ONCOFIT

Statistical Analysis Plan

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1. General information of the ONCOFIT study

1.1. Background and rationale

Most patients with colon cancer undergo surgery due to its potential for a cure. However, this procedure yields a stress response usually accompanied by postoperative complications. While prehabilitation programs are emerging as effective approaches to mitigate post-surgical disturbances, it is still unknown whether a multidisciplinary prehabilitation and postoperative rehabilitation can further reduce the incidence of postoperative complications.

1.2. Objective

The overall objective of the ONCOFIT randomized controlled trial is to determine the effects of a multidisciplinary prehabilitation (i.e., 4 weeks before surgery) plus postoperative rehabilitation (i.e., 3 months after surgery) program (PPP) on postoperative complications (as measured by Comprehensive Complication Index (CCI)) in patients undergoing resection of colon cancer.

The secondary aims are to:

(i) Examine the effects of PPP compared to usual care on clinical prognosis.
(ii) Exemine the effects of PPP compared to usual care on functional capacity.
(iii) Examine the effects of PPP compared to usual care on body composition.
(iv) Examine the effects of PPP compared to usual care on fecal microbiota composition.
(v) Examine the effects of PPP compared to usual care on patient-reported outcomes.
(vi) Examine the effects of PPP compared to usual care on clinical and tumor parameters.
(vii) Examine the effects of PPP compared to usual care on lifestyle patterns.
(viii) Examine the effects of PPP compared to usual care on sleep quality.

2. Study methods

2.1. Trial design

ONCOFIT is a single blinded one-arm randomized controlled trial (RCT). The RCT will include (i) a control group that will follow the usual care pathway (ERAS protocol) and

(ii) an intervention group, performing a multidisciplinary PPP including: (i) supervised concurrent training; (ii) dietary behavior change, and (iii) psychological support. A total of 90 patients with colon cancer undergoing surgery will be recruited. Measurements will be conducted at baseline, preoperatively (i.e., 1 day before surgery); 3 months after surgery and 1 year after surgery. More details are provided in the study protocol (1).

The ONCOFIT research group will perform the data quality control, data processing, writing analyses scripts/programs and statistical analysis.

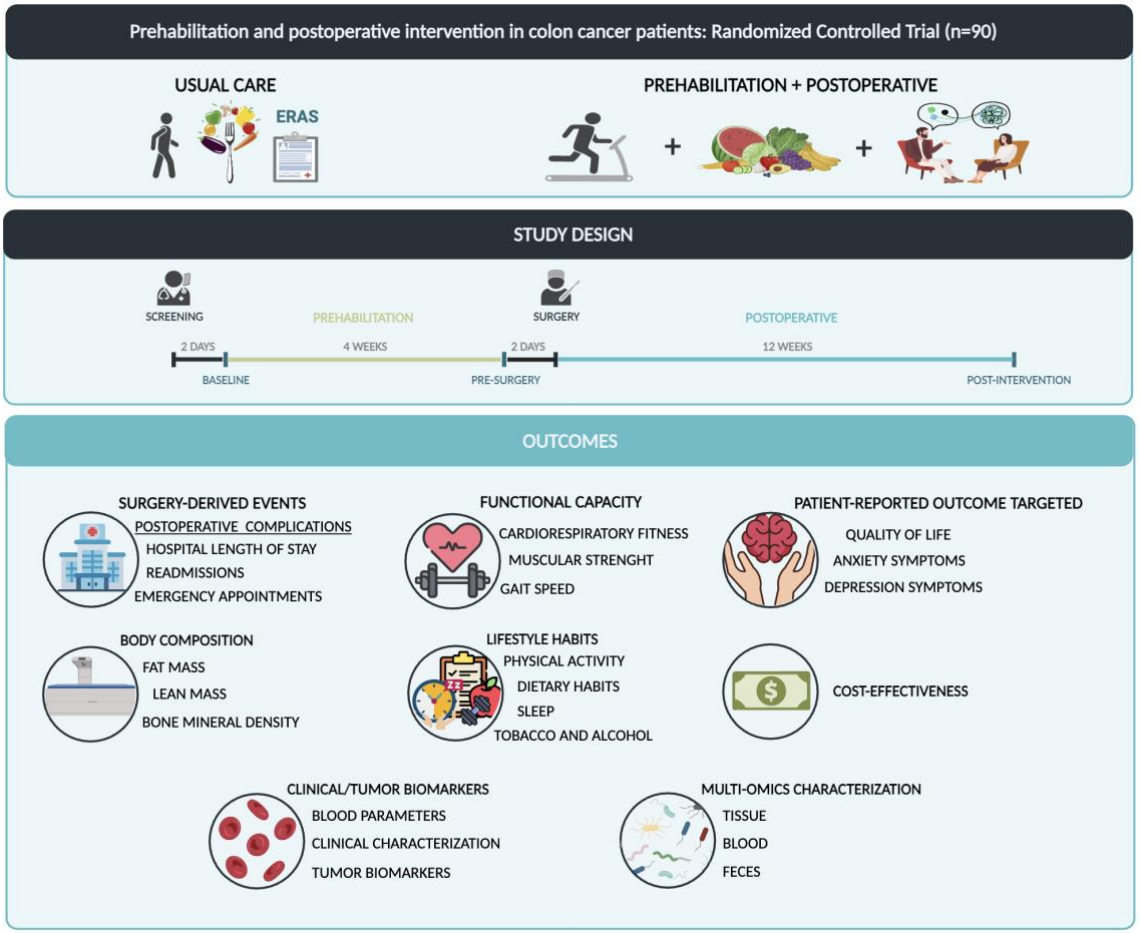

Figure 1. Overview of the ONCOFIT randomized controlled trial.

2.2. Randomization

After concluding baseline assessments, eligible participants will be randomly assigned (1:1 allocation) to either the control or intervention group by means of computer generated simple, unrestricted randomization.

2.3. Sample size

In a previous study, a 17,3-point mean difference was detected in CCI between a group of patients who were physically inactive and a group who were regularly active, both undergoing colorectal cancer surgery (2). Therefore, this study sample size was estimated for an α level of 0.05 and 90% power to detect a 17.3-point difference in CCI (primary outcome) between usual care and PPP groups, considering an σ of 25.2 (the CCI standard deviation of the group of participants who were physically inactive). Based on this data, a sample of 36 participants per group was considered sufficient for our analysis. However, assuming a 20% drop-out rate, we decided to recruit a total sample size of 45 participants for each study group. Thus, a total of 90 participants ($n \approx 45$ women) will be enrolled in the ONCOFIT study.

2.4. Framework

No superiority hypothesis testing framework will be used.

2.5. Statistical interim analyses and stopping guidance

No formal interim analyses of the primary outcome were pre-specified in the study protocol; thus, no formal stopping guidance has been established. However, basic descriptive analyses have been conducted after the inclusion of the first 20 participants, specifically to support grant applications and funding justification. These preliminary analyses were limited to feasibility indicators and secondary outcomes, and were not used to assess efficacy. Additionally, preliminary descriptive results for selected secondary outcomes were presented at national and international scientific conferences. Importantly, none of these analyses involved inferential testing of the primary outcome, and no modifications to the study protocol, including endpoints or procedures, were made because of these preliminary findings.

2.6. Timing of the final analyses

The final analyses will be performed after the finalization of the data collection and processing of the primary and some secondary outcomes.

2.7. Timing of outcome assessment

Primary outcome (CCI) and other clinical prognosis outcomes will be assessed 30 days postoperatively, 3 months after the surgery and 1 year after the surgical procedure. All other secondary outcomes will be measured at baseline, preoperatively (i.e., 1 day before surgery), 3 months after surgery, and 1 year after surgery.

3. Statistical principles

3.1. Confidence and P values

All statistical tests will be two-tailed, with a significance level set at $\alpha = 0.05$. Corresponding 95% confidence intervals will be reported. No adjustments for multiplicity will be applied, as only one primary outcome has been pre-specified and all secondary and exploratory analyses are considered hypothesis-generating.

3.2. Adherence, attendance, compliance and protocol deviation

Attendance will be defined as the % of sessions attended by the participants (recorded by trainers) divided by all the offered sessions (varying on patient preoperative and postoperative periods duration). Compliance will be defined as the % of sessions in which the amount of time in the target intensities is reached divided by the total exercise sessions with valid data excluding the familiarization and the early postoperative phases.

All protocol deviations will be reported and described.

3.3. Analysis population

We plan to use two analyses approaches:

(i) Intention-to-treat: This dataset will be used for the primary analysis of all outcomes and will include all the randomized participants. They will be analyzed based on the group they were originally allocated.

(ii) Per-protocol analyses: This dataset will be used for secondary analyses and will include participants with >80% of attendance.

4. Trial population

4.1. Screening data

Screening data will be based on patients that are defined as eligible by the clinical and research team. Screening will be performed in three phases:

(i) Screening based on the eligibility criteria (see '*4.2 Eligibility'*) by the clinicians/surgeons of the research team.

(ii) Screening based on phone call by the research team.

(iii) Screening during the baseline assessment.

The number of participants in each phase will be reported.

4.2. Eligibility

Eligible patients will be defined based on the inclusion and exclusion criteria. In general, colon cancer patients, older than 40 years old, undergoing a surgical procedure will be considered for eligibility. Specific inclusion and exclusion criteria are:

Inclusion criteria:

1. Patients older than 40 years old.
2. Diagnosis of nonmetastatic colon cancer (i.e., including right, transverse, left, sigmoid, subtotal, total and hemicolectomy).
3. Not participating in a nutritional/dietary intervention.
4. Being physical inactive (i.e., not to be participating in any physical exercise program in the last 3 months or performing less than 600 metabolic equivalents (METS)/week of moderate-vigorous physical activity).
5. To be capable and willing to provide informed consent.
6. Not to suffer from any specific condition that may impede testing the study hypothesis or make it unsafe to engage in the multidisciplinary intervention.

Exclusion criteria:

1. Medical contraindication for being engaged in an exercise or dietary program.
2. Additional surgery planned within the 3-month intervention.
3. History of another primary invasive cancer.
4. Planning to receive adjuvant chemotherapy.
5. To be pregnant.

6. To present any of the following clinical condition: (i) myocardial infarction or coronary revascularization procedure within prior 3 months; (ii) uncontrolled hypertension (i.e., systolic ≥ 180 mmHg or diastolic ≥ 100 mmHg); (iii) uncontrolled arrythmias; (iv) valvular disease clinically significant; (v) decompensated heart failure or (vi) to suffer from known aortic aneurysm.

4.3. Recruitment

Information concerning the CONSORT flow diagram will be collected. During the enrolment phase, we will record the number of patients assessed for eligibility, those excluded (with the specific reason), and the total number of participants randomized. In the allocation phase, we will report the number of participants in each group, as well as those who received or not the allocated intervention. During the follow-up, we will report the number of participants who lost to follow-up and the number who discontinued (plus reason). Finally, the number of participants included in the final analyses will be described.

4.4. Withdraw/follow-up

The number and reason of withdraw will be collected and reported by the research team.

4.5. Baseline patients' characteristics

A baseline table will be created to describe the characteristics of the study population. The characteristics include general characteristics (e.g., age or sex), anthropometry and body composition (e.g., body weight, height, body mass index or waist circumference), cardiometabolic health (e.g., glycaemic or lipid profile), physical fitness (e.g., cardiorespiratory fitness or muscular strength), tumour stage and surgery characteristics (e.g., type of surgery or duration of surgery). The characteristics of the total study population and each study arm will be summarized using mean (SD) or median (interquartile range) for normally and not normally distributed continuous variables, respectively, and as number (percentage) for categorical variables.

5. Analysis

5.1. Outcomes definition

Primary outcomes

CCI obtained by patients at 30 days and 3 months after the surgical procedure.

Secondary outcomes

1. **CCI** scores obtained at 1 year post-surgery.
2. **Proportion of patients experiencing at least one complication** within 30 days, 3 months and 1 year post-surgery.
3. **Proportion of patients experiencing at least one medical complication** within 30 days, 3 months and 1 year post-surgery.
4. **Proportion of patients experiencing at least one surgical complication** within 30 days, 3 months and 1 year post-surgery.
5. **Hospital readmissions occurring** within 30 days, 3 months and 1 year post-surgery.
6. **Urgency visits** within 30 days, 3 months and 1 year post-surgery.
7. **Hospital length of stay**, defined as the total number of days the patient remains admitted in the hospital following the surgical procedure.
8. **Quality of Recovery Short-form Questionnaire (QOR-15)** measured 24, 48 and 72 hours after the surgical procedure.
9. **Change in body weight** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Body weight will be measured by a digital scale.
10. **Change in body height** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Body height will be measured by a stadiometer.
11. **Change in fat mass** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Fat mass will be assessed by Dual-energy X-ray Absorptiometry (DXA).
12. **Change in fat free mass** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Fat free mass will be assessed by Dual-energy X-ray Absorptiometry (DXA).
13. **Change in bone mineral density** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Bone mineral density will be assessed by Dual-energy X-ray Absorptiometry (DXA).
14. **Change in waist circumference** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Waist circumference will be measured following the procedures outlined by the International Society for the Advancement of Kinanthropometry.
15. **Change in hip circumference** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Hip circumference will be measured following the procedures outlined by the International Society for the Advancement of Kinanthropometry.
16. **Change in neck circumference** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Neck circumference will be measured following the

procedures outlined by the International Society for the Advancement of Kinanthropometry.

17. **Change in systolic blood pressure** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Systolic blood pressure will be assessed by blood pressure monitor.
18. **Change in diastolic blood pressure** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Diastolic blood pressure will be assessed by blood pressure monitor.
19. **Change in cardiorespiratory fitness** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Cardiorespiratory fitness will be measured by the distance done in 6 minute walking test.
20. **Change in upper limb muscular strength** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Upper limb muscular strength will be measured using a digital hand dynamometer.
21. **Change in lower limb muscular strength** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Lower limb muscular strength will be assessed by the 30 seconds sit-to-stand test.
22. **Change in lower limb muscular strength** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Lower limb muscular strength will be assessed by the 5 times sit-to-stand test.
23. **Change in gait speed** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Gait speed will be determined by the 4-meter usual gait speed test.
24. **Change in Health-related quality of life** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Health-related quality of life will be assessed through The European Organisation for Research and Treatment of Cancer quality of life questionnaire for patients with colorectal cancer (EORT-QLQ-CR29).
25. **Change in self-reported depression levels** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Self-reported depression levels will be measured by Back Depression Inventory-II questionnaire and Hospital Anxiety and Depression Scale.
26. **Change in self-reported anxiety levels** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Self-reported anxiety levels will be measured by State-Trait Anxiety Inventory and Hospital Anxiety and Depression Scale.
27. **Change in mental adjustment to cancer** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Mental adjustment to cancer will be assessed using the Mini-Mental Adjustment to Cancer questionnaire.
28. **Change in glycaemic profile** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess glucose and insulin levels.
29. **Change in lipid profile** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess total cholesterol, HDL-Cholesterol, LDL-cholesterol and triglycerides levels.

30. **Change in immunological blood profiles** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess Leukocytes (10*3 uL), Neutrophils (10*3 uL), Lymphocytes (10*3 uL), Monocytes (10*3 uL), Eosinophils (10*3 uL), Basophils (10*3 uL) and LUC cells (10*3 uL).
31. **Change in inflammatory factors** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood simples will be used to assess C-Reactive protein, leptin, adiponectin, resistin, IL-6, IL-10
32. **Change in renal function parameters** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess creatinine and urea.
33. **Change in tumour markers** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess CA19-9, CA 242, CA 72-4 and CEA.
34. **Change in hormones** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Blood samples will be used to assess T3, T4, testosterone, cortisol, oestrogens and DHEAs.
35. **Change in energy intake** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Energy intake (kcal/day) will be assessed by food frequency questionnaire (FFQ).
36. **Change in carbohydrates intake** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Carbohydrates intake will be assessed by FFQ.
37. **Change in fat intake** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Fat intake will be assessed by FFQ.
38. **Change in protein intake** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Protein intake will be assessed by FFQ.
39. **Change in dietary habits** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Dietary habits will be assessed by food frequency questionnaire (FFQ).
40. **Change in subjective sleep quality** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Subjective sleep quality will be assessed by the Pittsburgh Sleep Quality Index (PSQI).
41. **Change in objective sleep quality** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Subjective sleep quality will be assessed by accelerometery.
42. **Change in physical activity patterns** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Physical activity patterns will be assessed by accelerometery.
43. **Change in faecal microbiota composition and diversity** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Faecal microbiota composition and diversity will be determined by DNA sequencing.
44. **Change in heart rate variability** (Baseline, preoperatively, 3 months after surgery and 1 year after surgery). Heart rate variability will be measured with the

participant lying in a supine position, with R–R signal being assessed for 15 min using the Polar Ignite 2.

45. **Tumour tissue analyses.** Colon tissue will be collected during the surgical procedure.

5.2. Analyses methods

The main analyses for primary and secondary outcomes will follow the intention-to-treat approach. For the primary outcome, given the commonly observed non-normality distribution of the data in this fields, a Mann-Whitney U test will be used to compare the median CCI between the intervention and control groups. For secondary outcomes with a single time-point (e.g., hospital lenght of stay), comparisons between groups will also be conducted using Mann-Whitney U test. To assess whether the group allocation (intervention/control) influences the risk of postoperative complications (yes/no), a binary logistic regression model will be conducted. Regarding secondary outcomes at multiple time points, a linear mixed-effects models with individual measures of growth being modelled as the function of randomly assigned group, assessment time, and the interaction between group and time. Estimations will be performed using the restricted maximum-likelihood method, including an unstructured covariance matrix to adjust for within-participant clustering resulting from the repeated-measures design. This model will assume that missing values are missing-at-random.

Secondary analyses will be performed using the per-protocol approach, defined as attending >80% of the sessions offered to the intervention group.

5.3. Missing data

The number of missing data will be reported, and patterns of missing data will be explored. Based on previous experience, no missing data are expected for prognostic variables, as these will be extracted from medical records and used unless the patient explicitly revokes consent. For other outcomes, we expect that missing data will be assumed as missing at random. Therefore, the linear mixed model analyses will appropriately account for missing data. Nonetheless, once data processing has been completed, we will formally re-evaluate this assumption.

5.4. Additional analyses

Changes in other outcomes will be analyzed using a similar protocol as described by '*5.2 Analysis methods*' unless others would be more appropriate depending on the outcome. Moreover, cross-sectional analyses will be performed using the baseline data of the RCT. For example, cross-sectional associations will be explored by bivariate and partial correlations. Then, further analyses will be performed using linear and logistic requestion depending on the nature of the study variables and research questions. ANOVAs will also be used to test differences in outcomes among groups.

5.5. Harms

The number and reasons of adverse events (e.g., falls, injuries, musculoskeletal problems, major cardiovascular disease events, or any other events potentially related to the implementation of the trial protocol) at each time point will be collected, reported, and described separately for each study arm. No formal statistical testing will be undertaken.

5.6. Statistical software

The analyses will be performed using R.

6. References

1. Amaro-Gahete FJ, Jurado J, Cisneros A, Corres P, Marmol-Perez A, Osuna-Prieto FJ, et al. Multidisciplinary Prehabilitation and Postoperative Rehabilitation for Avoiding Complications in Patients Undergoing Resection of Colon Cancer: Rationale, Design, and Methodology of the ONCOFIT Study. Vol. 14, Nutrients. MDPI; 2022.
2. Onerup A, Angenete E, Bonfre P, Börjesson M, Haglind E, Wessman C, et al. Self-assessed preoperative level of habitual physical activity predicted postoperative complications after colorectal cancer surgery: A prospective observational cohort study. European Journal of Surgical Oncology. 2019 Nov 1;45(11):2045–51.