Physical exercise during neoadjuvant chemotherapy for breast cancer as a means to increase pathological complete response rates: the randomized Neo-ACT trial

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Any relevant amendments to the protocol are first submitted to the responsible ethical committee and after approval disseminated to all participating sites. Changes that are not deemed relevant in accordance to guidance from the ethical committee, such as minor errors or discrepancies between the latest approved informed consent form and the protocol are released without ethical amendment and designated with a third number in the protocol version.

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SYNOPSIS

Title	Physical exercise during neoadjuvant chemotherapy for breast cancer as a means to increase pathological complete response rates
Short title	Neo-ACT trial (NCT05184582)
Trial design	Prospective randomized clinical trial
Trial rationale	Neoadjuvant chemotherapy (NACT) is the current standard of care for patients with breast tumours larger than 20 mm and/or lymph node metastases, particularly Human Epidermal growth factor Receptor 2 (HER2) and triple negative breast cancer (TNBC). The best proof of NACT efficacy is pathological complete response (pCR), i.e. the absence of residual invasive tumour in the breast and the axillary lymph nodes. Today, pCR is frequently used as surrogate endpoint in oncological pharmaceutical trials focusing on new compounds often in combination with the current standard of care. While NACT has the advantage to offer fast-track approval of new drug compounds in oncology, early expectations of improved survival rates have not been met. Additional systemic drug regimens come, however, at a cost, and associated side effects and toxicities are important to bear in mind. It is therefore utterly compelling to conceive that improved NACT efficacy – and thus de-escalated locoregional therapy – may be achieved by a non-toxic patient-driven life-style intervention such as physical exercise. Being physically active reduces the risk of breast cancer by 20-30%, and exercise induces reductions of tumour growth in animal models of breast cancer. Exercise may act through reduced systemic inflammation and enhanced anti-tumoural immune cell function, improve blood flow and perfusion and thus tumour susceptibility to systemic treatment, reduce systemic inflammation and enhance immune cell functions. The short-term stress of a single bout of physical exercise in healthy human subjects can induce a release of immune cells into the circulation, and primary tumour growth is reduced in mice exposed to voluntary running. Physical exercise during chemotherapy is feasible and safe. Observational studies show that exercise in breast cancer has a protective effect regarding recurrence and mortality. Long-term follow-up of aerobic and resistance exercise shows enhanced survival

	in patients with breast cancer. In the randomized OptiTrain trial, resistance and high-intensity interval training (HIIT) during postoperative chemotherapy had positive effects on fatigue and muscle strength, muscle mass and function. Chemotherapy completion rates are improved by exercise. Exercise may thus result in improved pCR rates through systemic anti-inflammatory effects and improved chemotherapy completion rates given at full dosage due to the favourable effects of exercise on treatment tolerability, compliance, and acceptability.
Endpoints	The **primary** endpoint is pathological complete response (pCR). The **secondary** endpoints are: - Residual Cancer Burden (RCB) - Objective radiological tumour response (RECIST) - All-cause, breast cancer-specific, and recurrence-free survival at 2, 5 and 10 years - Health-related quality of life assessed by the EORTC QLQ-C30 and BR23 questionnaires - Self-reported physical activity (Modified Godin Leisure Time Physical activity questionnaire) - Toxicity-related outcomes (chemotherapy completion rates, number of unplanned hospital admissions during NACT, objective cognitive dysfunction (Amsterdam Cognition Scan), cardiac toxicity, side effects, and sick leave) - Device-measured physical activity level (Fitbit activity tracker) - Muscle strength (handgrip strength test and hypothetical 1-RM maximal leg muscle strength tests) - Cardiorespiratory fitness (Ekblom-Bak submaximal cycle test)
Patient selection	Clinically T1-3, N0-2 breast cancer patients scheduled for NACT and surgery with curative intent. **Inclusion criteria**: • Patients with primary invasive breast cancer cT1-T3 cN0-2 • Tumour subtype (ER, HER2) available before initiation of NACT • Oral and written consent • Age ≥ 18 years **Exclusion criteria** • Bilateral invasive breast cancer • Pregnancy or breast-feeding

	• The presence of musculoskeletal, neurological, respiratory, metabolic or cardiovascular conditions that may prevent safe completion of the exercise and testing demands of the trial • Currently performing equal to or more than 150 mins of moderate to high intensity aerobic exercise plus 2 sessions per week of moderate intensity resistance exercise • Inability to complete baseline physical exercise test • Inability to access and/or use trial technology (app, activity tracker)
Intervention	Participants randomized to the exercise group will complete a total of 120 minutes home-based exercise sessions per week from initiation of NACT to surgery (approx. five months). Participants can choose exercise sessions that take 30, 45 or 60 minutes. Before initiation of the training sessions, the participants register personal information about their current physical health together with the physiotherapist to individually design the training program. In addition to the exercises described in detail below, participants are encouraged to accumulate further 150 minutes of physical activity each week. No exercise is recommended within 24 hours of chemotherapy administration. • Progressive home exercise program by an individualised mobile phone application, supported by local physiotherapists and centralised remote exercise support and voluntary online live and pre-recorded exercise sessions. • Initial exercise intensity individually tailored to each patient's fitness at baseline and rate of perceived exertion during the program and adapted if required. • All exercise sessions include 1) a 3-minute moderate intensity (12-13 on Borg's Rate of Perceived Exertion (RPE) scale) warm-up, b) a resistance training component targeting the major muscle groups, 2 x 12 repetitions of each exercise (RPE 14-16), where participants can choose to use equipment (resistance band or dumbbells) or their own bodyweight, c) a HIIT component including 1-minute intervals of bodyweight exercises that aims to increase the heart rate and improve cardiorespiratory fitness (RPE 16-18) with 1-minute active recovery in between (easy walking on

	the spot), and d) a short cooldown/stretching at the end of the session.
Control	Routine information on benefit of physical exercise as per clinical guidelines and local practice.
Follow-up	Each patient is followed up for two years after surgery regarding secondary endpoints. Survival and recurrence data are obtained via national registers or remote contact with the patients or their treating hospital at 5 and 10 years.
Statistical considerations	Patients will be randomized in a 1:1 fashion. In order to detect an increase of the pCR rate in the experimental arm by 10%, which is regarded clinically relevant, and using a power of 80% and an alpha of 5%, a total of 712 patients have to be included in the intention-to-treat population; 356 in each arm. Accounting for a drop-out of 10%, the trial will include 790 patients. Stratification at the moment of computerized randomization will be based on hospital and biological tumour subtype (ER+HER2-, ER+HER2+, ER-HER2+, ER-HER2-).
Time plan	Trial initiation: October 2022 Enrolment phase: October 2022 – December 2027 Reporting of primary endpoint: September 2028 End of 2-year follow-up: June 2030

BACKGROUND

Use of neoadjuvant (preoperative) chemotherapy in breast cancer

Neoadjuvant chemotherapy (NACT) has a history of being reserved for non-operable, locally advanced breast cancer (BC) but is increasingly used for patients with early BC. It is strongly recommended by national[1] and international[2] guidelines especially in the triple-negative (TNBC) and HER2-positive BC subtypes, and in case of lymph node metastases. In Sweden, 14% of all newly diagnosed early BC patients suitable for surgery (1041 out of 7289 individuals according to the National Breast Cancer Register) received NACT in 2019 (22% in Stockholm), while this figure was 35% (Stockholm 45%) for TNBC and HER2-positive BC, and 50% (Stockholm 62%) for all clinically node-positive BC. Combining lymph node metastases and tumour subtype, the rate of NACT was as high as 68% (Stockholm 80%) in clinically node-positive TNBC and HER2-positive BC in 2019.

The best proof of NACT efficacy is pathological complete response (pCR), i.e. the absence of residual invasive tumour in the breast and the axillary lymph nodes. Especially in the above-mentioned tumour subtypes TNBC and HER2-positive breast cancer, published pCR rates are high (50.7% in TNBC and 63.7% in HER2-positive, oestrogen receptor (ER)-negative BC) and strongly predict improved survival[3]. In luminal BC (ER-positive, HER2-negative), published pCR rates are substantially lower (10.8% if HER2 negative, 29.4% if HER2 positive)[3] which underlines the need for novel regimens including anti-endocrine strategies. Today, pCR is frequently used as surrogate endpoint in oncological pharmaceutical trials focusing on new compounds often in combination with the current standard of care (traditionally chemotherapy and/or targeted therapies with anti-HER2 and anti-ER drugs, but now also immunotherapy, CDK4/6 inhibitors and drug-antibody conjugates).

Need to improve pCR rates

While NACT has the advantage to offer fast-track approval of new drug compounds in oncology, such as the FDA approval of pertuzumab after the Neosphere trial published first in 2012[4], early expectations of improved survival rates simply by reversing the traditional order of treatments (surgery followed by adjuvant systemic therapy) have not been met[5]. Only recently, however, the Keynote 522 trial could show both a higher pCR rate and an improved event-free survival in TNBC patients receiving the anti-PD1 antibody pembrolizumab, which fuels hopes that specific subgroups will be identified who gain a survival benefit from NACT when compared to standard treatment[6]. Apart from improving outcomes in specific subgroups, NACT facilitates research into post-neoadjuvant systemic treatment strategies, which has already resulted in supplemental therapies such as adjuvant capecitabine in HER2-negative BC[7] and T-DM1 in HER2-positive BC[8]. It also allows for less extensive surgery by tumour shrinkage and conversion of node-positive BC into node-

negative disease, which may be of great benefit for the individual patient in terms of postoperative morbidity and quality of life. Additional systemic drug regimens come, however, at a cost, and associated side effects and toxicities are important to bear in mind. It is therefore utterly compelling to conceive that improved NACT efficacy – and thus de-escalated locoregional therapy – may be achieved by a non-toxic patient-driven life-style intervention such as physical exercise. Such patient empowerment is especially important in the view of the common clinical observation that most patients request to know how they themselves may contribute to a favourable course of their disease and treatment.

Evidence on physical exercise and cancer

Epidemiological research shows that being physically active reduces the risk for breast cancer by 20-30%[9] and has a similarly protective effect in women carrying high-risk BC mutations such as BRCA1 and BRCA2[10]. Since the 1960s, numerous intervention studies report exercise-induced reductions of tumour growth in animal models of breast cancer[11]. Mechanistically oriented preclinical trials suggest that exercise act through reduced systemic inflammation and enhanced anti-tumoural immune cell function[12,13]. Other studies show an altered phenotype of tumour vasculature with exercise, improving blood flow and perfusion, making the tumour more susceptible to systemic treatment[12,13]. Further suggested mechanisms for the anti-tumoural effects of exercise include weight control, endocrine effects, less systemic inflammation (reflected by lower CRP and pro-inflammatory cytokine levels in serum), improved immune cell functions such as increased recruitment and cytotoxic activity of CD8+ T-cells and NK cells, and a shift towards an anti-tumorigenic (Th1/M1) profile[12-14]. In the tumour microenvironment, the level of inflammatory cell infiltration increases markedly in response to physical exercise[15]. Here, immune cells can provide anti-tumour immune responses and thus improve survival outcomes, or instead facilitate tumour growth and metastasis. Infiltrating cytotoxic CD8+ T-cells and NK cells predict a favourable clinical outcome in several solid human cancers, including breast cancer; in contrast, high levels of infiltrating T-regulatory cells and myeloid cells are linked to tumour progression and poor prognosis[15]. The short-term stress of a single bout of physical exercise in healthy human subjects can induce a release of immune cells such as granulocytes, monocytes and NK cells, as well as CD4- and CD8-positive T-cells[16] into the circulation. In a recent study, primary tumour growth was reduced in multiple murine tumour models exposed to voluntary running[17]. This was attributed to enhanced tumour infiltration of NK cells, activated by an increase in systemic levels of epinephrine during exercise[18]. Very recently, a non-randomised prospective trial evaluated physical exercise during neoadjuvant treatment in patients with oesophageal cancer; participants in the exercise group had significantly more tumour regression at surgery than those in the control group[19].

Physical exercise and chemotherapy

Physical exercise during chemotherapy is deemed feasible and safe[20], even when performed via tailored home-based exercise during neoadjuvant chemotherapy[21]. We have evidence from observational studies showing that exercise following a BC diagnosis has a protective effect regarding recurrence, all-cause and cancer-specific mortality[22]. Furthermore, long-term follow-up of a randomized exercise intervention consisting of aerobic and resistance exercise showed enhanced recurrence-free and overall survival in patients with breast cancer[23,24]. In the randomized OptiTrain trial, resistance and high-intensity interval training (HIIT) during postoperative chemotherapy had positive effects on fatigue and muscle strength[25], muscle mass and function[26].
Chemotherapy completion at full dosage is strongly associated with an improved prognosis[27], but dose reductions occur in at least a third of BC patients. Importantly, chemotherapy completion rates can be improved by an exercise program of combined resistance and aerobic training[28]. Physical exercise may thus result in improved pCR rates after NACT not only through proposed systemic anti-inflammatory effects, but also through improved chemotherapy completion rates given at full dosage due to the favourable effects of exercise on fatigue, muscle strength and cardiorespiratory fitness which drive improvements in treatment tolerability. Thus, there is great potential for physical exercise to be put forward as a feasible and effective strategy to support patients to tolerate treatments, which needs to be corroborated in prospective trials.

Physical exercise during NACT and its effects on treatment response and on pCR rates has never been tested. According to clinicaltrials.gov (searched March 22, 2022), there are only two open randomized physical exercise trials in neoadjuvant treatment of BC with oncological outcomes, namely the BENEFIT trial (Germany, N=120) and the Neo-Train trial (Denmark, N=100). Both small trials have the reduction of tumour size through NACT as their primary endpoint. Another trial is registered but not yet recruiting, the NEOLIFE trial (no oncological endpoint, NCT04135586). The Neo-ACT trial goes one step further and explores physical exercise as a means to improve oncological outcomes on a clinically relevant scale.

PURPOSE AND AIMS

The Neo-ACT trial is a prospective randomized controlled multicentre trial testing the effect of a physical exercise intervention during neoadjuvant chemotherapy (NACT) on the primary endpoint pathological complete response (pCR). Secondary aims are response-related outcomes (RCB, RECIST), patient-related outcomes (health-related quality of life, self-reported physical activity), physiological outcomes (muscle strength, cardiorespiratory

fitness, device-measured physical activity), and toxicity-related outcomes (cognitive dysfunction, chemotherapy completion rates, unplanned hospital admissions, cardiac toxicity, sick leave). Furthermore, the trial will explore how physical exercise affects anti-tumoral mechanisms inherent to therapy or host by hypothesis-generating translational analyses in a patient subset.

HYPOTHESES

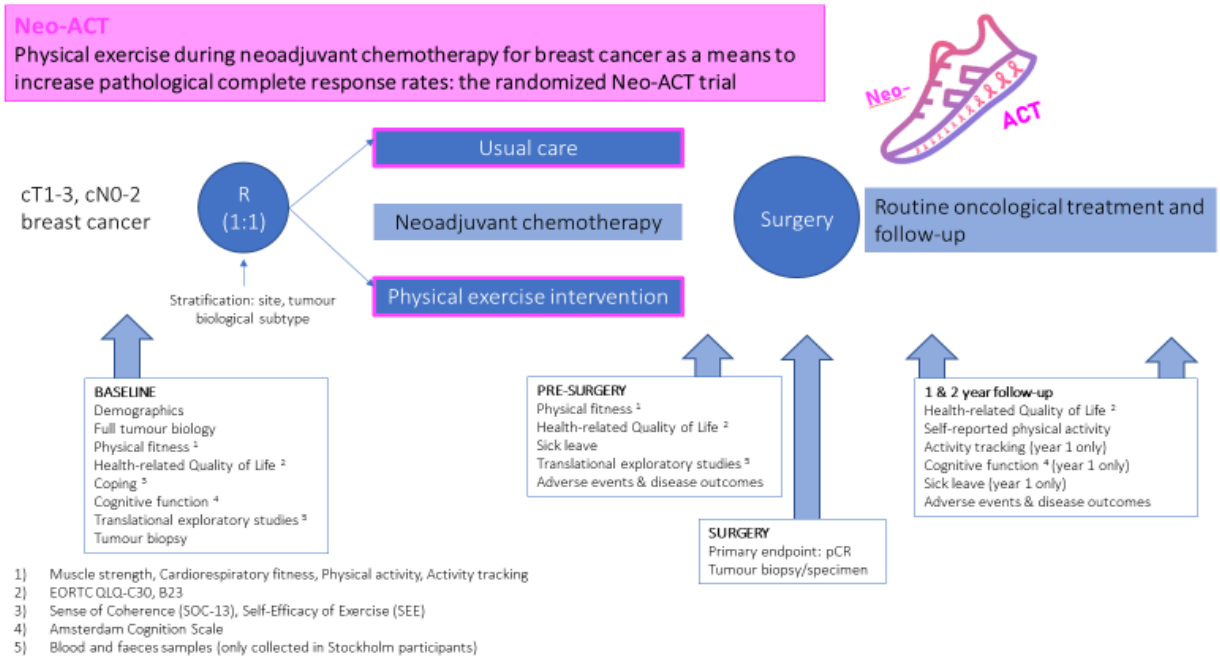
1. A physical exercise intervention improves pCR rates by 10% in the intervention group as compared with the control group.
2. A physical exercise intervention improves secondary outcomes such as response-related outcomes (RCB, RECIST), patient-related outcomes (health-related quality of life, self-reported physical activity), physiological outcomes (muscle strength, cardiorespiratory fitness, device-measured physical activity), and toxicity-related outcomes (cognitive dysfunction, chemotherapy completion rates, unplanned hospital admissions, cardiac toxicity, sick leave).

Translational hypotheses will be tested in subpopulations and will address which biological factors and mechanisms, assessed by contemporary translational studies from blood, faeces and tissue samples, are involved in anti-tumoral effects induced by physical exercise.

METHOD

STUDY DESIGN

The Neo-ACT trial is a prospective randomized trial with the primary endpoint pathological complete response (pCR) after neoadjuvant chemotherapy (NACT) for breast cancer (BC).



POPULATION

Clinically T1-3, N0-2 BC patients scheduled for NACT and surgery with curative intent and fulfilling all inclusion criteria and no exclusion criteria as listed below. Patients with oligometastases scheduled for treatment with curative intent are eligible. Baseline stratification is performed based on treating hospital and biological tumour subtype (ER+HER2-, ER+HER2+, ER-HER2+, ER-HER2-) prior to randomization.

Eligible patients are identified at pre-NACT multidisciplinary team conferences and then receive information about the trial by their oncologist or surgeon, avoiding undue delays. Informed consent is mandatory before randomization and can be obtained by physician or nurse with the appropriate delegation. All participants will undergo standardised tests of physical condition and strength before start of NACT and after NACT but before surgery.

Inclusion criteria	**Exclusion criteria**
• Patients with primary invasive breast cancer cT1-T3 cN0-2 • Tumour subtype available before initiation of NACT (ER, HER2) • Written informed consent • Age ≥ 18 years	• Bilateral invasive breast cancer • Pregnancy or breast-feeding • The presence of musculoskeletal, neurological, respiratory, metabolic or cardiovascular conditions that may prevent safe completion of the exercise and testing demands of the study • Currently performing equal to or more than 150 mins of moderate to high intensity aerobic exercise plus 2 sessions per week of moderate intensity resistance exercise (WHO criteria) • Inability to complete baseline physical exercise test • Inability to access and/or use trial technology (app, activity tracker)

In case of pre-term abortion of NACT either because of tumor progression or (cumulative) toxicity, participants undergo physical testing and other study-specific tests if they are willing to do so and it is deemed feasible by their treating physician. All participants will be included in the intention-to-treat population. Per-protocol analysis for the primary endpoint will only include participants who have received at least four treatment cycles (i.e. at least 12 weeks of treatment).

INTERVENTION

Participants randomized to the exercise group will complete a total of 120 minutes of home-based exercise sessions per week from initiation of NACT to surgery (approx. five months) via a mobile application, supported by on-demand and live online exercise sessions. In addition to the exercise intervention, patients will be encouraged to accumulate further 150 minutes of physical activity each week. Participants will receive initial instructions by an exercise physiologist or physiotherapist on how to perform exercise via the individualised training mobile application Vitala, and are supported by contact with a local physiotherapist/physiologist throughout the intervention. Participants can choose exercise sessions that last 30, 45 or 60 minutes.

No exercise is recommended within 24 hours of chemotherapy administration in accordance with the Swedish recommendations on physical activity (Fysisk aktivitet i

sjukdomsprevention och sjukdomsbehandling, FYSS 2021. Stockholm: Läkartidningen Förlag AB; 2021. ISBN: 978-91-985098-2-3).

To digitalise exercise interventions:

- The Vitala mobile phone application provides exercise instructions and support and measures program adherence. Videos of all exercises are included in the app.
- Initial exercise intensity will be individually tailored to each patient's fitness at baseline and rate of perceived exertion during the program and adapted if required.
- Initial exercise intensity individually tailored to each patient's fitness at baseline and rate of perceived exertion during the program and adapted if required.
- All exercise sessions include a) a 3-minute moderate intensity (12-13 on Borg's Rate of Perceived Exertion (RPE) scale) warm-up, b) a resistance training component targeting the major muscle groups, 2 x 12 repetitions of each exercise (RPE 14-16), where participants can choose to use equipment (resistance band or dumbbells) or their own bodyweight, c) a HIIT component including 1-minute intervals of bodyweight exercises that aims to increase the heart rate and improve cardiorespiratory fitness (RPE 16-18) with 1-minute active recovery in between (easy walking on the spot), and d) a short cooldown/stretching at the end of the session.
- Voluntary pre-recorded online and live online group exercise sessions are offered in addition to the app-based program. These sessions follow the same time frame and structure as the app-based exercise sessions.
- The aim of each exercise ession is for participants to reach a perceived exertion level of 14-16 RPE on the Borg Scale.

HIGH-INTENSITY INTERVAL TRAINING

The high intensity interval training will be performed in bouts of 6-10 x 1-minute bouts of bodyweight aerobic exercises interspersed with 1-minute active recovery. The number of intervals (6-10) depends on the length of the exercise session (30, 45 or 60 minutes). The HIIT protocol consists of a total of 8 different HIIT exercises and each individual exercise session will include and alternate between two different exercises. Examples of included exercises are running on the spot, jumping jacks, running with heel kicks and ski jumps. There are three variations with different intensity levels of each of the 8 HIIT exercises, which allows the exercise intensity to be individually adapted to each participant. During the HIIT exercise component, participants should reach 16-18 RPE on the Borg Scale.

RESISTANCE EXERCISE COMPONENT

Resistance exercises are automatically generated by the exercise app according to a predetermined pattern to target the whole body and get an even distribution between upper

body, core and lower body exercises. Each exercise session includes 4-8 different exercises, depending on the length of the session (30, 45 or 60 minutes), and each is performed at 2 x 12 repetitions. Each participant in the intervention group receives two elastic Therabands to use during exercise. Additional exercise equipment is not necessary, but if a participant has other equipment such as dumbbells available at home, this can be registered in the mobile exercise app before each exercise session. During the online live and the pre-recorded exercise sessions, the trainer selects the exercises are to be performed. Some examples of resistance exercises are squats, lunges, push-ups, seated row, leg raises and sit-ups. The aim of the resistance training component is for participants to reach a perceived exertion level of 14-16 RPE on the Borg Scale.

COMPLIANCE: SUPERVISED VERSUS HOME-BASED EXERCISE

In analogy to drug compliance, it is important for patients to adhere to the exercise prescription. Consequently, research has focused on strategies to enhance both attendance and adherence to exercise interventions. Technological support in the form of mobile apps is a potential sustainable strategy to improve attendance and adherence to exercise and rehabilitation programs[29]. On the other hand, the effects of exercise on health are increased if the exercise program at least initially includes supervision[30]. Health care systems unfortunately rarely have the resources to invite every patient undergoing NACT to supervised weekly exercises. To increase feasibility and reach out to as many patients as possible, distance-based approaches must therefore be included. Another advantage of home-based exercise is that it significantly reduces time and travel burden for patients who often have frequent appointments. It also improves access and equity in participation as those who live in rural and remote areas or are working or have family commitments throughout their treatment may still be able to participate. Thus, it is vital that the proposed trial investigates innovative and potentially sustainable strategies to implement exercise programs for patients with cancer.

THE MOBILE APPLICATION

Participants randomised to the intervention group will receive a prescription and instructions to download and use the training Vitala mobile application. Vitala has been developed to support patients with various physical limitations and diseases in need of medical exercise programs, in both rural and urban areas, and help them to independently create and maintain exercise routines.

The main features of the Vitala app include a Medical Exercise Generator specifically adapted to this trial and in-app self-monitoring services. Before using the app, participants receive instructions and guidance on how to set up and use the app; they also fill out an in-app health questionnaire to ensure that all of Vitala's functionalities and features are custom-tailored for each user.

The Vitala app has been developed in co-creation with patients and a cross-disciplinary research team consisting of researchers in physiotherapy, medicine, informatics and computer science, including experts in e-health. The app provides a large repository of evidence-based exercises in video formats and allows the user (in this case the trial team) to independently create an individualized medical exercise program based on preferences, disease, perceived energy levels and functional limitations. To help the patient to adhere to the program, the app offers feedback on exercise pattern, access to self-monitoring and possibility to submit questions regarding the program.

QUALITY CONTROL OF INTERVENTION

After approximately 300 included participants, Fitbit activity tracker data as well as Vitala app user data from a random sample of 50 participants from each randomization group will be extracted, 25 per group from the first two years of enrolment and 25 per group from the following two years. Groups will be compared regarding the following outcomes: 1) the proportion of exercise sessions rated by the participant as corresponding to an intensity level of at least 14-16 on the Borg scale in total and per week, 2) the number of minutes with at least moderate intensity activity levels per week ("zone minutes"), 3) the number of steps per week, and 4) group differences over time.

In Sweden only, a healthy group of 20 volunteers matched to participants such that eligibility criteria are fulfilled apart from being affected by breast cancer, will be recruited for a validation study of the exercise intervention using the Vitala app and Fitbit activity tracker twice during four consecutive weeks with several months in between. Physical testing will be performed before and after start of the four-week testing period. The same satisfaction questionnaire distributed to participants in the trial will also be completed by these healthy volunteers, and basic demographic data (age, BMI, comorbidity, medications) registered. A specific signed informed consent form will be mandatory for participants in this validation study. The outcomes of the validation study will be equal to the outcomes for the quality control described above. The performance of the exercise intervention is regarded successful if at least 75% of exercise sessions using the mobile app Vitala are estimated to result in an exercise intensity of at least 14-16 on the patient-rated Borg scale during the first week, and at least 80% of exercise sessions during the fourth week.

CONTROL

The aim of the trial is to compare the effects of an exercise program with routine care rather than to test a specific type of exercise. Thus, the control group is a routine care control group, which commonly implies brief verbal, general information about the benefits of physical activity from the treating physicians or breast cancer nurses. Participation in voluntary

training groups organised by the treating hospital is not encouraged for participants since this implies that physical activity is potentially increased to the level specified in exclusion criteria. It is important to acknowledge that individuals consenting to an exercise trial may be more predisposed to exercising and may thus continue performing physical exercise if they are allocated to the control group. To measure this potential effect, all physical activity of both groups will be digitally collected so any "contamination" in the control group can be accounted for in the analysis. Importantly, the trial pursues an active recruitment strategy in order to meet potential participants at their respective fitness level however little they may be used to exercise. The control group will not have access to trial-specific exercises via the mobile application during the duration of the trial but will be offered temporary free access after the completion of the 2-year follow-up period.

OUTCOMES, VARIABLES AND MEASURES

The **primary endpoint** pCR is measured by histopathological assessment according to the TNM classification of the American Joint Committee on Cancer (ypT0/is ypN0) after breast and axillary surgery (approx. 5 months after initiation of NACT, according to standard of care).

In order to ensure congruence in assessment of pCR between study sites, a central review of digitally scanned histopathological tumour slides will be performed at Karolinska University Hospital in Stockholm for all included cases.

The **secondary** endpoints are:

1. Residual Cancer Burden (RCB), calculated using primary tumour bed area (mm x mm), overall cancer cellularity (%), percentage of cancer that is in situ disease (%), number of positive lymph nodes and diameter of largest nodal metastasis (http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3). Categories are RCB-0 (pCR), RCB-I, RCB-II and RCB-III.
2. Objective radiological tumour response according to RECIST v1.1 criteria[31], measured as % change from largest radiological diameter of target lesion at baseline to pre-surgery (mammography or magnetic resonance tomography). No more than two target lesions are measured, which are the largest measurable lesions within the breast. Categories are: complete response (CR, disappearance of all target lesions), partial response (PR, at least a 30% decrease in the sum of the largest diameter (LD) of target lesions, taking as reference the baseline sum LD), progressive disease (PD, at least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions) and stable disease (SD,

neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started).

3. Overall, breast cancer-specific and recurrence-free survival at 2, 5 and 10 years. For overall survival, censuring is at death or at date for latest follow-up. For breast cancer-specific survival, censuring is at breast cancer-specific death or at date of last follow-up. For recurrence-free survival, censuring is at first local, regional or distant recurrence or at death or date of last follow-up. Contralateral invasive or in situ breast cancer or non-breast secondary malignancies are not counted as an event, in accordance with STEEP criteria[32]. Follow-up beyond 2 years postoperatively is collected via national or local registers or remote contact with the patient and/or the treating physician/hospital.
4. Health-related quality of life including fatigue, assessed by the EORTC QLQ-C30 and BR23 questionnaires (baseline, pre-surgery, 1- and 2-year follow-up)
5. Self-reported physical activity (Modified Godin Leisure Time Physical activity questionnaire) at baseline, pre-surgery, and 1- and 2-year follow-up, facilitating subsequent adjustment for any cross-contamination in the control group.
6. Toxicity-related outcomes:
    a. Chemotherapy completion rates, i.e. the proportion of participants receiving the planned number of treatments at full dosage regarding neoadjuvant chemotherapy (measured pre-surgery).
    b. Number of unplanned hospital admissions during neoadjuvant chemotherapy.
    c. Objective cognitive dysfunction measured by an online neuropsychological test (Amsterdam Cognition Scan)[33] (baseline and 1-year follow-up).
    d. Cardiac toxicity (defined as either left ventricular ejection fraction (LVEF) decline >15% or LVEF decline below an absolute value of 50% or clinical heart failure), to be measured by echocardiogram at baseline and after 3 months of NACT in the HER2-positive subgroup
    e. Sick leave (patient-reported percentage of sick leave as a single measurement pre-surgery and at 1- and 2-year follow-up)
7. Device-measured physical activity level/intensity and steps, assessed through the Fitbit activity tracker (baseline to 1-year follow-up).
8. Muscle strength assessed through the handgrip strength test and hypothetical 1-RM maximal leg muscle strength tests (baseline and pre-surgery).
9. Cardiorespiratory fitness assessed by the Ekblom-Bak submaximal cycle test (baseline and pre-surgery).

ADDITIONAL MEASURES

Attendance to the exercise sessions and adherence to the exercise prescription will be continually monitored and recorded. Sense of coherence measured by the SOC-13 scale and the Swedish version of the original Self-Efficacy for Exercise (SEE) scale will be used to better understand who adheres to and attends the exercise intervention.

Participant demographics, disease and medical history, body mass index, age, sex, education level, and smoking status will be recorded. Since this trial is not powered to assess survival and recurrence rates, follow-up will end two years after surgery. Register data will be used in order to investigate overall and BC-specific survival rates after 2, 5 and 10 years; if registers are not available, remote contact with the patient and/or the treating hospital/physician is planned.

At the end of the intervention, a satisfaction questionnaire is used to collect patient-reported information on the satisfaction with the intervention, satisfaction with the mobile exercise application and the exercise program.

STUDY CALENDAR

Time point	
Baseline (before initiation of NACT)	• Verify eligibility • Informed consent • Three 14G tumour biopsies* • Blood samples (50 ml for plasma, serum, whole blood) * • Faeces samples * • Full tumour biology (ER, PR, HER2, tumour grade, and Ki67) Baseline questionnaires and testing 1. EORTC QLQ-C30 (quality of life) 2. EORTC QLQ-B23 (quality of life, breast cancer) 3. Sense of coherence (SOC-13) 4. Godin Leisure Time Self-Reported Physical Activity questionnaire 5. Self-Efficacy of Exercise (SEE) questionnaire 6. Hand grip muscle strength test 7. 1-RM leg press test 8. Ekblom-Bak submaximal cardiorespiratory cycle test 9. Amsterdam Cognition Scan 10. Height/weight (BMI), body fat composition, blood pressure, patient and disease characteristics
Randomization	***Standard*: Usual care** ***Intervention*: Physical exercise intervention**

Pre-surgery	Repeat baseline questionnaires and testing (1.-2., 4.-8., 10.) Device-measured physical activity (Fitbit activity tracker) Blood (45 ml for plasma and serum) and faeces samples *. Record toxicity-related outcomes and treatment details (CRF) Satisfaction questionnaire	
Surgery	Breast and axillary surgery: endpoint pCR assessed and recorded. Tumour tissue collected and stored at trial biobank*	
Postoperative treatment	Adjuvant treatment as per clinical routine	
Follow-up (years after surgery)	**1**	**2**
EORTC QLQ-C30	x	x
EORTC QLQ-B23	x	x
Record recurrence and survival	x	x
Godin (physical activity)	x	x
SEE scale	x	
Amsterdam Cognition Scan	x	
Device-measured physical activity (Fitbit activity tracker)	x	
Self-reported sick leave	x	x

* Stockholm sites only

DATA MANAGEMENT

Data are registered using an electronic Case Report Form (eCRF) and supplementary documentation at physical testing (source data). Monitoring is performed according to Good Clinical Practice (GCP) guidelines. The eCRF provides data on patient and disease characteristics deriving from medical history, clinical, radiological and histopathological assessment, details concerning type, dose and duration of neoadjuvant and adjuvant systemic therapy, as well as histopathological results at surgery and data on follow-up. Data are managed by the Clinical Trial Office at Centre for Clinical Cancer Studies, Karolinska University Hospital, Stockholm, Sweden. Security is comparable to bank security with encrypted data.

Recorded information is pseudonymised and the key kept at each responsible site. Keys may however need to be transferred to trial staff for quality assurance purposes such as central review of pathology slides and central review of imaging. Information is confidential and the database is privacy-protected, i.e., no data can be traced back to the patient in research reports and no unauthorized individuals may have access to the data about individuals in the database. The database will be maintained until further notice (at least 20 years after inclusion of the last patient) and be reported in accordance with General Data Protection

Regulation (GDPR). The authority responsible for the database is Karolinska Institutet, Stockholm, Sweden.

The Neo-ACT trial is registered at www.clinicaltrials.gov (NCT05184582).

MONITORING AND FOLLOW-UP

This prospective trial is conducted according to GCP guidelines and monitored by the Clinical Trial Office at Centre for Clinical Cancer Studies, Karolinska University Hospital, Stockholm, Sweden, for Swedish sites, and further CROs in further countries participating in this trial. CROs will be monitoring inclusion and exclusion criteria as well as completeness and accuracy of data recorded in the eCRF by regular on-site and/or remote visits. To this end, participating units will grant access to patient medical records in due time on request. Patients are informed about monitoring procedures and medical record access in the patient information leaflet and grant their consent to these by signing the consent form.

Each patient is followed for two years after surgery regarding secondary endpoints. Follow-up can be conducted as telephone call or by post and includes access to the participant's medical record in order to check for survival and recurrence. Follow-up must be performed within +/- two months from the surgery date, and data are to be completed in the eCRF within one month from the follow-up date. Long-term follow-up regarding survival and recurrence is conducted via national registers. For patients enrolled in countries where no national registers may be used to this end, follow-up information is collected by contact with the patient and/or the treating hospital or primary care physician.

Participating sites that do not adhere to GCP guidelines or to the agreements stated in the agreements signed between the medically responsible and local investigator at the individual site and Karolinska Institutet may be excluded from this trial.

END OF TRIAL

The trial will end for each participant followed for two years after the date of surgery, but also for participants who die, withdraw consent or are lost to follow-up.

ADVERSE EVENTS

An Adverse Event (AE) is any untoward medical occurrence in a patient or a clinical investigation subject which does not necessarily have a causal relationship with the trial intervention. An AE can therefore be any unfavourable or unintended finding, symptom, or disease temporally associated with a trial intervention, whether or not related to it.

The local investigator will only document AEs of specific interest in relation to the intervention or any exercise that participants in the control groups perform without relation to the trial. **No AEs related to NACT should be reported.** Exercise-related AEs to be reported are e.g.:

- New exercise-related pain or muscle soreness (requiring a modification or interruption of the exercise intervention or causing the participant to be prescribed pain killers by a physician)

Changes in the exercise program due to recent oncological treatment or due to presence of metastases are *not* reported as AE. The local investigator assesses and records the AEs observed during the AE reporting period, which is from the date of patient consent signature until the date of surgery.

A Serious Adverse Event (SAE) (ClinO, Art. 63) is any untoward medical occurrence that results in death or is life-threatening, requires in-patient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or causes a congenital anomaly or birth defect. Local investigators make a causality assessment of the event to the trial intervention or any exercise performed unrelated to the trial in the control group (see table below). Any event assessed as possibly, probably or definitely related is classified as related to the trial intervention. **Only SAEs related to physical exercise, be it as trial intervention or as leisure activity, but not SAEs related to the underlying disease or NACT should be documented.**

Causality of AEs to trial intervention is assessed according to the following scale:

Not related	The AE is clearly not related to the trial intervention. It is independent of trial intervention, or evidence prevails that it is related to other aetiology.
Unlikely	The AE is doubtfully related to the trial intervention. Temporal association between the AE and the trial intervention and the nature of the AE is such that the trial intervention is not likely to have had any reasonable association with the observed AE (cause and effect relationship improbable but not impossible).
Possibly	The AE may be related to the trial intervention. Less clear temporal association; other aetiologies also possible.
Probably	The AE is likely related to the trial intervention. Clear-cut temporal association; a potential alternative aetiology is not apparent.
Definitely	The AE is clearly related to the trial intervention. Clear-cut temporal association, and no other possible cause.

Severity assessment: Local investigators make a severity assessment of the event according

to the Common Terminology Criteria for Adverse Events Version 5 published November 27, 2017[34].

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

A Semi-colon indicates 'or' within the description of the grade. ADL: activities of daily living
AEs have to be reported as SAE only when they are related (possibly, probably, definitely) to trial intervention. Trial intervention-related or other exercise-related SAEs in the control group are documented and reported immediately (within a maximum of 24 hours) to the Principal Investigators.

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 a general physician or a medical specialist.

ESTIMATED SAMPLE SIZE AND POWER

Patients will be randomized in a 1:1 fashion. It is anticipated that the rate of the primary endpoint pCR will be approximately 30% in the control arm. We aim to increase the rate of pCR in the experimental arm to 40%, i.e. a 10 percentage points increase, which is regarded clinically relevant since it would translate into improved disease-related outcomes. With a power of 80% and an alpha of 5%, a total of 712 patients have to be included; 356 in each arm. Accounting for a drop-out of 10%, we aim to include 790 patients. Stratification at the moment of computerized randomization will be done based on site of treatment (hospital) and biological tumour subtype (ER+HER2-, ER+HER2+, ER-HER2+, ER-HER2-).

STATISTICAL ANALYSIS PLAN

All outcomes will primarily be analysed using an intention-to-treat approach, i.e. all study subjects will belong to the treatment group (exercise intervention or control) they were assigned to, disregarding compliance. Participants who choose to withdraw consent

between randomization and the date of first NACT will however not be regarded as part of the ITT population and their data will not be reported in any analysis. As sensitivity analysis, all outcomes will also be analysed using a per-protocol approach, meaning that participants in the intervention group who comply with less than 75% of the prescribed physical exercise program or complete less than 40% of the planned neoadjuvant systemic therapy (around two 3-weekly courses) will be excluded from analysis.

Participants who cannot or do not wear the Fitbit activity tracker (technical problems, skin reaction) or who choose to not exercise using the mobile exercise app but employ own exercise sessions and methods are excluded from the per-protocol population since the adherence to the intervention cannot be assessed.

Covariate adjustment in statistical models

Guidelines from the European Medicines Agency (EMA) regarding statistics and covariate adjustments recommend that factors that are used to stratify the randomization, i.e. site of treatment and biological tumour type in this trial, should be accounted for in the statistical analysis[35,36]. However, it is also recognized that "limited numbers of subjects per centre will make it impracticable to include the centre effects in the statistical model". In this trial it is very likely that some of the centres will contribute few patients, which may cause problems in some of the statistical models, in particular for the categorical outcomes (such as the primary endpoint, pCR) where the variability in the outcome may be too small within each site. Hence, the statistical models, described below, will primarily be adjusted for biological tumour type and not for study site. If appropriate and if the data allows (i.e. no convergence problems and not too small subgroups), the site effect will also be incorporated in the models as a random effect in the mixed models and as a fixed factor in the remaining logistic regression models.

PRIMARY ENDPOINT (PCR)

The primary outcome in this trial (pCR) is dichotomous and will be assessed through histopathological examination of the surgical specimen after completed NACT. Hence, a multivariable logistic regression model, adjusting for biological tumour type (stratification factor in the randomization) will be used. The treatment effect will be assessed in terms of the resulting odds ratio between the two treatment arms together with a 95% confidence interval and a complementary Wald test. A two-sided statistical test with 5% significance level will be used. Furthermore, differences in pCR rates between the two treatment arms will be explored for each biological tumour type subgroup (ER+HER2-, ER+HER2+, ER-HER2+, ER-HER2-), using an interaction between treatment and biological tumour type.

SECONDARY ENDPOINTS

1. Residual Cancer Burden (RCB)

Residual Cancer Burden (RCB) is measured as four ordered categories (RCB-0 which corresponds to pCR, RCB-I, RCB-II, and RC-III) at histopathological assessment of the surgical specimen. An ordinal regression model, adjusting for biological tumour type (stratification factor in the randomization), will be used to analyse differences between the treatment arms. If the proportional odds assumption of the model is violated, a nominal regression model or a generalized logistic model will be used instead if appropriate.

2. Objective radiological tumour response

Response Evaluation Criteria in Solid Tumours (RECIST) will be applied in order to assess change in radiological tumour size (%) from baseline to pre-surgery imaging. RECIST criteria classify response into four ordinal outcome categories: complete response, partial response, stable disease and progressive disease (see page 18)[31]. An ordinal regression model, adjusting for biological tumour type (stratification factor in the randomization), will be used to analyse differences between the treatment arms. If the proportional odds assumption of the model is violated, a nominal regression model or a generalized logistic model will be used instead if appropriate. Tumour size at histopathological assessment of the surgical specimen may be included in comparative analyses. Mean difference in reduction of tumour size between treatment arms will be analysed with an ANOVA, including biological subtype as a factor in the model.

3. Survival outcomes

All-cause, breast cancer-specific and recurrence-free survival will be analysed at 2, 5 and 10 years. Contralateral breast cancer is not regarded as an event. Difference in time-to-event outcomes between treatment arms will be compared. The mortality outcomes are defined as time from date of randomisation to death (from any cause or breast cancer as underlying cause, respectively) or censoring at end of follow-up. For recurrence-free survival, the outcome is measured as time from date of randomisation to breast cancer relapse or censoring at end of follow-up. Survival estimates after 2, 5 and 10 years of follow-up will be compared using Kaplan-Meier estimates. Furthermore, a Cox regression model, adjusting for biological subtype will also be used.

4. Health-related quality of life

All health-related quality of life outcomes are measured as scores that range from 0 to 100, hence the outcomes are continuous. Both the global QoL score as well as the 17 subscales (8 scales reflecting symptoms and 9 reflecting function) will be assessed at four time points: baseline, pre-surgery, 1 and 2 years after surgery. To evaluate QoL scores at each time point both within and between the treatment groups, a mixed model for repeated measures

(MMRM) will be used. Treatment, visit, treatment visit interaction and stratification factor biological tumour type will be included in the model as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be evaluated, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry. Furthermore, differences in change from baseline between the treatment arms will be assessed at each time point by constructing relevant contrasts of the estimated regression coefficients.

5. *Self-reported physical activity*

Physical activity is reported using the Godin-Shephard Leisure-Time Physical Activity Questionnaire (GSLTPAQ) as total minutes per week of training in three intensity levels (light, moderate and intensive) and for weight training. Hence, the outcome is continuous. A mixed model for repeated measures (MMRM) will be used to assess physical activity at four different time points: baseline, pre-surgery, 1 and 2 years after surgery). The model will include treatment, visit, treatment-visit interaction and the stratification factor biological tumour type as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be evaluated, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry. Physical exercise will be assessed both within and between the treatment groups. Furthermore, differences in change from baseline between the treatment arms will be evaluated at each time point by constructing relevant contrasts of the estimated regression coefficients.

An additional analysis, using a dichotomous outcome that classifies patients as *active* and *insufficiently active* based on a score generated from GSLTPAQ, will be performed. Here, a generalized linear mixed model (GLMM) for repeated measures with a logit link function will be implemented. Activity will be assessed at baseline, pre-surgery and at 1- and 2-year follow-up, both within and between treatment arms. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be evaluated, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry.

6. Toxicity-related outcomes

The following toxicity-related outcomes will be assessed: a, chemotherapy completion rates, b, number of unplanned hospital admissions, c, cognitive function (measured at baseline and one year after surgery), d, cardiotoxicity and e, sick leave.

a. Chemotherapy completion rates, i.e. the proportion of participants receiving the planned number of treatments including potential adjuvant chemotherapy, is a dichotomous outcome (yes/no). Both neoadjuvant and adjuvant treatment will be assessed separately (regarded as two independent measurements) in multivariable logistic regression models, adjusting for biological tumour type (stratification factor in the randomization). The treatment effect will be assessed in terms of a resulting odds ratio and a complementary Wald test.
b. For the count outcome number of unplanned hospital admissions, we will use a Poisson regression model, adjusting biological tumour type. If overdispersion is present, a negative binomial regression will be considered instead. Results from the final model (Poisson or negative binomial, whichever fits the data best) will be presented as incidence ratios (IR) together with confidence intervals and a Wald test of the treatment effect.
c. Objective cognitive dysfunction is measured by an online neuropsychological test (Amsterdam Cognition Scan) at baseline and 1-year follow-up. The total Amsterdam Cognition Scan (ACS) score, calculated as the mean of the (reversed) z-scores of all main online neuropsychological outcome measures, will be of primary interest. Furthermore, separate z-scores of the cognitive tests will also be assessed. Hence, the measured cognitive dysfunction outcomes, measured at baseline and at 1-year follow-up, are continuous variables. A mixed model for repeated measures (MMRM) will be used to assess change from baseline of ACS scores, which is the primary interest. The model will include treatment, visit and the stratification factor biological tumour type as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be evaluated, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry. ACS scores will be assessed both within and between the treatment groups.
d. Cardiovascular toxicity is a dichotomous outcome (yes/no) that will be evaluated only in the subgroup of patients with HER2-positive tumours who are treated with anti-HER2 therapies (approximately 30% of the study population). A multivariable logistic regression model, adjusting for biological tumour type will be used to assess differences between the treatment arms.
e. Sick leave is a patient-reported singular measurement at pre-surgery and at 1- and 2-year follow-up (% sick leave). Mean sick leave proportions will be compared between the treatment arms at each time point using a mixed model for repeated measures (MMRM). Treatment, visit, treatment visit interaction and the stratification factor biological tumour type will be included in the model as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be

evaluated, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry. Additionally, since the outcome is most likely not normally distributed, a non-parametric Kruskal-Walllis test will be performed at each time as a sensitivity analysis.

7. *Device-measured physical activity level*

Several continuous outcome measures from the Fitbit activity tracker will be assessed: 1) METs (metabolic equivalents) in low, medium and high intensity activities, respectively, 2) step counts per day and week, and 3) daily active minutes at different intensity levels ("zone minutes"). The main interest is to see if there is a difference between the treatment arms regarding change of physical activity from baseline to pre-surgery (i.e. after intervention and chemotherapy), but also at 1-year follow-up. A mixed model for repeated measures (MMRM) with treatment, visit and the stratification factor biological subtype as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be tried, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry. Additionally, heart rates at equivalent exertion levels (assessed on the Borg scale) during physical activity will be explored.

8. *Muscle strength*

The muscle strength is measured on a continuous score on each arm and leg. The main interest is to see if there is a change in strength after intervention, i.e. change from baseline to pre-surgery. A mixed model for repeated measures (MMRM) with treatment, visit and the stratification factor biological tumour type as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be tried, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry.

9. *Cardiorespiratory fitness*

The Åstrand submaximal cycle test renders a continuous outcome. The main interest is to see if there is a change in cardiorespiratory fitness after intervention, i.e. change from baseline to pre-surgery. A mixed model for repeated measures (MMRM) with treatment, visit and the stratification factor biological tumour type as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the chosen structure fails to converge, the following covariance structures will be tried, in order, until convergence is reached: toeplitz with heterogeneity, autoregressive with heterogeneity, toeplitz, autoregressive and compound symmetry.

REPORTING

For the secondary outcomes, the differences between the treatment arms will be reported as an effect measure (e.g. odds ratio or mean difference) together with a complementary confidence interval and a two-sided statistical test of the effect parameter. A Bonferroni corrected significance level will be used.

HANDLING MISSING VALUES AND DEATHS

Experience from previous studies have shown that this group of patients are in general highly motivated to fully participate in trials, including answering questionnaires, undergo physical exams and other assessments. Hence, it is anticipated that the dropout rate and the number of missing values will be low in both treatment arms and that this will be a minor problem in this study. Furthermore, the number of deaths during this relatively short follow-up period is also expected to be low. Nevertheless, sensitivity analysis will be performed where missing values will be assumed to be missing at random, MAR. This assumption will be already accommodated in the mixed models where repeated measurement outcomes will be analysed. For the remaining outcomes multiple imputations, assuming MAR will be performed.

A data monitoring committee consisting of three independent experts with expertise in relevant areas, e.g., oncology, statistics and surgery or physiology/physiotherapy, will perform a blinded safety analysis after the recruitment of 400 patients or after four years, whatever occurs first. The aim of the safety analysis is to 1) assess patient safety by comparison of SAEs in both groups, 2) assess inclusion rates and trial feasibility, 3) assess the primary endpoint and give advice concerning the sample size calculation and 4) drop-out rates per group. The independent safety committee may recommend adjustments i.e., in the study protocol, outcomes and sample size, or terminating the study if a significant benefit in favour of one group is shown, such that the HR for intervention versus standard of care significantly ($p=0.001$) exceeds 1, or if the recruitment is so low that that the necessary number of events is unlikely to be reached, or if there are serious concerns about unexpected AEs in the intervention group. If the committee determines that it is safe to proceed with the study, the results of the analysis will remain unknown to everyone except the committee members.

ETHICAL CONSIDERATIONS

The original version of this trial protocol has been approved by the Ethics Committee of the Karolinska Institutet, Stockholm (Dnr 2022-02084-01).

Physical exercise during chemotherapy is deemed feasible and safe[19]. Potential adverse events, such as cardiovascular and musculoskeletal symptoms, will be recorded throughout

the NACT period and participants who experience serious adverse events will discontinue trial participation. Adverse events that are ascribed NACT but not the trial intervention will be handled in accordance to clinical routine by the responsible oncologists. There is no evidence that the intervention may lead to inferior results concerning the primary or secondary endpoints. Instead, there are data from studies in the adjuvant setting showing a preserved health-related quality of life, lower rate of self-reported symptoms related to treatment, and higher chemotherapy completion rates. In addition, this trial offers patient empowerment that is generally experienced as a positive aspect of treatment. Therefore, participation in this trial is judged ethically highly feasible.

WITHDRAWAL

Patients who wish to withdraw from the trial may do so at any time, without providing a reason. Data already included in previous eCRFs will be included in the analysis and linkage to national registers will be performed if the participant does not explicitly wish to have his/her data excluded from analysis. Ceasing participation will be recorded in the eCRF.

PUBLICATION POLICY

Before the collaborative publication of the main outcome from the entire cohort, no other publication regarding the primary outcome on the whole or parts of the cohort can be attempted. Publications of secondary endpoints or the trial protocol may be undertaken prior to the main publication. Each principal investigator is a co-author in any publication reporting on analyses from the Neo-ACT trial, that is, any report on primary or secondary outcomes, protocol, safety reports or translational substudies. Any further analysis using the full or parts of the clinical dataset from the Neo-ACT trial must first get the permission from the principal investigators. The principal investigators must in such case be permitted to take a more active part and thus fulfil the ICMJE criteria for authorship. Any investigators not fulfilling ICMJE criteria for authorship must be individually named in Acknowledgements. National coordinators are to be co-authors in any analysis as long as the data deriving from the country of their responsibility are included in such analysis. Local investigators may be invited to co-authorship based on their contribution to the trial.

TIME PLAN

Based on our experience in the OptiTrain trial, the rate of informed consent in patients up to the age of 70 years is about 50%. Participation rates may potentially rise in this trial since awareness of the benefits of physical exercise in the context of cancer has increased and since

Neo-ACT for the first time offers a real-time measurement of the anti-tumoral effects of physical exercise which may offer the participants prognostic gains. However, competing oncological trials investigating pCR rates may negatively affect participation rates: Currently, in spring of 2022, the Swedish PREDIX Luminal B trial is closed, while the ongoing Nordic Trip trial includes patients with T2-3 or node-positive triple-negative breast cancer. Further potential Swedish trials, such as the ARIADNE trial in HER2+ patients, may be initiated in the near future.

In 2020, the proposed trial sites (at the time of writing 10 Swedish and 1-7 Finnish sites) registered over 900 newly diagnosed BC patients receiving NACT. The proportion of NACT has been increasing over the last years, and a decline is not anticipated. The estimated participation rate is ca. 30%. In 2024 and 2025, international sites in Australia, Germany, Scotland, England and Canada will be opened. Accrual rates will be monitored each month and further sites may be opened if accrual drops below estimated rates. The Neo-ACT trial has opened for recruitment in December 2022, and it is estimated that inclusion may be completed in December 2027. The primary endpoint is analysed once all participants have had surgery. Follow-up will be two years per individual.

TRANSLATIONAL RESEARCH

The following translational projects are proposed. This list does not claim to be complete, and further translational projects may be added to the trial at any time point.

FAECES MICROBIOME

A growing research interest has arisen in the association between the composition of the intestinal microbiome and development and treatment-related outcomes of cancer. Especially the association between response to immune checkpoint inhibitors (ICI) and the intestinal microbiome is of great relevance, where pre-clinical and clinical studies have shown that presence of certain forms of microbiome components as well as recent and current use of antibiotics are associated with a lack of ICI treatment effect[37,38]. Intervention studies using faeces modification with the aim to change the intestinal microbiome and thereby potentially improve the response to ICI treatment are ongoing. First results are promising[39].

Physical exercise increases the number of beneficial microbial species, leads to an enrichment of the microflora diversity, and an improved development of commensal bacteria[40]. Proposed mechanisms include the release of neuroendocrine and immune-

modulatory factors, which in turn may lower inflammatory and oxidative stress, and thereby beneficially affect metabolic disorders[41].

The interplay between physical exercise, cancer and the intestinal microbiome is insufficiently explored. In a study in 15 patients who underwent resection for early-stage lung cancer, the gut-lung axis was investigated through paired faeces samples pre- and post-surgery. Changes in microbial community functional profiles were observed between both time points, as well as an association between functional capacity (VO2) and an increase in certain microbiotic species[42]. A randomised controlled trial was recently initiated exploring the impact of a three-month exercise programme for men with high-risk prostate cancer on androgen deprivation therapy on the intestinal microbiome and gut health. Gut health and gut function assessed via faecal samples is the primary endpoint whereas secondary endpoints include self-reported quality of life[43].

In Neo-ACT, clinical data on antibiotic and proton-pump inhibitor (self-reported) use up to six months pre-baseline will be registered. Faeces will be collected at baseline and before surgery. Changes in microbiome will be compared between the randomization groups and over time.

INTRATUMORAL AND CIRCULATING NATURAL KILLER (NK) CELLS

Transplantation studies show that NK cells are involved in tumor rejection and protection from relapse, supporting the therapeutic potential of NK cells in tumor eradication [44,45]. Despite these encouraging findings, NK cell therapies are limited by the lack of antigen specificity. Also, similar to T cells, resistance to NK cell-mediated killing may develop due to the recruitment and differentiation of immune suppressive cells, including regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC), and overexpression of immune inhibitory checkpoint proteins in the tumor microenvironment (TME). The adaptive NK (aNK) cell subset, defined by the expression of the maturation marker CD57, the activation receptor NKG2C, and the downregulation of several signaling molecules including PLZF, Syk, and FCεR1γ, is able to resist the TME suppression in hematological malignancies. Mechanisms that spare aNK cells from immune suppression by MDSC and Treg involves low expression of the checkpoint molecules T cell immunoglobulin and ITIM domain (TIGIT), programed death receptor (PD-1), NKG2A, and the IL-1R8 [46,47].

Samples from tumor and blood will be collected at two time points; at baseline prior to start of chemotherapy as well as at surgery. For this purpose, one fresh core biopsy and two tubes of heparin blood are utilized. If tumor biopsies are not collected, e.g., due to logistic reasons, the collection of blood samples may suffice.

The aim is to identify biomarkers/predictive markers associated with aNK cells in patients with breast cancer undergoing NACT with and without physical exercise. Examinations will enable identification of new biomarkers that determine the endpoint of NACT and long-term clinical responses. The specific aims are to characterize aNK cells in breast cancer and exploit whether NACT and physical exercise harness aNK cell memory and to investigate aNK cell interaction with other cells in the TME comparing the two trial groups.

TUMOUR GENE PROFILING

RNA and DNA will be extracted from RNAlater and/or FFPE-preserved biopsies from the primary tumour (preoperative core biopsies as well as surgically resected tumours after NACT), axillary metastases and distant/local recurrences for further analysis. Gene expression profiling will be performed to identify intrinsic subtypes, molecular signalling pathways and additional programs or genes associated with pathological response and with additional biomarkers and physical activity. DNA sequencing (exome, whole genome or targeted panels) will investigate any genomic variants.

TUMOUR MICROENVIRONMENT

Baseline presence and composition of tumour-infiltrating lymphocytes (TILs) are prognostic in triple-negative and HER2-positive breast cancers treated with neoadjuvant systemic therapy, and predictive for pCR[48,49]. TILs will be assessed by pathologists in the untreated primary tumour biopsy and the surgical specimen according to international guidelines (https://www.tilsinbreastcancer.org/). Even beyond TILs, the tumor microenvironment plays a crucial role in the response to neoadjuvant chemotherapy. Our hypothesis is that the study intervention (physical exercise) can improve pCR rates in tumors that are immunologically cold at baseline.

A study-specific tumor biopsy will be obtained from all study participants before start of neoadjuvant therapy (baseline biopsy) and will be formalin-fixed and paraffin embedded (FFPE). Similarly, tumor material will be obtained from the surgical specimen. Whole sections will be used for H&E staining and subsequently scanning for digital pathology and deep-learning image analyses. Tumor infiltrating lymphocytes (TILs) will be correlated with clinical characteristics and outcomes. Tissue microarrays (TMAs) will be fabricated and will be used for multiplex immunofluorescence assays for characterization of tumor cells, immune and other cell types in the tumor stroma both at baseline and at surgery. For this purpose. the 7-color IHC method (Opal 7 Solid Tumor Immunology Kit, PerkinElmer) using a panel of lymphocytic and macrophage markers and an additional panel of fibroblast, blood vessel and mesenchymal cell markers will be utilized. Multispectral fluorescent scanning (Vectra 3 Quantitative Pathology Systems) and image analysis with compatible software will

be used for the visualization and quantitation of the multiple markers in a spatial tissue context.

CIRCULATING TUMOUR CELLS AND TUMOUR DNA

DNA analysis will be performed in blood plasma specimens from the patients (liquid biopsy). Genomic variants will be compared in different localisations and sample types to investigate clonality and tumour evolution.

THE EFFECTS OF HUMAN ADIPOCYTES ON BREAST CANCER PROGRESSION AND METASTASIS

The worldwide prevalence of obesity nearly tripled during the past decades. Overall, in 2016 about 13% of the world's adult population were obese. Obesity rates in Sweden have also increased, with almost 20% of Swedes between 45-84 years old being obese. The global obesity rate in women is projected to reach 21% by 2025 and this is particularly alarming considering that 55% of all female cancers have an obesity-associated mechanism[50]. Obesity is defined as an abnormal excessive fat accumulation that causes a health risk. Body Mass Index (BMI) is a useful index of weight-for-height that is commonly used to classify this pathology. Obesity increases the risk for many types of cancer and is associated with poor outcomes. Despite a strong association with obesity, most current cancer treatments do not take into consideration the ongoing obesity epidemic. Whilst preventative measures, such as promoting weight loss should be conducted, often once cancer has been detected there may not be time to lose weight before treatment. As such, there is a need to develop specific drug targets that could be leveraged to address the obesity component of the disease.

In breast cancer obesity is only associated with an increased incidence of post-menopausal breast cancer, whilst obesity is a risk factor for progression in all breast cancer subtypes[51]. An important and negative predictor of cancer survival is metastasis. The mechanism underlying the metastatic spread of cancer including Epithelial-Mesenchymal-Transition (EMT), cell migration, progression, and dissemination remains unclear. The EMT is a critical tumour cell plasticity and dedifferentiation program, by which epithelial cells acquire pro-migratory and invasive mesenchymal properties. The effects of non-cancer associated adipocytes on promoting EMT and cancer progression in lean and obese individuals remain largely unknown[52].

In the Neo-ACT trial, we will acquire fresh fat tissues from the operated breast regardless of the NACT response of the individual patient. We will address the following questions as specific study aims:

1. Determine the potential effect of NACT on breast tissue fat cells senescence.
2. Determine whether adipocyte senescence level correlates with exercise and leads to different secretion patterns of various soluble factors and therefore enhances the attraction/infiltration of immune cells.
3. Determine whether factors secreted by adipocytes in resting versus exercising individuals are correlated with treatment response.
4. Determine whether patients from different BMI groups (both before and after exercises) have distinct response patterns and if adipose tissues play a role in it.
5. Identify possible biomarkers for breast cancer-associated adipocytes and their possible implications in breast cancer treatment regimen design (eg. CDK4/6 inhibitors).

The methods include basic cell and molecular experiment procedures including but not limited to culturing of patient adipocytes, staining, as well as RNAseq analysis. The results can be analysed together with the RNAseq results of biopsy/residual tumours, as well as the flow cytometry data to find connections with immune infiltration patterns. Blood testing results will be vital for subgroup patient populations and correlation analysis.

DEEP PROTEOMICS OF INFLAMMATORY REGULATORS IN CANCER

Inflammation and the escape from immune system are unavoidable events in cancer growth and progression, both in solid tumors and in hematological malignancies. Inflammatory cytokines and their receptors have an essential role as the regulator of tumor immunity and inflammation. They form a complex regulatory network where single factors play only minor roles in the broader context of inflammation-related carcinogenesis.

In most early-stage cancers, including breast cancer and colorectal cancer, perioperative inflammation has been studied traditionally by assessing a neutrophil-to-lymphocyte-ratio, which has shown significant potential as a prognostic marker. No studies combining hundreds of inflammatory biomarkers to prospective collected, highly data-intense material exist so far in breast cancer literature. This inflammatory index, consisting of hundreds of inflammatory-related regulators could have stronger predictive power than neutrophil-to-lymphocyte-ratio assessments. By studying a vast network of inflammatory cytokines, significant advantages in understanding the relationship between inflammation and cancer progression could be achieved. There are preliminary data from various cancer types that perioperative treatment with non-steroidal anti-inflammatory drugs could reduce invasion and increase survival rates in the patients with highest inflammatory status[53,54].

Through measurement of large cytokine regulatory networks from the blood stream, lung cancer and breast cancer patients have been recently reliably classified to different prognostic classes[55-57]. However, these studies used retrospective data and the number of

patients was limited. Although the published results are promising regarding their prognostic value, it has not been possible to evaluate the connection between cytokine networks and e.g. treatment toxicity in the published studies.

The unique aspect of this substudy to the Neo-ACT trial is to combine the network of hundreds of cancer-relevant inflammatory and anti-inflammatory cytokines and their receptors with a serially collected, data from the clinical trial, from patients receiving the modern oncological treatments.

Aim: Do inflammation-related and immunity-related cytokines, their receptors or other inflammation or immune response-regulating cytokines have an association with (a) the efficacy of the exercise intervention, (b) breast cancer subtype or stage, (c) the prognosis of the patients, (d) the structure of gut microbiome, or (e) the toxicity of the neoadjuvant chemotherapy?

Methods: The serum expression of inflammatory cytokines, their receptors and other cytokines will be studied using a high-throughput, multiplex Olink Proximity Extension Assay immunoassay (Olink, Uppsala, Sweden) enabling a simultaneous high-throughput analysis of 384 inflammatory biomarkers. This method allows us to study an exceptional specificity of multiple biomarkers and especially their networks. Currently, Olink offers the only commercially available Proximity Extension Assay in the market. Again, it is impossible to measure the expression of hundreds of proteins with conventional multiplexing. More specific details of the assay can be found at https://www.olink.com/products/olink-explore/olink-explore-384-inflammation/. Assessments will be done both from the baseline and pre-surgical blood samples, which allows us to evaluate not only the single values of inflammatory networks, but also the dynamic changes of the studied proteins to the outcomes defined above.

RADIOLABELED WHOLE BODY PET/CT IMAGING

This PET-imaging substudy is only performed in patients included at the University Hospitals of Turku and Helsinki. A separate patient information brochure and informed consent form is provided at these sites, and approval from the Finnish regulatory agencies will be mandatory for and all study-related procedures. The substudy is not performed in any other country and is therefore not mentioned in any other informed consent forms.

Molecular imaging offers a minimally invasive approach to visualizing physiological and tumour-specific processes throughout the body. Applications include assessing perfusion in tumours and other tissues, such as muscles, using total-body H2O-PET imaging. Examining perfusion changes in primary tumours following physical exercise may provide insights into therapy responsiveness. This technique enables comprehensive evaluation of various

tissues, including the heart, skeletal muscles, brain, fat, liver, kidneys, bones, and bone marrow. Additionally, PET imaging of vascular adhesion protein 1 (VAP-1) allows non-invasive quantification of luminal VAP-1 levels, indirectly measuring leukocyte trafficking.

The aim of this substudy is to assess changes in tumor perfusion (i.e. tumor blood flow) and local inflammation as well as in other organs assessed by whole body H2O- and VAP-1-PET imaging in relation to physical exercise.

A total of 30 patients included in the Neo-ACT trial at the university hospitals of Turku and Helsinki, Finland, are included in this substudy; 15 patients in the usual care arm and 15 in the intervention arm. PET-CT investigations are performed before the start of NACT and prior to surgery. The investigations are performed at the Department of Nuclear Medicine at the University Hospital of Turku in Finland.

At each time point, two whole-body PET/CT investigations are performed:

[^{15}O]O-H_2O

Blood flow will be measured using previously validated methods[58-60]. Positron-emitting tracer ([^{15}O]H_2O) will be produced according to the standard practises in at the Centre. A Siemens Biograph Vision Quadra total PET/CT scanner will be used for image acquisition. A dynamic scan (6 min) will be performed immediately following an intravenous injection 400 MBq of ([^{15}O]H_2O). Arterial input function will be obtained from the PET images from the heart left ventricle cavity or large artery (aorta, carotid artery) from the image. Tumor perfusion will be determined as mean tumor blood flow, and its heterogeneity as voxel variation in perfusion divided by the mean perfusion of the voxels. This imaging also yields information of arterial blood volume and blood mean transit time through tumor can also be calculated.

[^{68}Ga]Ga-DOTA-Siglec-9

The tracer, ^{68}Ga-DOTA-Siglec-9, is synthesized with automated module according to a method described by Käkelä et al., resulting to a molar activity of >20 GBq/μmol and a radiochemical purity of >95% in typical cases[61,62]. One catheter will be inserted in an antecubital vein for injection of the radiotracer. Another catheter will be inserted in the opposite antecubital vein for blood sampling to determine radioactivity concentration in plasma and metabolite-corrected input function. The patients will be placed in a supine position and arms down. After the tumor perfusion measurement, which will be performed first, ^{68}Ga-DOTA-Siglec-9 (140 MBq) will be intravenously injected as a bolus immediately followed by dynamic PET acquisition of one 104-cm field of view over 30 min. Thereafter, a static whole-body PET scan (2 bed positions; from head to toes) will be acquired. Blood samples will be taken at 1, 3, 5, 7, 10, 15, 20, 30 and 60 min after injection, plasma separated and analysed for radioactivity concentration and metabolite-corrected input.

To obtain the input function for the quantification of PET image data, radioactivity concentration in the plasma will be measured with automatic gamma counter cross-calibrated with the dose calibrator (VDC-202; Veenstra Instruments, Joure, the Netherlands) and the PET/CT scanner. For this purpose, arterialized venous blood samples will be collected into heparinized tubes before and during PET imaging, and analysed according to MET5001 of Turku PET Centre. Besides total radioactivity, plasma samples after ^{68}Ga-DOTA-Siglec-9 injections will be analyzed by radio–high-performance liquid chromatography to determine the proportion of intact tracer Other blood analyses associated with ^{68}Ga-DOTA-Siglec-9 will be determination of blood-plasma ratio and plasma free fraction (plasma protein binding) of radioactivity, which information will be taken into account for quantification of PET image data.

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