karisma II follow up questionnaire including general symptoms, tamoxifen related symptoms, compliance and health care contact connected to possible side effects
- Mammogram (one image per breast only for density measurement purposes)
- Measurement of weight, length and waist
- Meeting with Investigator (Group meeting)

The women will be informed that the study period now has ended and she will be called to mammography within the national screening program.

7.1.8 In between regular visits at the Karma Study Centre

In addition to the visits at the Karma Study Centre at baseline and 12 months, all participants will have access to web-based questionnaires at 1 and 3 months post baseline. The questionnaires will cover adverse events, change in concomitant medication and compliance.

The participants can at any time in between regular visit at the Karma Study Center contact the Karma Study Centre with questions and issues regarding adverse events, compliance and concomitant medications. The Karma Study Centre will give participants fast access to medical doctors in case of need.

7.1.9 Reminders

Women will receive e-mail/sms reminders regarding their upcoming appointment and/or web-questionnaire to complete.

Women not showing up at regular visits at the Karma Study Centre or fail to submit follow-up questionnaires will be contacted to ensure compliance and to ensure that no adverse events have occurred.

7.2 Dropouts

Participation in the Karisma II study is voluntary and the study participant may end her participation at any time. There may however be a scientific value to continue investigating the effects of tamoxifen after the participant discontinues her treatment to tamoxifen. Women that drop out after 1 week or more, will therefore be asked to still attend the planed follow-up questionnaires, blood sampling, exit-mammogram and biopsy if applicable. This is however not a mandatory request and the study participant is free to decline. The time limit of exit exams is set to 2 weeks after stop of medication.

7.3 The Karma Study Centre

Women participating in the Karisma II study will conduct all study specific activities, except the mammography, at the Karma Study Centre. The Karma Study Centre is located adjacent to the Breast and Mammography Unit at both Skånes Universitetssjukhus Lund and at Södersjukhuset. They were established in 2011 and many of the women invited to the Karisma II study are also part of the Karma Cohort and have thus visited the Karma Study Centre previously.

7.4 Choice of tamoxifen as risk-reducing medication

Tamoxifen is a selective estrogen receptor modulator (SERM), which means that it affects the way estrogen influences the breast tissue. Breast cancer development is closely linked to the exposure of the female breast tissue to female sex hormones, among them estrogen. The hormones increase the rate by which cells divide, proliferate, and thereby increase the risk of a deleterious mutation that could lead to cancer. Tamoxifen blocks the estrogen receptor and thereby estrogens effect on breast tissue.

The underlying mechanism driving tamoxifen to reduce breast density is not currently known, but rather one of the key scientific questions we struggle with right now. We do not know why more than 20% of the fibroglandular tissue “disappears” in approximately half of the woman treated with tamoxifen. It is not likely that the cells go into apoptosis but it could be that the reduced cell proliferation makes cells less radiolucent and therefore not seen on an x ray/mammogram.

Tamoxifen has been used in the treatment of breast cancer for more than 40 years and is generally well tolerated. There are several reasons why we chose to use tamoxifen in the preventive setting:

- The recently published American Association of Clinical Oncology (ASCO) clinical practice guideline on primary prevention of breast cancer, tamoxifen is one out of three suggested compounds and the only one suggested to be used in both pre- and post menopausal women [1].
- Tamoxifen is off patent and cheap (the cost is 10-20 that of aromatase inhibitors), thus is well suited from a health-economics perspective.
- Alternative drugs, e.g. aromatase inhibitors, have side effects such as severe joint pain that influence adherence to therapy.
- Aromatase inhibitors are not able to block the high production of estrogen seen in premenopausal women and are never used for younger women in the clinical setting. Thus aromatase inhibitors are not used for younger, premenopausal women. A woman identified with a very high risk of breast cancer at the age of 40 years should have the

possibility to start risk reducing medication and not have to wait until she enters menopause.

7.5 Choice of tamoxifen doses

In the *pilot study* we used 10 and 20 mg of tamoxifen. We choose the clinically accepted of 20 mg that has so far also been used in the preventive setting. By adding a dose of 10 mg we got an indication of both density response and severity of side effects.

There are currently no data supporting 10 mg of tamoxifen to have fewer and/or lower prevalence of side effects than 20 mg. This coincidence with what we saw in the *pilot study* but numbers were to small to make any significant conclusions. By adding 5 mg, 2.5 mg and 1 mg we believe that we will see a lower prevalence of side effects. The hypothesis that there is a linear dose – side effect relationship in tamoxifen therapy comes from the clinical experience in the early days of tamoxifen use. Initially the preferred dose was 40 mg/day but in the mid 1980s doses were lowered to 20 mg mainly because of the severe side effects of 40 mg. If we believe in a linear dose – side effects relationship, even lower doses should further reduce side effects.

7.6 Design of follow-up scheme

Experience from clinical practice using tamoxifen in the adjuvant setting and from the set up in the *pilot study,* indicates that side effects from tamoxifen typically occur within the first 3 months after initiation of treatment. The Karisma II study will therefore have frequent follow-ups during the first 3 months post baseline, including a follow up questionnaire after 1 and 3 month. Participants will also be instructed to contact Karma Study Center to report AE/side-effects whenever they occur.

7.7 Selection of study population

Eligible women will be identified from the Mammographic screening cohort, i.e. women age 40-74 years invited for screening at Södersjukhuset and University hospital in Lund during the inclusion period. Within this group of women, approximately 1/3 will be part of the Karma Cohort. The Karma Cohort is a prospective observational study including 70,877 women recruited in Stockholm and Region Skåne during 2010-2013. Women were recruited in to the Karma Cohort at screening centers in Lund, Landskrona, Helsingborg and at Södersjukhuset. Approximately 35 000 women were recruited from the Mammography department at Södersjukhuset and 10 000 from Mammography department in Lund. The only exclusion criteria were not being able to read and/or understand the informed consent form written in Swedish. For each participant we have gathered information on lifestyle factors, previous disorders, reproductive history, etc., through a web-based questionnaire. All participants have donated blood and allowed us to store mammograms. All Karma participants have signed an informed consent form allowing us to contact them to ask for participation in future studies.

Only including Karma participants in the study will slow down inclusion. We will therefore invite all women attending screening at Södersjukhuset and University hospital in Lund during the study period.

The rationale to exclude women with the lowest density is based on experience from our previous study of mammographic density reduction in patients on tamoxifen. In that study we had to exclude women with the lowest density since density change was not possible to measure in women with very low density. The cut off for inclusion is set to 4.5% density or more, measured by Volpara. The cut-off value is set to be sure that we will capture a change.

7.8 Inclusion criteria

To participate, the women must meet the following inclusion criteria:

- Attending the national mammography screening program, i.e. aged 40-74 and has performed a mammogram maximum 3 months prior to study inclusion
- Having a measurable mammographic density, i.e. ≥4.5 % density (volumetric) measured by Volpara
- Informed consent must be signed before any study specific assessments have been performed

7.9 Exclusion criteria

To participate, the women must not meet any of the following exclusion criteria:

- Being pregnant or planning to become pregnant during the study period
- Any previous or current diagnosis of breast cancer (including carcinoma in situ)
- Mammographic BI-RADS code 3 or above at baseline mammography, or at a diagnostic mammography during time of treatment (the first 6 months of the study)
- Any previous diagnosis of cancer with the exception of non-melanoma skin cancer and in situ cancer of the cervix
- Currently using *oral* oestrogen and progesterone based hormone replacement therapy
- Current use of hormone contraceptive with hormones, e.g. hormonal contraceptive pills, or progesterone implants. Hormonal intrauterine devices are accepted.
- A history of thrombo-embolic disease such as embolies, deep vein thrombosis, stroke, TIA or cardiac arrest.
- Known APC (Activated protein C)- resistance, an inherited hemostatic disorder
- A history of major surgery of the breast, e.g. reduction or enlargement, which might affect density measurements
- A history of immobilization, e.g. using wheelchair
- Known uncontrolled diabetes
- Hypertension at baseline, defined as systolic pressure higher than 140 mm Hg and diastolic higher than 90 mm Hg
- Use of drugs that interfere with CYP2D6 expression such as Seroxat (paroxetine), Fontex (fluoxetin) and Zyban / Voxra (bupropion)
- Use of Waran (warfarin)
- Non-medical approved drugs against hot-flashes including phytooestrogen
- Not able to understand study information and/or informed consent

7.10 Criteria for withdrawal

The Subject should be excluded from the study if, in the opinion of the Investigator, it is medically necessary, or if it is the expressed wish of the subject. Women are free to discontinue their participation in the study at any time.

Irrespective of the reason for withdrawal and whenever possible, the subject should be encouraged to return for a clinical visit at the time of or soon after discontinuation and complete

all evaluations which may be necessary to assure that the subject is free from any untoward effects and to perform appropriate follow-up for any continuing medical event. Relevant laboratory test samples should be obtained and all relevant assessments should be completed, preferably according to the scheme for the final assessment. The eCRF should be completed as far as possible.

A withdrawn subject is not allowed to re-enter into the study. The criteria for withdrawal are the following:

- A woman develops breast cancer, including ductal carcinoma in situ (DCIS).
- Subject wishes to discontinue
- Unacceptable adverse event/side effect
- Non-compliance with the study protocol
- Subject refuses to cooperate
- Other medical reasons
- Pregnancy

7.11 Treatments

7.11.1 Identity of Investigational Medicinal Product (IMP)

Tamoxifen 1, 2.5, 5, 10 , 20 mg and matching placebo are white to off white, flat, beveled edge uncoated tablets, plain on both sides.

All investigational product will be provided as a tablet for oral administration and provided in blisters.
All investigational product (tamoxifen and matching placebo) will be manufactured according to Good Manufacturing Practice (GMP).

7.11.2 Packaging, labelling and storage of IMP

The flow of the IMP:

1) Producer (Khandelwal Laboratories PVT Ltd)
2) Relabeling of CMO in Sweden (Galenica AB)
3) Release (Clinstorage AB)
4) Storage (Sjukhusapoteket Lund and Sjukhusapoteket Karolinska)
5) Distribution to Karma Study Center

At IMP producer (India):
The producer of the IMP produces the six doses of tamoxifen in different batches. Every dose will be produced during one series i.e. one batch. The tablets are placed in blisters (primary package) then 18 blisters will be put in a box (secondary package). All boxes of the same dose are placed in one of six tertiary packages (a tertiary package per dose). The tertiary packages are than sealed. All the (primary, secondary and tertiary) packaging is labelled with a batch

number in accordance with GMP. Labelling on blister is an adhesive label that can be removed. The six tertiary packages are sent to a CMO in Sweden (Galencia) where the primary and secondary packaging will be relabelled according to a list of randomization.

At CMO (Sweden):
Blister is labeled with:
Study: KarismaII
Sponsor: Karolinska Institutet
Investigator: Per Hall
Randomization No: [1,2, ..., 1200]
Batch No: xxx

Secondary packaging is labelled with complete information including Randomization No. QP of release in Sweden is ClinStorage AB (QP name Ola Camber). When released the IMP will be stored at Sjukhusapoteket Lund respectively Karolinska sjukhusapoteket Solna.

The investigational product (tamoxifen and matching placebo) should be stored in the containers in which they are received from the Sponsor's supplier, preferably at room temperature, but no specific storage conditions are needed.

7.11.3 Doses and treatment regimens

Participants will be randomised into:

- Tamoxifen 20 mg
- Tamoxifen 10 mg
- Tamoxifen 5 mg
- Tamoxifen 2.5 mg
- Tamoxifen 1 mg
- Placebo 0 mg

All treatments will be on a daily basis (1 tablet daily) for 6 months.

7.11.4 Investigational Medicinal Product accountability

The IMP will be dispensed to the study participant at baseline. The number of tablets given out will be recorded in both CRF and in the Product Accountability log in the Study file. We will ask the participant to bring back the container at next visit (after 6 months or by withdrawal/dropout) in order to count remaining tablets. Any unused tamoxifen (i.e. remaining tablets) should be saved for drug accountability and sent for destruction.

7.11.5 Method of assigning participants to treatment groups

Randomisation will be performed by a CMO (Galenica AB). The participants will be randomized in blocks in where the 6 arms are distributed evenly. The study participant emergency code envelopes are kept by a 24/7 service provider (Apoteket AB in Lund) during the whole study. Researchers do not have access to the randomisation list. This means that the study is triple blinded, i.e. neither study participant, Karma Study Centre, nor researchers know which medication product was used for each study participant.

7.11.6 Blinding

The study is double-blinded, placebo controlled randomised study and neither the investigators nor the participants will know who is on active treatment and at what doses (See 7.11.5 for method of assigning participants to treatment groups).

The randomization code (emergency envelopes) are held by Apoteket in Lund. The treatment code can only be broken if an investigator considers there is a valid medical or safety reason to break the blinding. The Investigator must have a reason to break the blinding, for example when the studied drug is likely to have a significant effect on the clinical management of the study participant. When the code is broken for an individual subject, a notification has to be given in the eCRF.

The study is also blinded for the person responsible for Mammographic Density assessments.

7.11.7 Prior and concomitant therapy

Other therapy that is considered necessary for the subject's welfare may be given at the discretion of the Investigator.

- Women using drugs that interfere with CYP2D6 expression such as Seroxat (paroxetine), Fontex (fluoxetin), Zyban / Voxra (bupropion) are not allowed during the study or used at study enrolment.
- Oestrogen and progesterone based hormone replacement therapy (oral), hormone contraceptive with hormones, e.g. hormonal contraceptive pills, or progesterone implants are not allowed during the study or used at study enrolment.
- Waran (warfarin) is not allowed during the study or used at study enrolment
- Non-medical approved drugs against hot-flushes including phytoestrogen are not allowed during the study or used at study enrolment.

All concomitant therapy must be recorded in the eCRF. No other drug under investigation may be used concomitantly with the study medication. Medication will be coded according to WHO's Anatomical Therapeutic Chemical (ATC) classification.

7.11.8 Treatment compliance

Compliance will be assessed throughout the study by *i*) calculating the number of tablets remaining at the final visit at the Karma Study Centre and comparing with the expected usage, *ii*) by web-questionnaires targeting adherence to therapy. The participant is defined as a complier if more than 80% of the tablets are taken.

8 SAFETY ASSESSMENTS

Safety assessment will consist of monitoring and recording Adverse Events (AE) and Serious Adverse Events (SAE). Women in fertile age will be asked to use high efficient birth control and pregnancy tests will be done if the women approve.

8.1 Participant Card

Participants will get a wallet sized Participant card containing essential information about the study and contact information to Karma Study Center (see appendix).

8.2 Birth control for women in fertile age

Premenopausal participants will be asked if they use any of the highly effective birth control methods mentioned below:

1. Hormonal contraception inhibiting ovulation.
    a. Women on using exogenous female sex hormones will be excluded from the study since hormones affects mammographic density.
2. Intrauterine devices
    a. Fully acceptable
3. Intrauterine hormone releasing systems
    a. Fully acceptable since we do not believe that the low systemic hormone levels affects mammographic density. They do not influence the risk of breast cancer.
4. Bilateral tubal exclusion
    a. Fully acceptable
5. Vasectomised partner
    a. Fully accepted
6. Sexual abstinence
    a. In most circumstances not an accepted method since the study continues for 6 months.

Methods #2-5 will be considered acceptable and in some few circumstances #6, depending on the wish of the participant.

9 ADVERSE EVENTS

9.1 Definitions

9.1.1 Adverse Event

Any untoward medical occurrence in a subject, who has received IMP, will be registered. The occurrence does not necessary have to have a causal relationship with the IMP. An AE can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, weather or not considered related to the IMP.

9.1.2 Serious Adverse Event

An SAE is any untoward medical occurrence or event, at any dose, that:

- Results in death
- Is life threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defects.

Or

- Other important medical event (example of such events are convulsions that do not result in hospitalisation)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject's health or may require intervention to prevent one of the other outcomes listed in the definitions above. These AEs should also usually be considered as *serious.*

9.1.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is a suspected unexpected serious adverse reaction, potentially causally related to the investigational medicinal product, and will be reported to the appropriate IEC and Regulatory Authorities. A Serious Adverse Reaction is expected if it is mentioned in the Summary of Product Characteristics (SPC) for Tamoxifen Mylan.

9.2 Reporting of SUSARs

Only SAEs that are both unexpected and assessed as related to IMP(s), i.e. SUSARs, are subject to expedited reporting by the Sponsor to the appropriate IEC and Regulatory Authorities, as per local requirements.

The sponsor must report all SUSARs that resulted in death or was life threatening to the authority and to the IEC within 7 days, and if necessary complete the follow up report within the following 8 days. Other SUSAR should be reported within 15 days.

9.3 Eliciting and recording of AEs

The method for collecting and recording AEs will be through the web-based questionnaire each study participant has to fill in at a regular basis during the study period. Additionally, the participants can at any time report AEs to Karma Staff by contacting Karma Study Centre. All AEs, serious and non-serious, will be recorded in the eCRFs. If no AE has occurred during the study period, this should also be recorded.

9.4 AEs of interest

In the questionnaire (Appendix 14.2) are AE:s of interest listed.

In the questionnaire it's also possible to address not stated adverse events by writing free text. These answers are continuously monitored by Karma staff in order to take immediate action if there are any signs of adverse events of interest or possible serious adverse event. Potential serious adverse events are signs of thromboembolism or gynaecological bleedings.

An alarm system in the e-CRF will alert personnel at the Karma Study Centre when an AE of interest is recorded so evaluation and follow up can be performed throughout the study period.

All AEs of interest will be coded according to Medical Dictionary for Regulatory Activities - MedDRA. The Investigator will perform the coding.

The following evaluations are to be done by the Investigator in connection with the AE of interest:

- Description of the AE. If possible the investigator uses the diagnosis for description of the event. If no diagnosis is decided, each symptom will be described.
- Seriousness

- Yes or No, see definition of a SAE for seriousness, as of above.

- Intensity (if the intensity changes during the period of the AE the maximum intensity should be recorded)

- Mild - symptoms which are easily tolerated (acceptable)
- Moderate - symptoms which interfere with usual activity (disturbing)
- Severe - symptoms which give incapacity to do usual activity (unacceptable)

- Duration of the AE (start - end)
- *Action taken* due to the AE

- None
- IMP stopped
- Additional details on action taken, including diagnostic procedures, will be recorded for any women with vaginal bleeding or discharge. Any women who report abnormal vaginal bleeding or discharge or pelvic pain or pressure, should be promptly investigated. It is not mandatory that the study code is broken in such cases, but this may be done if requested by the treating physician, necessary for further treatment.

- Causality rating with investigational medicinal product

- Not related: Indicates that the AE is definitely not related to the study drug.
- Unlikely: The adverse onset and the medicinal product administration is such that the medicinal product is not likely to have any reasonable association to the AE.
- Possible: The subject´s clinical status or the medicinal product could have produced the AE.
- Probable: The AE follows a reasonable temporal sequence from the time of administration of the medicinal product, abates upon discontinuation of the medicinal product and cannot be reasonably explained by the known characteristics of the subject´s clinical status.
- Not assessable: There is not sufficient information to assess causality between the medicinal product and the suspected reaction.

- Outcome of the AE

- Recovered/Resolved
- Recovering/Resolving
- Not recovered/Not resolved
- Recovered/Resolved with sequelae
- Fatal
- Unknown

9.5 Reporting of SAEs

It is important to distinguish between serious and severe adverse events. Severity is a measure of intensity whereas seriousness is defined by the criteria in the previous sections. An adverse event of severe intensity need not necessarily be considered serious. For example nausea, which persists for several hours, may be considered severe nausea but not a serious adverse event. On the other hand, a stroke resulting only in limited degree in disability may be considered a mild stroke but would be considered a serious adverse event.

All SAEs must be reported by the Investigator using phone or fax within 24 hours from the Investigator's knowledge of the event to the Sponsor regardless of the time that may have elapsed from the time the event occurred to when the Investigator first learns of it. The initial report should contain as a minimum the following information:

- Subject identification
- Treatment specification
- Diagnosis or symptoms
- Name of the original reporter

A SAE report form must also be completed, signed by the Investigator and submitted to the sponsor no later than five working days after the initial information was received. Apart from the information above, this follow-up report should also contain the following information:

- Assessment of intensity
- Assessment of causality

9.5.1 Follow-up of unresolved AEs

If a subject is withdrawn due to an AE, or if an AE persists at the end of the study treatment period, this should be followed up until the condition has ceased or until the subject is under professional medical care and a potential causality between IMP and the AE has been penetrated. An outcome assessment should be performed when an AE persists.

9.5.2 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the study treatment must be stopped immediately, and the subject discontinued from participation in the study. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (e.g. spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the subject was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. All outcomes of pregnancy must be reported to Karma Study Centre.

10 DATA QUALITY ASSURANCE

10.1 Electronic Case Report Forms (eCRFs)

The site personnel will enter the information required for the study in the eCRF. Data will also be imported from web questionnaire, medical records and laboratory findings.

10.2 Monitoring

The Karisma II study will be monitored according to ICH-GCP guidelines, Regulatory requirements and the study specific Monitoring plan. The Karisma II study will be monitored by an independent Monitor according to ICH GCP Guidelines. Study conductance, source data and adherence to the study protocol will be monitored. The Investigator's duty is to inform all personnel concerned about the trial and, if necessary, educate and train all persons participating in the study. This should be checked by the monitor.

10.3 Source data

Source data will be handled according to regulatory requirements. A source data location list will be kept at the investigational site for definition of source data.

10.4 Audit and inspections

The study site may be subject to quality assurance audit by the Sponsor or someone appointed for this task by the Sponsor. A Regulatory Authority may request to make an inspection of the study site. The procedures of such a visit would be similar to those of a monitoring visit, and data already checked by the Monitor may be checked again. The Investigator is required to inform Karisma II study immediately of an inspection requested by a Regulatory Authority. The investigator and other relevant personnel must be available during the audit/inspection and must devote sufficient time.

10.5 Changes in the approved Study Protocol

Study procedures must not be changed without the mutual agreement of the Investigator and Sponsor.

Any substantial change to the approved Final Study Protocol will be documented in a written and numbered Protocol Amendment. Any proposed substantial change to the Final Study Protocol must be discussed with and approved by Sponsor/Investigator before submitted to IEC and Regulatory Authority for approval, according to applicable national regulations.

10.6 Statistical methods and determination of sample size

All analyses will be performed after the study is completed and the database is released. All statistics, including tables, figures and listings, will be performed using SAS®, Version 9.3, and R, Version 3.x.

The statistical analysis are described in detail in the Statistical Analysis Plan (SAP) including a Data Display Plan (DDP), which will be in a final draft version prior to study start and finalised and approved before database lock.

10.6.1 Demographics and baseline data

All data will be presented using descriptive statistics. Results will be presented in total, by treatment group, age and as menopausal status. Continuous variables will be summarised using number of women, mean, standard deviation, median, minimum and maximum. Categorical variables will be summarised using the number and percentage of women.

10.6.2 Analysis of the primary endpoint (effectiveness)

The primary analysis will be performed on an intention to treat (ITT) basis.

The results of both papers [4] and [5] showed that a threshold value in mammographic density decrease needs to be exceeded in order for a woman to experience the beneficial effect of tamoxifen. This threshold value was different in [4] compared to [5]. A 10% or greater reduction was needed in [4] whereas a 20% reduction was needed in [5], a difference most likely explained by different methods of measuring mammographic density. However, the proportion of women exceeding this threshold was exactly 48% in both studies, implying that approximately 50% of the women experience the beneficial effect of tamoxifen irrespective of the way mammographic density is measured.

In the Karma II study, we will use area based mammographic density, which was used in both [4] or [5]. With support of the results in [4] and [5], we define the *response threshold* to be the *median decrease* in mammographic density of women in the 20 mg tamoxifen arm (this definition ensures that the same proportion of women exceeds the threshold as observed in

previous studies). Further, we define a *responder* to be a woman whose mammographic density decrease between baseline and 6 months exceeds the response threshold.

Compliance of study drug intake is defined as at least 80% of the prescribed numbers of tablets should be used during time of treatment (i.e. 6 months).

The primary efficacy endpoint is the *proportion of responders* (women with a reduction of mammographic density, from baseline to 6 months). We will test for non-inferiority with respect to the primary efficacy endpoint for groups of women treated with placebo, 1, 2.5, 5, and 10 mg compared to the group of women treated with 20 mg tamoxifen. The non-inferiority margin is defined to be 16.7 percentage points; that is, we define non-inferiority to mean that the fraction of responders is not less than one third of the treated individuals (50% minus 16.7% = 33.3%).

The null hypothesis is thus that the proportion of responders in women treated with placebo, 1, 2.5, 5, and 10 mg is 16.7 percentage points less than those women treated with 20 mg tamoxifen (i.e. the fraction of responders is less than one third).

We will also perform sensitivity analyses where the threshold is varied ±10 percentage points.

10.6.3 Sample size

With 168 patients in each treatment group, the lower limit of the observed one-sided 98.5% confidence limit for the difference will be expected to exceed the non-inferiority margin 16.7 percentage points with a 80% power, assuming that the expected difference in responders between two arms is 0 and the proportion responders in the 20 mg tamoxifen group is 50%. With an estimated 30% drop-out rate, 240 patients in each group need to be randomized. The computations are based on the following formula:

$$n = f(\alpha, \beta) \times [\pi_s \times (100 - \pi_s) + \pi_e \times (100 - \pi_e)] / (\pi_s - \pi_e - d)^2$$

where π_s and π_e are the true percent 'success' in the standard (20mg) and experimental treatment group respectively, and

$$f(\alpha, \beta) = [\Phi^{-1}(\alpha) + \Phi^{-1}(\beta)]^2$$

Φ^{-1} is the cumulative distribution function of a standardised normal deviate.

10.6.4 Analysis of the secondary outcome

We will assess the proportion of drop-outs at six months and test for differences in the proportion of drop-outs in the intervention arms compared with the 20 mg arm. We will use Fisher's exact test with an p-value of less than 0.05 (corrected for multiple testing, see SAP Section 6.7) as a threshold to declare statistical significance.

10.6.5 Analysis of tertiary outcomes

Tertiary outcomes will be analysed with statistical methods appropriate for the specific outcome. For example, the association of plasma concentrations of endoxifen with responder status will be analysed with logistic regression both within treatment groups and among treatments groups (treatment group will be handeled as a random effect in the latter case). Full details of the analysis of tertiary outcomes will be in the statistical analysis plan (SAP) prior to database release.

10.6.6 Examination of subgroups

We will perform subgroup analyses, where the subgroups are CYP2D6 polymorphism status

(excellent, intermediate, poor metaboliser). CYP2D6 polymorphism status will be handled as a categorical fixed effect variable.

10.6.7 Interim analysis

We will three months into the study perform an interim analysis on overall dropout level. If the proportion of drop out at three months, we will – at best – have 73% power for our primary analysis (see SAP Section 6.6). In this case, we will increase the number of women the study needs to accrue according to:

$n_{tot} = 168 \times 6/(1-d)$,

where d is the overall proportion of drop outs at three months.

10.7 Data Management

A quality control (QC) of data will be performed to ensure that data entry and verification have been performed correctly in accordance to pre-defined instructions. The QC will be performed as described in the Data Management Plan (DMP). The QC will be performed before data is declared clean.

11 STUDY MANAGEMENT

11.1 Study time table - End of study

The study is planned in two steps geographically (see 5.4). The first inclusion phase will start in November 2016 in Lund. Last participant in Lund will end medication in May 2017 and will do the final examinations in November 2017. In the overlapping second phase we will start inclusion at Södersjukhuset, Stockholm, by November 2016 and end the study by May 2018.

11.2 Insurance/indemnity

All participants are covered from accidents in connection with the trial by the Patient Injury Act insurance (Patientförsäkringslagen) and from injuries caused by study drug by the Swedish Pharmaceutical Drug Insurance.

The Sponsor agrees to indemnify (legal and financial coverage) and hold the Investigator free of harm from any claim, whether based on legal principles or on generally accepted liability standards within the pharmaceutical industry, made against him by reason of personal injury, including death, to any person arising out of or connected with the performance of the study to the extent that the injury is not caused by:

1) Failure by the Investigator to adhere to the terms of the Protocol;
2) Failure by the Investigator to comply with any applicable governmental regulations;
3) Malpractice, negligence or wilful malfeasance by the Investigator.

The Investigator agrees to notify the Karisma study personnel whenever he/she becomes aware of a claim or action, and to co-operate with and to authorise Karisma study personnel to carry out sole management of such claim or action.

The Sponsor's responsibility is covered by LIF Läkemedelsförsäkring via an association agreement with Kammarkollegiet. The insurance covers Karisma II liability under law and

generally accepted liability standards within the pharmaceutical industry towards any third parties, including participating women, as Sponsor of the study.

11.3 Study agreements

The Principal Investigator at the investigational site must comply with all the terms, conditions, and obligations of the Clinical Study Agreement (CTA) for this study.

11.4 Criteria for termination of the study

The Karisma II study personnel reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must contact all participating women within 2 weeks and inform the women as well as perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused study products and other study materials must be returned and all eCRFs completed as far as possible.

11.5 Reporting of results and publication policy

A clinical study report, in compliance with ICH E3; Guideline for Industry, will be prepared by the Karisma study personnel , describing the conduct of the study, the statistical analysis performed and the obtained results.

The data generated by this study are considered confidential information and the property of the Karisma Study. Said confidential information may be published only in collaboration with the Karisma study leadership. The Karisma study leadership reserves the right to review and comment on the proposed publication prior to being submitted and/or published.

11.6 Record retention

The Investigator must arrange for retention at the investigational site of a list of the women and their identifying code, subject files and other study documents. The archiving period must be adapted to regulations in force but should not be shorter than ten years after the termination of the study and the presentation of the final report.

It is the responsibility of the Karisma study personnel to inform the Investigator/institution as to when these documents no longer need to be retained

11.7 Disclosure and confidentiality

All unpublished information concerning the test product and research carried out within the study, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and the sole property of Karisma. Disclosure to third parties must be limited to those undertaking legitimate peer reviews of the scientific and ethical aspects of the study and to those participating, including the recipients of drugs, so that customary medical care and informed consent can be achieved.

12 EMERGENCY PROCEDURES

12.1 Emergency contacts

In case of a medical emergency, contact Karisma study personnel:

- Per Hall, Responsible Medical Officer, Coordinating and Principal Investigator

Mobile +46733960590, email: per.hall@ki.se

- Signe Borgqusit, Principal Investigator, Lund

Mobile +46704452558, email: Signe.Borgquist@med.lu.se

- Mattias Hammarström, Clinical Trial Manager

Mobile +46737121476, email: Mattias.Hammarstrom@ki.se

12.2 Emergency unblinding for safety reason

The randomization code (emergency envelopes) are held by Apoteket in Lund. In case of a rare emergency where, in the opinion of the Investigator, discontinuation of the study treatment is not sufficient and the study treatment must be unblinded in order to evaluate further course of action, the Investigator should contact Apoteket Lund (Phone numbers and routines are to be found in the Study Site File) to initiate patient unblinding. In the event the blind is broken, the sponsor should be informed as soon as possible. The date, time, and reason for unblinding must be documented in the appropriate section of the eCRF, and in the source document.

Information about the treatment assignment must be restricted to designated study site staff who are providing immediate care to the patient. Any documentation of the treatment assignment must be maintained separately (i.e. in a secured file). The information must not be included in the patient's source files to ensure the treatment assignment will remain blinded to the monitor and other study personnel not involved with the patient's immediate care.

12.3 Procedures in case of medical emergency

The Investigator is responsible for ensuring that there are procedures and expertise available to cope with medical emergencies during the study. The study is performed at the hospitals Södersjukhuset and Skånes Universitetssjukhus Lund, and any acute medical illness should be dealt with at the hospital.

12.4 Procedure in case of overdose

Overdosing tamoxifen may on theoretical grounds increase the pharmacological side effects of the drug. Any study participant with a suspected or confirmed overdose of tamoxifen should contact the Swedish Poison Information Center.

13 REFERENCES

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14 APPENDIXES

14.1 Signature pages

14.2 Questionnaire

14.3 Schedule of events

14.4 Additional translational protocol: Biopsy Karisma II

14.5 Participant Card

Appendix 14.1; Signature pages

Sponsor, Coordinating Investigator and Principal Investigator

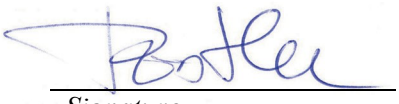
Study title:

Karisma II: A randomized, double blinded, six-armed placebo controlled study to investigate optimal dose of tamoxifen with the most favourable side effect spectra and with mammographic density reduction non-inferior to that of 20 mg tamoxifen.

By signing this page I acknowledge that I have read and understood the study protocol "Karisma II: A randomized, double blinded, six-armed placebo controlled study to investigate optimal dose of tamoxifen with the most favourable side effect spectra and with mammographic density reduction non-inferior to that of 20 mg tamoxifen.".

I also agree by signing this page that I will conduct the study in accordance with the procedures specified in the protocol, the ethical principles in the latest version of the Declaration of Helsinki, ICH Good Clinical Practice and applicable regulatory requirements.

Sponsor, Coordinating Investigator and Principal Investigator, Södersjukhuset

Per Hall		2017-01-12
Name	*Signature*	*Date*

Principal Investigator

Study title:

Karisma II: A randomized, double blinded, six-armed placebo controlled study to investigate optimal dose of tamoxifen with the most favourable side effect spectra and with mammographic density reduction non-inferior to that of 20 mg tamoxifen.

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I also agree by signing this page that I will conduct the study in accordance with the procedures specified in the protocol, the ethical principles in the latest version of the Declaration of Helsinki, ICH Good Clinical Practice and applicable regulatory requirements.

Principal Investigator, Lund

Signe Borgquist		2017-01-12
name	*Signature*	*Date*

Appendix 14.2; Questionnaires

Questionnaires sent to MPA and IRB are in Swedish and therefore removed from Clinical Trial version of protocol

Appendix 14.3

Schedule of events Karisma II

Events	Mx Screening	Baseline						End of treatment						End of study
Month		0	1	2	3	4	5	6	7	8	9	10	11	12
Selection of eligible study participants and invitation	✔													
Mammography within screeningprogram	✔													
Mammography for density measure								✔						✔
Visit at Karma Study centre		✔						✔						✔
Screening for inclusion (inclusion and exclusion criterias checked)		✔												
Informed consent signed		✔												
Blood pressure		✔												
Randomization		✔												
Blood sampling		✔						✔						
Biometrics; Length,weight, waist		✔						✔						✔
Pregnancy test (if applicable)		✔												
Biopsy (optional)		✔						✔						
Interview (optional)		✔						✔						✔
IMP administration		✔												
Drug acountability check								✔						
Karma Baseline questionnaire		✔												
Karisma Baseline questionnaire		✔												
Follow up questionnaire (sideeffects, compliance, concomitant medication)			✔		✔			✔						✔
AE-reporting (spontanously)		day2	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Check screen mx for exclusion (code 3,4 or 5)		day2												

Appendix 15.4

Additional translational study Protocol: Biopsy Karisma II

1. Objective

Relate changes in breast tissue to different tamoxifen doses and placebo

2. Endpoint

Breast tissue changes between baseline and 6 months of tamoxifen treatment

3. Rational

The molecular basis for the tamoxifen-induced changes in breast density is sparsely understood. Consequently, a secondary aim in the Karisma trial is to investigate changes in tissue markers by comparing breast tissue biopsies sampled prior to and after six months of treatment with different doses of tamoxifen or placebo.

Mammographic density is the white part of the mammogram and consist of glandular and connective tissue. The black, non-radiolucent part is fat. The more glandular and connective tissues, the whiter the breast, and thus; the higher the risk of breast cancer [1]. Factors that influence risk of breast cancer are known to influence density. Hormone replacement therapy increases density and risk of breast cancer, while tamoxifen has the opposite effect [2].

4. Design

Donation of breast biopsies is optional and not a criteria to participate in Karisma II. All Karisma II participants will be invited to donate a biopsy upon study entry. Based on the recruitment frequency of a previous study with breast biopsies conducted within the Karma project, we anticipate that approximately 10% of the Karisma II participants will donate biopsies. Breast biopsies will be collected at study baseline and at month 6 (end of treatment).

5. Investigational plan

5.1. Procedure at baseline

5.1.1. Women have to meet inclusion and not fulfil exclusion criteria of the Karisma II study. Women are informed about the study and accompanying procedures by the investigator. If consenting women are invited for breast biopsy.

5.1.2. Only participants with a negative mammogram, defined as BI-RADS score 1 or 2, at baseline are excluded from the study.

5.1.3. The responsible mammography physician performs an ultrasound-led breast biopsy, guided by the mammogram, in the densest part of the left breast under local anaesthesia.

5.1.4. Four core needle biopsies will be extracted from one single incision site. Two cores will be formalin-fixed and paraffin embedded (FFPE) and two cores will be freshly frozen. All four biopsies will be biobanked.

5.1.4.1. Biopsy #1 is the “clinical” FFPE biopsy

5.1.4.2. Biopsy #2 is the “research” FFPE biopsy

5.1.4.2.1. The biopsies for FFPE will be sent to Histocenter, Gothenburg, for preparation. HE-stained slides will be sent to the Department of Pathology, Skåne University Hospital, Lund or the South General Hospital, Stockholm, for clinical evaluation. In case of pathological histopathology, participants will be referred for routine clinical work up by the responsible Karisma physician. Diagnostic HE-stained slides will be stored at the relevant Department of Pathology, whereas remaining biopsies will be stored at the Karolinska Institutet Biobank.

5.1.4.2.2. Biopsy #3 and #4 are the fresh frozen biopsies for research. Biopsies will be stored at the Karolinska Institutet Biobank.

5.1.5. For Biopsy procedure details see *SOP Biopsy Karisma II.*

5.2. Procedure at month 6 (end of treatments)

Procedure according to 5.1.3 to 5.1.4.3.

The procedure at 6 month will follow the schedule of Karisma II and the mammography will not be part of the ordinary screening program.

6. Possible side effects

Breast biopsy is a routine procedure frequently used in clinical practice. The physicians performing the biopsies are all experienced medical professionals. Furthermore, the Karisma II participants are healthy women. The risk of side effects is thus relatively low. The most commonly associated side effects are tenderness at the incision site, contusion and bleeding. Clinical routines apply to deal with these issues.

7. References

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Appendix 15.5

Participant Card (in Swedish, therefore removed from Clinical Trial version)