Clinical Protocol Template for ClinO, Chapter 4 »Other Clinical Trials« Protocol Title

Study Type: Multicenter, observational prospective longitudinal study.

Risk Categorisation: Risk category B

Study Registration:

Sponsor:

Investigated Intervention: Early detection of cardiotoxicity from systemic and radiation therapy in breast cancer patients
Protocol ID

Version and Date: Version 3.0 (23.01.2020)

CONFIDENTIALITY STATEMENT

PROTOCOL SIGNATURE FORM

Study Title	EARLY DETECTION OF CARDIOTOXICITY FROM SYSTEMIC AND RADIATION THERAPY IN BREAST CANCER PATIENTS.
Study ID	

The Sponsor-Investigator has approved the protocol version 3 (23.01.2020) and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, and ICH-GCP guidelines as well as the local legally applicable requirements.

TABLE OF CONTENTS

GLOSSARY OF ABBREVATIONS

AE	*Adverse Event*
ASR/DSUR	*Annual Safety Repot / Development Safety Report*
BASEC	*Business Administration System for Ethical Committees*
CMR	*Cardiac Magnetic Resonance*
CRF	*Case Report Form*
CTCAE	*Common Terminology Criteria for Adverse Events*
ECHO	*Echocardiography*
FADP	*Federal Act on Data Protection (in German: DSG, in French: LPD, in Italian: LPD)*
eCRF	*electronic Case Report Form*
FOPH	*Federal Office of Public Health*
GCP	*Good Clinical Practice*
GLS	*Global Longitudinal Strain*
HRA	*Human Research Act (in German: HFG, in French: LRH, in Italian: LRUm)*
ICH	*International Conference on Harmonisation*
ClinO	*Ordinance on Clinical Trials in Human Research (in German: KlinV, in French: OClin, in Italian: OSRUm)*
SAE	*Serious Adverse Event*
LVEF	*Left Ventricular Ejection Fraction*

1 STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	Dr.ssa Mariacarla Valli
Study Title	EARLY DETECTION OF CARDIOTOXICITY FROM SYSTEMIC AND RADIATION THERAPY IN BREAST CANCER PATIENTS. A MULTICENTER OBSERVATIONAL PROSPECTIVE LONGITUDINAL STUDY
Short Title / Study ID	CARDIOTOX-BREAST
Protocol Version and Date	Version 3.0 (dated 23.01.2020)
Study Registration	
Study Category and Rationale	Multicenter, observational prospective longitudinal study.
Background and Rationale	Incidence of breast cancer is rising worldwide. Due to developments in systemic and local therapies, median survival of early breast cancer patients has increased (Waks et al. 2019). Treatment of early breast cancer is multimodal and usually consists of surgery, systemic therapy and radiation therapy. As patients are living longer, it is important to decrease the rate of side effects from treatment and increase patients quality of life. This includes prevention and early detection of cardiotoxicity as many of the current treatments are potentially cardiotoxic. Cardiovascular complications from cancer therapy are a very heterogenous group- e.g myocardial dysfunction, heart failure (HF), coronary artery disease, valvular disease, arrhythmias, arterial hypertension, etc. Myocardial dysfunction and HF are the most frequent cardiac problems with breast cancer treatment. Cardiotoxicity from breast cancer treatment is the main mortality reason after malignancy in these patients (Barish et al 2019). It is belived, that cardiotoxicity is a continuous phenomenon beginning with myocardial injury, changes in myocardial strain and followed by progressive LVEF decline that may gradually lead to symptomatic heart failure (Cardinale et al. 2018). Anthacyclines cause irreversible cardiac damage usually characterized by continuous progressive decline in LVEF, which is dose-dependant and can lead to dilatated heart failure. However, it is hard to predict which patients develop these side effects as some patients can tolerate standard-dose of anthracyclines without long-term complications. On the other hand treatment-related cardiotoxicity may occur as early as after the first dose in other patients. (ESC Position Paper 2016). Many affected patients may initially be asymtomatic and clinical manifestations appear years later, often in the context of other triggering factors (ESC Position Paper 2016). Anthracyclines induced cardiotoxicity may present during or immediately after the infusion (acute), within the first year of treatment (early) and years after treatment (late) (Curigliano et al 2012, Cardinale et al 2015). Trastuzumab-induced cardiotoxicity is usually reversible with drug interruption and/or treatment with HF therapies. Toxicity from type II agents are not cumulative-dose related and usually develops during treatment (ESC Position Paper 2016). The incidence of cardiotoxicity from trastuzumab is 1,7-20,1% and from anthracycline 3-48%. Radiation induced cardiotoxicity (RICT) usually develops years after RT. Interstitial myocardial fibrosis is common in RICT. Also RT may be associated with a higher incidence of ischaemic heart disease through the development of severe atherosclerotic and non-atherosclerotic disease, complicated by plaque rupture and thrombosis, and potentially with coronary spasm (ESC Position paper 2016). We have to be aware that the critical portions of the heart (e.g LV, LAD) can receive more than 40 Gy, although the mean heart dose may be low, because the majority of the heart is receiving almost 0 Gy, (Cahlon et al. 2017). The actual incidence of radiation-induced cardiotoxicity is difficult to evaluate. Some studies have found a relative risk of fatal cardiovascular events between 1 and 2.2 in patients with breast cancer. Studies have suggested a synergistic effect on cardiac risk with left breast RT and cardiotoxic chemotherapy.

	Systolic dysfunction is generally observed when RT is combined with anthracyclines (Jaworski et al 2013). The 2014 American Society of Echocardiograpy guidelines recommend baseline troponin at the initiation of both type I and II systemic therapy agents and the measurement of troponin before and 24 hours after each systemic therapy cycle to aid in detection of subclinical cardiotoxicity. Troponin and BNP are well studied in many trials, unfortunately with different results. Several studies have shown that elevation of TnI may predict the development of future LVEF depression and pro-BNP can predict the risk for radiation induced cardiotoxicity (Zagar et al. 2016, Cardinale et al. 2018). Of note, one study has showed that a reduction in longitudinal strain and an increase in high-sensitivity troponin, after the end of anthracycline therapy, predicted future left ventricular dysfunction (Cardinale et al. 2018). Several epidemiologic studies have shown a significant association between elevated plasma concentrations of high-sensitivity CRP and the prevalence of underlying atherosclerotic vascular disease, the risk of recurrent cardiovascular events among patients with established disease, and among apparently healthy individuals (Ridker et al. 2000, 2008 and 2017). Onitilo and his colleagues published a pilot study in 2012 about hs-CRP as a biomarker for trastuzumab-induced cardiotoxicity. They showed, that abnormal hs-CRP (≥3 mg/L) predicted decreased LVEF with a sensitivity of 92.9 % (Onitilo et al 2012). We are still in need of sensitive and specific biomarkers, which could diagnose preclinical cardiotoxicity induced by breast cancer therapies. Echocardiography (ECHO) is currently the standard method for detecting cardiotoxicity, usually by monitoring serial LVEF. Three dimensional is preferred over 2 dimensional because of better reproducibility (Galderisi et al. 2019). Unfortunately LVEF is not directly correlated to early toxicity and usually reduces months after myocardial cell injury has happened. The use of global longitudinal strain (GLS) by speckle tracking echocardiography is strongly recommended becuse of its feasibility and biological reproducibility (Galderisi et al. 2019). Also, GLS is changing earlier than LVEF, corresponding to myocardial deformation, so this technique could diagnose cardiotoxicity earlier, during subclinical myocardial dysfunction phase (Zagar et al. 2016, Charbonnel et al 2016). Current guidelines suggest ECHO at baseline- before potentially cardiotoxic treatment, after the end of anthracycline therapy/before the start of trastuzumab treatment and every 3 months during trastuzumab treatment. Cardiac Magnetic Resonance (CMR) is the most accurate methodology for the evaluation of volumes and function of heart chambers. Additionally, it is exquisitely capable of providing myocardial tissue characterization, including specifically the presence and extension of myocardial edema and fibrosis. Serial CMR imaging in women treated for breast cancer with anthracycline-based chemotherapy showed reduction in LVEF 12 to 24 months after initiating therapy. Few recent studies have suggested that LVEF could start to decline earlier, but the prognostic implications of these early changes are not yet known. Recent preliminary experimental data suggest that the decline in contractile function is preceded by CMR evidence of myocardial edema with T2 sequences and T2 mapping (Farhad et al. 2016, Arriola et al. 2019, Jordan et al. 2014). Moreover, breast cancer patients, who receive radiation therapy, have an increased risk for acute asymptomatic pericardial effusion, which can also be distinguished by CMR. CMR is the most sensitive and reproducible measure of LVEF. To our knowledge early radiation therapy cardiotoxicity has not been evaluated with CMR. When biomarkers and cardiac imaging methods (eg GLS on ECHO or oedema on CMR) are integrated, we can detect preclinical cardiotoxicity and prevent left ventricular dysfunction. Unfortunately so far the studies have had a small sample size and consequently we cannot evalute any practice chancing conclusion. This is why it is important to have baseline (before any cardiotoxic treatment) CMR together with biomarkers and LVEF with GLS measured on ECHO. Without baseline imaging, it is impossible to evaluate changes after systemic and radiation therapy as all individuals have different normal values. To our knowledge, our proposed study is the first longitudinal prospective one, which includes 200 patients. Its primary endpoint is to evalute myocardial oedema on CMR after cardiotoxic systemic and radiation therapy in predicting the incidence of cardiotoxicity.

	It is important to distinguish these high risk patients who need intensive cardiovascular screening during and after cardiotoxic treatment. Our purpose is find these risk group patients when the toxicity is still subclinical and reversible and prevent the subclinical toxicity with protective drugs administered to the right patient at the right time.
Risk / Benefit Assessment	Predicting the incidence of cardiotoxicity in stage I-III breast cancer patients treated with radiation therapy and neo/adjuvant chemo/immunotherapy +/- aromatase inhibitor/tamoxifen/LhRh agonist, as by International Guidelines.
Objective(s)	To assess the role of myocardial oedema on CMR (T2 mapping) after radiation therapy and cardiotoxic systemic therapy in predicting the incidence of cardiotoxicity, defined as by consensus guidelines* (decline of LVEF ≥10% points with a final LVEF <53%) measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy. *American Society of Echocardiography and European Association of Cardiovascular Imaging Expert Consensus
Endpoint(s)	Secondary objectives: 1. To detect GLS decrease >15% from baseline, measured on Echo over the time window of 12 months 2. To assess the incidence of myocardial oedema on CMR (T2 mapping) after radiation therapy and cardiotoxic systemic therapy measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy. 3. To see if the changes in biomarkers will correlate with LVEF measurements, assessed by ECHO and CMR 4. To see if the changes in biomarkers will correlate with GLS measurements, assessed by ECHO 5. To compare the time to the biomarkers positivity to the time to the decrease in GLS >15% and/or decline of LVEF ≥10% points with a final LVEF <53% measured on Echo. 6. To find out if patients with increased baseline biomarkers will develop cardiotoxicity, identify predictors of cardiotoxicity by multivariable analysis 7. To detect major cardiovascular events (defined as acute myocardial infarction, hospitalization due to heart failure, atrial flutter/fibrillation, ventricular tachycardia) or death due cardiac problems during the follow up 8. To assess the role of fibrosis on CMR (T1 mapping with evaluation of extracellular volume) after cardiotoxic radiation therapy and systemic therapy in predicting the incidence of cardiotoxicity. 9. To detect incidence of acute asymptomatic pericarditis after radiation therapy, measured on CMR 10. To investigate if the area of the edema on CRM correlates with RT dose distribution
Study Design	This is a multicenter, observational prospective longitudinal study.
Statistical Considerations	Sample size of this multicenter study is evaluated based on feasibility. We hypothesize to be able to enroll 150-200 patients satisfying the enrollment criteria and giving consent to participate into the study. We base calculations on the primary endpoint. From previous experience we expect a rate of cardiac toxicity around 17% at 12 months. We use a alpha level of 10% one-sided, given the lack of strong evidence in the literature (“early evidence” study). We used Stata 15 (StataCorp, College Station, TX, USA) for computation. Analyses will be performed using Stata 15 or later versions by the study statistician. Data at enrollment will be described as mean and standard deviation or median and 25th-75th percentiles if continuous and as counts and percent if categorical. They will be compared between groups of patients with and without oedema at the end of RT with the Student t test (or the Mann Whitney U test, based on the distribution) and with the Fisher exact test, respectively. Analysis of the primary endpoint. The proportion of patients with cardiac toxicity at 12 months will be compared between groups. The mean difference in proportions of cardiac toxicity at 12 months and its 80% confidence interval (CI) will be reported. Logistic regression will be used to adjust for potential confounders. Patients who die or are lost before the 12th month assessment will be considered as having toxicity. We do not expect losses to follow-up. A sensitivity analysis will classify them as no cardiac toxicity patients. If the mortality and/or

	losses to follow-up are above 10% will also compare groups using survival analysis methods to account for the different follow-up between patients, using the same strategies. Analysis of the secondary endpoints. Proportions will be compared as described above. Time to event endpoints will be compared using survival analysis methods. Correlation between changes in biomarkers and changes cardiac in cardiac function will be assessed with linear regression models. Data transformation will be applied as needed. Multivariable analysis to identify potential predictors of cardiac toxicity will be performed if allowed, given the 1:10 thumb rule between predictors and events. An ancillary study will enroll also stage 0 patients (appendix 2).
Inclusion- / Exclusion Criteria	Participants eligible for this study will include female patients aged ≥ 18 years, stage I-III breast cancer, treated with potentially cardiotoxic therapies at the participating centers over 2-3 years Inclusion criteria: Patients eligible for inclusion in this study must fulfill all of the following criteria: 1. Written informed consent must be obtained before any assessment is performed 2. Age ≥ 18 years at visit 1 3. Performance status ECOG 0-1 4. *Stage I-III histology proven breast cancer 5. Treated with adjuvant radiotherapy and neo/adjuvant anthracycline and/or trastuzumab-based therapy +/- hormonal therapy 6. Negative pregnancy test (plasma HCG) for all females of childbearing potential (i.e not permanently sterilised- post hysterectomy or tubal ligation status) Patients will be excluded if they meet any of the following criteria: 1. Known metastatic spread of any cancer 2. Known active or recurrent hepatic disorder (cirrhosis, hepatitis), ASAT/ALAT 2xULN 3. Renal function decrease (eGFR < 30 ml/min) 4. Known coronary artery disease 5. Angina pectoris 6. Positive or missing pregnancy test (pre- and perimenopausal women) at enrolment visit 7. Patients with baseline LVEF <53% and GLS <15% 8. Patients with pacemaker *In the ancillary study patients with stage 0 (DCIS) histology proven breast cancer will also be included.
Number of Participants with Rationale	Sample size of this multicenter study is evaluated based on feasibility. We hypothesize to be able to enroll 150-200 patients satisfying the enrollment criteria and giving consent to participate into the study. We base calculations on the primary endpoint.
Study Interventio	NA
Control Intervention	This study does not include control group.
Study procedures	**Overall study schedule** The Overall Study Schedule is summarized in the assessment schedule (appendix 1). This study is composed of three subsequent phases: a Run-In Phase, a RT/Systemic Therapy Phase, a Follow-Up Phase. Run-In Phase The Run-In Phase starts with the first visit (before any cancer treatment), when Screening/Enrollment procedure is performed. This phase will start once a patient has provided WIC to participate in the study and ends the day of treatment start. Screening / Enrolment Visit Visit will be performed before the expected starting date of treatment. After a WIC has been obtained from the patient, the patient will be visited by the Investigators and the following information will be gathered: • Demographic Data (age, height, weight, BMI); • Medical history (previous and concomitant diseases, previous therapies, family history of CVD);

	• Concomitant Medication; • Physical examination & overall health assessment (including vital signs). • Pregnancy test (pre- and perimenopausal women). The inclusion and exclusion criteria will be checked and, if the patient complies with all the Inclusion and Exclusion criteria, she will be enrolled into the study A baseline assessment will be performed by the Investigator: CMR, ECG and ECHO will be done at the participating centers The patient will be assigned to specific treatment (chemo/immunotherapy and adjuvant radiation therapy +/- aromatase inhibitor/tamoxifen/LhRh agonist). A standard of care treatment will be administered. Radiotherapy/Systemic therapy Treatment Phase (specific Visit descriptions) *SYSTEMIC TREATMENT* Blood sample will be scheduled before and, if possible, 24 hours after chemotherapy administration. -Patients treated with antracyclines regimens will be checked with ECG and ECHO at the end of treatment. -Blood sample will be scheduled before Trastuzumab administration every three weeks and ECHO will be done after every 4 cycles (3 months). *RADIOTHERAPY* For Technical details see appendix 3. Before starting RT patients will be checked clinically the first day of treatment and baseline tests will be done. Biomarkers will be checked the first day and in the middle of RT. If a patient gets symptomatic heart failure during the treatment, or if LVEF decline greater than 10% points with a final LVEF <53% measured on Echo, the patient will be referred to the cardiologist for a specific treatment as described by guidelines End of RT Group Patients treated with trastuzumab, will continue the treatment up to 1 year. Blood tests will be taken every three weeks and Echos will be done after every 4 cycles (3-week cycles). Follow-Up Phase 2 weeks+/-3 days after the end of RT, blood sample will be taken. An ECHO and CMR will be done. All patients will be checked 6 weeks after the end of radiotherapy for the study visit. The following activities will be performed: Blood sample for biomarkers. If hs-CRP ≥3mg/l, ECHO will be done. All patients will be followed at least until 10 years after the end of RT. Blood samples for measuring biomarkers and ECHO and CMR will be done 12 months after the end of RT. Unscheduled Visit An unscheduled visit may occur at any time during the study, only for safety reason or for a premature discontinuation from the study.
Study Duration and Schedule	Planned 02/2020 of First-Participant-In Planned 12/2022 of Last-Participant-Out
Investigator(s)	
	4 centers in 3 countries will be involved: 1) Istituto Oncologico della Svizzera Italiana Lugano/Bellinzona (IOSI), Via Ospedale, 6500 Bellinzona, Switzerland 2) Cardiocentro Ticino Lugano (CCT), Via Tesserete 48, 6900 Lugano, Switzerland 3) North Estonia Medical Center (NEMC), Sutise tee 19, 13419 Tallinn, Estonia 4) Ospedale Universitario S. Matteo, Pavia, Italia
Data privacy	Data will be collected in a dedicated database in Filemaker 11. The database will be resident on a Server at CCT. Backup will be performed according to the local operating procedures.

	Data will be pseudoanonymized with a unique personal number (UPN). It is the responsability of the PI at each Center to keep a logbook to maintain the link of patient ID and the code. No identifiers will be included in the database. Data entry will be performed via web. Access will be regulated by a center username and account. Each Center will be able to see and modify its own records only. The study PI will be able to see all records. Export of the database will be allowed to the study statistician.
Ethical consideration	Patients are treated according to current best practice and International Guidelines. Based on the assumptions of the study and the sponsor's previous clinical experience as well as current scientific knowledge, it is possible that by participating in the study patients may benefit from a diagnostic evaluation with CMR. Our aim is to diagnose a possible event when it is still subclinical and reversible and to prevent its evolution with protective drugs. Distinguish asymptomatic patients who are at risk of cardiotoxicity and, if possible, avoid side effects with protective treatment. This study does not involve any risks to patients.
GCP Statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP, the HRA as well as other locally relevant legal and regulatory requirements.

2 BACKGROUND AND RATIONALE

Incidence of breast cancer is rising worldwide. Due to developments in systemic and local therapies, median survival of early breast cancer patients has increased (Waks et al. 2019). Treatment of early breast cancer is multimodal and usually consists of surgery, systemic therapy and radiation therapy.

Local therapy for nonmetastatic breast cancer patients consists of surgical operation (lumpectomy or mastectomy and sentinel lymph node biopsy or axillary lymph node dissection). Depending on the type of operation and stage, adjuvant radiation therapy is prescribed to decrease the risk of disease recurrence and reducing the risk for breast cancer death (EBCTCG et al 2018).

Systemic therapy is an essential part of treatment for preventing recurrence in many patients with stage I-III breast cancer. It includes hormonal-, chemo- and/or biological therapy. Anthracycline-based regimens are one of the most effective treatments for patients with breast cancer. Early Breast Cancer Trialists' Collaborative Group meta-analysis showed that these regimens decrease breast cancer mortality by about 20% (EBCTCG et al. 2012). Not all early stage breast cancer patients need chemotherapy: the decision depends on the stage, tumor grade, molecular subtypes, genomic risk score and patients preference. 20% of breast cancers has ErbB-2 protein overexpression or ErbB-2 gene amplification. These patients benefit from ErbB-2-targeted therapy (e.g 1 year of trastuzumab) (Waks et al. 2019).

As patients are living longer, it is important to decrease the rate of side effects from treatment and increase patients quality of life. This includes prevention and early detection of cardiotoxicity as many of the current treatments are potentially cardiotoxic.

Cardiovascular complications from cancer therapy are a very heterogenous group- e.g myocardial dysfunction, heart failure (HF), coronary artery disease, valvular disease, arrhythmias, arterial hypertension, etc. Myocardial dysfunction and HF are the most frequent cardiac problems with breast cancer treatment. Cardiotoxicity from breast cancer treatment is the main mortality reason after malignancy in these patients (Barish et al 2019). It is belived, that cardiotoxicity is a continuous phenomenon beginning with myocardial injury, changes in myocardial strain and followed by progressive LVEF decline that may gradually lead to symptomatic heart failure (Cardinale et al. 2018).

When heart failure treatment in these patients is delayed, it is relatively possible that their cardiac function will never restores as it was baseline (Zagar et al. 2016). That is why it is important to distinguish asymptomatic patients who are at risk for cardiotoxicity and if possible, avoid side effects with protective treatment.

Anthacyclines are considered as the prototype of type I cardiotoxic agents (Curigliano et al 2012). It is belived that agents, belonging to this group, cause irreversible cardiac damage usually

characterized by continuous progressive decline in LVEF, which is dose-dependant and can lead to dilatated heart failure. However, it is hard to predict which patients develop these side effects as some patients can tolerate standard-dose of anthracyclines without long-term complications. On the other hand treatment-related cardiotoxicity may occur as early as after the first dose in other patients. (ESC Position Paper 2016). Many affected patients may initially be asymtomatic and clinical manifestations appear years later, often in the context of other triggering factors (ESC Position Paper 2016,). Anthracyclines induced cardiotoxicity may present during or immediately after the infusion (acute), within the first year of treatment (early) and years after treatment (late) (Curigliano et al 2012, Cardinale et al 2015).

Trastuzumab is considered to be type II cardiotoxic agent (Curigliano et al 2012). Trastuzumab-induced cardiotoxicity is usually reversible with drug interruption and/or treatment with HF therapies. Toxicity from type II agents are not cumulative-dose related and usually develops during treatment (ESC Position Paper 2016).

The incidence of cardiotoxicity from trastuzumab is 1,7-20,1% and from anthracycline 3-48%.

It varies between studies and so does the definition of cardiotoxicity. American Society of Echocardiography and European Association of Cardiovascular Imaging Expert Consensus defines cardiotoxicity as a decline of left ventricular ejection fraction (LVEF) ≥10% points with a final LVEF <53%.

Due to their potential cardiotoxicity, anthracycline and trastuzumab are usually not administred concurrently. Trastuzumab can be combined with taxanes (ESC Position Paper 2016).

If the patient needs adjuvant chest wall or breast radiation therapy (RT), it is usually given together with trastuzumab. Studies have not found any increased risk of cardiotoxicity due to concurrent use of these treatments (Sari et al. 2016).

Radiation induced cardiotoxicity (RICT) usually develops years after RT. Interstitial myocardial fibrosis is common in RICT. Also RT may be associated with a higher incidence of ischaemic heart disease through the development of severe atherosclerotic and non-atherosclerotic disease, complicated by plaque rupture and thrombosis, and potentially with coronary spasm (ESC Position paper 2016). Older studies have suggested that this phenomen manifests more than 10 years after the end of radiation therapy (Zagar et al 2016). New tehniques (e.g deep inspiration breath hold, 3D CT-based planning) should lower the risk for cardiotoxicity. Darby et al. metaanalysis evidenced that there are no “safe” radiotherapy dose to the heart. Also, there is a 7,4% increase in relative risk of major cardiac event for each additional 1 Gy of mean heart dose. This metaanalysis also confirmed that RICT begins in the first few years after treatment (Darby et al 2013, Cahlon et al 2017). We have to be aware that the critical portions of the heart (e.g LV, LAD) can receive more than 40 Gy, although the mean heart dose may be low, because the majority of the heart is receiving almost 0 Gy, (Cahlon et al. 2017). The actual incidence of radiation-induced cardiotoxicity is difficult to evaluate. Some studies have found a relative risk of fatal cardiovascular events between 1 and 2.2 in patients with breast cancer. Studies have suggested a synergistic effect on cardiac risk with left breast RT and cardiotoxic chemotherapy. Systolic dysfunction is generally observed when RT is combined with anthracyclines (Jaworski et al 2013).

The 2014 American Society of Echocardiograpy guidelines recommend baseline troponin at the initiation of both type I and II systemic therapy agents and the measurement of troponin before and 24 hours after each systemic therapy cycle to aid in detection of subclinical cardiotoxicity.

Troponin and BNP are well studied in many trials, unfortunately with different results. Several studies have shown that elevation of TnI may predict the development of future LVEF depression and pro-BNP can predict the risk for radiation induced cardiotoxicity (Zagar et al. 2016, Cardinale et al. 2018). Of note one study has showned that a reduction in longitudinal strain and an increase in high-sensitivity troponin, after the end of anthracycline therapy, predicted future left ventricular dysfunction (Cardinale et al. 2018).

Several epidemiologic studies have shown a significant association between elevated plasma concentrations of high-sensitivity CRP and the prevalence of underlying atherosclerotic vascular disease, the risk of recurrent cardiovascular events among patients with established disease, and among apparently healthy individuals (Ridker et al. 2000, 2008 and 2017).

Onitilo and his colleagues published a pilot study in 2012 about hs-CRP as a biomarker for trastuzumab-induced cardiotoxicity. They showed, that abnormal hs-CRP (≥3 mg/L) predicted decreased LVEF with a sensitivity of 92.9 %.
Still we are in need of sensitive and specific biomarkers, which could diagnose preclinical cardiotoxicity induced by breast cancer therapies.
Echocardiography (ECHO) is currently the standard method for detecting cardiotoxicity, usually by monitoring serial LVEF. Three dimensional is preferred over 2 dimensional because of better reproducibility (Galderisi et al. 2019). Unfortunately LVEF is not directly correlated to early toxicity and usually reduces months after myocardial cell injury has happened.
The use of global longitudinal strain (GLS) by speckle tracking echocardiography is strongly recommended becuse of its feasibility and biological reproducibility (Galderisi et al. 2019).
Also, GLS is changing earlier than LVEF, corresponding to myocardial deformation, so this tehnique could diagnose cardiotoxicity earlier, during subclinical myocardial dysfunction phase (Zagar et al. 2016, Charbonnel et al 2016).
Current guidelines suggest ECHO at baseline- before potentially cardiotoxic treatment, after the end of anthracycline therapy/before the start of trastuzumab treatment and every 3 months during trastuzumab treatment.
Cardiac Magnetic Resonance (CMR) is the most accurate methodology for the evaluation of volumes and function of heart chambers. Additionally, it is exquisitely capable of providing myocardial tissue characterization, including specifically the presence and extension of myocardial edema and fibrosis.
Serial CMR imaging in women treated for breast cancer with anthracycline-based chemotherapy showed reduction in LVEF 12 to 24 months after initiating therapy. Few recent studies have suggested that LVEF could start to decline earlier but the prognostic implications of these early changes are not yet known.Recent preliminary experimental data suggest that the decline in contractile function is preceded by CMR evidence of myocardial edema with T2 sequences and T2 mapping (Farhad et al. 2016, Arriola et al. 2019, Jordan et al. 2014).
Moreover breast cancer patients, who receive radiation therapy, have an increased risk for acute asymptomatic pericardial effusion, which can also be distinguished by CMR.
CMR is the most sensitive and reproducible measure of LVEF. To our knowledge early radiation therapy cardiotoxicity has not been evaluated with CMR.
When biomarkers and cardiac imaging methods (eg GLS on ECHO or oedema on CMR) are integrated, we can detect preclinical cardiotoxicity and prevent left ventricular dysfunction.
Unfortunately so far the studies have had a small sample size and consequently we cannot evalute any practice chancing conclusion.
This is why it is important to have baseline (before any cardiotoxic treatment) CMR together with biomarkers and LVEF with GLS measured on ECHO. Without baseline imaging, it is impossible to evaluate changes after systemic and radiation therapy as all individuals have different normal values.
To our knowledge, our proposed study is the first longitudinal prospective one which includes 200 patients. Its primary endpoint is to evalute myocardial oedema on CMR after cardiotoxic systemic therapy and radiation therapy in predicting the incidence of cardiotoxicity.
It is important to distinguish these high risk patients who need intensive cardiovascular screening during and after cardiotoxic treatment. Our purpose is find these risk group patients when the toxicity is still subclinical and reversible and prevent the subclinical toxicity with protective drugs administered to the right patient at the right time.

3 STUDY OBJECTIVES AND DESIGN

3.1 Hypothesis and primary objective

Describe a clear hypothesis that will be answered through the study and the primary objective. In rare cases a hypothesis might not be required (e.g. exploratory studies).

The primary objective of this trial is to find out if myocardial oedema on CMR (T2 mapping) after radiation and cardiotoxic systemic therapy is predicting the incidence of early cardiotoxicity, defined as by consensus guidelines (decline of LVEF ≥10% points with a final LVEF <53%).

3.2 Primary and secondary endpoints

The primary endpoint is the main result that is measured during or at the end of an intervention to verify whether the intervention (i.e. the administrated treatment, the surgical procedure, the physiotherapy etc.) was successful or not. Describe the variable of primary interest, the rationale for its selection, the method and as applicable the time point of assessment. In general, a single variable and a single time point are used for the primary endpoint. Under certain circumstances, a combined primary endpoint is possible, but should be carefully determined together with trial statistician.

If applicable, provide a description of all secondary endpoint variables to be assessed. The secondary endpoint(s) are used to answer the secondary objectives.

If applicable, provide a description of the safety endpoint variables referring to e.g. specific adverse events (AEs), the rate of adverse events in general, laboratory parameters/vital signs, etc.

Describe baseline factors that may have an influence on the endpoints (e.g. age, gender, history, morbidity etc.).

Primary Endpoint

To assess the role of myocardial oedema on CMR (T2 mapping) after radiation and cardiotoxic systemic therapy in predicting the incidence of cardiotoxicity, defined as by consensus guidelines* (decline of LVEF ≥10% points with a final LVEF <53%) measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy.

*American Society of Echocardiography and European Association of Cardiovascular Imaging Expert Consensus

Secondary Endpoints

1. To detect GLS decrease >15% from baseline, measured on Echo over the time window of 12 months
2. To see if the changes in biomarkers will correlate with LVEF measurements, assessed by ECHO and CMR
3. To see if the changes in biomarkers will correlate with GLS measurements, assessed by ECHO
4. To compare the time to the biomarkers positivity to the time to the decrease in GLS >15% and/or decline of LVEF ≥10% points with a final LVEF <53% measured on Echo.
5. To find out if patients with increased baseline biomarkers will develop cardiotoxicity, identify predictors of cardiotoxicity by multivariable analysis
6. To detect major cardiovascular events (defined as acute myocardial infarction, hospitalization due to heart failure, atrial flutter/fibrillation, ventricular tachycardia) or death due cardiac problems during the follow up
7. To assess the role of fibrosis on CMR (T1 mapping with evaluation of extracellular volume) after cardiotoxic radiation therapy and /or systemic therapy in predicting the incidence of cardiotoxicity.
8. To detect incidence of acute asymptomatic pericarditis after radiation therapy, measured on CMR
9. To investigate if the area of the edema on CRM correlates with RT dose distribution

10. To assess the incidence of myocardial oedema on CMR (T2 mapping) after radiation therapy and cardiotoxic systemic therapy measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy.

3.3 Study design

Both the study design and the selected methods should be appropriate to answer the research question and address the hypothesis.

Describe the general study design (e.g. confirmatory, non-randomised / randomised, one-arm / two-arms, open / single-blinded / cross-over) and the study setup (monocentric / multicenter, national / international). Discuss known or potential problems associated with the trial design.

Describe the methods of minimising bias (e.g. randomisation or other methods of minimising bias, such as the use of validated questionnaires).

This is a multicentric, observational prospective longitudinal study.

The study will be submitted for an approval by Ethical Committees and will be conducted in agreement with the principles of Good Clinical Practice and the Declaration of Helsinki.

This study is designed to evaluate myocardial oedema on CMR after radiation therapy and cardiotoxic systemic therapy in predicting the incidence of cardiotoxicity.

Stage I-III female breast cancer patients, who are planning on starting neo/adjuvant therapy for breast cancer at the participating centers (appendix 4) are asked to join the study. After the eligible patient has signed her written informed consent, she will start with scheduled visits and examinations as shown in the study assessment schedule.

Baseline blood tests will be taken, physical examination, CMR, ECG and ECHO will be done, proper medical history, including current medications of the patient and family history of CVD will be taken. If baseline hs-CRP ≥10 mg/l, another blood test will be checked after 2 weeks.

ECHO will be done on Philips Epiq or General Electic Vivid E95 and Cardiac Magnetic Resonance will be performed with Siemens Skyra 3T or a 1.5T scanner (MAGNETOM Aera, Siemens AG). Parameters measured and protocol is listed in appendix 5.

Patients receiving cardiotoxic chemotherapy will be drown blood before and if possible 24 hours after chemotherapy administration.

Patients who get anthracycline, have an ECHO and ECG after the end of this treatment.

During trastuzumab, blood will be taken before every administration (every 3 weeks) and ECHO will be done after every 4 cycles (every 3 months).

Before the beginning of radiotherapy, blood tests will be taken, CMR and ECHO will be done.

Patients who receive trastuzumab concurrently with RT will continue visits as described above.

Biomarkers will be taken in the middle of RT.

2 weeks +/-3 days after the end of RT, blood tests will be taken, CMR and ECHO will be done.

Six weeks after the end of RT, biomarkers will be measured. If hs-CRP ≥3mg/l, ECHO will be done.

Patients will be followed at least until 10 years after the end of RT.

12 months after the end of RT blood tests for measuring biomarkers and ECHO and CMR will be checked.

During treatment, if a patient gets symptomatic heart failure or decline of LVEF greater than 10% points, with a final LVEF <53% on ECHO, patient will be referred to cardiologist and specific treatment as described by guidelines will be prescribed.

3.4. Study intervention

Patients are treated by standard of care treatment. This study includes extra diagnostics (CMR,

ECHO and blood tests). Study procedures are discussed at section 4.3.

4 STUDY POPULATION AND STUDY PROCEDURES

4.1 Inclusion and exclusion criteria, justification of study population

Describe the study population and specify the total number of participants, including the control groups. Justify the choice of study population.

The study will enroll about 150- 200 female patients aged ≥ 18 years with stage I-III breast cancer treated with radiation therapy and neo/adjuvant chemo/immunotherapy +/- aromatase inhibitor/tamoxifen/LhRh agonist, as by International Guidelines.

An ancillary study will enroll also stage 0 patients (appendix 2).

This study does not include control group.

Inclusion criteria:

Patients eligible for inclusion in this study must fulfill all of the following criteria:

1. Written informed consent must be obtained before any assessment is performed
2. Female, Age ≥ 18 years at visit 1
3. Performance status ECOG 0-1
4. *Stage I-III histology proven breast cancer
5. Treated with adjuvant radiotherapy and neo/adjuvant anthracycline and/or trastuzumab-based therapy +/- hormonal therapy
6. Negative pregnancy test (plasma HCG) for all females of childbearing potential (i.e not permanently sterilised- post hysterectomy or tubal ligation status-)

Patients will be excluded if they meet any of the following criteria:

1. Known metastatic spread of any cancer
2. Known active or recurrent hepatic disorder (cirrhosis, hepatitis), ASAT/ALAT 2xULN
3. Renal function decrease (eGFR < 30 ml/min)
4. Known coronary artery disease
5. Angina pectoris
6. Positive or missing pregnancy test (pre- and perimenopausal women) at enrolment visit
7. Patients with baseline LVEF <53% and GLS <15%
8. Patients with pacemaker

*In the ancillary study patients with stage 0 (DCIS) histology proven breast cancer will also be included.

4.2 Recruitment, screening and informed consent procedure

Patients will be recruited in participating centers, where they are planning on starting treatment with anthracycline and/or trastuzumab and radiation therapy for stage I-III breast cancer. The enrollment will be ongoing for 2-3 years until the needed number of patients are recruited. The study investigators will recruit patients during their daily clinical practice.

Eligible patients may only be included in the study after providing written, IEC-approved informed consent (WIC) for the use of their data in the study. She will be informed that data will only be communicated in aggregated form.

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that she may withdraw from the study at any time and that withdrawal of consent will not affect his or her subsequent medical assistance and treatment.

The participant will be informed that her medical records may be examined by authorised individuals other than their treating physician.

All participants for the study will be provided a participant information sheet and a consent form (appendix 6, 7) describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. Patients have one week to decide whether to participate in the study or not.

The formal consent of a participant, using the approved consent form, will be obtained before the participant is submitted to any study procedure.

The consent form will be signed and dated by the investigator or his designee at the same time as the participant sign. A copy of the signed informed consent will be given to the study participant. The consent form will be retained as part of the study records.

The screening visit will consist of collecting this data:

• Demographic Data (age, height, weight, BMI)

• Medical history (previous and concomitant diseases, previous therapies, family history of CVD)

• Concomitant Medication

• Physical examination & overall health assessment (including vital signs)

• Pregnancy test (pre- and perimenopausal women).

The inclusion and exclusion criteria will be checked and, if the patient complies with all the Inclusion and Exclusion criteria, she will be enrolled into the study

A baseline assessment will be performed by the Investigator:

CMR, ECG and ECHO will be done at the participating centers

The patient will be assigned to specific treatment (chemo/immunotherapy and adjuvant radiation therapy +/- aromatase inhibitor/tamoxifen/LhRh agonist.). A standard of care treatment will be administered.

Patients are treated according to current best practice and International Guidelines. No additional costs are foreseen in relation to the study.

4.3 Study procedures

The recruitment period is 2-3 years until the needed number of patients have been enrolled. Each patient will be followed until 12 months after the end of radiation therapy. If possible, patients will be followed for 10 years.

Study Procedures

Overall study schedule

The Overall Study Schedule is summarized in the assessment schedule (appendix 1).

This study is composed of three subsequent phases: a Run-In Phase, a RT/Systemic Therapy Phase, a Follow-Up Phase.

Run-In Phase

The Run-In Phase starts with the first visit (before any cancer treatment), when Screening/Enrollment procedure is performed. This phase will start once a patient has provided WIC to participate in the study and ends the day of treatment start.

Screening / Enrolment Visit

Visit will be performed before the expected starting date of treatment.

After a WIC has been obtained from the patient, the patient will be visited by the Investigators and the following information will be gathered:

• Demographic Data (age, height, weight, BMI);

• Medical history (previous and concomitant diseases, previous therapies, family history of CVD);

• Concomitant Medication;

• Physical examination & overall health assessment (including vital signs).

• Pregnancy test (pre- and perimenopausal women).

The inclusion and exclusion criteria will be checked and, if the patient complies with all the Inclusion and Exclusion criteria, she will be enrolled into the study

A baseline assessment will be performed by the Investigator:

CMR, ECG and ECHO will be done at the participating centers

The patient will be assigned to specific treatment (chemo/immunotherapy and adjuvant radiation therapy +/- aromatase inhibitor/tamoxifen/LhRh agonist.). A standard of care treatment will be administered.

Radiotherapy/Systemic therapy Treatment Phase

SYSTEMIC TREATMENT

-Blood sample will be scheduled before and, if possible, 24 hours after chemotherapy administration.

-Patients treated with antracyclines regimens will be checked with ECG and ECHO at the end of treatment.

-Blood sample will be scheduled before Trastuzumab administration every three weeks and ECHO will be done after every 4 cycles (3 months).

RADIOTHERAPY

For Technical details see appendix 3.

-Before starting RT patients will be checked clinically the first day of treatment and baseline tests will be done.

-Biomarkers will be checked the first day and in the middle of RT.

-If a patient gets symptomatic heart failure during the treatment, or if LVEF decline greater than 10% points with a final LVEF <53% on ECHO, the patient will be referred to the cardiologist for a specific treatment as described by guidelines

End of RT Group

Patients treated with trastuzumab, will continue the treatment up to 1 year. Blood tests will be taken every three weeks and ECHOs will be done after every 4 cycles (3-week cycles).

Follow-Up Phase

2 weeks+/-3 days after the end of RT, blood sample will be taken. An ECHO and CMR will be done.

All patients will be checked 6 weeks after the end of radiotherapy for the study visit.

The following activities will be performed:

-Blood sample for biomarkers.

If hs-CRP ≥3mg/l, ECHO will be done.

-All patients will be followed at least until 10 years after the end of RT.

-Blood samples for measuring biomarkers and ECHO and CMR will be done 12 months after the end of RT.

Unscheduled Visit

An unscheduled visit may occur at any time during the study, only for safety reason or for a premature discontinuation from the study.

Data will be collected in a dedicated database in Filemaker 11. The database will be resident on a Server at CCT. Backup will be performed according to the local operating procedures. Data will be pseudoanonymized with a unique personal number (UPN). It is the responsability of the PI at each Center to keep a logbook to maintain the link of patient iD and the code. No identifiers will be included in the database. Data entry will be performed via web. Access will be regulated by a center username and account. Each Center will be able to see and modify its own records only. The study PI will be able to see all records. Export of the database will be allowed to the study statistician.

4.4 Withdrawal and discontinuation

Subject has the right to withdraw from the study at any time for any reason, including

personal reasons. Subjects prematurely withdrawing from the study will not be replaced.

The reason for withdrawal and discontinuation of any subject from the investigation shall be recorded.

A patient's participation in the investigation is to terminate immediately upon her request. Every patient has the right to refuse further participation in the study at any time and without providing reasons. We will register the reason only in case of withdraw due to disconfort with diagnostic tools. The Investigator should anyway seek to obtain the reason and record this on the eCRF.

5 STATISTICS AND METHODOLOGY

5.1. Statistical analysis plan and sample size calculation

Study statistician is dr.ssa Catherine Klersy.

Sample size

Sample size of this multicenter study is evaluated based on feasibility. We hypothesize to be able to enroll 150-200 patients satisfying the enrollment criteria and giving consent to participate into the study. We base calculations on the primary endpoint.

Primary endpoint: To assess the role of myocardial oedema on CMR (T2 mapping) after cardiotoxic systemic therapy and radiation therapy in predicting the incidence of cardiotoxicity, defined as by consensus guidelines* (decline of LVEF ≥10% points with a final LVEF <53%) measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy.

Table 2. summarizes the effect size for the primary endpoint that can be detected for the expected sample size, given a power of 80% and different rates of prevalence of myocardial edema after cardiotoxic systemic therapy and/or radiation therapy. From previous experience we expect a rate of cardiac toxicity around 17% at 12 months. We use a alpha level of 10% one-sided, given the lack of strong evidence in the literature (“early evidence” study).

We used Stata 15 (StataCorp, College Station, TX, USA) for computation.

Table 2: Effect size computation assuming a rate of cardiac toxicity at 12 months of p1=17% in the cohort without edema at MR. Power 80%, alpha (2-sided) 20%

Hypotesized % of pts with MR oedema at the end of RT	Detectable effect size in the edema population P2 [Difference] N=150	Detectable effect size in the edema population P2 [Difference] N=200
5%	52% [35]	48% [31]
10%	43% [26]	39% [22]
15%	39% [22]	36% [19]
20%	37% [20]	34% [17]
25%	35% [18]	33% [16]

Statistical Analysis

Analyses will be performed using Stata 15 or later versions by the study statistician. Data at enrollment will be described as mean and standard deviation or median and 25th-75th percentiles if continuous and as counts and percent if categorical. They will be compared between groups of patients with and without oedema at the end of RT with the Student t test (or the Mann Whitney U test, based on the distribution) and with the Fisher exact test, respectively.

Analysis of the primary endpoint. The proportion of patients with cardiac toxicity at 12 months will be compared between groups. The mean difference in proportions of cardiac toxicity at 12 months and its 80% confidence interval (CI) will be reported. Logistic regression will be used to adjust for potential confounders. Patients who die or are lost before the 12th month assessment will be considered as having toxicity. We do not expect losses to follow-up. A sensitivity analysis will classify them as no cardiac toxicity patients. If the mortality and/or losses to follow-up are above 10% will will also compare groups using survival analysis methods to account for the different follow-up between patients, using the same strategies.

Analysis of the secondary endpoints. Proportions will be compared as described above. Time to event endpoints will be compared using survival analysis methods. Correlation between changes in biomarkers and changes cardiac in cardiac function will be assessed with linear regression models. Data transformation will be applied as needed.

Multivariable analysis to identify potential predictors of cardiac toxicity will be performed if allowed, given the 1:10 thumb rule between predictors and events.

CRF and data management

Data will be collected in a dedicated database in Filemaker 11. The database will be resident on a Server at CCT. Backup will be performed according to the local operating procedures. Data will be pseudoanonymized with a unique personal number (UPN). It is the responsability of the PI at each Center to keep a logbook to maintain the link of patient ID and the code. No identifiers will be included in the database. Data entry will be performed via web. Access will be regulated by a center username and account. Each Center will be able to see and modify its own records only. The study PI will be able to see all records. Export of the database will be allowed to the study statistician.

5.2. Handling of missing data and drop-outs

NA

6 REGULATORY ASPECTS AND SAFETY

6.1 Local regulations / Declaration of Helsinki

This study is conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP, the HRA as well as other locally relevant legal and regulatory requirements.

6.2 (Serious) Adverse Events-

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

A Serious Adverse Event (SAE) (ClinO, Art. 63) is any untoward medical occurrence that

- Results in death or is life-threatening,
- Requires in-patient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity, or
- Causes a congenital anomaly or birth defect

Both Investigator and Sponsor-Investigator make a causality assessment of the event to the trial invervention, (see table below based on the terms given in ICH E2A guidelines). Any event assessed as possibly, probably or definitely related is classified as related to the trial intervention.

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Both Investigator and Sponsor-Investigator make a severity assessment of the event as mild, moderate or severe. Mild means the complication is tolerable, moderate means it interferes with daily activities and severe means it renders daily activities impossible.

Reporting of SAEs (see ClinO, Art. 63)

All SAEs are documented and reported immediately (within a maximum of 24 hours) to the Sponsor-Investigator of the study.

All reported SAEs, independently from their classification, will be recorded in the eCRF by filling in the “adverse event” section.

If it cannot be excluded that the SAE occurring in Switzerland is attributable to the intervention under investigation, the Investigator reports it to the Ethics Committee via BASEC within 15 days.

If the SAE occurs at one of the study sites, the coordinating Investigator reports the events to the Ethics Committee concerned, within 15 days.

Follow up of (Serious) Adverse Events

In case participants terminating the study (either regularly or prematurely) with reported ongoing SAE, or any ongoing AEs of laboratory values or of vital signs, the patient will be followed by the cardiologist or the specialist.

All AEs will be followed in order to elucidate as completely and practical as possible their nature and/or causality. The follow-up information will be submitted until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, until progression has been stabilized or till the conditions are considered out of threaten and acceptable or until the patient is lost to follow-up. Follow-up may therefore continue until after the patient has left the investigation.
Notwithstanding, any SAE will be followed-up after the study end until resolution or stabilization or till patient's health is considered no more at risk at Investigator's discretion.
In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the Sponsor Safety Contact by fax together with the SAE form, retaining a copy on site with the CRF. Any efforts should be performed in order to contextualize the circumstances leading to fatal outcome.

6.3 (Periodic) safety reporting

An annual safety report (ASR/DSUR) is submitted once a year to the Swiss Ethics Committee by the Investigator (ClinO, Art. 43 Abs).

In international multicentric studies the ASR/DSUR contains information from all sites including information from sites outside of Switzerland. The Sponsor-Investigator distributes the ASR/DSUR to all the participating Investigators.

6.4 Radiation

No extra radiation is administered to patients during this study. Patients will receive standard of care treatment for breast cancer.

6.5 Pregnancy

Pregnancy in an exclusion criterion.

6.6 Amendments

Substantial changes to the study setup and study organization, the protocol and relevant study documents are submitted to the Ethics Committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Ethics Committee. Such deviations shall be documented and reported to the Ethics Committee as soon as possible.

Substantial amendments are changes that affect the safety, health, rights and obligations of participants, changes in the protocol that affect study objective(s) or central research topic, changes of study site(s) or of study leader and sponsor (ClinO, Art. 29).

A list of substantial changes is also available on www.swissethics.ch.

A list of all non-substantial amendements will be submitted once a year to the competent EC together with the ASR.

6.7 (Premature) termination of study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.

- Ethical concerns,
- Insufficient participant recruitment,
- When the safety of the participants is doubtful or at risk (e.g. when the benefit-risk assessment is no longer positive),
- Alterations in accepted clinical practice that make the continuation of the study unwise, or
- Early evidence of harm or benefit of the experimental intervention

Upon regular study termination, the Ethics Committee is notified via BASEC within 90 days (ClinO, Art. 38).

Upon premature study termination or study interruption, the Ethics Committee is notified via BASEC within 15 days (ClinO, Art. 38).

Health-related data are anonymised upon end of data analysis.

6.8 Insurance

An Insurance policy has been undersigned to cover subjects who enter the trial.

FURTHER ASPECTS

7.1 Overall ethical considerations

To our knowledge, our proposed study is the first longitudinal prospective one which includes 150-200 patients. Its primary endpoint is to evalute myocardial oedema on CMR after cardiotoxic systemic and radiation therapy in predicting the incidence of cardiotoxicity. It is important to distinguish these high risk patients who need intensive cardiovascular screening during and after cardiotoxic treatment. Our purpose is find these risk group patients when the toxicity is still subclinical and reversible and prevent the subclinical toxicity with protective drugs administered to the right patient at the right time.

7.2 Risk-benefit assessment

Patients are treated according to current best practice and International Guidelines. Based on the assumptions of the study and the sponsor's previous clinical experience as well as current scientific knowledge, it is possible that by participating in the study patients benefit from a diagnostic evaluation with CMR. Our aim is to diagnose a possible event when it is still subclinical and reversible and to prevent its evolution with protective drugs. Distinguish asymptomatic patients who are at risk of cardiotoxicity and, if possible, avoid side effects with protective treatment. This study does not involve any risks to patients.

8 QUALITY CONTROL AND DATA PROTECTION

8.1 Quality measures

For quality assurance the sponsor, the Ethics Committee or an independent trial monitor may visit the research sites. Direct access to the source data and all study related files is granted on such occasions. All involved parties keep the participant data strictly confidential.

8.2 Data recording and source data

Data will be collected in a dedicated database in Filemaker 11. The database will be resident on a Server at CCT. Backup will be performed according to the local operating procedures. Data will be pseudoanonymized with a unique personal number (UPN). It is the responsability of the PI at each Center to keep a logbook to maintain the link of patient iD and the code. No identifiers will be included in the database. Data entry will be performed via web. Access will be regulated by a center username and account. Each Center will be able to see and modify its own records only. The study PI will be able to see all records. Export of the database will be allowed to the study statistician.

8.3 Confidentiality and coding

Trial and participant data will be handled with uttermost discretion and is only accessible to authorised personnel who require the data to fulfil their duties within the scope of the study. On the CRFs and other study specific documents, participants are only identified by a unique participant number.

8.4 Retention and destruction of study data and biological material

All study data are archived for 10 years after study termination or premature termination of the study.

9 MONITORING AND REGISTRATION

Radiation Oncology Clinic is responsible of monitoring and registration in a national language in the Swiss National Clinical trial Portal (SNCTP via BASEC)

The study, after approval, will be registered in a registry listed in the WHO International Clinical Trials Registry Platform (ICTRP; http://www.who.int/ictrp/en/), if it satisfies the definition given therein.

10. FUNDING / PUBLICATION / DECLARATION OF INTEREST

Funding has not appointed for this study.

The data collected during the study will be the property of the Investigators.

Results of the present study will not be disseminated or published until the study data are mature for the final analysis of the primary study endpoint. An interim-analysis could be performed when 50% of patients have been enrolled and have completed the 6 months follow-up.

This study is intended for publication irrespective of its results. The final publication of the main trial results will be written by Study Coordinators on the basis of the final analysis report. It will be published in a peer-reviewed scientific journal within 1.5 year of the end of the study.

The publications must conform to the CONSORT guidelines and to the International Committee of Medical Journal Editors guidelines on authorship.

Authorship is decided in accordance with the recommendations of the International Committee of Medical Journal Editors defining the roles of authors and contributors (http://icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html).

The first and last authors of publication of primary study results are the Study Coordinators who initiated the study design. Other Study Coordinators are usually second, third or fourth author. Specialists responsible for the collection and analysis of patient reported outcomes also qualify as co-author.

For publication of ancillary studies, the first author is the coordinator of the corresponding research, co-authors will include the study coordinators and scientific collaborators who made

substantial contribution to the research.

Other investigators and scientific contributors to the publication who do not qualify for authorship will be acknowledged in the publication.

Revision of posters, abstracts or any other kind of publication should be carried out within 15 days.

10 REFERENCES

1) Waks AG, Winer EP, Breast Cancer Treatment: A Review. JAMA. 2019 Jan 22;321(3):288-300
2) Barish, R, Lynce F, Unger K, Barac A, Management of Cardiovascular Disease in Women With Breast Cancer, Circulation. 2019 Feb 19;139(8):1110-1120
3) Cardinale D, Ciceri F, Latini R, et al. Anthracycline-induced cardiotoxicity: A multicenter randomised trial comparing two strategies for guiding prevention with enalapril: The International CardioOncology Society-one trial. Eur J Cancer. 2018 May;94:126-137
4) Zamorano JL, Lancellotti P, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). European Heart Journal, Volume 37, Issue 36, 21 September 2016, Pages 2768–2801
5) Curigliano G, Cardinale D, Dent S, et al. Cardiotoxicity of Anticancer Treatments: Epidemiology, Detection, and Management. CA Cancer J Clin 2016;66:309-325
6) Cardinale D, Colombo A, Bacchiani G, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation. 2015 Jun 2;131(22):1981-8
7) Cahlon O, Khan AJ. Cardiac Toxicity: The More We Learn, the Less We Know. Int J Radiat Oncol Biol Phys. 2017 Dec 1;99(5):1162-1165
8) Jaworski C, Mariani JA, et al .Cardiac Complications of Thoracic Irradiation. J Am Coll Cardiol. 2013 Jun 11;61(23):2319-28
9) Zagar TM; Cardinale DM, Marks LB. Breast cancer therapy-associated cardiovascular disease. Nat Rev Clin Oncol. 2016 Mar;13(3):172-84
10) Ridker PM, Hennekes CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000 Mar 23;342(12):836-43.
11) Ridker PM, Danielson E, Fonseca FAH, et al. Rosuvastatin to Prevent Vascular Events in Men and Women with Elevated C-Reactive Protein. N Engl J Med 2008; 359:2195-2207
12) Ridker PM, MacFadyen JG, Everett BM, et al. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet. 2018 Jan 27;391(10118):319-328.
13) Ontilo AA, Stankowski RV, Berg RL. High-sensitivity C-reactive protein (hs-CRP) as a biomarker for trastuzumab-induced cardiotoxicity in HER2-positive early-stage breast cancer: a pilot study. Breast Cancer Res Treat (2012) 134:291-298
14) Galderisi M, Lancellotti P. What is the best imaging tool in cardio-oncology? E-Journal of Cardiology Practice. 2019 Feb, Vol 16, N 39.
15) Charbonnel C, Convers-Domart R, Rigaudeau S, et al. Assessment of global longitudinal strain at low-dose anthracycline-based chemotherapy for the prediction of subsequent cardiotoxicity. Eur Heart J Cardiovasc Imaging. 2017 Apr 1;18(4):392-401
16) Farhad H, Staziaki PV, Addison D, et al. Characterization of the Changes in Cardiac Structure and Function in Mice Treated With Anthracyclines Using serial Cardiac Magnetic Resonance Imaging. Circ Cardiovasc Imaging. 2016 Dec;9(12).
17) Galán-Arriola C, Lobo M, Vílchez-Tschischke JP, et al. Serial Magnetic Resonance Imaging to Identify Early Stages of Anthracycline-Induced Cardiotoxicity. J Am Coll Cardiol. 2019 Feb 26;73(7):779-791

18) Jordan JH, D'Agostino Jr RB, Hamilton CA, et al. Longitudinal Assessment of Concurrent Changes in Left Ventricular Ejection Fraction and Left Ventricular Myocardial Tissue Characteristics After Administration of Cardiotoxic Chemotherapies Using T1-Weighted and T2-Weighted Cardiovascular Magnetic Resonance. Circ Cardiovasc Imaging. 2014 Nov;7(6):872-9
19) EBCTCG Peto R, Davies C, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012 Feb 4;379(9814):432-44
20) Sari SY, Yazici G, Sendur MA, et al. Concurrent Trastuzumab Use With Radiation Therapy and the Risk for Cardiotoxicity. Int J Radiat Oncol Biol Phys. 2016 Oct 1;96(2):E10-E11
21) Darby SC, Ewertz M, McGale P, et al. Risk of Ischemic Heart Disease in Women after Radiotherapy for Breast Cancer. N Engl J Med 2013;368:987-98.
22) Meyersohn NM, Pursnani A, Neilan TG. Detection of Cardiac Toxicity Due to Cancer Treatment: Role of Cardiac MRI. Curr Treat Options Cardio Med (2015) 17: 38.
23) Common Terminology Criteria for Adverse Events (CTCAE) https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf
24) Declaration of Helsinki https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
25) Federal Act on Data Protection (FADP) https://www.admin.ch/opc/en/classified-compilation/19920153/index.html
26) Human Research Act (HRA) https://www.admin.ch/opc/de/classified-compilation/20061313/index.html
27) International Conference on Harmonization (ICH) E6(R2) Guideline for Good Clinical Practice http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf
28) International Conference on Harmonization (ICH) E2A Clinical Safety Data Management: Definitions and Standards for Expedited Reporting http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002749.pdf
29) Ordinance on Clinical Trials in Human Research (ClinO) https://www.admin.ch/opc/de/classified-compilation/20121176/index.html

Appendix 1

Schedule of assessments

Visit	V1	V2	V3.X	V4.X	V5	V6.X (only in patients receiving trastuzumab)	V7	V8	V9	V10	V11
Obtain informed consent	X										
Age		X									
Height		X									
Weight		X									
Inclusion/exclusion		X									
Labs: Hs-CRP, troponin, proBNP		X	X	X		X	X	X	X	X	X
Labs: ASAT, ALAT, Creat, eGFR		X	X								
Labs: Hb1Ac, LDL, HDL, Chol		X									
Labs: HCT		X					X		X		
History of CV disease		X									
Medical History/Current Condition		X									
Family History of CVD		X									
Smoking and Alcohol History		X									
Current CV Medications		X	X			X					
Current DM Medications		X	X			X					
Concomitant Medications		X	X			X					
Physical Examination		X	X			X					
ECG		X			X						
ECHO		X			X	every 3 months during treatment	X		X	X[a]	X
CMR		X					X		X		X
Serum pregnancy test (HCG) for pre-and perimenopausal pt		X									
V1. Enrollment											
V2. Before operation or before starting neoadjuvant chemotherapy, whichever is done first											
V3-X. Before administration (on the same day) of every chemotherapy cycle (every 3 or every 2 weeks depending on the anthracycline based chemotherapy schedule; every 3 weeks during taxane-based chemotherapy)											
V4-X. 24 hours after every chemotherapy administration, if possible (every 3 or every 2 weeks depending on the anthracycline based chemotherapy schedule; every 3 weeks during taxane-based chemotherapy)											
V5. After the end of anthracycline regimen (if RT administered just after anthracycline chemo V5=V7)											
V6-X. Before administration (on the same day) of every trastuzumab cycle (every 3 weeks also for weekly schedule of trastuzumab)											
V7. Before starting adjuvant radiation therapy											
V8. In the middle of radiation therapy											
V9. 2 weeks +/-3 days after the end of radiation therapy											
V10. 6 weeks after the end of radiation therapy											
V11. 12 months after the end of radiation therapy											

* if V10 hs-CRP ≥3mg/l

Appendix 2

Ancillary Study

An Ancillary study will be performed at Istituto Oncologico della Svizzera Italiana (IOSI), Bellinzona (CH) and North Estonia Medical Center, Tallinn (EST).
The study population consists of stage 0 breast cancer patients who will be treated with surgery, adjuvant radiation therapy +/- hormonal therapy.

Primary endpoint:
To assess the role of myocardial oedema on CMR (T2 mapping) after radiation therapy in predicting the incidence of cardiotoxicity, defined as by consensus guidelines* (decline of LVEF ≥10% points with a final LVEF <53%) measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy.
*American Society of Echocardiography and European Association of Cardiovascular Imaging Expert Consensus

Secondary objectives:

1. To detect GLS decrease >15% from baseline, measured on Echo over the time window of 12 months
2. To see if the changes in biomarkers will correlate with LVEF measurements, assessed by ECHO and CMR
3. To see if the changes in biomarkers will correlate with GLS measurements, assessed by ECHO
4. To compare the time to the biomarkers positivity to the time to the decrase in GLS >15% and/or decline of LVEF ≥10% points with a final LVEF <53% measured on Echo.
5. To find out if patients with increased baseline biomarkers will develop cardiotoxicity; identify predictors of cardiotoxicity by multivariable analysis
6. To detect major cardiovascular events (defined as acute myocardial infarction, hospitalization due to heart failure, atrial flutter/fibrillation, ventricular tachycardia) or death due cardiac problems during the follow up
7. To assess the role of fibrosis on CMR (T1 mapping with evaluation of extracellular volume) after cardiotoxic systemic therapy and/or radiation therapy in predicting the incidence of cardiotoxicity.
8. To detect incidence of acute asymptomatic pericarditis after radiation therapy, measured on CMR
9. To investigate if the area of the edema on CRM correlates with RT dose distribution
10. To assess the incidence of myocardial oedema on CMR (T2 mapping) after radiation therapy and cardiotoxic systemic therapy measured on CMR and ECHO over the time window of 12 months from the end of radiation therapy.

Inclusion criteria:
Patients eligible for inclusion in this study must fulfill all of the following criteria:

1. Written informed consent must be obtained before any assessment is performed
2. Female, age ≥ 18 years at visit 1
3. Performance status ECOG 0-1
4. Stage 0-II histology proven breast cancer
5. Treated with operation, adjuvant radiation therapy +/- hormonal therapy
6. Negative pregnancy test (plasma HCG) for all females of childbearing potential (i.e not permanently sterilised- post hysterectomy or tubal ligation status)

Patients will be excluded if they meet any of the following criteria:

1. Known metastatic spread of any cancer
2. Breast cancer patients treated with chemo/immunotherapy
3. Known active or recurrent hepatic disorder (cirrhosis, hepatitis), ASAT/ALAT 2xULN
4. Renal function decrease (eGFR < 30 ml/min)
5. Known coronary artery disease
6. Positive or missing pregnancy test (pre- and perimenopausal women) at enrolment visit
7. Patients with baseline LVEF <53% and GLS <15%
8. Patients with pacemaker

Patients in this ancillary study will follow the procedures of the main study.
A signed consent will be obtained from every participant in the ancillary study.

Appendix 3
Technical aspects of radiation therapy

A) IOSI

1. Tumori della mammella

1.1. CTV Mammario (CTV II)

Il disegno del CTV II si basa sulle seguenti informazioni:

- limiti palpabili del corpo mammario identificati con reperi radio-opachi
- anatomia TC della ghiandola mammaria (tenendo conto che il tessuto ghiandolare visibile su TC può non corrispondere esattamente alla sua reale estensione)
- anatomia TC di strutture anatomiche extramammarie di riferimento.

Il CTV II è costituito dall'intera mammella, fino a 0,3-0,5 cm al di sotto della superficie cutanea.
L'espansione a PTV II deve tener conto delle escursioni respiratorie e delle incertezze di posizionamento. Espansione 0,7-1 cm, "crop" dalla cute 0,3-0,5 cm.

3.1.1 *Dosi e frazionamenti*

Dopo chirurgia conservativa la mammella può essere trattata impiegando un frazionamento convenzionale,1.8-2 Gy/die in 5 frazioni settimanali fino alla dose totale di 50-50.4 Gy, o schemi ipofrazionati, con la stessa equivalenza in termini di efficacia e tossicità.

1.2. Boost (CTV I)

La presenza di clips nel letto operatorio permette una migliore definizione dell'area da irradiare.
Il volume bersaglio (PTV I) del boost deve comprendere:

- il letto operatorio (CTVI) con 1,5 cm di margine se l'escissione è completa
- il letto operatorio (CTVI) con 3 cm di margine se l'escissione è incompleta e non si prevede un re-intervento.

L'espansione a PTV I deve tener conto delle escursioni respiratorie e delle incertezze di posizionamento. Espansione 0,7-1 cm, crop dalla cute 0,3-0,5 cm.

3.2.1 *Dosi e frazionamento*

Di norma sono previste, in caso di margini di resezione istologicamente negativi, dosi totali al letto operatorio di 10-16 Gy in 5-8 frazioni (2 Gy/die).

1.3. CTV Parete toracica (dopo mastectomia)

Il disegno del CTV si basa sulle seguenti informazioni:

- limiti anatomici della parete toracica identificati con reperi radio-opachi, incluso repere sulla cicatrice di mastectomia
- anatomia TC della parete toracica
- anatomia TC di strutture anatomiche extramammarie di riferimento.

L'espansione a PTV deve tener conto delle escursioni respiratorie e delle incertezze di posizionamento. Espansione 0,7-1 cm, "crop" dalla cute 0,3-0,5 cm se T1-T3.
Nel caso di T4, la cute sarà compresa nel CTV e PTV e dovrà essere previsto un bolus (spessore 0,5-1 cm) per ottimizzare la distribuzione di dose.

3.3.1 *Dosi e frazionamento*

Dopo mastectomia la parete toracica può essere trattata impiegando un frazionamento convenzionale, 2 Gy/die in 5 frazioni settimanali fino alla dose totale di 50 Gy +/- boost su cicatrice chirurgica se indicato.

1.4. CTV/PTV Stazioni linfonodali di I drenaggio

Regione sovraclaveare, catena mammaria interna, ascella omolaterali.
La decisione di irradiare le stazioni linfonodali dipende dallo stadio di malattia, dal numero di linfonodi metastatici e dalle caratteristiche immuno-istochimiche del tumore.

3.4.1 *Dosi e frazionamento*

Dopo chirurgia conservativa o mastectomia le aree linfonodali possono essere trattate impiegando un frazionamento convenzionale,1.8-2 Gy/die in 5 frazioni settimanali fino alla dose totale di 50-50.4 Gy.

1.5. Organi a rischio

- mammella controlaterale
- cuore
- polmone omolaterale
- Arteria Coronarica Discendente Anteriore (LAD)
- tiroide
- midollo spinale
- plesso brachiale

Dose constraints for breast/chest wall +/- lymph nodes (IM,SC,Axilla) target volumes and organs at risk:

Cuore	V5Gy ≤ 40.0% (≤ 50.0% for left-sided tumors) V20Gy ≤ 20.0% QUANTEC 25Gy (V25) <10%
Polmone omolaterale	V20Gy<25% solo breast V20Gy<35% breast + lymph nodes Mean <18Gy
Polmoni	Mean 20Gy V20Gy ≤ 25.0% V30Gy ≤ 20.0%

Mammella controlaterale	V5Gy ≤ 15.0%
Midollo	Max 45/47/50/50.5Gy 0.03cc<48Gy
LAD	V20Gy = 0%
Tiroide (se RT su sola mammella) Tiroide (se RT su SC)	V26Gy<20% Accettare 50 Gy su lobo omolaterale
Plesso brachiale	Max 50Gy

Appendix 5
ECHO and CMR measured parameters and protocol

On ECHO the following parameters will be defined:
• Left ventricular (LV) End Diastolic and Systolic diameter (mm)
• Septal and Posterior Wall Thickness (mm)
• 2D with or without 3D LV end diastolic and systolic volume index (LV EDVi, LV ESVi, mL/m2)
• 2D with or without 3D LV ejection fraction (LV EF, %)
• LV Global longitudinal strain (GLS, %)
• Left atrial volume (ml/m2)
• Mitral Valve (MV) E/A ratio, MV Deceleration Time (DT, ms)
• Pulsed-wave TDI e' velocity (cm/s), Mitral E/e'
• Pulmonary vein's flow
• Aortic root and annulus diameters (mm)
• Right ventricular (RV) diameter (mm), TAPSE (mm)
• RV fractional area change (%)
• Tricuspid regurgitation (TR) and Systolic pulmonary artery pressure (SPAP, mmHg)
• Valvular apparatus
• Inferior Vena Cava (IVC) diameter (mm) and Collapsibility

CMR will be performed as above: All patients will undergo a standard protocol, including late gadolinium enhancement for eGFR >30 ml/min. For native T1 and post-contrast mapping, basal, midventricular, apical short-axis and 4-chamber long-axis images will be acquired by "ECG triggered the modified Look-Locker inversion recovery" (MOLLI) sequence. Additionally, T2 mapping (T2-prepared Treu-FISP) will be acquired on the same plane. At the time of insertion of the iv cannula, blood will be drawn for hematocrit measurement. All CMR images and maps will be analyzed offline. Late gadolinium enhancement (LGE) will be quantified on short-axis stacks using a semiautomatic approach.

Appendix 6
DOCUMENTO INFORMATIVO SULLO STUDIO

Appendix 7
Consent form