STATISTICAL ANALYSIS PLAN for EBBA-II (Energy and Breast Cancer Aspects)

Administrative information:

Sponsor name	Oslo University Hospital
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SIGNATURE PAGE

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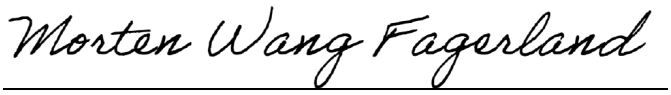

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ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CI	Confidence Interval
CLS	Crown Like Structures
CRP	C-Reactive Protein
DLco	Diffusing Capacity of the Lung for Carbon Monoxide
FEV1	Forced Expiratory Volume in 1 second
HDL	High Density Lipoprotein
HR	Hazard Ratio
IGF	Insulin-like Growth Factor
IGFBP	Insulin-like Growth Factor Binding Protein
LDL	Low Density Lipoprotein
MetS	Metabolic Syndrome
OR	Odds Ratio
PEF	Peak Expiratory Flow
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
TILS	Tumor Infiltrating Lymphocytes
WHR	Waist to Hip Ratio

TABLE OF CONTENTS

1 Introduction

1.1 Background and rationale

Physical activity may affect several specific biological processes involved in the primary and secondary occurrence of breast cancer and general health. Many studies have observed that women who are physical active both before and after diagnosis have a greater chance of surviving breast cancer. However, other factors may explain this link. The purpose of this study, the EBBA-II trial, is to determine whether a 12-month exercise program comprised of endurance and strength training among newly diagnosed breast cancer patients undergoing adjuvant therapy, are safe and feasible during adjuvant breast cancer treatment, and will influence biological mechanisms that play a role in breast cancer development. Factors associated with cardiopulmonary function, metabolic profile, hormones, prothrombotic markers and growth factors will be studied. Secondary aims are to determine whether the 12-month exercise program will influence disease-free survival, overall mortality and breast cancer specific mortality. Furthermore, whether this 12-month exercise program will influence metabolites, gut microbiota, dietary factors and quality of life, will be assessed and evaluated. If such a trial demonstrates that physical activity during adjuvant breast cancer treatment improve physical function and metabolic profile, physical activity should be prescribed as an integral part of breast cancer therapy.

1.2 Trial Objectives

1.2.1 Primary Objective

The primary objective of this trial is to determine whether a 12-month exercise program comprised of strength and endurance training among newly diagnosed breast cancer patients undergoing adjuvant therapy is superior to standard care regime, regarding cardiopulmonary function.

1.2.2 Secondary Objectives

The secondary objectives of this trial are to determine whether the 12-month exercise program, as compared with standard care regime, improves:

- Metabolic profile (lipids, lipoproteins, glucose, blood pressure, body composition, insulin)
- Forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF), diffusing capacity of the lung for carbon monoxide (DLco)
- inflammatory markers, prothrombotic markers, urinary markers, adipokines
- Levels of sex steroid hormones
- Disease-free survival, overall mortality, and breast cancer-specific mortality
- Growth factors (Insulin-like growth factor [IGF], insulin, insulin-like growth factor binding protein [IGFBP])
- Microenvironment of the breast, tumor infiltrating lymphocytes (TILS), Crown like structures (CLS)
- Microbiota

1.2.3 Exploratory Objectives (if applicable)

The exploratory objective of this trial is

- To compare quality of life and dietary factors between patients randomized to the 12-month exercise program and patients who follow standard care regime

2 Trial Methods

2.1 Trial Design

The EBBA-II study is designed as a randomized, assessor blinded, controlled, parallel-group, multi-center, single-country, superiority study. Treatment allocation is a 1:1 ratio. Patients are randomized to either a 12-month exercise program or standard care regime, without any restriction regarding physical activity in the control group.

2.2 Randomization

Eligible patients are allocated in a 1:1 ratio between a 12-month exercise program and standard care regime, using a computer randomization procedure stratified by pre- vs postmenopausal status. Block randomization is used with block sizes 2, 4, and 10, in random order.

2.3 Sample size

The original sample size calculation was based on change in metabolic profile, for the variables BMI, HDL cholesterol, and total cholesterol/HDL cholesterol. Clinically relevant differences in changes from baseline to 12 months between the groups were decided to be 0.8 kg/m^2 (SD 3.2 kg/m^2) for BMI, 0.09 mmol/l (SD 0.42 mmol/l) for HDL cholesterol, and 0.31 (SD 1.03) for total cholesterol/HDL cholesterol[1]. The numbers were supported by previous randomized trials in postmenopausal women, which observed weigh differences of 1.4 kg between intervention and control groups[2], and a difference in the level of HDL cholesterol of 1.64 vs 1.20 mmol/l [3]. A sample size in the range of 175-350 patients in each group was found to be sufficient to detect these differences with 80% power, using a two-sample T test. The target sample size was set to 300 in each group, a total of 600 patients.

In the original registration on clinicaltrials.gov (submitted Sept. 12, 2014), the primary outcome measures included cardiopulmonary function assessed by CPET, VO_{2max} , a measure of cardiopulmonary function. However, this was changed Sept 23, 2014 to include only metabolic profile. During the course of the study, however, VO_{2max} , emerged as the variable of primary interest. A sample size/power calculation was performed with change in VO_{2max} as the primary outcome measure to ensure that the study was adequately powered for this outcome (see below). This change in the definition of the primary outcome measure was subsequently made to the study's record on clinicaltrials.gov.

The amendment to clincaltrials.gov and the writing and signing off of the SAP occurred before database lock and before last patient last visit.

For the primary outcome measure of change in VO_{2max}, we assume (based on the results presented in ref #4) a baseline value of VO_{2max} = 32 in both groups, no change in the exercise group, a decrease in the control group of -0.5, and a standard deviation of the change of 2.0 in both groups. With 300 patients in each group, the study has 86% power to detect a difference of this size or larger, with a two-sample T test. To reach 80% power, at least 253 patients in each group have to be included.

2.4 Statistical Framework

2.4.1 Hypothesis Test

This trial is designed to establish superiority of a 12-month exercise program compared to standard care regime with regard to cardiopulmonary function in newly diagnosed breast cancer patients undergoing adjuvant therapy.

- The primary null hypothesis is that the change in VO_{2max} from baseline to 12 months is the same for patients in the exercise group and patients in the standard care group
- The alternative hypothesis is that the positive change in VO_{2max} from baseline to 12 months is greater in either the exercise group or the standard care group (two-sided hypothesis testing)

There is only one identified primary analysis in this trial. All other efficacy analyses will be regarded as supportive or exploratory.

2.4.2 Decision Rule

This trial is designed to address a single primary outcome. Superiority is claimed if the primary null hypothesis is rejected on the significance level (alpha) of 0.05 (two-sided). That is, if the 95% two-sided confidence interval for the between-group difference in changes in VO_{2max} from baseline to 12 months does not contain 0.

2.5 Statistical Interim Analyses and Stopping Guidance

There will be no interim analyses in this trial.

2.6 Timing of Final Analysis

The main analysis is planned when all patients have completed the 12 months (from baseline) assessments, all data up to 12 months have been entered, verified and validated, and the primary database has been locked.

2.7 Timing of Outcome Assessments

Outcome assessment occurred at baseline (V1, prior to surgery and randomization), 6 months after randomization (V2), and 12 months after randomization (V3).

Visit Label	Target Day	Definition (Day window)
(Screening)	1 day before consent & baseline	
V1 (Consent & Baseline)	2-8 days before operation	
(Surgery)	2-3 weeks before randomization	
(Randomization)	0	Day 0
V2 (6 months)	182	Target day ± 14 days
V3 (12 months)	365	Target day ± 14 days

Additional outcome assessments of patients are planned at 2, 3, 5, and 10 years after randomization.

3 Statistical Principles

3.1 Confidence Intervals and p-values

All calculated p-values will be two-sided and compared to a 5% significance level. If a p-value is less than 0.05, the corresponding treatment group difference will be denoted as statistically significant. All efficacy estimates will be presented with two-sided 95% confidence intervals. As there is only one primary null hypothesis to be tested in this trial, there will be no adjustments for multiplicity.

3.2 Adherence and Protocol Deviations

3.2.1 Adherence to Allocated Treatment

Adherence to the exercise program is defined as the number of weeks each patient participated in the group exercise divided by the total number of weeks with organized group exercise (40 weeks). For instance, a patient that participated in 34 weeks of organized training has an adherence of 34/40 = 85%.

The mean (SD) adherence in the exercise group will be reported, along with the number and % of patients with adherence less than 40%, between 40% and 60%, and more than 60%. The patients that are included in the full analysis data set (see section 3.3) will be used as the denominator to calculate the percentages.

3.2.2 Protocol Deviations

Major protocol deviations:

- Entering the trial when the eligibility criteria should have prevented trial entry
- Adherence less than 40%

Minor protocol deviations:

- Adherence between 40% and 60%

The number (and percentage) of patients with major and minor protocol deviations will be summarized with details of type of deviation provided. The patients that are included in the full analysis data set (see section 3.3) will be used as the denominator to calculate the percentages.

3.3 Analysis Populations

The Enrolled set will include all patients who have provided informed consent and have been included into the study data base.

The Full Analysis Set (FAS) will be defined as all patients randomly assigned to a treatment group and having completed at least one post-baseline outcome assessment.

The Per Protocol Analysis Set (PPS) will include all randomized patients meeting the study eligibility criteria and with no major or minor protocol deviations affecting the treatment efficacy.

4 Trial Population

4.1 Screening Data, Eligibility and Recruitment

The total number of screened patients and reasons for not entering the trial will be summarized and tabulated.

A CONSORT flow diagram will be used to summarize the number of patients who were:

- Eligible at screening and invited
- Measured at baseline
- Ineligible after operation*
- Randomized
- Allocated to control
- Allocated to exercise
- Lost to follow-up (by treatment group)*
- Included in the primary analysis (by treatment group)

*reasons will be provided.

4.2 Withdrawal/Follow-up

4.3 Baseline Patient Characteristics

Patient demographics and baseline characteristics will be summarized by randomized treatment arm and overall using descriptive statistics (N, mean, standard deviation, median, 25/75 percentiles, minimum, and maximum) for continuous variables, and number and percentages of patients for categorical variables. There will be no statistical analysis of treatment difference. Any clinical important imbalance between the treatment groups will be noted.

The patient demographics and baseline characteristics to be summarized include:

- The patient demographics and baseline characteristics to be summarized include age in years, ethnicity, education, birth weight, age at menarche, menopausal status, parity, age at first birth, breast feeding, blood pressure, heart rate, Body composition (height, weight, waist circumference, waist to hip ratio, total fat, truncal fat), hormone use, smoking habit, energy and fat intake, alcohol intake, Cardiopulmonary function (VO2 max) Forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF), diffusing capacity of the lung for carbon monoxide (DLco),
- serum levels of lipids, glucose, HbA1c, glucose, hormones, inflammatory markers, prothrombotic markers, adipokines
- Growth factors (Insulin-like growth factor [IGF], insulin, insulin-like growth factor binding protein [IGFBP])
- Microbiota (fecal samples)
- Urinary markers

5 Analysis

5.1 Outcome Definitions

5.1.1 General Definitions and Derived Variables

5.1.1.1 Body Mass Index

Body Mass Index (BMI) = Body weight in kilograms (kg) divided by the square of the height in meters.

5.1.1.2 Waist to Hip Ratio

Waist to Hip Ratio (WHR) = Waist circumference in centimeters divided by hip circumference in centimeters.

5.1.1.3 VO_{2max}

Aerobe capacity (VO_{2max}) will be calculated relative to body weight with the following formula:

VO_{2max} in mL/kg/min = (absolute VO_{2max} in L/min * 1000)/body weight in kg

5.1.1.4 Metabolic Syndrome

Metabolic Syndrome (MetS) is defined using the ATP III criteria[4] as having at least 3 of the 5 listed characteristics:

- Waist circumference > 88 cm
- Triglycerides ≥ 150 mg/dL or currently receiving drug treatment for an elevated triglyceride level
- HDL cholesterol < 50 mg/dL
- Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or currently receiving antihypertensive drug treatment
- Fasting glucose ≥ 100 mg/dL or currently receiving drug treatment for elevated glucose

5.1.2 Primary Outcome Definition

The primary outcome is the change in VO_{2max} from baseline to 12 months. The primary outcome is continuous.

5.1.3 Secondary Outcomes Definitions

5.1.3.1 Continuous outcomes

The following secondary outcomes are continuous and are defined as the change from baseline to 12 months: BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol/HDL cholesterol, glucose, Hb1Ac, insulin, triglycerides, CRP, heart rate (HR), total fat, truncated fat, waist circumference, energy from food diary.

The following continuous outcomes will be presented in a supplementary document: ratio between the amount of CO_2 produced in metabolism and O_2 used (RQ=respiratory quotient), blood lactate from fingertip one minute after test (lactate), maximal heart rate (HR_{max}), subjective measure of exhaustion ($BORG_{max}$).

5.1.3.2 Dichotomous outcomes

The following secondary outcomes are dichotomous and are defined at the 12-month assessment: Metabolic Syndrome.

5.1.3.3 Time to event outcomes

Disease-free survival is defined as the time from baseline to the date of cancer symptoms or death. Patients who survive the duration of the trial without cancer symptoms will be censored at the patient's last visit.

Time to overall mortality is defined as the time from baseline until the date of death, regardless of the cause of death. Patients who survive the duration of the trial will be censored at the patient's last visit. This is a time to event outcome.

Time to breast cancer-specific mortality is defined as the time from baseline until the date of breast cancer-specific death. Patients who survive the duration of the trial and patients who die of other causes will be censored at the patient's last visit. This is a time to event outcome.

5.2 Analysis Methods

5.2.1 Primary Outcome

The primary outcome of change in VO_{2max} from baseline to 12 months will be analyzed with a linear mixed model. The model will be fitted to all the measurements of VO_{2max} (baseline, 6 months, and 12 months) and will contain fixed effects for treatment, time, treatment x time interaction, and pre-/postmenopause status (the stratification factor in the randomization), and a random intercept. Time will be modeled as piecewise linear with a knot at 6 months.

Based on the fitted model, the change in VO_{2max} from baseline to 12 months with a 95% confidence interval (CI) will be estimated for each treatment group. The primary outcome measure will be the

between-group difference in change from baseline to 12 months, which will be estimated with a 95% CI and a test for the null hypothesis of no difference.

The primary analysis will be performed on the full analysis set (modified intention to treat analysis). A secondary analysis will be performed on the per protocol analysis set.

The linear mixed models will include all patients with at least one post-baseline measurement, thus complying with the requirement for analysis on the full analysis set without the need to impute missing values.

The linear mixed models will be checked for fit by comparing observed and model-predicted values in plots of VO_{2max} on the y-axis and time on the x-axis.

5.2.2 Subgroup Analysis of the Primary Outcome

The primary outcome will be analyzed in the following subgroups: chemotherapy (yes/no), pre-/postmenopause status, taxane use (yes/no), trastuzumab use (yes/no), left sided radiation (yes/no), chemotherapy and left sided radiation (yes/no), above 55 years of age (yes/no), above 60 years of age (yes/no), and above 65 years of age (yes/no).

The subgroup analysis will be performed by including FACTOR (the subgroup-defining variable) and treatment x FACTOR interaction terms as fixed effects in the linear mixed model, and the resulting treatment effect by FACTOR will be presented using a forest plot.

5.2.3 Continuous Secondary Outcomes

The continuous secondary outcomes will be analyzed with linear mixed models, using the same model setup as the primary outcome. The analyses will be performed on the full analysis set (modified intention to treat analyses).

5.2.4 Dichotomous Secondary Outcomes

The dichotomous secondary outcomes will be analyzed with logistic regression, where the 12-month assessment is the dependent variable, and treatment and pre-/postmenopause status (the stratification factor in the randomization) are the explanatory (independent) variables. Model fit will be tested with the Hosmer-Lemeshow goodness-of-fit test. The estimated odds ratio (OR) for treatment with a 95% CI will be reported, together with a test for the null hypothesis of a zero OR. The results for pre-/postmenopause status will also be reported.

5.2.5 Time to Event Secondary Outcomes

The time to event secondary outcomes of disease-free survival, overall mortality, and breast cancer-specific mortality will be analyzed with Cox regression models with treatment and pre-/post-menopause status (the stratification factor in the randomization) as explanatory variables. The proportional hazard assumption will be checked with a test based on Schoenfeld residuals. The

estimated Hazard Ratio (HR) for treatment with a 95% CI will be reported, together with a test for the null hypothesis of a zero HR. The results for pre-/postmenopause status will also be reported.

The Kaplan-Meier survivor function will be plotted for each of the two treatment groups, and the log-rank test for the null hypothesis of equal survivor functions will be reported.

We expect few events to occur during the first 12 months of follow-up. Thus, for the reporting of the results of the trial after the first 12 months only (the primary publication), the time to event outcomes will only be analyzed as described above if there are more than 15 events. If there are 15 or less events, descriptive statistics (number and % per treatment group) will be used to present these outcomes.

6 Adverse Events

Adverse events (AE) and serious adverse events (SAE) will be tabulated (with number and %) by type and treatment group. No specific AEs and no SAE are expected.

No analysis of AEs and SAEs will be performed.

7 Statistical Software

All statistical analyses will be done in Stata version 15 (StataCorp LLC*,* College Station, TX, USA).

8 References

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