SPRINT- feasibility trial

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PROTOCOL TITLE '**S**upervised exercise to **PR**omote **I**nfiltration of **N**K-cells into the **T**umor? – a feasibility trial.' (**SPRINT**-feasibility trial)

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Observational studies showed that higher levels of physical activity compared to lower levels are associated with a 38% reduction in risk of breast cancer-specific mortality [1]. However, the causality and underlying mechanisms have not been elucidated. Recent studies in rodents have shown that exercise can directly affect cancer outcomes. Exercise training (voluntary wheel running) in mice results in an increase in intratumoral Natural Killer (NK) and T-cells, which contributed to a 50-60% reduction in tumor growth [2]. Further analyses showed that exercise induced an increase in epinephrine which resulted in mobilization of NK-cells into the circulation which were then activated and redistributed to the tumor as a result of the production of IL-6 released by contracting muscles [2].
It is unclear whether a physical exercise program that has been proven feasible and effective for improving physical fitness, fatigue, quality of life and chemotherapy completion in patients with breast cancer receiving (neo)adjuvant chemotherapy [3-7] can yield similar results on tumor responses in patients. A randomized controlled trial (RCT) in patients with breast cancer receiving neoadjuvant chemotherapy is needed to formally assess whether physical exercise would improve antitumor responses and patient outcome. Such a study would provide an opportunity to directly assess the effects of exercise on the tumor in situ. However, the latter requires an additional tumor biopsy which might be a great barrier for patients to participate in such a study. Therefore, a pilot study is needed to determine the feasibility of conducting a sufficiently powered RCT examining the direct effect of exercise on the tumor.
Objective: To 1) ***study trial feasibility*** in terms of patient enrollment and the percentage of tumor biopsies that can be examined successfully, and 2) ***generate preliminary data*** on the potential effects of exercise on immune function assessed in the tumor and in blood.
Study design: Multicenter randomised controlled feasibility trial
Study population: 20 adult patients who are scheduled for neoadjuvant chemotherapy consisting of 4 cycles of 2 or 3-weekly adriamycin/cyclofosfamide (AC), followed by 12 weekly cycles of paclitaxel (T) weekly +/- trastuzumab, for early (stage I-III) breast cancer.
Intervention: 6-week (during 2 or 3 treatment cycles) moderate to high intensity aerobic and resistance exercise program supervised by a specifically educated physical therapist.
Main study parameters/endpoints: Trial feasibility and preliminary effects on immune function. Measurements will take place at baseline (prior to chemotherapy) and after 2 or 3 cycles (6 weeks).
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Prior to start of treatment, a diagnostic biopsy (part of routine care), a blood sample and a fitness test will be performed in all participants. At this time point the

blood sample collection and fitness test will be combined in one visit. After two (3-weekly) or three (2-weekly) cycles of AC chemotherapy an additional biopsy and blood sample will be taken and patients will perform a fitness test. The risks of the blood drawing and fitness test are negligible. The risk of clinically relevant complications of a breast tumor biopsy was around 0,2% in a multi-institutional study in 1994 [8]. Nowadays, the risk of complications is even lower, due to technological improvements that are implemented. The biopsies are necessary to study the effect of an exercise intervention on tumor and infiltrating immune cells. The safety of the exercise intervention has well been documented [9].

1. INTRODUCTION AND RATIONALE

Evidence from randomized controlled trials, including our own, shows that exercise during cancer treatment benefits physical fitness, fatigue and quality of life [4, 5]. Additionally, women with breast cancer who exercised during chemotherapy appeared to have fewer dose adjustments [3, 7]. However, the effect of exercise on tumor growth is largely unknown, which limits implementation in clinical practice [10].
Observational studies showed that higher levels of physical activity compared to lower levels are associated with a 38% reduction in risk of breast cancer-specific mortality[1]. However, the causality and underlying mechanisms have not been elucidated.

Recent studies in rodents have shown that exercise can directly affect cancer outcomes. Exercise training (voluntary wheel running) in mice results in an increase in intratumoral NK and T-cells, which contributed to a 50-60% reduction in tumor growth [2] . Further analyses showed that exercise induced an increase in epinephrine which resulted in mobilization of NK-cells into the circulation which were then activated and redistributed to the tumor as a result of the production of IL-6 released by contracting muscles [2]. Importantly, studies in healthy people showed that epinephrine also results in NK-cell mobilization into the circulation within minutes of initiating exercise [11]. Whether this mechanism of action (Figure 1) of exercise can also increase NK-cell infiltration into the human tumor is unknown.

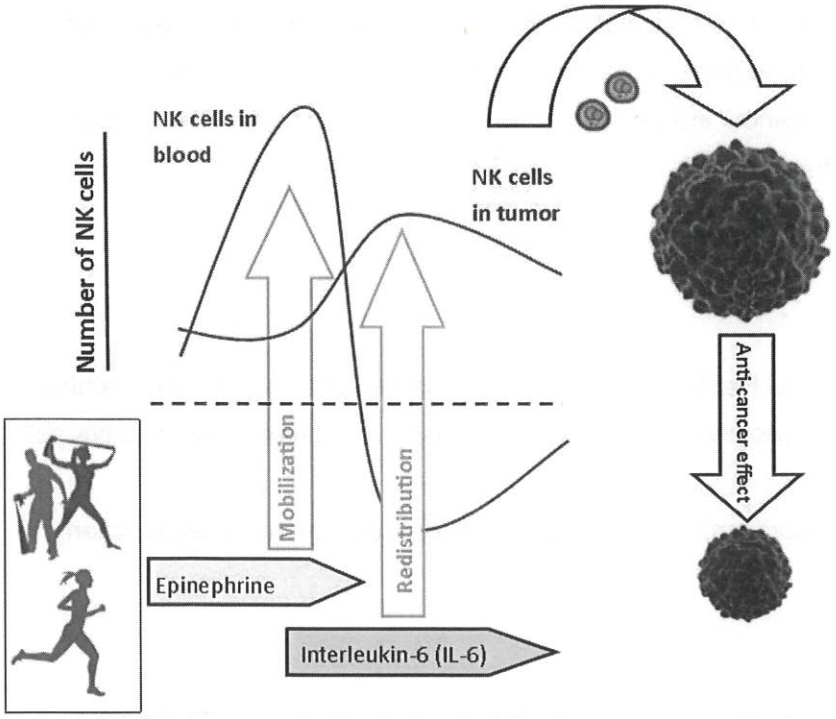

Figure 1. Mechanism of action of how exercise may improve immune response and reduce tumor growth [2].

Currently, no sufficiently powered randomized controlled trial among patients has investigated whether a supervised aerobic and resistance exercise intervention during chemotherapy would benefit the tumor response [12]. A study among patients undergoing neoadjuvant chemotherapy provides the unique opportunity to assess the direct effects of exercise on the tumor. Considering the non-invasive nature and relatively low costs of exercise, knowledge of its potential to boost immune function can – if effective - improve clinical practice.

In preparing for a future sufficiently powered randomized controlled trial, it is essential to 1) ***study trial feasibility*** in terms of patient enrollment and the percentage of tumor biopsies that can be examined successfully, and 2) ***generate preliminary data*** on the potential efficacy of exercise on immune function (immune cell infiltration into the tumor and immune cell functionality in blood).

Tumor tissue will be investigated by immunohistochemistry (IHC) to assess immune cell infiltration into the tumor with a specific focus on NK-cell frequency and phenotype. Tumor tissue will be obtained with tumor biopsies. ***Tumor biopsies*** at diagnosis are performed as part of usual care. An additional tumor biopsy will be performed after 2 or 3 cycles of chemotherapy (6 weeks). Other clinical trials among patients during neo-adjuvant treatment for breast cancer have also incorporated additional tumor biopsies (e.g., NCT03283384). A timeframe of 6 weeks was chosen to outweigh the presence of sufficient tumor tissue with sufficient physiological responses to exercise. Results from pre-surgical exercise interventions in patients with cancer indicate that physiological changes can already occur within 4 weeks of exercise training [13].

2. OBJECTIVES

Primary Objective: To study trial feasibility in terms of patient enrollment and the percentage of tumor biopsies that can be successfully assessed for changes in immune cell and NK cell content.
Secondary Objective(s): To study preliminary effects of exercise on immune cell function.

3. STUDY DESIGN

This is a multicenter randomized controlled feasibility trial, in which 20 women with breast cancer scheduled for neoadjuvant 2 or 3 weekly AC-T(H) chemotherapy will be randomized into a combined aerobic and resistance exercise intervention group or a usual care (no exercise)

control group, during the first 6 weeks (2 or 3 cycles) of chemotherapy. Patients from the control group will receive care as usual and are requested to maintain their usual daily physical activities. In order to limit contamination (increase of exercise in the control group), non-participation and prevent dropout, the control group will be offered the same 6-week exercise intervention after the tumor biopsy has been taken after 6 weeks. The study design and timepoints of measurements are presented in Figure 2.

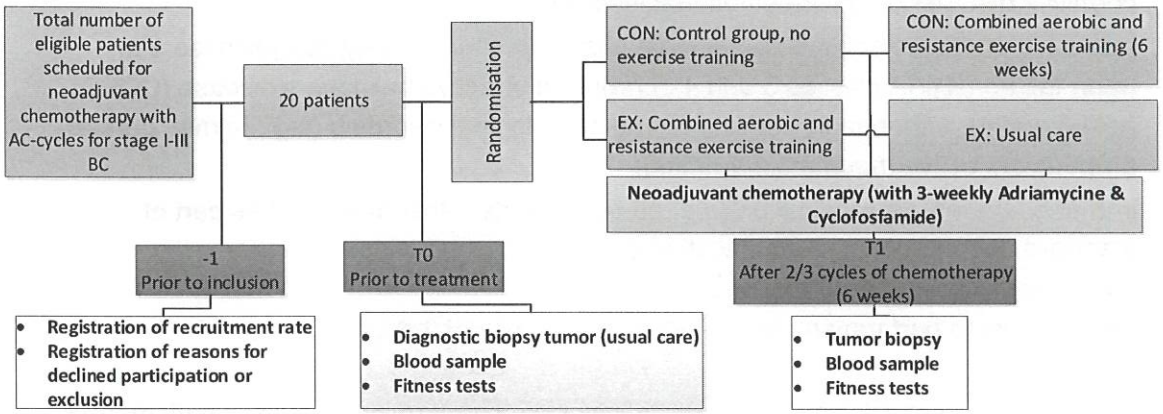

Figure 2. Study design and time-point of measurements

4. STUDY POPULATION

4.1 Population (base)

Eligible patients will be recruited from Amsterdam UMC and 2 other sites.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- stage I-III breast cancer
- scheduled for neoadjuvant chemotherapy with 2 or 3-weekly Adriamycin/Cyclofosfamide, followed by Paclitaxel weekly +/- trastuzumab
- willing to undergo an additional ultrasound guided biopsy
- ECOG-performance score ≤ 2 (able to perform basic activities of daily living such as walking or biking)

4.3 Exclusion criteria

A patient who meets any of the following criteria will be excluded from participation in this study:

- addition of immuno- or targeted therapy at start of neoadjuvant chemotherapy
- currently participating in structured vigorous aerobic exercise and/or resistance exercise (≥2 days per week).
- cognitive disorder or severe emotional instability
- presence of other disabling co-morbidity that might hamper physical exercise e.g. heart failure (NYHA classes 3 and 4), chronic obstructive pulmonary disease (COPD, gold 3 and 4), orthopaedic conditions and neurological disorders (e.g., hernia, paresis, amputation, active rheumatoid arthritis);
- immunosuppressive medication (e.g. corticosteroids (other than used as part of standard chemotherapy premedication protocol), cyclosporine)
- immunodeficiency (primary or secondary)
- impossibility to perform an ultrasound-guided biopsy of the tumor

In case the treating physician experiences difficulties to determine whether a patient fulfils the physical exercise related criteria mentioned above, an experienced physiotherapist (M.R. ten Tusscher) will be requested for consultation.

4.4 Sample size calculation

Eligible patients will be recruited from Amsterdam UMC and 2 other sites. In these hospitals, over 100 patients receive neoadjuvant chemotherapy with Adriamycin/Cyclofosfamide treatment for breast cancer every year. With an anticipated accrual rate of 37% (corresponding to our previous REACT [14] and METRIC (NTR 4105) trials) recruitment will be completed after 7 months. In case accrual rates drop to 25%, patient enrollment will be completed after 10 months.
This study is a pilot trial focusing on evaluating study feasibility and preliminary effects of exercise on immune function. For the preliminary effects, the feasibility trial is underpowered by design. We will primarily explore the average size of the effect to identify whether it may have the potential to be clinically relevant, instead of observing statistical significance only ($p \leq 0.05$). Additionally, we will calculate the sample size required to detect the same difference in effect with 80% power (alpha 5%).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Exercise intervention
Patients in the exercise-group will follow a combined resistance and aerobic exercise program that was proven beneficial for physical fitness, fatigue, quality of life and chemotherapy completion rates in patients with breast cancer receiving adjuvant treatment [3]. This program meets the American College of Sports Medicine's exercise recommendations for patients with cancer[11]. Exercise sessions take place 2 times per week for 60 min and include aerobic and resistance exercises at moderate-to-high intensity, in which the adequate training load will be tailored to a patient's individual fitness level throughout the intervention [3]. After a short warming-up (5 min), patients perform muscle strength exercises for 20 min per session, starting with two series of 12 repetitions at 70% of the one repetition maximum (1RM) per exercise and increasing gradually to two series of eight repetitions at 80% 1RM. The resistance program consists of at least six exercises targeting the large muscle groups with vertical row, squat, bench press, pull over, abdominal crunch and lunge. Aerobic exercises are performed for 30 minutes per session (with a minimal duration of ten minutes per exercise), with an intensity of 50% to 80% of the maximal workload (Wmax) as estimated by the Steep Ramp Test (SRT). The intensity of aerobic exercises is increased if a patients scores a 12 or lower on the Borg scale of perceived exertion, while the intensity is decreased at a score of 16 or higher. The heart rate should be

within a heart rate zone of 60% to 90% of the maximal heart rate, which is conventionally estimated as 220 minus age [3].
Exercise sessions are supervised by a specially educated physical therapist via the OncoNet network (www.onconet.nu) allowing patients to train close to their homes [3].
Additionally, patients are encouraged to be physically active for 30 minutes on at least 3 other days in the week at Borg level 12-14.
Patients in the exercise group are asked to keep an exercise log to register home-based physical activity.

Usual care group

Patients from the control group will receive care as usual and are requested to maintain their usual daily physical activities. In order to limit contamination (increase of exercise in the control group), non-participation and prevent dropout, the control group will be offered the same 6-week exercise intervention after the tumor biopsy at T1 at the expense of the study-budget.

6. METHODS

6.1 Study parameters/endpoints

6.1.1 Main study parameter/endpoint

Feasibility will be judged according to two key questions:

1) Recruitment
   What is the proportion of eligible patients who are willing to participate in this study and undergo an additional tumor biopsy?
2) Quality of tumor material
   What is the percentage of tumor biopsies that can be examined successfully both at baseline and after 6 weeks of neo-adjuvant treatment?
   We define a biopsy as successful if we collected enough tumor tissue and the quality of the tissue enables us to perform IHC-analysis to determine the infiltration of NK-cells into the tumor.

We conclude that our feasibility-study is successful if:

1. we achieve a recruitment rate of at least 20%.
2. we can examine biopsies successfully on infiltration of NK-cells (both on T0 and T1) in 14 of 20 patients (70%).

6.1.2 Secondary study parameters/endpoints

Preliminary treatment effects:

1. *Changes in immune cell infiltration measured with tumor biopsy*

Primary end-point for the preliminary effects is the alteration in number of NK-cells per total number of lymphocytes and in NK-phenotype.
Tumor tissue will be investigated by immunohistochemistry (IHC) to assess immune cell infiltration into the tumor with a specific focus on NK-cell frequency and phenotype.
Tumor tissue will be obtained with tumor biopsies. Tumor biopsies at diagnosis are performed as part of usual care. An additional tumor biopsy will be performed after 2 or 3 cycles of chemotherapy (6 weeks).

2. *Immune function measured from blood.*

Venous blood samples will be drawn prior to the start of chemotherapy and randomization (T0), and after 2 (3-weekly) or 3 (2-weekly) cycles of chemotherapy (T1). At T0 we will collect 20ml blood and at T1 we will collect 24ml blood to measure immune function. This 4ml extra blood on T1 is used to determine levels of leukocytes and thrombocytes prior to the biopsy. Immune cells will be investigated in whole blood using fluorescence activated cell sorting (FACS), to determine the frequency of $CD4^+$ T cells, $CD8^+$ T cells, regulatory T cells, B cells, monocytes, granulocytes, circulating dendritic cell (DC) subsets, myeloid derived suppressor cells, and the frequency, phenotype and function (i.e. cytotoxic potential, cytokine production capacity, and IL-6 and epinephrine receptor expression) of NK cells as demonstrated and reported previously [15].

3. *IL-6 levels measured from blood*

Patients included from the VUmc and randomized to the intervention group will be asked for consent to draw venous blood samples prior to and directly after one of the exercise sessions to examine acute responses to exercise. At both measurements we will collect 5ml blood. This will be done to measure Interleukine-6 levels (IL-6) which are supposed to be present in elevated concentrations during- and directly after exercise as outlined in the rationale for this study [16].

4. *Physical fitness:*

This will be assessed as secondary end-point to determine whether the exercise intervention yielded the expected physiological responses in 6 weeks.

- Aerobic fitness will be assessed during a submaximal endurance test performed on a cycle ergometer assessing the time that a patient can cycle at 70% of the estimated work load based on the steep ramp test, until a maximum of 30 min.[3]
- Upper and lower body muscle strength will be assessed with the 1RM leg press and bench press according to standardized protocols.

5. *BMI*

BMI will be calculated by dividing body weight by height (in meters) squared. Height will only be measured at baseline on a calibrated scale to the nearest 0.1 mm. Weight will be measured at baseline (T0) and T1 on a calibrated scale to the nearest of 0.1 kg.

6. *Physical activity and health-related quality of life*

Physical activity will be assessed with the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) and health-related quality of life with the EORTC QLQ-C30 (30 items) at baseline and after 6 weeks (T1).

6.1.3 Other study parameters (if applicable)

Exercise adherence:

Session attendance and compliance to the prescribed intensity, frequency and duration of exercises is monitored in exercise logs registered by the physical therapist. Patients in the exercise group are asked to keep an exercise log to register home-based exercise.

Demographic and clinical covariates

Information on smoking, alcohol intake, sociodemographic characteristics (e.g. age, ethnicity, education level, marital status), and exercise history will be obtained at baseline.

Clinical data:

- tumor characteristics (TNM-classification, degree of differentiation, hormone and Her2 receptor status)
- disease, treatment and treatment-relatedtoxicity
- hospitalization
- performance status (ECOG)
- comorbidities (Charlson comorbidity index) and co-medication

Contamination is assessed from the control group at follow-up by a 3-item questionnaire, whether they changed their physical activity behavior and participated in structured exercise.

6.2 Randomisation, blinding and treatment allocation

After baseline assessment, patients will be randomized into the exercise or the control group by the use of a computerized random generator. Randomization is stratified for TNBC (yes/no), extent of disease (N+/N-) and treatment (regular versus dose-dense).
The researcher that examines the physical fitness will not be blinded to group allocation.
The pathologist and analyst that will examine the blood and biopsy tissue will be blinded to group allocation.

6.3 Study procedures

After providing informed consent (IC), patients will be randomized in either the intervention or waitlist control group.
Participants in the intervention group will attend supervised exercise sessions twice a week at a local physical therapist and are encouraged to be physically active for at least 30 minutes per day at Borg level 12-14 (see paragraph 5.1).
Participants in the control group are requested to maintain their usual daily physical activities and are offered supervised exercise sessions after 6 weeks at the expense of the study budget (see paragraph 5.1).

Study parameters will be assessed at baseline (before start of chemotherapy) and after 6 weeks of chemotherapy (2 3-weekly or 3 2-weekly cycles) in the patient's own treating hospital. When patients are included from the Amstelland hospital they will be asked beforehand to come to the Amsterdam UMC – location VUmc for the physical test, blood samples and biopsy. Consequently, Amstelland will be a recruiting hospital in this study.

During the visits, information on physical fitness and blood samples will be collected. In addition, during the visit on T1 a tumor biopsy will be obtained. The T1 biopsy and physical fitness test will not be performed on the same day, as this might lead to contamination of the results on immunoreactivity in blood and tumor. We will strive to perform the physical fitness tests prior to the 3th (3-weekly) or 4th (2-weekly) chemotherapy administration so patients don't have to come to the hospital an extra time.
The visit on T0 will last approximately 75 minutes, the first visit on T1 (biopsy and blood sample) approximately 30 minutes and the second visit on T1 (physical fitness test) approximately 45 min.
Questionnaires will be sent to the participant's home and will take up to 10 minutes to complete, based on 6 items per minute. Questionnaires will be sent in paper only. Table 1 presents a summary of all study parameters.

	Measurement tool	-1	T0	T1
Primary outcome measurement				
Recruitment		X		
Quality of tumor material	Tumor biopsy		X	X
Secondary outcome measurement				
Clinical data				
Extent of the tumor	Medical records		X	
Disease, treatment and toxicity	Medical records		X	X
Performance status	Medical records		X	X
Co-morbidities and co-medication	Medical records		X	
Physical tests				
Aerobic fitness	Steep ramp test & endurance test		X	X
Muscle strength	1 RM legpress and 1 RM bench press		X	X
BMI	Height		X	
	Weight		X	X
Blood sampling	Venous blood sample (20ml on T0, 24 ml on T1l)		X	X
Tumor biopsy for determining preliminary effect	Tumor biopsy[1]		X	X
Questionnaires				
Physical activity	SQUASH (11-items)		X	X
Health-related QoL and symptoms	EORTC QLQ-C30 (30-items)		X	X
Sociodemographics, smoking & alcohol	6-items		X	
Contamination of control group[2]	1-item			X
Exercise logs				
Adherence[3]	Exercise log			X
Compliance[3]	Exercise log			X

Table 1; Summary of measurements

[1] This is the same biopsy as mentioned above (quality of tumor material)

[2] Parameter will only be assessed in the control group

[3] Parameter will only be examined in physical therapists

Recruitment

The physician and/or nurse practitioner responsible for recruitment per participating center will be asked to keep a log and register the number of eligible patients (stage I-III breast cancer and scheduled for 2 or 3 weekly AC-treatment), the number of eligible patients invited for participation in the study and the number of eligible patients who declined participation. The physician and/or nurse practitioner will notify the coordinating researcher if patients received the study information and agreed to be approached by the coordinating researcher. The coordinating researcher will register the number of patients that declined to participate. Reasons for declined participation will be reported, as well as reasons for exclusion for study par-ticipation by both the physician/nurse practitioner and the coordinating researcher.

Tumor biopsies

The first tumor biopsy at diagnosis is performed as part of usual care.

The second tumor biopsy is for research purposes only and will be performed after 2 (3-weekly) or 3 (2-weekly) cycles of chemotherapy (6 weeks). Both biopsies will be performed in the patient's own treating hospital, except for patients from the Amstelland hospital which will take place at location VUmc.

***Blood samples* – immune system**

The first blood sample of 20ml will be drawn prior to the start of chemotherapy and randomization (T0), and the second blood sample of 24ml after 2 (3-weekly) or 3 (2-weekly) cycles of chemotherapy (T1). The second blood sample will be taken before the biopsy to be able to determine levels of thrombocytes and neutrophils. In case values of thrombocytes are $\leq 50 \times 10^9$/L and/or neutrophils (ANC) are $\leq 0.5 \times 10^9$/L, patients will be excluded for the second biopsy because of an increased risk of procedure related hemorrhage or infection. Blood samples will be collected in 10ml heparin tubes without gel. Both blood samples will be drawn in the patient's own treating hospital, except for patients from the Amstelland hospital.

Blood samples will be centrifuged and PBMC's will be isolated before storage at -80° in the Tumor Immunotherapy laboratory of the Cancer Center Amsterdam for the duration of this study.

Blood samples – IL-6

Patients included from the Amsterdam UMC and randomized to the intervention group will be asked for consent to draw blood samples prior to and directly after one of the exercise sessions to examine acute exercise responses. This will be done with an extra informed

consent form. If patients prefer not to participate in this extra blood sampling they will not be excluded from the study.

Patients will be asked to perform one of their exercise sessions in the VUmc. This session will be guided by an experienced physical therapist. Both before and after the exercise we will collect 5ml blood in a serum tube. This will be done to measure Interleukine-6 levels (IL-6) from serum which is supposed to be present in elevated concentrations during- and directly after exercise and is part of the rationale for this study.

Fitness tests

Physical fitness will be assessed both prior to randomization and after two cycles of chemotherapy in the patient's own treating hospital, except for the patients from the Amstelland hospital.

- Aerobic fitness will be assessed during a submaximal endurance test performed on a cycle ergometer assessing the time that a patient can cycle at 70% of the estimated work load based on the steep ramp test, until a maximum of 30 min [3].
- Upper and lower body muscle strength will be assessed using the 1-repetition maximum for leg press and bench press according to standardized protocols.

Questionnaires

- Physical activity will be assessed by the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH). The SQUASH contains 11 items and measures the frequency, duration and intensity of 4 different physical activities (actively commuting, physical activity at work or school, household activities and leisure time activities).
- Health-related Quality of Life (HRQoL) will be assessed by the European Organization Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ) Core on baseline and after the intervention (T1).
- A questionnaire on demographic and clinical covariates will be assessed on baseline. This questionnaire includes questions about smoking, alcohol, exercise history and sociodemographic characteristics (e.g. age, ethnicity, education level, living situation).

Clinical data

Clinical data will be obtained from medical records on baseline, during and/or after the intervention. This includes information on extent of the tumor, disease, treatment and treatment-related toxicity, hospitalization, performance status (ECOG), comorbidities and co-medication.

Exercise logs

To collect data on adherence and compliance to the exercise, physical therapists will be asked to register data in exercise logs. These exercise logs will be collected at T1 by the researcher.

If this pilot-trial determines feasibility, we will strive to set up a sufficiently powered RCT. In that case, data collected in this pilot trial might be used as part of the data collection of this next RCT. Patients will be asked for consent to use the coded data (not the human material) for this next RCT.

6.4 Withdrawal of individual subjects

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

6.5 Replacement of individual subjects after withdrawal

In case of drop-outs, patients will not be replaced by including extra patients because feasibility of patient enrollment is one of the primary outcomes of this study. This also includes patients with an infection at T1 and thereby an exclusion for the biopsy.

7. SAFETY REPORTING

7.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

7.2 AEs, SAEs and SUSARs

7.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention. For this study, all exercise-related AE's and AE's related to the biopsy (T1) and blood sampling (T0 en T1) reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

In case a subject (with 3-weekly AC chemotherapy cycles) receives a prescription for G-CSF (pegfilgrastim) prior to the second cycle of chemotherapy (in case of neutropenia) this

will be reported by the coordinating researcher, because this might interfere with the secondary outcomes on immune function.

7.2.2 Serious adverse events (SAEs)

In this study, the sponsor will report only the SAEs related to the exercise intervention, the blood sampling (T0 en T1) and the biopsy (T1). The sponsor will report these specific SAE's through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

7.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

8. STATISTICAL ANALYSIS

8.1 Primary study parameter(s)

Information on trial feasibility and preliminary effects are crucial to set up a randomized controlled trial with sufficient statistical power to evaluate the exercise effects on immune cell infiltration into the tumor.

The *feasibility* will be assessed using the following descriptive statistics:

- The proportion of eligible patients who are willing to participate in this study and undergo an additional tumor biopsy.
- The percentage of tumor biopsies at baseline and after 6 weeks of neo-adjuvant treatment that can be assessed successfully.

8.2 Secondary study parameter(s)

Analyses of the *preliminary effects* on the outlined immune parameters will be conducted using an intention-to-treat principle. The exercise intervention effect on markers of immune cell infiltration and activity will be evaluated using linear regression analyses in which the intervention is regressed on the post-test value of the marker, adjusting for the baseline value (ANCOVA). Additionally, we will explore whether the exercise effect on NK-cell infiltration is moderated by receptor expression of epinephrine and IL-6 on NK-cells with interaction tests. As this feasibility trial is underpowered by design, we will primarily explore the average size of the effect to identify whether it may have the potential to be clinically relevant, instead of observing statistical significance only ($p \leq 0.05$). Additionally, we will calculate the sample size required to detect the same difference in effect with 80% power (alpha 5%).

Preliminary effects of the intervention on physical fitness and QoL will be explored using linear (continuous outcomes), logistic (dichotomous outcomes) and cox (survival data) regression analyses. In the linear regression analyses, we will adjust for baseline values. We will use a multilevel model to take account for the clustering of patients within hospitals.

8.3 Other study parameters

Descriptive statistics are calculated for covariates (smoking, sociodemographics and clinical data). The population characteristics of the subjects will be shown as averages or percentages (± standard deviation).

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version, date, see for the most recent version: www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

9.2 Recruitment and consent

The patients will be recruited and informed about the study by the treating physician or nurse from the patient's own hospital. They will receive the patient information in which the study is outlined and the practical implication of study participation will be explained. Patients are asked if the study coordinator is allowed to make a call to the patient to further explain the study. The study coordinator will call the patients to answer any remaining questions and to set up a first appointment if they want to participate in the study. Since time between diagnosis of breast cancer and start of chemotherapy is short, patients will have two days to consider their decision for participation in this study All patients will sign an IC statement prior to participation in the study. Patients will be recruited from the department of Medical Oncology from the Amsterdam UMC and 2 other sites.

9.3 Benefits and risks assessment, group relatedness

Patients randomized to the exercise group will be guided by a physical therapist that is educated specifically on the exercise protocol for this study and is used to working with oncology patients. The safety of the exercise intervention has well been documented and the program meets the American College of Sports Medicine's exercise recommendations for patients with cancer [17].
Therefore, we expect a negligible risk for participants with regard to the exercises. In addition, evidence from randomized controlled trials, including our own, shows that exercise during cancer treatment benefits physical fitness, fatigue and quality of life [4, 5]. Additionally, women with breast cancer who exercised during chemotherapy appeared to have fewer dose adjustments[3]. For this reason, the control group will be offered the same intervention after 6 weeks. Even though this evidence exists, referral to a physical therapist during chemotherapy is not standard care because costs for physical therapy are not covered with basic insurance.

The risks of the blood drawing and fitness test are negligible. The patients that perform one of their exercise sessions in the VUmc (to be able to collect blood samples for IL-6 monitoring) will be guided by an experienced physical therapist. In case of emergency during this specific training, the emergency procedure of the department of rehabilitation medicine will be followed.

The risk of clinically relevant complications of a breast biopsy was around 0,2% in a multi-institutional study in 1994 [8]. Nowadays, the risk of complications is even lower, due to technological improvements that are implemented. The biopsies are very important to study the effect of an exercise intervention on infiltration of immune cells in the tumor.

9.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

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

9.5 Incentives (if applicable)

Patients will receive compensation for their travel costs for the measurements.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

All data and human material will be handled confidentially and treated coded. All subjects will receive an unique code and only the investigators will know the key to this code. The coded data are stored in a digital database on an intern network, which is protected by a password that only the investigators have access to. The handling of personal data should comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

The data will be stored for a period of 15 years. The biopsy material will be stored and analyzed at the biobank unit of the pathology department. The blood samples will be stored and analyzed in the department of clinical chemistry and partly in the Cancer Center Amsterdam. The human material will be used for immune monitoring during this trial and will be destroyed when the analyses for this trial are completed and the manuscript has been published (if applicable).

The human material will not be exchanged with other countries.

10.2 Monitoring and Quality Assurance

From the risk classification of our study we determined a negligible risk and therefore the use of a certified data monitor is not necessary. Data monitoring will be performed the Clinical Research Bureau of our institute. He or she will compare the data entered into the database with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the database are known to the investigational staff and are accessible for verification. At a minimum, source documentation must be available to substantiate: subject identification, eligibility and participation; proper informed consent procedures; data of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of adverse events; date of subject completion, discontinuation from treatment, or

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the database are consistent with the original source data.

10.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

10.4 Annual progress report

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

10.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

10.6 Public disclosure and publication policy

No restrictions regarding to public disclosure and publication of the research data have been, or will be made by the funding agency. Publications from this project will be submitted to scientific journals and publications will be accessible to public.

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