1. Neo-ACT Standard Operating Procedure

This Neo-ACT trial SOP 2.0 includes updates regarding eCRF data collection, Fitbit registration, and training modalities. Please review these changes carefully.
The SOP does not contain detailed descriptions of documents and manuals. You can always find the newest version of relevant manuals and documents on the Neo-ACT homepage.

2. RECRUITMENT

Recruitment strategies description and referral processes

- Potential candidates can be identified at multidisciplinary team conferences. The first physician to meet the patient, when the neoadjuvant chemotherapy (NACT) is decided, informs them about Neo-ACT. Informed consent can be obtained by either a physician or a nurse. Observe that there needs to be enough time to perform physical testing and obtain completed questionnaires prior to randomisation. Randomisation must take place before the first NACT administration.
- Information brochures and posters may be made available in the waiting rooms at the outpatient clinics.

2.1. The physician, contact or research nurse checks for eligibility

Use the *Patient screening form Neo-ACT* located on the homepage.

Inclusion checklist (must have all checked)

- ☐ Patient with primary invasive breast cancer cT1-T3 cN0-2 M0
- ☐ Full tumour biology (ER, HER2) available before initiation of NACT
- ☐ Written consent
- ☐ Age ≥18 years

Exclusion criteria (if one checked, then ineligible)

- ☐ 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 two 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)

Mandatory: Fill in the document *Subject Screening and Enrolment Log* (located on the homepage) for all individuals screened. Please observe that each participant is assigned a study ID at this stage; the first three digits are for your individual site, the next three digits are assigned

in order of subject screening with 001, 002, etc. This number will serve as study ID for each participant at randomisation.

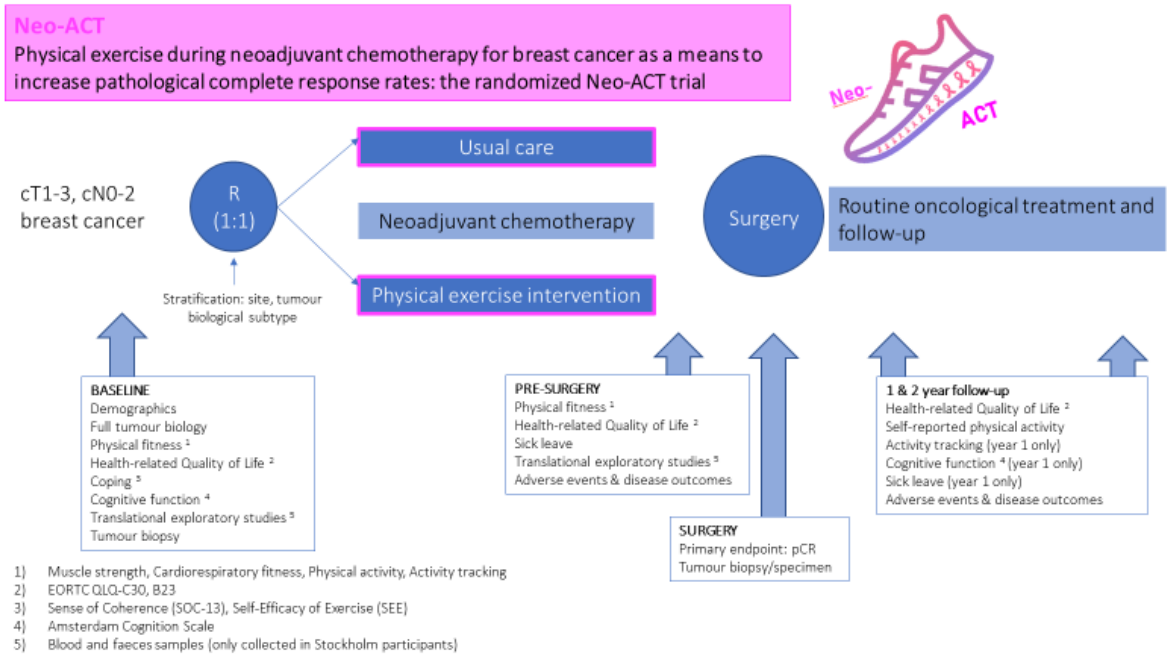
If the participant is eligible and interested in participation, hand out two copies of the participant information and informed consent form (ICF), one of which is for the participant's own records. Ask her/him to read it carefully and address any questions before signing. The ICF must then be countersigned by a physician or nurse, according to the relevant ethics permission in your country. Please note that in Sweden, there are different ICFs for sites in Stockholm (including biobanking) and outside Stockholm (no biobanking). For the sites in Finland (Helsinki and Turku), a consent form for the use of biobanking samples must be completed. In Stockholm, **blood, tumour, and faeces** samples are collected; in Finland, **blood and faeces** samples are collected.

2.2. Booking for baseline testing

After the ICF has been signed by both participant and nurse/physician, hand out the baseline questionnaire link to Dynareg and the link for the Amsterdam Cognition Scan to the participant and book her for baseline physical testing. The date for baseline physical testing must be before the first administration of neoadjuvant therapy.

Explain that the testing session will include physical tests. Inform that they need to come in suitable clothes, be well hydrated and avoid vigorous activity and alcohol 24 hours prior. Explain that they will be randomized upon completion of the testing session.

ASSESSMENT TIME POINTS



	Baseline	Pre-surgery	1 year	2 years
Demographics	X			
Full tumour biology and other disease characteristics	X			
Questionnaires Online				
Health-related quality of life (EORTC-QLQ-C30+BR23)	X	X	X	X
Sense of Coherence (SOC-13)	X			
Self-efficacy for exercise (SEE)	X	X	X	
Godin Leisure Time Self-Reported Physical Activity questionnaire	X	X	X	X
Amsterdam Cognition Scan*	X		X	
Satisfaction questionnaire – intervention group only		X		
Physical measurements				
Resting heart rate / blood pressure	X	X		
Height and weight	X	X		
Bioimpedance and body mass (optional)	X	X		
Muscle strength; Grip strength test	X	X		
Muscle strength; Hypothetical-12-RM Leg press test (optional)	X	X		
Cardiorespiratory fitness; Exercise capacity test – Ekblom-Bak	X	X		
Sick leave		X	X	
Adverse events		X	X	X
Disease outcomes		X	X	X

* Amsterdam Cognition Scan is only used in Sweden and English-speaking countries.

3. BASELINE

3.1. QUESTIONNAIRES AND PHYSICAL TESTING

3.1.1. Dynareg

The link to the baseline questionnaires (created via Dynareg, see below) is distributed immediately after receipt of the fully signed ICF. The study ID assigned each participant in the *Subject Screening and Enrolment Log* is used for registration in Dynareg. The participant receives two links via email: one link for all questionnaires (which is automatically linked to the participant's study ID) and another link for the Amsterdam Cognition Scan.

🔎 For full instructions of Dynareg, please consult the *Instructions Dynareg ePROM Neo-ACT* on the Neo-ACT homepage.

To create the baseline questionnaire link for the participant:

- Log in to Dynareg https://data.dynareg.se/neoact
- Organisation: XXX (code for site)
- Username and password are individual (distributed by an administrator at Dynareg after the SIV meeting)
- Fill in the participant's Study ID in the field **Patient ID** (XXX001, XXX002 etc) and click **OK**, then choose **Fill in new form *Baseline*** then click **SAVE**
- Click on **Information letter baseline** and copy the text and link to the questionnaire into an email and send to the patient. The patient is then able to access all questionnaires via this link.
- Completion of all questionnaires requires about 20 minutes and can be done on any digital device.

3.1.2. Amsterdam Cognition Scan

The research nurse will in conjunction with trial initiation receive an Excel sheet from the study team containing individual links for patients, each pre-named with the patient's study ID. These links can be copied from the Excel sheet and pasted into a Word document. The link can then be sent to the patient via email or printed out and handed directly to them.
It is important to explain to the patient that the test must be completed on a computer with a mouse or trackpad, not on a mobile phone or tablet. The participant should sit quietly and undisturbed while taking the test. The test takes approximately 60-80 minutes to complete and should be done without breaks.

🔎 For full instructions of the Amsterdam cognition scan, please consult the test manuals *ACS-Neo-ACT User Manual* on the Neo-ACT homepage

3.1.3. Physical testing outline

Physical testing will be conducted by a physiotherapist or exercise specialist at each site.

Equipment needed:

- Testing Recording Sheet (see homepage)
- Pen
- Computer with internet access for randomisation
- Scale for body weight (and body composition, only if available)
- Blood pressure monitor/ sphygmomanometer
- Pulse oximeter
- Pulse monitor to use during exercise capacity test
- Ergometer bike (electronic or mechanically braked)
- Chair without arm rests with a straight back (preferably placed against a wall to prevent it from moving)

- Grip strength dynamometer
- Leg press (not mandatory)
- Stopwatch to keep track of rest time for leg press test

3.1.4. Order at physical testing visit

3.2. Before the participant arrives:

1. **Check that the participant has completed the online questionnaires** in Dynareg and the Amsterdam Cognition Scan (ACS) (only **Sweden** and English-speaking sites)
   1.1 Dynareg
   - Log in to **Dynareg** https://data.dynareg.se/neoact
   - Organisation: XXX (code for site)
   - Username and password are individual for staff
   - Fill in Patient ID (same as study ID - XXX001, XXX002 etc) and klick OK to view if Baseline questionnaire has been completed ("Finished"). If not, remind the patient to answer the questionnaires or let them complete the questionnaires just before starting the physical testing.

2. **Log in to ACS**
   ACS is only performed at English- and Swedish-speaking sites.
   In the ACS online portal, you can create and manage the cognition test and generate personalized links to be given to participants.
   Use the following link to log in to the portal, using the credentials you received via email:
   https://acs-neo-act.neurotask.com/management/login

   Once logged in, you will see the ACS homepage, which contains five main tabs. The relevant tab for study sites to monitor participant status is:
   1. Tokens-name of a single test administration – listing (view), create, and upload:
      1.1 If 'results' = 0 and 'active' = 1, the participant did not open the ACS yet
      1.2 If 'results' = 32 or 33 and 'active' = 0, the participant has completed the ACS.
      1.3 If 'results' = higher than 0 and lower than 32, the participant has started but not yet completed the ACS → **It is important that participants complete the ACS before physical testing:** for the baseline ACS, that may be completed during the baseline visit. For the 1-year ACS, patients need a reminder by telephone or Email since no physical visit is planned.
   2. It is strongly recommended to check the status for a trial participant within a week after the tokens are shared. Depending on the results, we encourage contacting the participant to increase the data completeness of this test.

   Note: Some tabs or options might not be visible depending on your account's authorization

🔎 Please follow the instructions in the manual *ACS-NEO-ACT- User Manual EN* for more details.

3.3. When the participant arrives:

1. **Initial check** - Check in with the participant to see how they are feeling today. Provide an overview of the session and ask if they have any questions. If the participant has not yet completed the questionnaires and the ACS testing, provide a calm and private space where they can complete these tasks.

2. **Ask pre-testing questions** according to the *testing recording sheet*, located on the Neo-ACT homepage.

3. Physical testing should **not** be performed if the participant presents with any of the following: fever (≥38.0 °C during the last 24 hours), open wounds with or without pus, new swelling in the extremities, severe skin infections, or acute bleeding.

4. **Take blood pressure and resting heart rate**

- Participant positioned with feet flat, seated, not talking, taken after 5 mins of seated rest.
- If the patient has hypertension (systolic blood pressure >140mmHg and/or diastolic blood pressure >90mmHg), wait 1 minute and repeat the testing. Record the lower value on the testing sheet.
- Patients with severe hypertension at rest (systolic blood pressure >180mmHg and/or diastolic blood pressure >110mmHg) must not undergo the physical test and should be advised to contact their doctor.

5. **Measure height**

- Patients will stand in front of the stadiometer without shoes with their head in the horizontal plane (eyes in line with upper part of the ears)
- Assessor will slide the rod downwards until it rests on the patient's head to read the height.

6. **Body weight (and body composition if available)**

- **Weight**
  If the site does not have a bioimpedance scale, a regular scale can be used to measure weight only.
- **Body composition (bioelectrical impedance analysis)**
  - Contraindications for this test include having a pacemaker.
  - To measure body composition, follow specific guidelines for the scale provided at each site.

7. **Exercise capacity test**

Contraindications – in addition to testing contraindications described in section 3 and 4: The exercise capacity test should not be performed if any of the following are present:

oxygen saturation <95%, unstable angina or other myocardial problems within the past month, hypertension (as outlined above), or a resting heart rate above 120 bpm.

- Exercise capacity will be assessed on a cycle ergometer (electronic or mechanically braked) using the Ekblom-Bak submaximal cycle test to calculate VO2max.
- Begin by measuring the participant's resting oxygen saturation (in percent). If <95% stop testing here.

🔎 For full instructions of the Ekblom-Bak submaximal cycle test, please consult the test manuals (electronic or mechanically braked) - *Test Manual EBtest Eng electronic bike / Test Manual EBtest Eng mechanically braked bike* on the Neo-ACT homepage.

8. **Grip strength test**

Contraindications are fresh hand injuries and painful hand/foot syndrome

🔎 For full instructions on the grip strength test, please consult the test manual *Grip strength test manual* available on the Neo-ACT homepage.

9. **Hypothetical 1RM leg press test**

Contraindications are musculoskeletal injuries that may be exacerbated and severe osteoporosis.

🔎 For full instructions of the hypothetical 1RM leg press test, please consult the test manual *Hypothetical 1RM leg press test* available on the Neo-ACT homepage.

4. RANDOMISATION AND DOCUMENTATION

4.1. Randomisation

On **completion of the physical testing session,** randomise the participant directly using **ALEA Data Management (Link to ALEA on homepage or here: https://prod.tenalea.net/karolinska/DM/**). Inform the participant of randomisation assignment and provide appropriate information according to group allocation (see below). Randomisation will occur on a 1:1 basis.

🔎 For full instructions of the randomisation, please consult the *ALEA manual general English* available on the Neo-ACT homepage.

All participants, regardless of whether they are randomized to the control or intervention group, need to be provided with:

4.2. Fitbit activity tracker

Fitbit- setup

- Create a Google account (Gmail) based on the standardized instructions in the *Neo-ACT Fitbit Set-up Guide* available on the Neo-ACT homepage. Use meb-neoACT@ki.se as

recovery email address. Make sure to record and save all participants' addresses and passwords in a digital file (including a backup copy) that is stored according to GDPR and Good Clinical Practice (GCP) guidelines. Remind participants not to change their password until earliest one year after surgery.

- Help the participant to download the Fitbit mobile app and set up a Fitbit account according to the *Neo-ACT Fitbit Set-up Guide.* Connect the participant's Fitbit app with the Fitbit device.

- Provide the participant with the document *Neo-ACT Fitbit Inspire 3 Participant Information* and fill in the individual study-ID and Gmail account details. This document is available on the homepage.

- Participants are required to wear their Fitbit during the daytime; nighttime use is optional. This information is also included in the *Neo-ACT Fitbit Inspire 3 Participant Information.*

- Register the participants on the KI tool with details about the Gmail account used for the Fitbit device on this registration page: https://register.neoact.meb.ki.se/. **This step is crucial to ensure we can securely download and access participants' data.** Make sure you have the study-specific Gmail login details ready (standard format; Gmail: NeoACTxxxxxx@gmail.com, where xxxxxx = study ID; Password: NeoACT%xxxxxx%)

🔎 See the *Neo-ACT Fitbit Registration manual* for detailed instructions.

5. INTERVENTION/ CONTROL GROUP

Before the participant leaves the baseline session **and regardless of whether they are randomised to the control or the intervention group**, remind them that they will be invited back for re-testing after neoadjuvant therapy but before surgery (pre-surgery visit). Prior to this visit they will receive a link to complete questionnaires online.

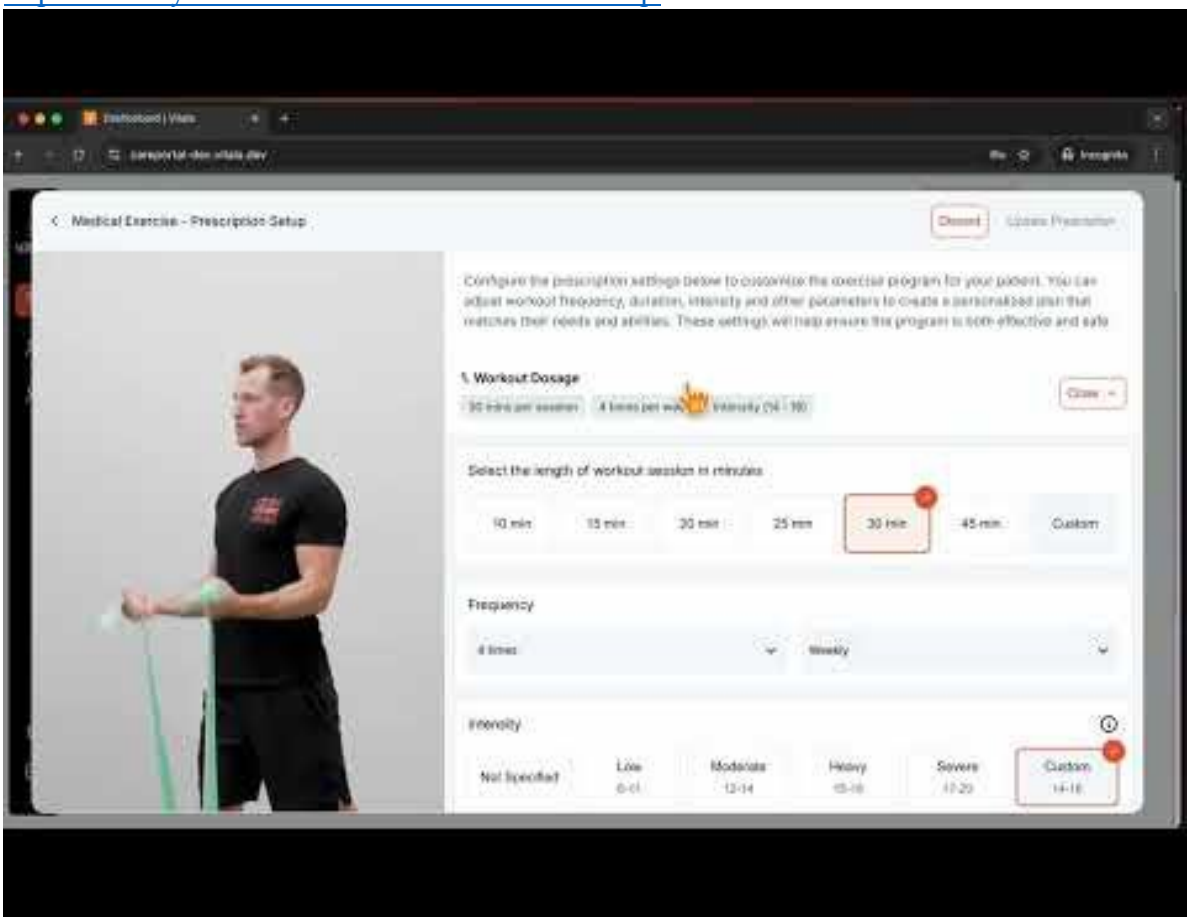
5.1. If randomised to the intervention group:

Go through the different exercise intervention modalities and provide the participant with the intervention package including:

5.1.1. Vitala App

- Help the participant to download the Vitala app to their mobile phone.

🔎Prescribe exercise to the participant in the Care portal of Vitala according to the manual: https://www.youtube.com/watch?v=xYFNdw-Lsqs



- Briefly show how to use the Vitala App.

5.1.2. Online pre-recorded sessions and online live sessions

Encourage participants to make use of the online sessions (both pre-recorded and live remote sessions), where they can receive personalised instruction and support by a trainer (live remote session). Instruct them to record all exercise sessions that are performed without the Vitala app in the training diary. Provide the participant with the written information document and send an email with the links to the study-specific Gmail account (see section "Documents to Share with the Intervention Group" below).

Provide the following to participants in the intervention group

5.1.3. Resistance bands

At the baseline visit, each participant in the intervention group will receive two green resistance bands (~1.65m each) to take home. The resistance bands are packaged in containers with approx. 5-m band per container. Cut each 5-m band into three equally long pieces, approx. 1.65 m each. In case a participant loses or damages a band, replace it.

5.1.4. Borg RPE scale

Explain that the target exercise intensity in the trial corresponds to levels 14–18 on the *Borg RPE scale.* All participants in the exercise intervention group will receive a printed version of the Borg RPE scale to take home (included in the document "*Training Diary for Online Sessions,*" see section below or on the homepage under the name *Borg RPE scale*).

5.1.5. Adverse events

Please inform participants about what to do in case of an adverse event:

- Provide each participant with the Adverse Event Logbook. Instruct them to document any adverse events related to intentional exercise that occur between inclusion and the pre-surgery follow-up in this logbook — for example, muscle strains, joint pain, falls, dizziness, etc. Participants should be asked to bring the logbook to the pre-surgery follow-up visit.
- In case of a Serious Adverse Event (SAE) - defined as any untoward medical occurrence during exercising that results in death or is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or causes a congenital anomaly or birth defect—local study site staff must be informed immediately.
- Local study site staff should assess and record the SAE; document it, using the designated form (located on the homepage: *Neo-Act SAE form Final 2022-09-26)*, and send it to Yvonne Wengström, Coordinating Investigator for Exercise, at Karolinska Institutet (meb-neoACT@ki.se) within 24 hours after becoming aware of the event.

🔎 More detailed information can be found in the SAE Manual on the Neo-ACT homepage *Neo-ACT SAE Instruction Site Final_20220928_EN.*

Documents to share with the intervention group:

- Exercise Intervention Information is available in the following languages: English, Swedish, German and Finnish.
- Remember to send an Email to the participant's study-specific Gmail address with the links to the online sessions. An Email template is available in a separate document titled "*Email Template – Links to Participants in the Exercise Intervention Group*."
- Training diary for online sessions (including the Borg RPE scale)
- Adverse Event Logbook
- Neo-ACT Fitbit Inspire 3 Participant Information
- Informative flyers if available, e.g., about live sessions

5.2. If randomised to the control group

Inform participants that they will receive free access to the app for 8 weeks starting at the 1-year follow-up (one year after surgery). The local study nurse can repeat this information and give access at the same time as the participant receives the links to the 1-year questionnaires. If

the research nurse does not have access to the Vitala prescription, the physiotherapist at the local study site should be contacted to facilitate this.

6. DOCUMENTATION

After the participant has left, enter all data into the baseline eCRF.
A manual with instructions for data entry can be found on the Neo-ACT homepage under *eCRF Data Entry Instructions.* Please note that the paper copy of the *testing recording sheet* (all pages marked with the participant's study ID and date) must be stored locally.
Please note that three variables have been added under the Exercise Capacity Test in the eCRF in May 2025. Details about these additional measures can be found in the latest version: *eCRF Data Entry Instructions v1.5.*

7. INTERVENTION

7.1. Exercise program

Participants in the intervention group will complete ≥120 min structured physical exercise per week from enrolment to surgery (approx. five months). The exercise program consists of resistance training and aerobic high-intensity interval training (HIIT) at a moderate to high intensity, targeting 14-18 on the Borg RPE Scale. All training sessions (Vitala, Youtube-Sessions, remote live sessions) have the same structure:

1) Warm-up
2) Resistance training: Exercises for the upper body, lower body and core. There are always 2x12 repetitions per exercise and 4-8 different exercises, depending on the length of the session.
3) High-Intensity Interval Training (HIIT): Exercises with a focus on training the cardiovascular system (raising your heart rate). This section consists of 1-minute-high intensity intervals with 1-minute rest in between. Three different intensity levels for each exercise are available. There are 6-10 intervals in each session, depending on its total duration.
4) Cool down and stretch

The Neo-ACT exercise training intervention can be completed in three ways:

- Via the mobile training app Vitala (30, 45 or 60 min)
- Via online live-remote training sessions (instructor-led in real time: 30 and 45 min)
- Via pre-recorded online training sessions (30 and 45 min sessions)

Participants can combine the three different exercise options and freely choose their exercise days to achieve the target of ≥120 minutes per week. **In addition to the ≥120 minutes of structured training, participants are instructed to complete an additional 150 minutes of moderate physical activity per week, such as walking, cycling, or similar activities**.

Participants are advised to avoid moderate-to-vigorous exercise for 24 hours following chemotherapy administration, although light activities such as walking are permitted.

7.2. Adherence

On a weekly basis, the local physiotherapist or exercise specialist should monitor participants' adherence to the exercise intervention. Completed exercise sessions in the Vitala app are automatically recorded and can be viewed in the Vitala Care Portal (under "Patients list" → select the participant → "Compliance X%" is displayed in the "Medical Exercise" box).
To monitor adherence to the online exercise sessions (both live-remote and pre-recorded), participants should be personally contacted to verify their logged sessions. For now, participants are instructed to fill out a training diary for live-remote and pre-recorded sessions and bring the completed form to the pre-surgery follow-up. In Germany and Finland, trainers of the live-remote sessions send the attendance list (Study ID/ date of session) + Borg RPE scale to meb-neoACT@ki.se. Trainers have been provided with a form for this purpose.
If a participant has lower than desired adherence to the exercise intervention, contact the participant to find out the cause and if the participant needs any help or support. If participants require adjustments to the training plan, please review the prescription with them. You can remove specific exercises or refer them to the online live sessions with a trainer for advice.

8. Pre-surgery

8.1. Questionnaires

- **One week before the physical testing**, questionnaire links corresponding to the pre-surgery visit must be emailed to the participant. **Confirm that questionnaires have been completed before the participant comes for physical testing.** If the questionnaires have not been completed, remind the participant to complete them before arriving for the physical test or provide an opportunity to do so on site just before starting the physical testing.

Dynareg:

- Log in to **Dynareg questionnaire database**, select the participant by entering the **Patient ID** and choose the correct questionnaire link. Open the **information letter**, copy the text and link, and include them into a mail to be sent to the participant.
- Also, per Email (or phone), remind participants to bring the adverse event (AE) logbook and the training diary to the pre-surgery physical testing visit.

8.2. Submission of Training Diary and AE Logbook

Please upload a copy of the training diary and the AE logbook on SharePoint, a secure cloud service provided by KI, after the pre-surgery visit. You will receive access to a specific folder via a link sent by email. Please ensure all documents are named with the correct study ID and date. The paper copy of the training diary and AE-logbook (all pages marked with the participant's study ID) must be stored locally.

8.3. Testing

The pre-surgery visit involves the same physical tests as at baseline.

9. 1-year follow-up

9.1. Questionnaires

- It is recommended that each site designates a staff member responsible for tracking the one-year follow-up investigations, as these are not linked to a physical clinic visit.
- Questionnaire link corresponding to 1-year follow-up must be emailed to all the participants.

Dynareg:

- Log in to **Dynareg questionnaire database**, select the participant by entering the **Patient ID** and choose the correct questionnaire. Open the information **letter**, copy the text and link, and include them into an Email to be sent to the participant.
- **Amsterdam Cognition Scan (if applicable at your site):** The code for the test is retrieved from the Excel file and provided to the patient. Each patient thus receives two codes corresponding to the two time points when the ACS is performed, baseline and 1-year follow-up.
  It is recommended to monitor the completion of the ACS and send a reminder to the participant if the test has not yet been completed.
- Check whether participants have completed the questionnaires and ACS if applicable within two months before or after their due date (i.e. one year after surgery) and send reminders if needed.

9.2. Password for Fitbit

At the one-year follow-up, participants may change their Fitbit password. However, it must be ensured that the central study team has already accessed sufficient activity tracking data before any password changes. If in doubt, please contact the central study team before changing the password. After this point, the study team will no longer download or access Fitbit data. The Fitbit remains in the participant's possession.

9.3. Give access to the Vitala app to participants in the control group

- Provide participants in the control group with 8 weeks of access to the Vitala app. The exercise prescription for control group participants is created in the Vitala Care Portal following the intervention manual (https://www.youtube.com/watch?v=xYFNdw-Lsqs), **with a few modifications:**

  - In the "researchID" field, enter the participant's study ID followed by "CONTROL" (e.g., xxxxxxCONTROL).
  - At step 5 – Prescriptions, click "Edit" under "Medical exercise," set the prescription period to 8 weeks, and uncheck the box labeled "Allow the patient to renew this prescription independently when expired."

10. 2-year follow-up

- Questionnaire link corresponding to 2-year follow-up must be sent out by email:

10.1. Dynareg

- Log in to **Dynareg questionnaire database**, select the participant by filling in the **Patient ID** and select the correct questionnaire. Open the information **letter**, copy the text and link, and include them into an Email to be sent to the participants.
- Check whether participants have completed the questionnaires within two months before or after their due date (i.e. two years after surgery) and send reminders if needed.