D4.1. – First study subject approvals package for the prospective study

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3	PANEPISTIMIO IOANNINON	UOI	GREECE
4	ETHNIKO KAI KAPODISTRIAKO PANEPISTIMIO ATHINON	NKUA	GREECE
5	REGION STOCKHOLM (KSBC)	KSBC	SWEDEN
6	ONKOLOSKI INSTITUT LJUBLJANA	IOL	SLOVENIA
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Statement of Originality

This deliverable contains original unpublished work except where clearly indicated otherwise. Acknowledgement of previously published material and of the work of others has been made through appropriate citation, quotation or both.

Disclaimer

Compliance of Deliverable 4.1 with the Description of Action

DoA Task description[1]	Addressed by D4.1
Task 4.1. Design of the prospective study, Ethics application and approval **Subtask 4.1.1: Study protocol finalization.** Subtask 4.1.2 (M6): Submission of the documentation package to competent ethical and regulatory Boards. More specifically, from the Essential Information for clinical studies: Annex 1: Mandatory deliverables for clinical studies **'First study subject approvals package'** (prior to enrolment of first study subject): Final version of study protocol as approved by first regulator / ethics committee(s)	• Section 2. Prospective Clinical Study protocol, pages 10-36
a. Registration number of clinical study in a WHO- or ICMJE- approved registry that also allows later posting of study results.	• Section 5. Registration of the Prospective Clinical Study, page 41
b. Approvals required for invitation / enrolment of first subject in at least one clinical centre: ethics committees, and copies of opinion or confirmation by the competent Institutional Data Protection Officer. If the position of a Data Protection Officer is established, its opinion/confirmation that all data collection and processing will be carried out according to EU and national legislation	• Section 6. Approvals required for enrolment of the first study subject, page 42

[1] The information that the First study subject approvals package shall provide is described in Annex 1: Mandatory deliverables for clinical studies of the Essential Information for clinical studies template: https://ec.europa.eu/research/participants/data/ref/h2020/other/legal/templ/h2020_tmpl-clinical-studies_2018-2020_en.pdf

Executive summary

The current document (**D4.1**) reflects the work done within the framework of **Task 4.1** of the CARDIOCARE project, "**Design of the prospective study, Ethics application and approval**" (M1-M12). The task aims at preparing all the documents requested by competent, ethical boards and obtaining the ethical clearance to conduct the study from regulators and ethics committees of the clinical study partners.

In particular, D4.1 concerns the prospective clinical study protocol finalization. With the support of IMS and the clinical partners, the study Principal Investigator (IEO) finalized the study protocol based on preliminary information already provided in the document "Essential Information for clinical studies". Analytical definitions of all aspects of the clinical study have been defined in close collaboration and interaction with WP1 and the technical partners (UOI. FORTH, HMU). Indeed, the prospective study will collect data decided coherently with the retrospective study and with the Psychological Outcome Minimum Data Set created in Task 1.2 and implemented in the ePsycHeart mobile application (Task 1.3); also, the prospective study will provide the new integrated interventions defined in Task 1.4 and implemented in eHealtHeart mobile application (Task 1.5).

Furthermore, several meetings were conducted among clinical partners to discuss any potential drawbacks (clinical, organizational, and ethical) that could undermine the success of the study. On this point, issues related to the natural difference of clinical centers were discussed, in particular related to the type of data usually collected from patients by the clinical partners and the most suitable time points of data collection to have a more complete patient's trajectory without burdening patients. Ethical issues, in particular associated to data privacy protection have been discussed in order to share data within the consortium.

Coherently, solutions were reached in order to have a homogeneous data collection process among all clinical partners, despite the existing differences.

Due to the initial difficulties to find a consensus on the points described above, the study protocol was finalized with a delay compared to the initial expected date and approval from the Ethics Committee has to be obtained. With IMS' support, each clinical site will submit the prospective

study protocol for approval and follow up this activity until the ethical and administrative approvals are obtained.

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List of Abbreviations

Abbreviation	Explanation
BNP	Brain natriuretic peptide
CRP	C- reactive protein
CT	Computed Tomography
DPIA	Data Protection Impact Assessment
DPO	Data Protection Officer
ECG	Electrocardiogram
ECHO	Echocardiography
eCRF	Electronic Case Report Form
EORTC	European Organisation for Research and Treatment of Cancer
ESMO	European Society of Medical Oncology
GDPR	General Data Protection Regulation
HER2	human epidermal growth factor receptor 2
HRV	Heart Rate Variability
ICF	Informed Consent Form
ICMJE	International Committee of Medical Journal Editors
IL-6	Interleukin-6
LVEF	Left Ventricular Ejection Fraction
QoL	Quality of Life
TNF-α	Tumor Necrosis Factor-alpha
WHO	World Health Organization
LVDd	Left Ventricular Diastolic diameter
LVDs	Left Ventricular Systolic diameter
IVSd	Interventricular septal thickness at end-diastole
LVPWd	Left Ventricular Posterior Wall thickness at end-diastole
FS%	Fractional Shortening
RWT	Relative Wall Thickness
TAPSE	Tricuspid Annular Plane Systolic Excursion
LVOT	Left Ventricular Outflow Tract
VTI	Velocity Time Integral
Vmax	Max Velocity
E/A ratio	Early to Atrial filling velocity ratio
E/E' med	Early mitral inflow velocity to Early diastolic mitral annulus velocity ratio
S/D ratio	Systolic to Diastolic Velocity ratio
TR Vmax	Maximal Tricuspid Regurgitation Velocity
RAP	Right Atrial Pressure
LA vol	Left Atrial Volume
RA vol	Right Atrial Volume
GWI	Global Work Index
GCW	Global Constructive Work

GWW	Global Wasted Work
GWE	Global Work Efficiency
SNP	Single Nucleotide Polymorphisms
miRNAs	Micro RiboNucleic Acid
GLS	Global Longitudinal Strain
AEs	Adverse Events

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1 Introduction

The information provided in this deliverable concerns the first study subject approvals package for the prospective study of the CARDIOCARE project, performed under Task 4.1 and led by IEO. The deliverable provides information on:

- The final version of the prospective clinical study protocol.
- The registration of the prospective clinical study in a WHO- or ICMJE- approved registry that also allows later posting of study results.
- The approvals required for invitation / enrolment of the first subject in at least one clinical centre: ethics committee approval, and copies of the Data Protection Officer opinion.

Additional information on the implementation of the ethical standards and guidelines of Horizon 2020 that must be applied in the CARDIOCARE project and the GDPR implementation plan is provided in **Deliverable D6.1**.

2 Prospective Clinical Study protocol

2.1 Introduction

As already described in previous deliverables of **CARDIOCARE project**, a high percentage (more than 50%) of the newly diagnosed breast cancer patients are older than 65 years and particularly susceptible to the cardiotoxic effects of cancer treatment due to age-related risk factors, preexisting cardiovascular disease and a high prevalence of multiple co-morbidities. The cumulative effect of risk factors in the elderly breast cancer patient resembles a "snowball effect", where baseline age-related factors and cancer-related changes are further exacerbated by direct therapy-induced cardiotoxicity, resulting ultimately to severe multi-morbid states and mortality. Frailty and high risk of cardiotoxicity in this vulnerable group may lead to inappropriate interventions, but also undertreatment, resulting in poorer outcomes, deterioration of quality of life (QoL) and increased healthcare costs for patients and healthcare systems. Considering that older cancer patients are systematically underrepresented in clinical trials, there are currently limited means to effectively address the complex needs of these patients and their caregivers. In this context, broader and interdisciplinary clinical trials able to provide evidence-based best practices, specifically directed towards the monitoring and management of the elderly breast cancer patients, are urgently needed. The most effective approach to minimize cardiotoxicity is early identification and early onset of a prophylactic treatment. However, the current standard of cardiac monitoring identifies cardiotoxicity only when a functional impairment has already occurred, precluding any chance of effective prevention.

Cardio-Oncology guidelines recommend that serial examinations, ECGs, echocardiograms, circulating biomarkers (e.g. troponin I, BNP) and detailed assessment of the intrinsic capacity of the individuals, defined as the composite of all physical and mental capacities (Integrated Care for Older People – ICOPE guidelines) have to be included in a standard follow-up protocol to establish best practices for the management of the elderly cancer patient at risk of cardiotoxic adverse effects from cancer treatment (Chianca et al., 2022).

In this perspective, together with biological information, psychological, emotional and functional factors need to be a focus of the patient's assessment, since they can be both an outcome and a risk factor of CVD.

Emotional states can be a facilitator of the cardiovascular pathology. In such sense, personal disposition, depression, stress and anxiety (also typical cancer patient reactions) can increase the risk of cardiovascular complications (Bussotti and Sommalunga, 2018). Besides emotional states that might be elicited by cancer diagnosis and treatments, personality traits or dispositions existing before cancer and related treatments can influence how the patient adapts to, and copes, with this condition. Denollet and colleagues (Denollet et al., 2003) demonstrated that people with negative affectivity and social inhibition (Type-D personality) are exposed to a higher risk of cardiovascular disease. In the same line, the prolonged exposition to stressful life circumstances (i.e., stress at work, general stress) may predict subsequent hypertension and cardiovascular disease (Rosengren et al., 2004). Currently there are no large studies that allow the collection of all the data needed for a meaningful assessment of the patient intrinsic capacity, also due to the lack of tools to simplify the collection of patients' reported data.

In the CARDIOCARE project, **we aim to better identify elderly patients at risk of developing cardiotoxicity, by using multidimensional tools for monitoring intrinsic capacity and QoL,** to improve the general wellbeing and follow-up during their "survivorship".

The risk stratification models will be built using clinical and imaging data from the CARDIOCARE Retrospective Study, however available dataset does not include behavioral/psychological factors nor "omics data". Therefore, in the CARDIOCARE Prospective Clinical Study, which is the subject of the present Deliverable, we will refine and validate the models developed and we will enrich them with novel biochemical biomarkers, psychomarkers to better evaluate the patient intrinsic capacity, and -omics markers (metagenomics, miRNAs, SNPs).

2.2 The Methodology of the Prospective Data Collection

In this section, revealing aspects of the prospective data procedure and analysis are presented.

2.2.1 Objectives

The main objective of the CARDIOCARE project is to identify potential risk factors associated with cardiotoxicity and deterioration of QoL in breast cancer patients with previous baseline cardiovascular disease and in patients with no cardiovascular disease, to train and develop the first version of a risk prediction model for the future prediction of cardiotoxicity and QoL, and to provide actionable insights for best practices and cost-effective healthcare pathways by helping clinicians in identifying patient care gaps. In order to train and validate the risk prediction model developed on the retrospective observational study (see D1.1 for the Retrospective Study), a prospective clinical study has been designed (**objective of this deliverable**).

Specifically, this prospective clinical study will aim at:

- Collecting prospective multidimensional data of elderly breast cancer patients from diverse data sources and determine the epidemiological characteristics of cardiotoxicity in these patients.
- Determining the efficacy of integrated patient-oriented behavioral and psychological interventions, to counteract cardiotoxic effects of cancer treatment, improve intrinsic capacity and QoL and improve cost-effectiveness of healthcare pathways in the management of the elderly breast cancer patients.
- Providing prospective patient data to refine and validate the CARDIOCARE risk stratification model to improve early diagnosis, prevention and therapy of cardio-toxicity and improve patients' intrinsic capacity and QoL.

2.2.2 Primary Endpoint

- The study primary objective is to evaluate the onset of cardiotoxicity, where: subclinical cardiotoxicity is defined as preserved Left Ventricular Ejection Fraction (LVEF) (i.e. LVEF ≥

50%) and >15% reduction in GLS relative to baseline and/or new rise in cardiac Troponin I or Natriuretic peptides levels in plasma, and clinical cardiotoxicity is defined as the reduction of LVEF by ≥ 10% from baseline to a value below 50%, incurred at any timepoint during study FU (Čelutkienė J, et al., 2020; Herrmann J et al., 2022).

2.2.3 Secondary Endpoints

- Intra-patient assessment of plasma Troponin I during follow up and association/agreement with LVEF change;
- Intra-patient assessment of plasma BNP elevation;
- Hospital admissions from cardiovascular causes or falls;
- Cardiovascular death;
- Non-Cardiovascular death;
- Health Related Quality of Life assessed by EORTC QLQ-BR23, validated breast cancer Patient Reported Outcome Measure (PROM);
- Cost-effectiveness of provided healthcare pathways determined by costs combined with quality-adjusted life-years (QALYs). Costs will consider healthcare provided, numbers of admission and days spent in hospital and patient costs for out-of-pocket expenses associated with their condition (i.e., travel expenses (of both patient and caregiver), over-the-counter medicines and supplements, complementary therapies not supported by Health care system, home help, and time away from work);
- Psychological and behavioural variables:
  a) Intrinsic (mental and physical) capacity as evaluated by Comprehensive Geriatric Assessment (CGA) using standardized PROMs, where applicable sensors and wearables, and performance tests. More specifically the following variables will be measured: i) mobility and locomotion (distance, balance, gait speed), ii) sensory ability (Snellen test, whisper test), and iii) vitality status (exercise duration, ECG characteristics, HRV, grip strength, nutritional/energy state, sleep, fatigue), together with complementary frailty screening (using Geriatric 8 frailty screening tool declared as the preferred tool by SIOG for identifying frailty in older cancer patients including breast cancer patients) and frailty assessment (comorbidity, mental health, cognition, functional status, polypharmacy, geriatric syndromes, socioeconomic and nutritional status tools);

b) psycho-cognitive status (personal traits, cognitive factors, emotional states);

c) social and socio-economic factors;

d) general QoL measured using standardized validated questionnaires to make comparison with the more specific psychological and behavioural measures listed above.

- The following continuous variables will also be measured to identify predictive factors of disease trajectories and cardiac toxicity related to the objective of developing, refining and validating the risk stratification model:
  a) Imaging markers of cardiac structural and functional variables by echocardiography, including:
     - LVEF% – Simpson Biplane (LV end-diastolic and end-systolic Volyme)
     - LVDd + LVDs
     - IVSd
     - LVPWd
     - FS%
     - Mass index + RWT
     - TAPSE + S' Tricuspid annulus
     - LVOT VTI + LVOT Vmax + Ao VTI + Ao Vmax + aorta dimension
     - E/A + E/E' med + S/D ratio
     - TR Vmax
     - RAP
     - LA vol index + RA vol index
     - Global Longitudinal Strain (of both ventricles if possible)
     - Myocardial Work (GWI, GCW, GWW, GWE) (if available) + Systolic & Diastolic Pressure
  b) 2D apical 4-chambers (A4C) and 2-chambers (A2C) echocardiographic images at end-diastole (ED) and end-systole (ES) and corresponding manual annotations of the left ventricle (or left endocardium), left epicardium and left atrium from a subset of the provided images.
  c) Mammography (conventional or tomosynthesis).
  d) Whole blood and plasma biomarkers including biochemical (Troponin I, BNP), inflammatory/psychological (platelet activation, IL-6, TNF-α, HRV, CRP, Fibrinogen, Ferritin) and -omics (SNPs, extracellular vesicles miRNAs) biomarkers.
  e) Microbiome biomarkers from metagenomic analysis of stool samples:

- Species diversity indices
- Relative abundance at Family, Genus and Species levels
- Presence of pathogenic species

2.3 Patient Selection: Criteria for Eligibility/Ineligibility

2.3.1 Participants population and sample size consideration

The prospective clinical study will enroll a total of 750 breast cancer patients over 12 months. Six clinical centres in Italy, Greece, Cyprus, Sweden and Slovenia will recruit breast cancer patients with a pre-planned recruitment number per country and rate per month/per centre. Taking into account that 40%-50% of newly diagnosed breast cancer patients in Europe are women older than 65 years of age, together with the estimated numbers of attending patients/per site/per year, the sample size in each clinical centre will be as follows:

- At the European Institute of Oncology (IEO), Italy, a recruitment target of 125 patients ≥ 65 years of age is set (Estimation of 35 patients in Neoadjuvant or Adjuvant setting and 90 patients in metastatic setting).
- At the Bank of Cyprus Oncology Center (BOCOC), Cyprus, a recruitment target of 120 patients ≥ 65 years of age is set (Estimation of 70 patients in Neoadjuvant or Adjuvant setting and 50 patients in metastatic setting).
- At the Breast Centre, Karolinska University Hospital (KSBC), Sweden, a recruitment target of 125 patients ≥ 65 years of age is set (Estimation of 100 patients in Neoadjuvant or Adjuvant setting and 25 patients in metastatic setting).
- At the University Hospital of Ioannina (UOI), Greece, a recruitment target of 60 patients ≥ 65 years of age is set (Estimation of 40 patients in Neoadjuvant or Adjuvant setting and 20 patients in metastatic setting).
- At the National and Kapodistrian University of Athens (NKUA), Department of Surgery and Heart Failure and Cardio-oncolgy Unit, Greece, a recruitment target of 195 patients ≥ 65 years of age is set (Estimation of 110 patients in Neoadjuvant or Adjuvant setting and 85 patients in metastatic setting).

- At the Institute of Oncology Ljubljana (IOL), Slovenia, a recruitment target of 125 patients ≥ 65 years of age is set (70 patients in Neoadjuvant or Adjuvant setting and 55 patients in metastatic setting).

2.3.2 Inclusion criteria

To be eligible for inclusion in the study, each patient must fulfill any of the criteria below:

1. Women ≥ 65 years with a diagnosis of locoregional breast cancer who will undergo neoadjuvant and/or adjuvant treatment with regimens including anthracyclines.
2. Women ≥ 65 years with a diagnosis of HER2 positive locoregional breast cancer who will undergo neoadjuvant and/or adjuvant treatment with anti-HER2 therapy (trastuzumab or trastuzumab and pertuzumab).
3. Women ≥ 65 years with HER2 positive metastatic breast cancer who will undergo first-line therapy with anti-HER2 therapy (trastuzumab or trastuzumab and pertuzumab in combination with taxanes).
4. Women with age ≥ 65 years before starting the aforementioned treatment for breast cancer.
5. Women eligible ≥ 65 years who will undergo first-line therapy in the metastatic setting with any type of treatment (chemotherapy, immunotherapy, biological agents).
6. Willingness and ability to comply with scheduled visits, laboratory tests, and other trial procedures
7. Written informed consent.
8. Participant affiliated to a social security system.
9. Life expectancy of at least 12 months.

2.3.3 Exclusion criteria

Patients who meet any of the following criteria will be excluded:

1. Age < 65 years.
2. Diagnosed severe psychiatric or neurological disorders that might impair the ability to give informed consent.

2.4 Methods and Study Design

The prospective study is a multicentre clinical study. For all patients, multi-modal data, including clinical data, cardiac imaging, biochemical and psychological biomarkers, -omics as well as intrinsic (mental and physical) capacity indicators and QoL data will be measured after breast cancer diagnosis, before cancer treatment and at selected follow-up time points every 3 months from the start of treatment for 18 months. A sub-population of 500 patients will be randomly selected (IEO: 100, BOCOC: 100, KSBC: 80, NKUA: 130, IOL: 90) to assess gut microbiome changes due to cancer treatment in fecal (stool) samples collected at baseline (T1) and within 14 to 25 days after the end of each treatment (Tn).

Along the prospective observational study, patients will be assigned to intervention and control arms in 1:1 ratio (*see figure 1*), in order to test the feasibility and efficacy of intervention defined in Task 1.4 and implemented in eHealtHeart developed in Task 1.5 of the **CARDIOCARE Project**.

All patients will receive, in addition to standard of care, supportive care. Patients in the control and in the intervention group will receive wearables and will be invited to complete the mobile ePsycHeart evaluation (developed in Task 1.3). ePsycHeart will assess patients' intrinsic capacity indicators that include (a) psycho-cognitive states (psychological and emotional states, QoL, cognitive function and memory, perceptions of aging, environ-mental and social factors), (b) mobility and locomotion (distance, balance, gait speed), (c) sensory screening (Snellen test, whisper test), and (d) vitality (exercise duration, ECG characteristics, HRV, grip strength, nutritional/energy state, sleep, fatigue).

In addition, patients in the intervention group will receive access to the eHealtHeart mobile application: behavioral and psychological interventions will be delivered to the intervention arm via the eHealtHeart mobile application to mitigate potential risk factors associated with cardiotoxicity and deterioration of QoL. eHealtHeart interventions will target patients and care givers aiming at improving patients' intrinsic capacity including psychological interventions (e.g. emotional, dispositional states, biofeedback self-regulation, Best Possible Self), cognitive stimulation (e.g. cognitive restructuring plus card games to improve memory and executive functions, SOC strategy), physical activity and performance exercises, vision and hearing suggestions, dietary guidance on nutrition, guidance to improve management of urinary incontinence (e.g. alerts, self-monitoring) and falls (e.g. remove home hazards, manage polypharmacy) together with providing education

and support to caregivers. At the end of the follow up the patient will have to return the wearables used for the study.

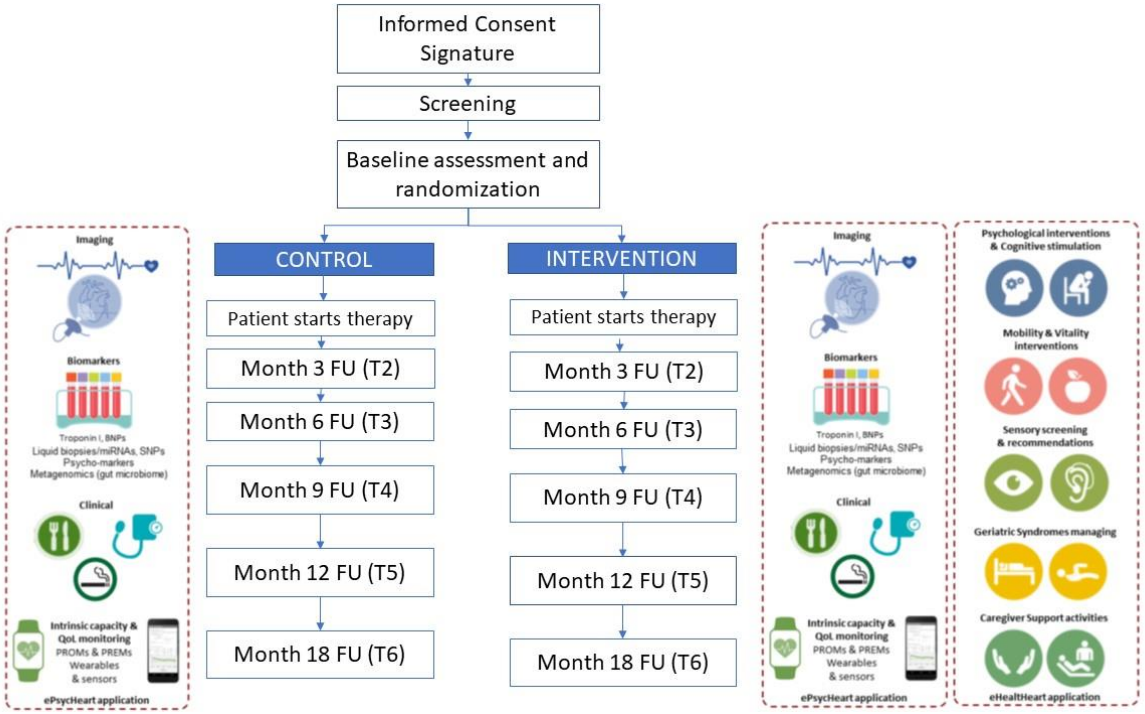

Figure 1** - Procedure specific of the intervention-control study. Intervention is related to psychological and behavioral interventions implemented in the **eHealtHeart platform.

2.4.1 Evaluations and Procedure

2.4.1.1 Written Informed Consent

Written Informed Consent must be obtained from the patient at screening prior to initiating any study procedure required by the protocol. The details of the study should be discussed with the patient prior to obtaining informed consent, and the ICF will be provided in the center local language together with a short summary of the study in lay language, and with the phone contact of the Recruiting Center Investigators. The ICF must be signed and dated by the patient and by the Investigator or his/her designee. A copy of the signed ICF will be provided to the patient, and the original will be retained with the source documents.

2.4.1.2 Study Conduct

2.4.1.2.1 Screening (T0)

Patients ≥ 65 years old with breast cancer who are candidates to neoadjuvant and/or adjuvant treatment with regimens including *anthracyclines, or anti HER2 therapy* (trastuzumab or trastuzumab and pertuzumab) AND breast cancer patients eligible for a first-line treatment in the metastatic setting with any type of treatment (chemotherapy, immunotherapy, endocrine therapy plus biological agents) will be invited to participate to the trial. After providing consent, as described in Section 10.1, each patient will undergo a screening procedure, during which demographic information and additional information on the Personal and Family Medical History, will be collected for all patients.

2.4.1.2.2 Baseline (T1)

The patient will undergo clinical examination during an oncological visit, a psychological visit and routine blood testing (including Troponin I, BNP plasma levels), collection of blood samples for genetic analysis and miRNA analysis, stool collection, echocardiography and ECG assessment, BP and HR measurement, and will be asked to complete validated self-administered questionnaires and scales (ePsycHeart App) (*see Table 2 for the measures that will be used and related timeline*). Adherence to inclusion/exclusion criteria will be verified and information on Adverse Events will be collected. Expected time points are reported in *Table 1*.

Fully eligible patients will be randomized to one treatment arm and will receive:

- Control group (ARM 1): Weareables/sensors and ePsycHeart App
- Intervention group (ARM 2): Weareables/sensors and ePsycHeart App and eHealtHeart

2.4.1.2.3 Treatment Start (Day 0)

The patient will start an oncological treatment cycle according to the doctor's instructions and will be checked for concomitant medications.

Information on Adverse Events will be collected.

2.4.1.2.4 Home Monitoring

The patient will be asked to wear the *Garmin Venu SQ* and the *Polar h10* as much as possible until the follow-up at month 12 (T5) of the study, after which the wearable devices will be given back to the researchers. They will be then disinfected and washed and given to a new study participant. If possible, it will be recommended to wear the devices for at least 48 continuative hours each week.

In addition, the patients will be requested to perform a Hand Grip test every 6 months.

They will be also asked to provide information through the ePsyHeart App (*see Table 2 for the measures timeline*).

Table 1 - *Summary of procedures scheduled*

Procedure	May be one single or two visits		Day 0	Month 3	Month 6	Month 9	Month 12	Month 18	14-25 days after end of treatment ***
	Day -15 T0 (screening)	T1 (baseline)	Treatment Start	T2	T3	T4	T5	T6	Tn
ICF signature	X								
Diagnosis	X								

Personal and Family Medical History (including diagnosis of severe psychiatric disorders)	X								
Smoking habits		X							X***
Co-morbidities	X								
Oncological visit		X			X		X	X	
Psychological visit ¥ (if the center has a psycological support service)		X		X	X	X§	X	X	
Cardiological visit (if needed)	X	X		X	X	X§	X	X	
BP and HR measure-ment		X		X	X	X§	X	X	
Routine blood analy-sis (i.e. hematology and biochemistry)*		X		X	X	X	X	X	
ECHO assessment**		X		X	X	X§	X	X	
ECG assessment		X		X	X	X§	X	X	
Plasma troponin I level assessment	X	X		X	X	X	X	X	
Plasma BNP assess-ment		X		X	X	X	X	X	
Plasma myeloperoxi-dase and high-sensi-tivity CRP (if availa-ble)		X		X	X	X	X	X	
I/E checklist		X							
Collect blood sample for genetic analysis		X							
Collect plasma sam-ple for miRNA analy-sis		X							X***
Collect stool sample		X							X***

Oncological treatment (plan/changes), cycle			X	X	X	X	X	X	X***
Information on treatment status (ongoing/completed)				X	X	X	X	X	
Concomitant medications				X	X	X§	X	X	X***
Gastrointestinal Symptom Rating Score (GSRS)		X							X***
Patient LifeStyle questionnaire		X							X***
Verify completion of self-administered scales/questionnaires (ePsycHeart App)#		X	X	X	X	X§	X	X	
Cognitive effect assessment		X			X			X	
Verify collection of data from wearable devices		X		X	X	X§	X		
Collect information on patient's out-of-pocket expenses					X		X	X	
AEs assessment		X	X	X	X	X	X	X	X***

Legend:
ICF: Informed Consent Form
BP: Blood Pressure
HR: Heart Rate
ECHO assessment: Echocardiographic assessment
ECG: Electrocardiogram
I/E checklist: Inclusion/Exclusion criteria checklist
AE: Adverse Events

§ Only if possible.

¥ These visits can be conducted in presence or remotely, if the center can involve the required experts. The psychological visit consists in meeting the patient in person (if already in the clinical centre) or remotely to touch base about the study: how they feel about it and whether they would like something to be different / modified. Within the visit, a motivational intervention will also be carried out with the aim of minimising drop-outs as much as possible.

* Including cholesterols, glucose levels and renal and liver function tests, platelet activation, IL-6, TNF-α, HRV, CRP, Fibrinogen, Ferritin (if available).

**Including right and left ventricles dimensions and functions and diastolic function.

***at the first visit after the end of each treatment which should be within 14-25 days. The time of these samples collection will vary in relation to the therapeutic schedule of the patient

**** To be repeated for each treatment if the patient undergoes a second treatment (including anthracyclines or anti-HER2 therapy) within the CARDIOCARE study

Please refer to Table 2 for the precise list of the questionnaires and scales requested at each timepoint.

Table 2 *- The timeline for the psychological and behavioral assessment.*

Psychological and behavioral assessment	**May be one single or two visits**		**Day 0**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 18**
	Day -15 T0	**T1 (baseline)**	**Treatment start**	**T2**	**T3**	**T4**	**T5**	**T6**
Quality of life (EORTC-QLQ-30/BR23)		✓		✓	✓	✓	✓	✓
Fatigue FACIT-Fatigue		✓		✓	✓	✓	✓	✓
Resilience (Brief Resilience Scale)		✓		✓				✓
Anxiety and Depression (PHQ4)		✓		✓	✓	✓	✓	✓
Emotion Regulation Questionnaire (ERQI)		✓		✓				✓
Life Orientation Test Revised (LOT-R)		✓		✓				✓
Life Satisfaction (Single-item life satisfaction Measure)		✓	✓	✓	✓	✓	✓	✓
Perceived social support (MSPSS)		✓		✓				✓
Self-control and self-management (SCMS)		✓		✓				✓

Cancer Behavior Inventory short form (CBI-B)		✓		✓	✓	✓	✓	✓
Impact of Event Scale (IES-R)		✓		✓				✓
Perceived stress scale (PSS)		✓		✓	✓	✓	✓	✓
Positive and Negative Affect (B-PANAS)		✓		✓				✓
Burden for Family Caregivers (BSFC-s)		✓		✓	✓	✓	✓	✓
Nutritional questionnaire		✓		✓				✓

***Table 3** - Frequency of interventions and eHealtHeart mobile application*

Modules	Interventions	Frequency
Psychological Support Module	Biofeedback	If the patient reports a high anxiety and depression value in PsyHearth (PHQ-4) an alert will suggest to complete the module
	BPS	20 minutes each day for 4 consecutive days (every 3 months)
	EW	Every 3 months
	ABCDE	Every 3 months
Cognitive stimulation and training	Find the word	5 consecutive days/month until the end of the study
	Tic Tac Toe	
	Color Beans	
	Drawing	
Improve mobility and vitality	Physical activity/exercise	At least 48 continuative hours each week
Vision & Hearing guide assessment Module	Vision test	Every 6 months
	Hearing test	
	Pelvic floor exercises	Daily for 3 months

Geriatric Syndromes assessment Module	Fall detection and fall prevention	Twice a week for 6 weeks
Education and training module for patients and caregivers	Psychological and social support, training and educational material	Link to educational material and training videos/support to better cope with difficult emotions. If the patient reports a low value of caregiver support or the caregiver reports a high burden in ePsycHeart (MSPSS; BSFC-s), an alert will suggest to access the related educational material and training module.

2.4.1.2.5 Month 3 Follow-up visit (T2)

The presence of co-morbidities in the patient will be evaluated. The subject will undergo BP and HR measurement, blood analysis (including hematology, biochemistry, plasma troponin I level and BNP assessments), ECHO and ECG assessments. There may be a plan or changes to the oncological treatment and information on treatment status and Concomitant Medications will be collected. The completion of self-administered scales (ePsycHeart App) and the collection of data from wearable devices will be verified. Information on Adverse Events will be collected.

2.4.1.2.6 Month 6 Follow-up visit (T3)

The presence of co-morbidities in the patient will be evaluated. The subject will undergo clinical examination during an oncological visit, a psychological visit, BP and HR measurement, routine blood analysis (including hematology, biochemistry, plasma troponin I level and BNP assessments), echocardiography and ECG assessments. Information on treatment status and concomitant medications will be collected. The completion of self-administered scales (ePsycHeart App) and the collection of data from wearable devices will be verified. Information on Adverse Events will be collected.

2.4.1.2.7 Month 9 Follow-up visit (T4)

The presence of co-morbidities in the patient will be evaluated. The subject will undergo BP and HR measurement, routine blood analysis (including hematology, biochemistry, plasma troponin I level and BNP assessments), ECHO and ECG assessments. Information on treatment status and concomitant medications will be collected. The completion of self-administered scales

(ePsycHeart App) and the collection of data from wearable devices will be verified. Information on Adverse Events will be collected.

2.4.1.2.8 Month 12 Follow-up visit (T5)

The presence of co-morbidities in the patient will be evaluated. The subject will undergo clinical examination during an oncological visit, a psychological visit, BP and HR measurement, routine blood analysis (including hematology, biochemistry, plasma troponin I level and BNP assessments), ECHO and ECG assessments. Information on treatment status and concomitant medications will be collected. The completion of self-administered scales (ePsycHeart App) and the collection of data from wearable devices will be verified. Information on Adverse Events will be collected.

2.4.1.2.9 Month 18 Follow-up visit (T6)

The presence of co-morbidities in the patient will be evaluated. The subject will undergo clinical examination during an oncological visit, a psychological visit, BP and HR measurement, routine blood analysis (including hematology, biochemistry, plasma troponin I level and BNP assessments), ECHO and ECG assessments. Information on treatment status and concomitant medications will be collected. The completion of self-administered scales (ePsycHeart App) and will be verified. Information on Adverse Events will be collected.

2.4.1.2.10 Post-treatment visit/s

After 14-25 days from the end of the first treatment planned the patient will return to the hospital for the collection of stool and plasma samples for omics analysis. The patient will be asked to provide information on smoking habits, gastrointestinal symptoms (GSRS) as well as information of the oncological treatment status and future plans and concomitant medications.

Post treatment visit will be repeated for each treatment if the patient undergoes a second treatment (including anthracyclines or anti-HER2 therapy) within the CARDIOCARE study.

Post-treatment visit/s can be scheduled to be more convenient for patients (for example to coincide with planned clinical visits).

2.4.1.3 Schedule of Visits and Assessments

2.4.1.3.1 Screening (T0)

At Screening, the following procedures will be performed to establish eligibility for the study:

1. Obtaining written informed consent (before any study procedures)
2. Demographic
3. Personal and Family Medical History (including diagnosis of severe psychiatric disorders and co-morbidities)
4. Review of Concomitant Medications
5. Plasma troponin I level assessment

2.4.1.3.2 Baseline (T1)

The following procedures will be performed:

1. Oncological visit
2. Cardiological visit (if needed)
3. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
4. Collection of information on smoking habits
5. BP and HR measurement
6. Routine blood analysis (i.e., hematology and biochemistry)
7. ECHO assessment
8. ECG assessment
9. Plasma troponin I level assessment
10. Plasma BNP assessment
11. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
12. Definition of the oncological treatment plan

13. Assessment of the Inclusion/Exclusion checklist and patient enrolment
14. Randomization
15. Short training on the use of the Apps and wearables
16. Gastrointestinal Symptom Rating Score (GSRS)
17. Patient Lifestyle questionnaire
18. Blood sample for genetic analysis
19. Plasma sample for miRNA analysis
20. Stool sample collection
21. Verify completion of self-administered scales/questionnaires (ePsycHeart App)
22. Cognitive effect assessment
23. Review of Concomitant Medications
24. Adverse Events (AEs) assessment

2.4.1.3.3 Treatment Start (Day 0)

1. Date of oncological treatment start
2. Adverse Events (AEs) assessment

2.4.1.3.4 Month 3 Follow-up visit (T2)

1. Cardiological visit (if needed)
2. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
3. BP and HR measurement
4. Routine blood analysis (i.e., hematology and biochemistry)
5. ECHO assessment (if possible)
6. ECG assessment (if possible)
7. Plasma troponin I level assessment
8. Plasma BNP assessment
9. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
10. Collection of information on oncological treatment status and changes if any
11. Review of Concomitant Medications

12. Verify completion of self-administered scales/questionnaires (ePsycHeart App)
13. Verify collection of data from wearable devices
14. Adverse Events (AEs) assessment

2.4.1.3.5 Month 6 Follow-up visit (T3)

1. Oncological visit
2. Cardiological visit (if needed)
3. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
4. BP and HR measurement
5. Routine blood analysis (i.e., hematology and biochemistry)
6. ECHO assessment
7. ECG assessment
8. Plasma troponin I level assessment
9. Plasma BNP assessment
10. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
11. Information on oncological treatment status and changes if any
12. Review of Concomitant Medications
13. Verify completion of self-administered scales/questionnaires (ePsycHeart App)
14. Cognitive effect assessment
15. Verify collection of data from wearable devices
16. Adverse Events (AEs) assessment

2.4.1.3.6 Month 9 Follow-up visit (T4)

1. Cardiological visit (if needed)
2. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
3. BP and HR measurement
4. Routine blood analysis (i.e., hematology and biochemistry)
5. ECHO assessment (if possible)
6. ECG assessment (if possible)

7. Plasma troponin I level assessment
8. Plasma BNP assessment
9. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
10. Information on oncological treatment status and changes if any
11. Review of Concomitant Medications
12. Verify completion of self-administered scales/questionnaires (ePsycHeart App)
13. Verify collection of data from wearable devices
14. Adverse Events (AEs) assessment

2.4.1.3.7 Month 12 Follow-up visit (T5)

1. Oncological visit
2. Cardiological visit (if needed)
3. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
4. BP and HR measurement
5. Routine blood analysis (i.e., hematology and biochemistry)
6. ECHO assessment
7. ECG assessment
8. Plasma troponin I level assessment
9. Plasma BNP assessment
10. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
11. Information on treatment status (ongoing/completed) and changes if any
12. Review of Concomitant Medications
13. Verify completion of self-administered scales/questionnaires (ePsycHeart App)
14. Verify collection of data from wearable devices
15. Adverse Events (AEs) assessment

2.4.1.3.8 Month 18 Follow-up visit (T6)

1. Oncological visit
2. Cardiological visit (if needed)

3. Psychological visit (to be conducted in presence or remotely) if the center has specialized personnel
4. BP and HR measurement
5. Routine blood analyses (i.e., hematology and biochemistry)
6. ECHO assessment
7. ECG assessment
8. Plasma troponin I level assessment
9. Plasma BNP assessment
10. Plasma myeloperoxidase and high-sensitivity CRP (if possible)
11. Information on treatment status (ongoing/completed) and changes if any
12. Review of Concomitant Medications
13. Verify completion of self-administered scales (ePsycHeart App)
14. Cognitive effect assessment
15. Adverse Events (AEs) assessment

2.4.1.3.9 Post-treatment visit/s (Tn: 14-25 days from the end of the treatment)

1. Collection of information on smoking habits
2. Gastrointestinal Symptom Rating Score (GSRS)
3. Patient Lifestyle Questionnaire
4. Plasma sample for miRNA analysis
5. Stool sample collection
6. Oncological treatment (plan/changes), cycle
7. Check concomitant medications
8. Adverse events (AEs) assessment

The visit will be repeated for each treatment if the patient undergoes a second treatment (including anthracyclines or anti-HER2 therapy) within the CARDIOCARE study. The following procedures will be conducted:

- Collection of information on smoking habits
- Gastrointestinal Symptom Rating Score (GSRS)

- Patient Lifestyle questionnaire
- Plasma sample for miRNA analysis
- Stool sample collection
- Oncological treatment (plan/changes), cycle
- Check concomitant medications
- Adverse events (Aes) assessment

2.5 Statistical Design and Analysis

2.5.1 Statistical analysis and power calculation

According to previous clinical trials in IEO (ICOS-ONE clinical trial; NCT01968200), an increase of circulating troponin I levels is estimated to be 20%-26% in breast cancer patients, younger than 63 years of age, treated with anthracyclines at conventional dosage. Moreover, according to the ASCO guidelines on the risk of cardiac dysfunction in older patients with cancer treated with anthracyclines and/or trastuzumab, an increased risk for CHF is independently associated with older age (>65 years), the presence of comorbidities (e.g., hypertension, diabetes, dyslipidemia) and compromised cardiac function and more likely to occur within the first 12 months after initiation of treatment.

Based on power analysis, considering a conservative incidence of cardiotoxicity (for definition see section 8) of 21%, a number of 368 patients in each arm (total sample size of 736 patients) would suffice to detect a 40% relative risk reduction in the incidence of elevated troponin I levels at a 2-sided alpha=0.05 and a 1-beta=0.80 taking into account a 10% dropout rate. This endpoint will allow comparing whether integrated patient-oriented behavioral and psychological interventions can mitigate, prevent, or delay the onset of, cardiac damage and toxicity from chemotherapy during treatment and follow-up period. Sample size calculation was performed by the Fisher's exact test for the comparison of the inequality in the proportions of two independent-groups test of equal exponential survival, with a fixed length of follow-up of 9 months, using the software G*Power version 3.1.9.4. Taking into account the results of the power analysis and the number of attending breast cancer patients per year, in each of the participating centres, a **recruitment target of 750 patients (375 in each arm)** has been set.

The main analysis will be performed according to intention-to-treat (ITT) approach, therefore all patients randomized in the study will be included in the final analysis. Based on the evidence shown by Keramida et al. 2019; Sawaya et al., 2011, patients who do not reach at least 3 months of follow-up from the start of treatment will not be considered in the analysis. Results will be presented using descriptive statistics (mean, standard deviation, median and range for continuous variables and proportions for nominal variables). Baseline characteristics will be compared using a two-samples t-test or a nonparametric test for continuous variables and Pearson's Chi Squared test for qualitative variables. All hypothesis tests will be performed using two-sided tests at the 5% significance level. Interventions effect on the primary endpoint will be analyzed by means of a Cox proportional hazards model. Point estimates along with 95% confidence intervals for the hazard ratio of the control arm versus the intervention arm will be calculated. Any statistically significant unbalance of the baseline characteristics between the randomized groups will be considered for multivariable adjustment. Plots of the Kaplan-Meier estimates for time to first occurrence of the primary endpoint will be presented. Logistic regression or Cox proportional hazards model will be adopted to test for interventions effect between the two arms on the secondary endpoints. Continuous variables such as those obtained from imaging, biochemical and molecular biomarkers will be summarized descriptively at baseline and at serial follow up visits. Differences between the two arms at the various follow-up visits will be tested by analysis of covariance taking into account their respective baseline measurement as covariate. Other covariates found to be unbalanced between the two groups will be added to the model.

Finally, for cost effectiveness analysis health outcomes and QoL assessed in the clinical study will be used to calculate quality-adjusted life-years (QALYs). Costs will be combined with QALYs for cost-utility analysis to estimate the cost-per-QALY associated with the implementation of the CARDIOCARE model and provided interventions. The incremental cost-effectiveness ratio (ICER) will be calculated serving as a major means for evaluation compared to current care practices. ICER will be then utilized to indicate where healthcare resources should be allocated, using a threshold approach. Given that treatment will be based on standard clinical practice and guidelines no interim analysis is foreseen. Safety aspects will be monitored throughout the study by the Data and Safety Monitoring Board (DSMB). In case, not expected, safety issues arise a recommendation by

the DSMB to stop the clinical study will be based on the pattern of the interventions effect across all end-points, as well as the overall benefit/risk ratio of proposed interventions.

2.5.2 Assessment of data quality

Periodic controls are planned to verify the quality of data collected (verifying missing data, out-of-range values and misalignments). At the end of the data collection a final data quality check will be performed, including the following checks:

1. Extraction of the entire database and manual inspection of the data;
2. Statistical analysis of each variable:
    a. Distribution analysis;
    b. The outliers will be identified according to a statistical analysis of patients' distribution. For categorical data, frequency analysis will be performed to see the incidence;
    c. Identification of putative errors and outliers and queries to the clinical sites;
3. Random selection of patients, for key variables, and confirmation of data set with source documents.
4. Clear identification of missing data (missing values vs. non-available data vs non-collected data).

2.5.3 Data Collection and Management

The following data will be collected:

- Cardiac structural and functional data by echocardiography and ECG.
- 2D apical 4-chambers (A4C) and 2-chambers (A2C) echocardiographic images at end-diastole (ED) and end-systole (ES) and corresponding manual annotations of the left ventricle (or left endocardium), left epicardium and left atrium from a subset of the provided images.
- Mammography (conventional and tomosynthesis)

- Whole blood and plasma biomarker data including biochemical (Troponin I, BNP), inflammatory (e.g., platelet activation, IL-6, TNF-α, HRV, CRP, Fibrinogen, Ferritin) psychological and -omics (SNPs, extracellular vesicles miRNAs) data.
- Microbiome biomarker data from metagenomic analysis of collected stool samples.
- Clinical data including medical record, demographics, risk factors, medication, lab tests, hospital admissions and death from any cause will be collected by the I.
- Health Related Quality of Life assessed by EORTC QLQ-C30 and EORTC QLQ-BR23, validated breast cancer Patient Reported Outcomes Measures (PROMs).
- Cost-effectiveness of provided healthcare pathways determined by costs combined with quality adjusted life-years (QALYs). Costs will consider healthcare provided, number of hospital admissions, and patient costs for out-of-pocket expenses associated with their condition (i.e., travel expenses (of both patient and caregiver), over-the-counter medicines and supplements, complementary therapies not supported by health care system, home help, and time away from work).
- Psychological and behavioural variables:

  a) Intrinsic (mental and physical) capacity as evaluated by Comprehensive Geriatric Assessment (CGA) using standardized PROMs, where applicable sensors and wearables, and performance tests. More specifically the following variables will be measured: i) mobility and locomotion (distance, balance, gait speed), ii) sensory ability (Snellen test, whisper test), and iii) vitality status (exercise, ECG, HRV, grip strength, nutritional/energy state, sleep, fatigue), together with complementary frailty screening (using Geriatric 8 frailty screening tool declared as the preferred tool by SIOG for identifying frailty in older cancer patients including breast cancer patients) and frailty assessment (comorbidity, mental health, cognition, functional status, polypharmacy, geriatric syndromes, socioeconomic and nutritional status tools);

  b) psycho-cognitive status (personal traits, cognitive factors, emotional states);

  c) social and socio-economic factors;

  d) general quality of life measured using standardized validated questionnaires to make comparison with the more specific psychological and behavioural measures listed above.
- Online-continuous monitoring of intrinsic capacity indicators a) mobility and locomotion (distance, balance, gait speed) and b) vitality status (ECG, HRV, exercise, energy state,

sleep/fatigue, grip strength) will be performed by means of wearable sensors (Polar H10 Heart Rate Chest Sensor) and hand grip dynamometer.

A well-defined Data Management Plan has been submitted (see Deliverable D5.1), within the first 6 months of the project, providing detailed information on the procedures that will be implemented for data collection, storage, protection, retention, re-use and/or destruction complying to the General Data Protection Regulation (GDPR).

An electronic case report form (eCRF) has been delivered to serve as the data entry tool for all patient data, blood and imaging examination, characteristics, efficacy and safety endpoints for the trial (all except from data coming from the CARDIOCARE mobile app). The eCRF will be accessible to all participating centers. The leading investigator in each clinical centers will be responsible for the collection of individual patient data, their accuracy and validity. Data will be collected and transferred to the eCRF developed in the CARDIOCARE Data Management and HPC Platform (WP5). A robust data protection and security strategy, by design, will be implemented in all procedures of data collection and storage in the CARDIOCARE platform. eCRF data will be uploaded and stored encrypted to the platform, and systematically backed-up in additional external RAID drives. Audit Log operation will be implemented to allow viewing the users' access history to the system enabling the detection of any potential security or data breaches. Dr. Maria Angela Massaro (IEO), as the Clinical Study Manager of the study, will have full responsibility for the collection, distribution and the secure storage of data from each of the participating centers by the end of the trial. The eCRF will be frozen after final quality control, and then exported for the statistical analysis of the primary and secondary objectives. All members of the consortium will take appropriate organizational and technical measures in order to prevent any event of abuse, accidental loss, destruction or damage by a physical or technical incident, enabling to reinstate the system in a timely manner.

Data quality, completeness and integrity will be ensured by visits of members of the IDMC who will visit the participating centers in order to certify consistency of the files, adherence to the study protocol and Good Clinical Practice Guidelines, accuracy of the CRF forms and compliance with safety reporting. The investigators will meet the members of the IDMC, co-operate and permit direct access to all source data needed in order to facilitate verification. Lead clinical

investigators are responsible for keeping up to date the CRF forms and should provide them to the clinical trial manager by the end of the study. Strict confidentiality of all personal and research data will be ensured. All recruiting centers will be required to sign a clinical study agreement document detailing their commitment towards complying with the relevant laws, regulations, codes of practice and obligations to publication.

2.5.4 Sample Management

Whole blood, plasma and stool sampling will be performed for the purposes of the clinical study. Whole blood and plasma will be used for routine lab tests, biochemical biomarkers (Troponin I, BNP), inflammatory (platelet activation, IL-6, TNF-α, HRV, CRP, Fibrinogen, Ferritin) and -omics biomarkers (SNPs, liquid biopsies, extracellular vesicles miRNAs). Stool samples will be collected (n=500) in a subpopulation of 500 patients (IEO: 100, BOCOC: 100, KSBC: 80, NKUA: 130, IOL: 90) at baseline (T1) and within 14 to 25 days after the end of each treatment.

For DNA analysis, whole blood and stool samples will be collected, labelled and stored at -20oC by each clinical partner. Plasma samples will be prepared by the clinical partners and stored at -80oC. Batches of samples will be shipped through a cold-chain courier on dry ice to the technical partner in charge of "omics" analysis (STREMBLE). STREMBLE will store and prepare the blood samples for DNA sequencing of SNPs and plasma for qPCR of a miRNA panel. Stool samples will be stored and prepared for DNA sequencing and 16S metagenomic analysis to identify microbiome biomarkers.

In order to avoid accidental loss, destruction or damage of the biological samples by a physical or technical incident, secure biological sample storage conditions will be applied including storage equipment maintenance and authorized access to and processing by trained personnel only.

For sample collection, handling and storage please refer to Annex 1 "D2.1. Experimental Design and relevant protocols of omics study – Detecting miRNA and gut microbiome changes induced by cancer treatment".

3 Regulatory Approval Procedures

This protocol will be submitted for approval to the Ethics Committees of all clinical sites involved in the study. Any amendments to the protocol or to study documents, other than administrative ones, must be approved by EC/IRB.

3.1 Protection of the Data Subjects

Each clinical site will have access to personal patient's data and will be able to associate clinical data with patient identifiers, while only anonymized data will be shared with the CARDIOCARE partners for the purposes of the study.

3.2 Patient Identification

As per GCP, patients have the right to confidentiality. Therefore, no patients' names will be used in any documentation transmitted to the European Institute of Oncology. Items that are used to identify a patient include the year of birth and registration number.

The local data manager will keep an identification log for all patients entered in this trial including:

- Patient's name
- Patient's initials
- Registration number
- Date of birth
- Date of registration

4 Administrative Considerations

4.1 Statement of Compliance

This study will be conducted in accordance with the Declaration of Helsinki and the ICH E6 Guideline (Good Clinical Practice). To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation, including subjects' hospital files (the source documents), by authorized individuals. The protocol was developed in compliance with the ISO 14155:2020. By signing this protocol, the investigator declares to conduct the trial in accordance with that regulation and norms. All procedures will comply with National law and the European Union's General Data Protection Regulation (GDPR).

4.2 Deviation from clinical protocol

A Protocol Deviation is defined as any change, divergence or departure from the study design or procedures defined in the study protocol, as approved by the Ethical Committee and the Competent Authority. A Protocol Deviation Handling Plan will be prepared by the study sponsor (responsible for the clinical trial, i.e., IEO), together with the study Contract Research Organization (CRO), to describe the approach for detecting, documenting, assessing, tracking and closing protocol deviations.

4.3 Monitoring Plan

An Investigators Meeting involving all participating investigators and co-investigators will be organized prior to the start of the prospective study. Appropriate training on procedures, protocol and eCRF use will be delivered during this meeting. A light remote monitoring to ensure the authenticity and credibility of data in accordance with the Good Clinical Practice will be performed, including a) verification of the informed consent (confirmation will be asked to each woman in the first questionnaire), b) verification that the eCRF data is consistent and in agreement with the source documents and c) if required (several inconsistencies), audit of the participating clinical centers. Finally, the European Society of Cardiology (ESC) will have a regulatory role, overlooking the clinical trial from the very beginning (since protocol design), taking care of ethical and legal

issues and respecting principles of good clinical practice. ESC is a CARDIOCARE partner independent from the clinical partners. Selected ESC members together with ICOS members comprise the Independent Data Monitoring Committee (IDMC). IDMC will be responsible for overseeing the procedures implemented for patient recruitment and quality of the acquired data by the six clinical sites. Monitoring will be performed in a minimum of three visits, in each clinical centre. A first visit upon Ethical Approval, a second visit after recruitment of 10 patients and a last visit upon study completion. For some centers additional visits may be mandatory under local regulations e.g., for every year of the study. Visits on each clinical site will be performed depending on the availability of the respective ESC and ICOS members. In addition, the lead clinical investigator, co-investigators, the local ethics committees, or any other supervisory body may audit or inspect the local trial sites at any time.

4.4 Suspension or Premature Termination of the Clinical Investigation

The Sponsor (IEO) may suspend/terminate the entire study, or the study at an individual site, at any time, for any of the following reasons:

- failure to enroll subjects;
- protocol violations or deviations;
- inaccurate or incomplete data;
- non GCP compliance;
- completion of enrolment;
- administrative reasons.

The Investigator may terminate his/her participation in the study in consultation with the Sponsor due to the occurrence of severe adverse events and/or adverse drug reactions endangering the health of subjects, which make it ethically unacceptable to continue.

4.5 Data property and publication policy

Property of data will be treated in accordance with the Consortium Agreement of the Horizon 2020 CARDIOCARE project. The main results of the clinical trial may be published in a peer-reviewed scientific journal and presented in scientific workshops only in an anonymous and aggregated way.

Co-authors will be the principal investigators of the Study who participate in the design and draw up of the research project, the contributing CARDIOCARE technical partners, the investigators of the clinical centers who add their patient data, and a representative of the European Institute of Oncology Data Management.

5 Registration of the Prospective Clinical Study

The Prospective Clinical Study will be registered in a WHO- or ICMJE- approved registry, such as the www.clinicaltrials.gov registry allowing later posting of study results. The sponsor of the study is IEO and is responsible for the study registration which requires an Ethics Approval by the competent Ethics Committee (EC). IEO has submitted the protocol to IEO EC, however ethics approval is still pending since the next EC meeting will be held on September, the 28th, 2022.

6 Approvals required for enrolment of the first study subject

The approvals required for the enrolment of the first data subject in at least one clinical centre are the following:

a. Ethical approval by the competent ethics committee,
b. The opinion or confirmation by the competent Institutional Data Protection Officer (DPO), that a Data Protection Impact Assessment (DPIA) has been performed and all data processing activities can proceed in compliance with the GDPR and national legislation.

To this end, all clinical centers participating in the prospective clinical study should have submitted the study protocol to their institution's ethics committees to obtain ethical approval and a comprehensive DPIA to their institutional DPO.

Due to issues related to the natural difference among the clinical centers several meetings were necessary to discuss and reach a consensus, in particular related to the type of data usually collected from patients by the clinical partners, the best time points of data collection to have a more complete patient's trajectory without burdening patients. Solutions were reached in deciding a homogeneous data collection process among all the clinical centers. However, this process slowed down the process of the study protocol finalization, with a delay of its submission to the Ethics Committees. The risk of this delay however was described in the **Description of Risks in the DoA**. As also described, in order to mitigate the risk of poor recruitment due to a delay in Ethical Approval, the recruitment period will be extended to guarantee a 12 months recruitment. This will slightly affect the rest WPs, since retrospective data will be already available for the technical developments.

The current status of these approvals in each center is presented in **Table 4**.

Table 4 *- Approvals status for each clinical center.*

Country	Centre	Ethics Approval	DPIA Approval	Expected date
Italy	IEO	Pending (Next IEO EC meeting scheduled for the 28th of Sept.)	Pending	October 26th 2022
Cyprus	BOCOC	Pending (next EC meeting scheduled on the 29th of Sept.)	Pending	October 2022
Sweden	KSBC	Expected: November	Pending	September
Greece	UOI	Expected: November	Pending	November
Greece	NKUA	Expected: November	Pending	November
Slovenia	IOL	Pending (Medical Ethics Committee of Republic of Slovenia scheduled for Nov 15,2022)	Pending	November 2022

7 Conclusions

Based on the protocol, in the prospective study multi-dimensional data will be collected to identify the cardiotoxicity trajectory of breast cancer patients. In particular the collected data will be analyzed using machine learning approaches to improve and validate the risk stratification model initially developed in WP3 with the data from the retrospective study.

Simultaneously, the prospective study will provide information on the mitigation effect of intervention on breast cancer patients' cardiotoxicity.

8 References

1. Bussotti and Sommaruga. Anxiety and depression in patients with pulmonary hypertension: impact and management challenges. Vasc Health Risk Manag. 2018; 14: 349–360.
2. Čelutkienė J, et al. Role of cardiovascular imaging in cancer patients receiving cardiotoxic therapies: a position statement on behalf of the Heart Failure Association (HFA), the European Association of Cardiovascular Imaging (EACVI) and the Cardio-Oncology Council of the European Society of Cardiology (ESC). Eur J Heart Fail. 2020 Sep;22(9):1504-1524.
3. Chianca M, Panichella G, Fabiani I, Giannoni A, L'Abbate S, Aimo A, Del Franco A, Vergaro G, Grigoratos C, Castiglione V, Cipolla CM, Fedele A, Passino C, Emdin M and Cardinale DM (2022) Bidirectional Relationship Between Cancer and Heart Failure: Insights on Circulating Biomarkers. Front. Cardiovasc. Med. 9:936654. doi: 10.3389/fcvm.2022.936654
4. Denollet J et al. Cytokines and immune activation in systolic heart failure: the role of Type D personality. Brain Behav Immun. 2003;17(4):304-9.
5. Herrmann J, et al. Defining cardiovascular toxicities of cancer therapies: an International Cardio-Oncology Society (IC-OS) consensus statement. Eur Heart J. 2022 Jan 31;43(4):280-299.
6. Keramida K, Farmakis D, Bingcang J, Sulemane S, Sutherland S, Bingcang RA, Ramachandran K, Tzavara C, Charalampopoulos G, Filippiadis D, Kouris N, Nihoyannopoulos P. Longitudinal changes of right ventricular deformation mechanics during trastuzumab therapy in breast cancer patients. Eur J Heart Fail. 2019 Apr;21(4):529-535. doi: 10.1002/ejhf.1385. Epub 2019 Feb 27. PMID: 30811091.
7. Rosengren A, Hawken S, Ounpuu S, Sliwa K, Zubaid M, Almahmeed WA, Blackett KN, Sitthi-amorn C, Sato H, Yusuf S; INTERHEART investigators. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. Lancet. 2004 Sep 11-17;364(9438):953-62.
8. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Cohen V, Gosavi S, Carver JR, Wiegers SE, Martin RP, Picard MH, Gerszten RE, Halpern EF, Passeri J, Kuter I, Scherrer-Crosbie M. Early detection and prediction of cardiotoxicity in chemotherapy-treated patients. Am J Cardiol. 2011 May 1;107(9):1375-80. doi: 10.1016/j.amjcard.2011.01.006. Epub 2011 Mar 2. PMID: 21371685; PMCID: PMC3703314.