Is Empagliflozin equivalent and/or synergistic with ACE inhibition in the prevention of chemotherapy mediated cardiotoxicity?

by

Tim Rozovsky

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Physiology and Pathophysiology

Max Rady College of Medicine, Rady Faculty of Health Sciences

University of Manitoba

Winnipeg, Manitoba, Canada

Abstract

Background: Breast cancer is a major public health concern in Canada. Doxorubicin and Trastuzumab (DOX+TRZ) are two of the most common anti-cancer drugs used in the treatment of breast cancer. While these two anti-cancer drugs improve overall survival in women with breast cancer, they increase the risk of developing heart failure. Several randomized controlled trials have demonstrated that sodium-glucose co-transporter 2 (SGLT2) inhibitors, including Empagliflozin (EMPA), which is a novel anti-diabetic medication, reduce the risk of heart failure associated hospitalization and mortality in patients with and without diabetes. Little is known, however, about whether SGLT2 inhibitors are cardioprotective in the setting of chemotherapy mediated cardiotoxicity.

Objective: The specific aim of this thesis is to evaluate whether the prophylactic use of SGLT2 inhibition with EMPA will be comparable and/or synergistic to standard pharmacological therapy using the renin-angiotensin system (RAS) antagonist perindopril (PER) in preventing DOX+TRZ mediated cardiotoxicity in a chronic *in vivo* female murine model.

Methods: In a chronic in *vivo* murine model of chemotherapy mediated cardiotoxicity, a total of 160 C57Bl/6 female mice received prophylactic treatment with PER (3 mg/kg), EMPA (10 mg/kg), or EMPA+PER orally for a total of 3 weeks as a run-in period prior to weekly administration of DOX+TRZ (8 mg/kg and 3 mg/kg, respectively) intraperitoneally for an additional 3 weeks (total of 6 weeks). Serial echocardiography was performed on a weekly basis and at the end of week 6, cardiac tissue was collected for histological, biochemical, and lipidomic analyses.

Results: In mice treated with DOX+TRZ, the left ventricular ejection fraction (LVEF) decreased from 75±3% at baseline to 41±4% at week 6. Prophylactic treatment with either PER, EMPA, or EMPA+PER improved LVEF to 57±3%, 66±3%, and 68±4% at week 6, respectively ($p<0.05$). Histological analyses confirmed significant disruption of myofibrils, vacuolization, and loss of sarcomere integrity in the DOX+TRZ treated mice. Prophylactic administration with PER, EMPA, or EMPA+PER, however, improved myofibril integrity at week 6 in mice receiving DOX+TRZ. Additionally, the Bax/Bcl-xL ratio was significantly elevated by 1.5x fold in mice receiving DOX+TRZ. This marker of apoptosis was attenuated by prophylactic treatment with either PER, EMPA, or EMPA+PER, to a level comparable with control mice. Finally, in DOX+TRZ treated mice, an increase in two oxidized phosphatidylethanolamine (OxPE) species was observed. Preliminary data demonstrates there is a possible trend in which prophylactic treatment with EMPA+PER attenuates these markers of lipid oxidation.

Conclusion: In a chronic *in vivo* murine model of DOX+TRZ induced cardiotoxicity, the prophylactic administration of PER, EMPA or EMPA+PER was effective in preventing adverse cardiovascular remodeling, attenuating histopathological changes in cardiomyocyte structure, and improving markers of oxidative stress and apoptosis.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Davinder Jassal, for his continuous support and mentorship over the years. Back when I interviewed for the Master's student position, I sat outside Dr. Jassal's office, the Section Head of Cardiology, and thought to myself "I am just a random undergrad student, this will probably be a very short conversation and I will be on my way, he must be too busy for me". Instead, he opened his door, shook my hand, and had a long conversation with me, inquiring about my background, goals and aspirations. Once we finished, he offered to go to his laboratory and introduce me to the lab members. There, I met with two of his master's students, as well as the lab technician David Cheung. When speaking with them, they had nothing but positive things to say about Dr. Jassal, acknowledging his high expectations, but that this is only for the benefit of the growth of his graduate students, as well as producing research that is of the highest standards. This is the kind of mentor a graduate student needs. Tough but fair. Demanding but accommodating. He maintains a professional environment in which we feel comfortable sharing and asking for his advice. He never leaves me waiting for an answer, both when it comes to research work and for personal matters. He cares for my well-being and makes sure I have all the tools I need to succeed. Dr. Jassal intensified my passion to become a doctor, and supported me on my journey to getting accepted into medical school.

Next, I would like to acknowledge the deepest gratitude I have for Mr. David Cheung, the lab manager/technician at the Cardiovascular Imaging (CVI) Laboratory. David was always highly accommodating, fun, engaging, and a great person to talk to about anything and everything. He taught me all the lab techniques, including handling animals, analyzing echos, performing Western Blots, and many more. I can say that I really lucked out with having him not only as my direct manager, but also as a great friend for life.

A special thank-you is extended to Allison Ledingham, who was a big part of welcoming me into the lab in the Fall of 2023 and helping me adjust to being a graduate student, as well as assisting me with my project, and teaching me many valuable skills while being kind, considerate, and genuinely friendly. I would also like to thank past and present CVI lab members Sumha Ali, Adrian Siapno, Lauren Castagna, Leah Schwartz, Dr. Akshi Malik, Sara Telles-Langdon, and Vibhuti Arya for their friendship, many great laughs, and for providing support and advice.

I feel truly honoured to have brilliant committee members, Dr. Sanjiv Dhingra, Dr. Amir Ravandi, and Dr. Jeffrey Wigle, who have provided innovative ideas for my project and pushed me to expand my knowledge in both the basic sciences and in the clinical context of heart failure prevention. Their research expertise and teaching skills are of the highest calibre, and I am very grateful that they have agreed to being part of my examining committee. In addition, I thank Dr. Ian Dixon for his leadership and support throughout the years, Dr. James Thliveris for his histological expertise, as well as Craig Resch and Dr. Aleksandra Stamenkovic for their invaluable help with lipidomic analyses.

On another note, I thank my family and my dear wife for their support throughout the years. I would not be where I am today without your unconditional love and the sacrifices you make to help me advance towards my ultimate goal of becoming a physician. THANK YOU.

Lastly, I would like to pay tribute to Dr. Pawan Singal who graciously agreed to serve as my co-supervisor for my Master's project back in 2022. My interview with him was unlike any other I experienced. While his passion for excellent research was very apparent, he was also genuinely interested in talking about life beyond the laboratory. Sadly, Dr. Singal passed away before the commencement of my project, but his pioneering spirit and kindness will be cherished forever.

Table of Contents

List of Tables

List of Figures

List of Equations

List of Abbreviations

AC	Adriamycin and Cyclophosphamide
ACEi	Angiotensin converting enzyme inhibitor
AMPK	Adenosine monophosphate-activated protein kinase
ANG-II	Angiotensin-II
ANOVA	Analysis of variance
ARB	Angiotensin receptor blocker
ATF4	Activating transcription factor 4
ATP	Adenosine triphosphate
Bax	Bcl-2 associated X protein
β-blocker	Beta-adrenergic receptor blocker
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra-large
Bcl-XS	B-cell lymphoma extra-small
BHT	Butylated hydroxytoluene
BNP	Brain-type natriuretic peptide
BRCA	Breast cancer tumor suppressor genes
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
BSA	Bovine serum albumin
CCS	Canadian Cardiovascular Society
CHF	Congestive heart failure
CHOP	CCAAT/enhancer-binding protein homologous protein

CM	Chloroform:methanol
CMR	Cardiac magnetic resonance
CRP	C-reactive protein
CRS	Cardiorenal syndrome
cTnT	Cardiac troponin-T
CTRCD	Cancer therapy related cardiac dysfunction
DBP	Diastolic blood pressure
DCL	Docetaxel
DOX	Doxorubicin
EBC	Early-stage breast cancer
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EPR	Epirubicin
ER	Endoplasmic reticulum
FEC	5-Flurouracil, Epirubicin, and Cyclophosphamide
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLS	Global longitudinal strain
GLUT	Glucose transporter
GPX4	Glutathione peroxidase 4
GRP78	78-kDa glucose-regulated protein
HEC	Hydroxyethyl-cellulose
HER2	Human epidermal growth factor receptor 2
HFpEF	Heart failure with preserved ejection fraction

HFrEF	Heart failure with reduced ejection fraction
HPLC	High performance liquid chromatography
HR	Heart rate
IL-1β	Interleukin 1-beta
IL-6	Interleukin-6
IL-8	Interleukin-8
INF-α	Interferon-alpha
i.p.	Intraperitoneal
IVS	Interventricular septal wall thickness
JNK	c-Jun N-terminal kinase
LV	Left ventricle/ventricular
LVEDD	Left ventricular end-diastolic diameter
LVEF	Left ventricular ejection fraction
LVESD	Left ventricular end-systolic diameter
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MRA	Mineralocorticoid receptor antagonist
MRI	Magnetic resonance imaging
MUGA	Multiple-gated radionuclide angiography
NF-κβ	Nuclear factor kappa beta
NLRP3	Nucleotide-binding oligomerization domain-like receptor 3
NRF2	Nuclear factor erythroid 2-related factor 2

NT-pro-BNP	N-terminal pro-brain type natriuretic peptide
OxPE	Oxidized phosphatidylethanolamine
OxPL	Oxidized phospholipids
PALB2	Partner and localizer of BRCA2
PBS	Phosphate buffered saline
PCL	Paclitaxel
PER	Perindopril
PERK	RNA-like ER kinase
PLAX	Parasternal long axis
PP	Pulse pressure
PSAX	Parasternal short axis
PVDF	Polyvinylidene difluoride
PWT	Posterior wall thickness
RAS	Renin-angiotensin system
RIPA	Radioimmunoprecipitation assay
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SERM	Selective estrogen receptor modulator
SGLT2	Sodium-glucose co-transporter 2
SLC7A11	Cystine transporter solute carrier family 7 member 11

SMP	Skim milk powder
TDI	Tissue doppler imaging
TLR4	Toll-like receptor 4
TnI	Troponin I
TNF-α	Tumor necrosis factor alpha
TRAF2	TNF receptor associated factor 2
TRZ	Trastuzumab
TTE	Transthoracic echocardiography
UPR	Unfolded protein response
XBP1	X-box binding protein 1

Chapter 1: Introduction

1.1 Breast Cancer

1.1.1 Epidemiology

Breast cancer is a major public health concern in Canada. In fact, it is the second most common cancer and is one of the leading causes of death from cancer for women living in Canada.[1] According to the Canadian Cancer Society, in 2024, an estimated 30,500 Canadian women will receive a breast cancer diagnosis.[1] Even though the breast cancer death rate has decreased by over 40% since 1986, and the 5-year survival rate is estimated at 89%, more than 5,500 Canadian women will die from breast cancer in 2024, representing 13% of all cancer deaths.[1,2] As a testament to its prevalence, it is approximated that 1 in 8 Canadian women will develop breast cancer during their lifetime, and 1 in 36 will die from the disease.[1] While breast cancer mostly affects post-menopausal women, nearly 20% of diagnoses occur before the age of 50, signifying the importance of early detection and screening.[3] Breast cancer is the leading cause of the ongoing increase in cancer-related healthcare expenses, which has a significant financial impact worldwide, amounting to an estimated figure of $1,531 billion in any given year, which includes direct medical costs, as well as indirect costs related to loss of productivity and unemployment. Although the survival rate for breast cancer patients has improved, the growing disease burden highlights the need for continued scientific research to enhance breast cancer treatment and improve outcomes.[4]

1.1.2 Risk Factors

In order to aid the development of screening guidelines and improve early detection efforts, researchers worldwide have established a list of risk factors that can increase an individual's susceptibility to developing breast cancer.[5] There are two main groups of risk factors, including

biological risk factors and lifestyle/socioeconomic risk factors, the former being associated with a more significant risk.[5-9] Having a mutation in the breast cancer tumor suppressor genes (BRCA), specifically breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2), can increase the lifetime risk of developing breast cancer by over 80%.[7,9,10] A more rare but significant risk factor is having a mutation in the partner and localizer of BRCA2 (PALB2) gene, which is increasingly being recognized in clinical practice.[1,5,11,12] Additionally, a family history of breast and/or ovarian cancer can significantly increase the odds of developing breast cancer, especially if these are first-degree relatives.[1] Biological factors including increased breast density, early menarche, and late menopause may play a role in increased breast cancer risk.[1] Certain lifestyle factors can contribute to that risk including high levels of alcohol consumption, smoking, usage of oral contraceptives, obesity, and physical inactivity. High socioeconomic status increases the risk as well, which is linked to having children later in life and/or having fewer children, which increases the exposure of breast cells to circulating estrogen and progesterone.[1,6,8,13-15]

1.1.3 Diagnosis

The steady decrease in breast cancer mortality rates over the past four decades can partially be attributed to increased screening allowing for earlier detection and targeted treatment.[1] The Canadian Cancer Society suggests that all women between the ages of 40 and 74 should undergo a mammography every 2 years, regardless of the presence of risk factors.[1] In order to decrease mortality rates, the World Health Organization suggests that appropriate screening programs should be implemented worldwide.[5] Non-invasive imaging modalities including mammography, ultrasound, and magnetic resonance imaging (MRI) are effective tools in detection of tumors and other abnormalities in the breast tissue. Once breast cancer is identified, due to the heterogeneity

of the disease, a core needle biopsy is performed to identify the presence of various biomarkers, including estrogen and progesterone hormone receptors, and the human epidermal growth factor receptor 2 (HER2) protein. This test helps determine the specific type and grade of breast cancer present. These diagnostic steps guide the process of developing an appropriate and effective treatment plan for the patient with breast cancer.[16]

1.1.4 Treatment

Breast cancer death rates in Canadian women between the ages of 35 and 69 have declined by nearly 62% between 1950 and 2015, depending on age group. These improved survival rates are mainly attributed to advances in treatment as well as improved detection technologies.[1,2] Early diagnosis is often associated with improved prognosis, reliant on prompt initiation of an effective treatment protocol. The treatment for breast cancer is individualized and depends on several factors, and may involve surgery, radiation, hormone therapy, chemotherapy, and/or targeted biological therapy.[1]

Surgery has been the primary, gold-standard treatment approach for breast cancer since the 1800s. Mastectomy, a surgical approach through which the entire breast and involved lymph nodes are removed has played a critical role in the treatment of breast cancer for centuries. However, the medical field has partially transitioned to the less radical type of surgery, lumpectomy, starting in the second half of the 20th century. Lumpectomy involves a more targeted, conservative approach in which only the tumor is removed, avoiding the often-unnecessary removal of the entire breast, leading to improved recovery and higher patient satisfaction.[17,18] However, mastectomies are still required in many cases, dependant on the size and location of the cancerous tissue.[17,18] Following

the removal of the tumor mass, recurrence of cancer is possible, and therefore it is often recommended that the patient undergoes radiation therapy and/or chemotherapy to eliminate the remaining cancer cells that my persist in the chest wall, surrounding skin, or neighboring tissue.[19,20]

Radiation therapy, which utilizes high-energy rays or particles to target cancer cells and kill them is often used both in the adjuvant and neoadjuvant setting.[1] Adjuvant radiation therapy is often administered following surgical intervention and/or chemotherapy. This intervention decreases the risk of post-lumpectomy cancer recurrence by up to 50% and improves breast cancer survival rate by approximately 17%.[20] Neoadjuvant radiation therapy, administered before surgery, can be used to shrink the tumor, which helps reduce the extent of the surgery, increasing the chances of undergoing breast-conserving surgery rather than a mastectomy. Pre-surgical radiotherapy improves pathologic response, decreases recurrence rate, and improves overall survival in comparison to adjuvant radiotherapy.[21,22]

Early-stage breast cancer (EBC) is most commonly treated with chemotherapy. There are multiple chemotherapy agents and regimens, administration of which depends on breast cancer stage and subtype.[23] In the treatment of EBC, third generation regimens are most commonly prescribed, which often combine two classes of cytotoxic agents; anthracyclines and taxanes. Anthracyclines include Doxorubicin (DOX) and Epirubicin (EPR). Taxane class agents include Paclitaxel (PCL) and Docetaxel (DCL).[23,24] Although highly effective for the treatment of breast cancer, anthracyclines pose a 10% risk of developing cardiotoxicity in a dose-dependent manner. [25,26]

Breast cancer can possess varying characteristics that can be specifically targeted in order to achieve the best treatment outcomes. For this reason, accurate diagnosis is critical for the selection of the appropriate targeted biological therapy. An important factor in discerning breast cancer progression is the hormone receptor status. This marker indicates whether the cancer cells possess estrogen and/or progesterone receptors, which in the presence of these hormones, may stimulate the neoplastic cells to survive and proliferate.[27] Approximately 75% of breast cancers are estrogen receptor and/or progesterone receptor positive.[28] Another characteristic that the cancer cells may exhibit is the hormone epidermal growth factor receptor 2 (HER2), which may be overexpressed in 20-25% of breast cancers. While tumors that possess these receptors grow quicker and worsen patient prognosis, a positive receptor status provides focused therapeutic opportunities.[27]

Breast cancer tissue positive for estrogen receptor is treated using varying agents to limit cancer progression and survival of the neoplastic cells. Selective estrogen receptor modulators (SERMs) suppress estrogen signaling by blocking the receptor and preventing the hormone from binding. Estrogen-receptor down-regulators work in a similar fashion, but can also induce degradation of the estrogen receptors.[29] Aromatase inhibitors inhibit the enzyme responsible for catalyzing the conversion of androgens to estrogen in post-menopausal women. Luteinizing hormone-releasing hormone agonists can be used to induce menopause and inhibit ovarian production of estrogen.[30] These treatments can be used alone or in combination to inhibit the effects of estrogen on the tumor cells.

Breast cancer positive for the HER2 receptor exhibits accelerated growth and proliferation of the cancer cells, resulting in a more aggressive disease state with a poorer prognosis. Approximately

7000 Canadian women will be diagnosed with HER2 positive breast cancer in 2024 alone.[1] Trastuzumab (TRZ), also known under the brand name Herceptin, is a humanized anti-HER2 receptor monoclonal antibody, used in the adjuvant and metastatic breast cancer setting. It works by binding to the HER2 receptors extracellularly to block the downstream pathways required for accelerated proliferation of cancer cells. TRZ has been shown to improve patient survival and reduce risk of recurrence and is the most common agent used in the HER2 positive breast cancer setting.[31-34]

In the adjuvant setting, TRZ is administered once anthracycline-based chemotherapy treatment is completed, intravenously, as a loading dose of up to 8 mg/kg followed by doses of 6 mg/kg every three weeks for a total of up to 18 cycles.[35] The use of TRZ in combination with anthracyclines has been validated by multiple studies.[31,33,35-45] Several multi-centre randomized clinical trials have evaluated the efficacy of TRZ in the clinical setting. In these trials, patients received a cumulative dose of DOX or EPR of 360 mg/m^2 and 720 mg/m^2, respectively, over a 12-week period, and were then treated with a one-year adjuvant therapy using TRZ.[37-44] The trials showed a 50% reduction in overall rate of recurrence and 33% decrease in cancer mortality in women with HER2 positive breast cancer.[37-44]

For metastatic breast cancer, TRZ is administered as a loading dose of 4 mg/kg intravenously followed by a maintenance dose of 2 mg/kg weekly. The duration of TRZ administration varies considerably dependant on disease progression and other clinical indicators, ranging from 9 to 30 months.[46] Metastatic breast cancer continues to be one of the top causes of cancer-related death among women. In fact, approximately 50% of individuals afflicted with HER2 positive breast

cancer receive a diagnosis once the disease has already metastasized.[47-48] While prognosis is extremely poor in this patient population, chemotherapy treatment in combination with TRZ has been shown to extend survival, averaging 4 to 5 years.[47] A small but considerable proportion of patients obtain long-term disease control and live more than ten years following diagnosis.[31,47-50] A clinical trial evaluating the efficacy of monotherapy with TRZ (in patients with no history of previous cytotoxic chemotherapy) demonstrated a 26% response rate.[51] A total of 48% of patients with the highest level of HER2 overexpression experienced a significant clinical benefit.[51] Although TRZ has been shown to be a powerful anti-cancer agent, it can also trigger adverse cardiotoxic side effects.[31,52-53]

1.2 Cardio-Oncology

Cardio-Oncology is an evolving discipline that focuses on the prevention, diagnosis, and management of cancer in patients who are at risk of developing cardiovascular complications as a result of their anti-cancer treatment. Over the past several decades, the prevalence of breast cancer patients developing cardiovascular disease, including heart failure, has increased. This is partly due to the development of efficient anti-cancer drugs, which can inadvertently affect the heart as well, and partly due to increased awareness and screening of cardiac function before, during, and after chemotherapy treatment. Despite the beneficial effects of surgery, radiation, chemotherapy, and/or targeted biological therapy for improving overall survival in women with breast cancer, cardiotoxicity remains a serious complication for many anti-cancer therapies, serving as the leading cause of morbidity and mortality in cancer patients.[25,26,37,38,52-54] Cancer therapy related cardiac dysfunction (CTRCD) can be diagnosed via cardiac imaging modalities in combination with assessment of cardiac biomarkers.[54-56] Once CTRCD is suspected or confirmed, the anti-

cancer therapy may be modified or discontinued and patients are prescribed medications with the goal of reversing the existing injury and prevent further cardiac damage.[57]

1.2.1 Chemotherapy & Targeted Biological Therapy

There are two common chemotherapy regimens presently employed for treating breast cancer. One is known as FEC, which combines 5-Flurouracil, EPR, and Cyclophosphamide. The other regimen, called AC, includes Adriamycin (DOX) and Cyclophosphamide.[23] Both chemotherapeutic regimens incorporate anthracyclines. In the context of breast cancer treatment, FEC chemotherapy is commonly given intravenously every three weeks, for a total of four to six cycles. The cumulative dosages usually reach up to 500 mg/m^2 of 5-Flurouracil, 100 mg/m^2 of EPR, and 500 mg/m^2 of Cyclophosphamide, unless there are contraindications. The AC regimen is normally administered every three weeks as an intravenous infusion. It consists of 60 mg/m^2 of Adriamycin and 600 mg/m^2 of Cyclophosphamide. This treatment is offered for four consecutive cycles, either before or after surgical removal of the tumor.[23] The total duration that either FEC or AC is administered is 3-5 months, on average. AC chemotherapy is discontinued if there is evidence of disease progression or the onset of cardiac dysfunction.[23]

Although all chemotherapy agents carry risks and side effects, anthracyclines—such as DOX and EPR—are the primary contributors to chemotherapy-induced cardiotoxicity. These agents cause type-I, irreversible cardiotoxicity in a dose-dependent manner, which can affect approximately 10% of patients. Anthracyclines lead to apoptosis and necrosis of cardiomyocytes, with subsequent myocardial fibrosis.[26,54] The cumulative administrated dose of anthracyclines is limited by their dose-dependent cardiotoxicity, which constrains their use as heart-safe anticancer agents.[26] The

current recommended total cumulative dose of DOX is limited to 550 mg/m^2, while EPR's cumulative dosing should not exceed 900-1000 mg/m^2.[58,59] However, patients with known cardiac risk factors are at higher risk of developing cardiotoxicity at lower cumulative doses. In this subset of cancer patients, the cumulative dose of DOX and EPR should be limited to 400 and 650 mg/m^2, respectively.[58,59] The cardiotoxic side effects of DOX include up-regulation of the following pathways including: i) inflammation; ii) endoplasmic reticulum (ER) stress; and iii) oxidative stress-induced cell damage and/or death (Table 1).

Inflammation is one of the three regulatory pathways that lead to DOX mediated cardiotoxicity. Nuclear factor kappa beta (NF-κβ) is a molecular mediator that functions as a regulator of the initial stage of inflammation, resulting in the upregulation of its active form, phospho- NF-κB (p65 subunit), and increasing the expression of pro-inflammatory markers such as tumor necrosis factor alpha (TNF-α), interferon-alpha (INF-α), interleukin 1-beta (IL-1β), and interleukin-6 (IL-6). This cascade of events can contribute to the development of cardiac fibrosis and heart failure.[60-64] NF-κB can also be stimulated by TNF-α and IL-1β through a feedback mechanism.[63] Upregulation of these inflammatory markers are suggestive of an inflammatory response in the heart caused by DOX mediated cardiotoxicity.[60-66]

The role of ER stress in the development of cardiotoxicity from DOX is well established.[67-71] The ER is one of the largest organelles in cardiomyocytes. It has many roles including protein synthesis, folding of polypeptides into biologically active proteins and enzymes, as well as lipid and steroid biosynthesis. Cardiotoxic agents, including anthracyclines, can disrupt the intricate ER processes, leading to accumulation of misfolded proteins, inducing ER stress.[71] When there is a

disruption in the balance of ER homeostasis, certain signalling pathways are triggered, which in turn initiate an adaptive response called the unfolded protein response (UPR). The main objective of the UPR is to rectify the protein equilibrium by inhibiting protein translation, enhancing the removal of unfolded or misfolded proteins, and fostering cell viability. The UPR attempts to restore homeostasis by triggering the activation of transmembrane proteins like protein kinase RNA-like ER kinase (PERK). Normally, PERK exists in an inactivated state, bound to the 78-kDa glucose-regulated protein (GRP78). Under ER stress conditions, GRP78 binds to misfolded proteins and dissociates from PERK. This triggers activation of downstream pathways and molecules such as activating transcription factor 4 (ATF4), TNF receptor associated factor 2 (TRAF2), X-box binding protein 1 (XBP1), CCAAT/enhancer-binding protein homologous protein (CHOP), and caspase-12, leading to initiation of apoptosis (programmed cell death) in the cardiomyocyte (Table 1).[71]

In addition to inflammation and ER stress, anthracyclines are known to induce oxidative stress in the heart. Oxidative stress occurs when there is an excessive buildup of reactive oxygen species (ROS) that surpasses the biological system's capacity to counteract these reactive substances. These compounds are generated when oxygen is not fully reduced, leading to the formation of singlet oxygen molecules, superoxide radicals, and hydroxide radicals. ROS are harmful to nearby tissues due to their short half-lives, unstable properties, and high reactivity.[72,73] Cardiomyocyte dysfunction can occur as a result of oxidative stress, which is caused by either the auto-oxidation of catecholamines and/or the interference of ROS with the calcium transport in the ER.[60,72]

Anthracyclines induce oxidative stress via increasing the activity of the mitogen-activated protein kinase (MAPK) signaling pathway.[72-75] The MAPK cascade transmits intracellular signals from the cell surface to the nucleus, exerting a direct influence on the genetically programmed cell death mechanism called apoptosis.[75] Anthracyclines stimulate this pathway, leading to upregulation of pro-apoptotic proteins such as c-Jun N-terminal kinase (JNK), Caspases 3 and 9, and Bcl-2 associated X protein (Bax), while downregulating anti-apoptotic proteins like B-cell lymphoma extra-large (Bcl-xL) (Table 1).[74-79] Anthracyclines exert harmful effects on the heart, leading to an elevated myocardial energy demand. This results in increased oxygen consumption, which disrupts the equilibrium between the creation and neutralization of ROS.[72] Oxidative stress in cardiomyocytes can lead to mitochondrial damage, and trigger cell death.[79]

An additional mechanism by which oxidative stress is harmful for the cardiomyocytes is the oxidation of lipid molecules. Excess oxygen radicals can react with unsaturated lipids which are widespread in both the plasma membrane and mitochondrial membranes of the cell.[72] This lipid peroxidation can negatively affect membrane permeability, induce the loss of high energy phosphates, and contribute to contractile dysfunction of the cardiomyocytes.[73] Increased lipid peroxidation has been linked to increased severity of heart failure, and therefore there is a growing focus on exploring the mechanisms of excess ROS production due to anthracycline use and their downstream effects on various lipid species.[72,77,79]

Since DOX works through a mechanism of nonselective cytotoxicity, it is employed to treat several types of primary cancers and malignancies, including solid tumors, hematologic cancers, and breast cancer.[79-81] To enhance the effectiveness of the anti-cancer therapy in HER2 positive breast

cancer patients, the addition of the monoclonal antibody TRZ has positive results as a targeted biological therapy, but it is also linked to a higher risk of cardiotoxicity.[82-84] This anti-cancer agent induces cardiac dysfunction, which manifests in two distinct forms. When used in combination with anthracyclines, TRZ exacerbates type-I cardiotoxicity, significantly increasing the risk of irreversible myocardial damage—raising the incidence of long-term cardiac injury by up to 25%.[83,84] This synergistic toxicity imposes further limitations on the maximum cumulative chemotherapy dose in breast cancer treatment.[85,86] In contrast, when TRZ is administered alone, it leads to type-II cardiotoxicity, which typically results in dilated cardiomyopathy and a reduction in LVEF. However, these cardiac effects are often reversible upon temporary discontinuation of therapy.[79,82] Although both DOX and TRZ have extended the lives of many breast cancer patients, the cardiovascular adverse effects significantly reduce the noticeable therapeutic advantages of these treatments.

The main mechanisms by which TRZ is cardiotoxic are through an elevation in apoptosis and oxidative stress.[87-90] TRZ binding to HER2 receptors in cardiomyocytes modifies the expression levels of pro- and anti-apoptotic proteins, including the B-cell lymphoma 2 (Bcl-2) family of proteins which include pro-apoptotic Bax and B-cell lymphoma extra-small (Bcl-XS), as well as anti-apoptotic Bcl-xL (Table 1).[87-88] As the level of pro-apoptotic expression rises and the level of anti-apoptotic expression declines, the change in protein ratios triggers apoptosis. Oxidative stress causes damage to cardiomyocytes by increasing the activity of the renin-angiotensin system (RAS) and inhibiting the formation of dimers by TRZ. Both of these processes are linked to the suppression of cell survival pathways.[89,90] TRZ, when binding to the HER2 receptors, hinders the cell survival pathways, thereby exerting stress on the heart.[91] This, in turn, causes a notable rise in

the levels of angiotensin-II (ANG-II) in the bloodstream. ANG-II can then attach to the angiotensin-1 receptor, triggering a series of reactions that generate ROS, resulting in oxidative stress.[92-96] The oxidative stress caused by ANG-II also results in elevated amounts of pro-apoptotic genes, including Bax and Caspase-3, and reduced levels of anti-apoptotic genes, such as Bcl-xL.[72-75,97,98] TRZ binds to the HER2 receptor and prevents its dimerization with other HER family receptors, thereby inhibiting downstream cell survival pathways. This renders the cardiomyocytes vulnerable to harm.[90,99-105] Active cardiomyocytes have heightened electron transport chain activity in their mitochondria to meet the high metabolic demand for adenosine triphosphate (ATP), resulting in increased production of ROS.[92,102] The diminished ability of the cells to activate their anti-apoptotic pathways results in the inability of cardiomyocytes to counteract the oxidative stress. This leads to apoptosis, which, if extensive, ultimately causes cardiac dysfunction.[102,105-107]

The complex and multifaceted nature of cardiotoxicity caused by the combination of DOX and TRZ, which involves the activation of inflammatory pathways, induction of ER stress, and elevated production of ROS, is a challenge in terms of managing and reducing this harmful side effect. This study aims to clarify the molecular roles of inflammation, ER stress and oxidative stress in the cardiotoxicity caused by the combination of DOX and TRZ.

Pathway	Protein
Inflammation	Nuclear factor kappa beta (NF-κB)
	phospho- NF-κβ
	Nucleotide-binding oligomerization domain-like receptor 3 (NLRP3)
	Tumor necrosis factor alpha (TNF-α)
	Interferon-alpha (INF-α)
	Interleukins 1-beta (IL-1β), 6 (IL-6), 8 (IL-8)
Endoplasmic Reticulum Stress	78-kDa glucose-regulated protein (GRP78)
	Protein kinase RNA-like ER kinase (PERK)
	Activating transcription factor 4 (ATF4)
	CCAAT/enhancer-binding protein homologous protein (CHOP)
	Caspase-12
	TNF receptor associated factor 2 (TRAF2)
	X-box binding protein 1 (XBP1)
Oxidative Stress	Bcl-2 associated X protein (Bax)
	B-cell lymphoma extra-large (Bcl-XL)
	Caspase-3,9
	c-Jun N-terminal kinase (JNK)

Table 1. Protein markers of cell damage mediated by DOX+TRZ.

1.2.2 Cancer Therapy Related Cardiac Dysfunction

Chemotherapy-induced cardiotoxicity commonly appears clinically in the form of congestive heart failure (CHF).[108,109] A detailed review of the literature revealed that the percentage of patients who experience CHF as a result of DOX treatment ranges from 5% when the cumulative dose reaches 400 mg/m^2 to 48% when the cumulative dose reaches 700 mg/m^2.[81] However, to mitigate this high risk, the current recommended cumulative dose of DOX is limited to 500 mg/m^2 over an individual's lifetime.[81] A retrospective clinical study on the combination of DOX+TRZ revealed that 22% of patients had to stop their anti-cancer treatment because they developed left ventricular (LV) systolic dysfunction.[84] Additionally, 40% of patients who experienced cardiotoxicity showed either no improvement or a decline in their left ventricular ejection fraction (LVEF) over time, despite receiving appropriate medical therapy for CHF.[84]

1.2.3 Cardiovascular Imaging

The main imaging modalities employed for the diagnosis and surveillance of cardiotoxicity in breast cancer patients include multiple-gated radionuclide angiography (MUGA), cardiac magnetic resonance imaging (CMR), and transthoracic echocardiography (TTE).[54] Although the selection of imaging modality may differ depending on the availability of resources, it is important to maintain consistency in the choice of imaging modality for the same patient across time. This ensures that images can be compared and changes in cardiac function can be measured with greater accuracy.[54]

MUGA scans are a type of nuclear imaging technique utilized for the purpose of quantifying LVEF. The procedure involves administration of a radionucleotide tracer via intravenous

injection, which is subsequently visualized using a gamma camera to obtain images of the heart at particular time points during the cardiac cycle. MUGA scans are utilized to evaluate cardiac function in breast cancer patients due to its accessibility and accurate reliability as an imaging technique. These characteristics enable convenient repetition of imaging during cancer therapy and precise evaluation of changes in LVEF.[110] Nevertheless, MUGA scans do not offer any insights into concomitant valvular heart disease, and also subject patients to radiation, typically around 5-10 mSv (equivalent to 50-100 chest x-rays) per scan.[54] Furthermore, MUGA scans have limitations as they rely solely on LVEF as a marker of cardiac function, which is inadequate for the early diagnosis of CTRCD.[111]

CMR is considered the most reliable method for evaluating LV volumes and ejection fraction. CMR provides high-quality images and has excellent intra-observer and inter-observer variabilities.[112] CMR is regarded as an appropriate method for early diagnosis of CTRCD because of its strong tissue characterization capabilities and sensitivity to detect early alterations in tissue composition, such as changes in LV mass, inflammation, fibrosis, and myocardial strain.[113] Nevertheless, the limited accessibility and increased expense of CMR pose obstacles that presently restrict its feasibility in the field of Cardio-Oncology in Canada, where the ability to conduct repeated imaging is crucial for monitoring patient progression.

TTE is currently the established method for evaluating serial cardiac function during treatment with chemotherapy and for promptly identifying CTRCD. TTE is an optimal method of cardiac imaging due to its widespread availability, versatility, ease of repetition, and lack of radiation exposure for the patient. TTE enables the assessment of both the contraction and relaxation of the

ventricles, as well as the measurement of pulmonary pressures, detection of valvular anomalies, examination of the pericardium, and measurement of global longitudinal strain (GLS), an early marker of cardiac dysfunction.[114] Three-dimensional TTE provides more precision in comparison to two-dimensional TTE, resulting in a reduction of the error in LVEF calculation from 10% to below 5%.[115]

Serial TTE, MUGA, and CMR are all non-invasive imaging methods employed to monitor cardiac dysfunction by assessing changes in LVEF. CTRCD is defined as decrease of >10% in LVEF or an absolute LVEF of <53%.[54] However, it is important to note that LVEF is a delayed marker of myocardial injury. There is a greater focus on innovative echocardiographic methods that are more efficient in identifying chemotherapy-induced heart damage at an early stage. GLS, as measured using speckle tracking echocardiography, expresses longitudinal shortening of the LV cardiomyocytes as they contract during systole, and is represented as a percentage. The GLS value (expressed as a negative number) reflects the change in length of the contractile cells during each cardiac cycle, compared to their baseline length.[116,117] A more negative value reflects improved cardiac function, indicating stronger and more efficient myocardial contraction. GLS using TTE is an early marker of chemotherapy-induced cardiotoxicity and can predict future decreases in LVEF.[116-121] CTRCD is identified by a greater than 15% change in GLS compared to baseline in individuals undergoing cancer treatment.[118] GLS has demonstrated efficacy in predicting cardiotoxicity induced by DOX+TRZ. Patients who go on to develop cardiotoxicity exhibit significant increases in GLS as early as three months into treatment, whereas reductions in LVEF typically do not appear until six months. Although the LVEF drops at 6 months, this is a late marker and cardiotoxicity may have already developed. Therefore, GLS alterations serve as an

earlier echocardiographic indicator of subclinical cardiac dysfunction before changes in LVEF occur.[118,120] Early detection of reduced GLS in patients undergoing chemotherapy treatment is vital for prompt identification of myocardial damage, allowing clinicians the opportunity to halt or adjust cancer treatment regimens and initiate administration of cardioprotective therapies, with the goal of preventing more deleterious changes in the functional ability of the left ventricle (LV) to contract.[120,121]

1.2.4 Cardiac biomarkers

In clinical practice, the examination of cardiac biomarkers is primarily restricted to serum samples. Early elevations in cardiac biomarkers such as troponins, natriuretic peptides, and inflammatory proteins have been identified as predictors of the development of CTRCD.[122-124] Troponins are thin-filament contractile proteins found in the myocardium. Following myocardial injury, troponins are released from the sarcomeres (contractile units) and the cytoplasm into the blood.[124] Cardiac troponins are the most widely used diagnostic tool for assessing myocardial damage following myocardial infarction, and their use has been extended to other cardiac conditions such as heart failure, LV hypertrophy, and myocarditis.[124,125] In the Cardio-Oncology setting, cardiac troponins T (cTnT) and I (cTnI) are the most studied cardiac biomarkers in the setting of chemotherapy-mediated cardiac injury.[124] Their use for early identification of cardiac toxicity associated with anthracyclines has been validated in both animal and human models, often predicting future decreases in LVEF.[125-131] In breast cancer patients treated with TRZ, an increase in cTnI was observed shortly following the first treatment cycle.[128] In fact, LV systolic dysfunction occurred in 62% of patients who had an increase in cTnI during TRZ therapy, compared to just 5% of those with normal cTnI values.[128]

Natriuretic peptides are synthesized in the heart and are secreted in response to increased wall stretch, elevated pressure and volume overload.[122,132] The biologically active hormone B-type natriuretic peptide (BNP) and its inactive form N-terminal pro-brain type natriuretic peptide (NT-pro-BNP) are important regulators of cardiovascular homeostasis. Abnormally high levels of these cardiac biomarkers can be indicative of heart failure, aiding diagnosis when clinical uncertainty exists.[129] While there is conflicting evidence in the present literature, several studies indicate an association between raised levels BNP and/or NT-pro-BNP, and a higher likelihood of developing CTRCD.[132-134]

C-reactive protein (CRP), released by hepatocytes, and galectin-3, secreted by activated macrophages, have been linked to inflammation and fibrosis in the heart. These biomarkers have demonstrated promise in predicting CTRCD due to anthracyclines and TRZ.[135,136] Women receiving EPR as a treatment for breast cancer had markedly increased CRP and galectin-3 levels, although upregulation of these biomarkers was not associated with early decline in LV function.[135] During a clinical trial including patients with HER2 positive breast cancer who were administered TRZ, higher levels of serum high-sensitivity CRP (≥3mg/L) were indicative of future reductions in LVEF.[136] However, these biomarkers are not highly specific for cardiomyopathy, and can become elevated due to infections and other inflammatory conditions. Therefore, troponins and natriuretic peptides are more widely used in the monitoring of chemotherapy-induced cardiotoxicity.[136]

1.2.5 Prevention of Chemotherapy Mediated Cardiotoxicity

In the field of Cardio-Oncology, patients who are diagnosed with CTRCD either during or after completion of chemotherapy and/or targeted therapy have various pharmacological therapies that can be initiated in order to attempt to treat the cardiotoxicity. Currently, the Canadian Cardiovascular Society (CCS) recommends institution of an ACEi and a β-blocker in the settings of both asymptomatic and symptomatic declines in LVEF of patients experiencing chemotherapy mediated cardiotoxicity.[119] In clinical practice, when CTRCD is suspected or confirmed, the anti-cancer therapy may be adjusted or stopped, and patients are given low doses of an ACEi, a β-blocker, and/or a mineralocorticoid receptor antagonist (MRA) to try to reverse the current damage and prevent additional damage to the heart.[57]

Since reversing cardiac damage resulting from anti-cancer treatment is only partially effective, there has been increased focus on preventing cardiotoxicity from occurring in the first place. Prior to initiation of potentially cardiotoxic cancer treatments, the CCS suggests that the prophylactic use of an ACEi, and/or β-blocker, and/or statin can be considered in high-risk patients for the prevention of chemotherapy mediated cardiotoxicity.[119] In pre-clinical models, agents such as anti-oxidants, vitamin C, and flaxseed have also shown promise in chemotherapy-induced cardiotoxicity prophylaxis, attenuating LV remodeling, preserving LVEF, and reducing markers of oxidative stress, apoptosis, and inflammation.[137-144]

While not always well-tolerated, statins were shown to be cardioprotective in the setting of chemotherapy-induced cardiotoxicity.[145-147] A retrospective study identified women with HER2 positive breast cancer who underwent TRZ treatment with or without anthracyclines and found

that concomitant use of statins resulted in lower incidence of cardiotoxicity.[146] A randomized, single-blind, placebo-controlled clinical trial enrolled 83 newly diagnosed breast patients to receive rosuvastatin or a placebo for 6 months while undergoing AC chemotherapy treatment.[147] At the end of the study, individuals who received rosuvastatin (n=41) had preserved LVEF and GLS parameters, while those in the placebo group (n=42) showed significant LV dysfunction.[147]

In pre-clinical studies, RAS antagonists have been observed to reduce the harmful cardiotoxic side effects of chemotherapy and lower the risk of death.[148] In a chronic *in vivo* mouse model of DOX+TRZ induced cardiotoxicity, the prophylactic administration of the ACEi perindopril (PER) or the angiotensin receptor blocker (ARB) valsartan was shown to reduce adverse cardiac remodelling and decrease overall mortality.[149] The decreased mortality associated with the use of ARBs can be due to the cardioprotective effects of lowered blood pressure (BP), enhanced contraction of cardiomyocytes, reduced oxidative stress, decreased proportion of cells undergoing apoptosis, conserved LVEF, and prevention of adverse remodeling of the LV.[148-152] Initiating cardioprotective therapy earlier is associated with greater effectiveness, indicating that the protective benefits of RAS antagonists may be more significant than their treatment effects.[148]

Retrospective studies and randomized controlled trials have been employed in the clinical setting to investigate the effect of RAS inhibition in Cardio-Oncology.[151-156] In type I cardiotoxicity, initiating ACEi at least 24 hours prior to DOX therapy and maintaining ACEi for 6 months resulted in a reduction in the incidence of decreased LVEF, heart failure, and mortality in different cancer settings.[152,153] The administration of ARBs during chemotherapy has shown efficacy in preventing early signs of heart failure, as evidenced by echocardiographic assessments. Additionally, a

reduction in oxidative stress was indicated by decreased circulating ROS levels, while diminished inflammation was reflected by lower concentrations of IL-6, IL-1β, and TNF-α.[148,153,156]

When studying the cardiotoxicity caused by DOX+TRZ, it was shown that both systolic and diastolic dysfunction were somewhat mitigated by administering RAS antagonists and β-blockers as a preventive measure.[153,157] The PRADA study recruited EBC patients and randomized them to receive the ARB candesartan or the β-blocker metoprolol while undergoing adjuvant anticancer therapy. The study aimed to evaluate whether administration of an ARB or a β-blocker in parallel with anticancer treatment would prevent declines in LVEF. The PRADA study demonstrated the efficacy of candesartan in preventing deterioration of LV function resulting from both type I and type II cardiotoxicity, associated with anthracyclines and TRZ, respectively. No short-term beneficial effect was observed with the prophylactic treatment of metoprolol in the PRADA study.[157] The MANTICORE study specifically investigated the prevention of TRZ induced type II cardiotoxicity. HER2 positive EBC patients were assigned to receive the ACEi PER, or the β-blocker bisoprolol while receiving TRZ treatment. The trial concluded that both PER and bisoprolol attenuated trastuzumab-associated declines in LVEF; however, neither agent prevented increases in indexed left ventricular end-diastolic volume (LVEDVi), indicating a lack of effect on adverse LV remodeling.[86] The OVERCOME trial randomized cancer patients to receive both the ACEi enalapril and the β-blocker carvedilol, or placebo, starting at least 24 hours before the first cycle of chemotherapy and for the entire duration of treatment. This study demonstrated that combined treatment with ACEi and beta blockade has preserved LVEF but no significant differences in morbidity or mortality were observed.[153] Conversely, The CECCY trial found that the prophylactic use of the β-blocker carvedilol in HER2 positive EBC patients had no impact on

preventing early reductions in LVEF, but their results illustrated it may protect against myocardial injury through reductions in cTnI and diastolic dysfunction.[158]

Due to the limited amount of evidence and modest results of preventing chemotherapy mediated cardiotoxicity prior to initiation of DOX+TRZ anti-cancer treatment using ACEi and β-blockade for CTRCD, as described above,[86,153,157,158] further research is required for alternative pharmaceutical agents. While ACEi's, β-blockers, and statins are somewhat effective, they may present with side effects such as bradycardia, hypotension, fatigue, and/or myalgias that may not be tolerated well by some patients.[119] Therefore, we seek to explore the efficacy of a novel class of drugs in the prevention of chemotherapy induced cardiotoxicity.

1.3 SGLT2 Inhibitors

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, also known as gliflozins, are a novel class of oral prescription medicines originally developed and approved for the treatment of type 2 diabetes mellitus (T2DM) in 2013.[159] While they proved effective in patients with diabetes, they were found to be cardioprotective in the heart failure setting as well, based on large-scale clinical trials, in both diabetic and non-diabetic patients.[160-164] After demonstrating their clinical efficacy, SGLT2 inhibitors are now recommended as first-line agents in the management of individuals with heart failure in conjunction with ACEi's, β-blockers, and MRAs.[165] Currently, the Cardio-Oncology field is exploring the cardioprotective role of SGLT2 inhibitors in the setting of chemotherapy mediated cardiotoxicity, as a replacement or an add-on therapy to pharmaceuticals currently used in this patient population, including ACEi's, β-blockers, and statins.[166,167]

1.3.1 Overview

SGLT2 inhibitors act primarily on the proximal tubule of the kidney, where the vast majority of the corresponding receptors are expressed. These agents work by blocking SGLT2 receptors which are responsible for reabsorbing glucose from the kidneys back to the blood. This promotes urinary excretion of glucose, helping lower blood glucose levels (independent of insulin), reduce water retention, and reduce whole-body sympathetic tone.[168]

In addition to their original designation as anti-diabetic medications, over the past decade, emerging evidence has shown that these agents have well-established cardioprotective properties, irrespective of diabetes status.[166] The benefits are derived from the down-regulation of the inflammatory, ER stress, and oxidative stress pathways (Figure 1).[169-179] Limited and emerging evidence from pre-clinical and epidemiologic studies have shown that SGLT2 inhibitors suppress the growth of tumors across multiple cancer models, including breast cancer. However, randomized clinical trials are needed to explore the anti-cancer effects of those drugs.[166] SGLT2 inhibitors are associated with some side effects, including urinary tract infections, genital infections, and hypovolemia. However, these adverse reactions are well-tolerated and managed.[180,181] Current treatment medications for heart failure are often accompanied by hypotension, hyperkalemia and bradycardia, which are not seen with SGLT2 inhibition. Furthermore, SGLT2 inhibitors do not require up-titration, unlike most heart failure drugs which require gradual increase of dosage in order to achieve the desirable clinical benefits.[181-182] Therefore, the use of SGLT2 inhibition in the setting of chemotherapy induced cardiotoxicity as a standalone preventative intervention or in conjunction with standard pharmacological therapy warrants investigation.

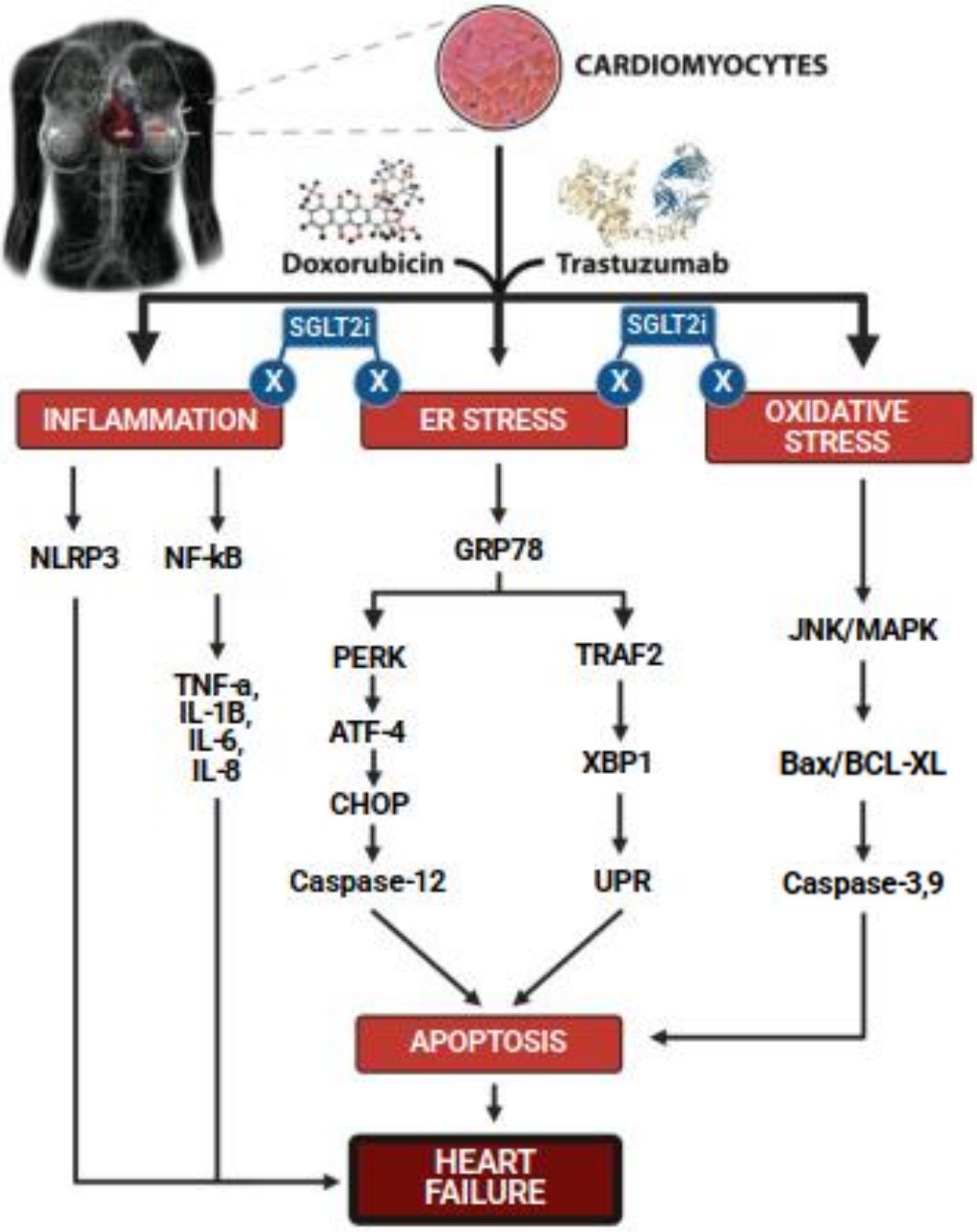


Figure 1. Pathogenesis of DOX+TRZ mediated cardiotoxicity and the potential cardioprotective mechanisms of SGLT2 inhibition. In experimental models of cardiac injury due to DOX+TRZ, upregulation of pro-inflammatory, ER stress, and oxidative stress pathways has been reported, leading to apoptosis and heart failure. SGLT2 inhibition (SGLT2i) with empagliflozin (EMPA) may mitigate the cardiotoxic side effects of DOX+TRZ via down-regulation of these pathways.

1.3.2 SGLT2 Inhibitors and Diabetes

The original designation of SGLT2 inhibitors was for the management of T2DM. In the setting of T2DM, glucose is unable to enter the body cells due to lack of insulin sensitivity, which results in accumulation of high glucose levels in the blood. This leads to detrimental multi-systemic effects, affecting over 3 million Canadians.[183] Normally, SGLTs are highly efficient at reabsorbing glucose in the kidney, with only a negligible amount being excreted through urine under standard physiological conditions and normal renal function. By blocking those glucose-reabsorbing channels, SGLT2 inhibitors promote glycosuria, accompanied by natriuresis, reductions in plasma volume and BP, and a whole-body metabolic shift away from glycolysis and towards fatty acid oxidation. These effects have been found to provide favorable outcomes for those living with diabetes, especially when paired with reported improvements in insulin sensitivity, likely due to reduced glycemic burden.[184] Specific therapeutic effects include lowering of HbA_{1c} by 0.6-0.9%, reduction in systolic blood pressure (SBP) of 4-6 mmHg, and a decrease in body weight of 2-4 kg. These effects appear to have long-term durability based on clinical trials.[160,185-191]

1.3.3 SGLT2 Inhibitors and Cardiovascular Disease

The benefits of SGLT2 inhibition are not limited to diabetes alone. In fact, as of 2021, SGLT2 inhibitors are one of four foundational therapies recommended by the CCS and the Canadian Heart Failure Society for the management of heart failure with a reduced ejection fraction (HFrEF).[165] These guidelines are based on the latest evidence obtained by a number of landmark randomized placebo-controlled clinical trials.[160-163] The DAPA-HF trial found that dapagliflozin reduced the risk of worsening heart failure or death from cardiovascular causes, regardless of diabetes status.[163] The EMPA-REG trial was performed in patients with type 2 diabetes who were at high risk for

cardiovascular events. Researchers found a significantly lower rate of death from cardiovascular causes, hospitalization for heart failure, and death from any cause.[160] The EMPEROR-Reduced and EMPEROR-Preserved trials both showed lower risk of cardiovascular death or hospitalization for heart failure than those in the placebo group, regardless of the presence or absence of diabetes.[161,162] Empagliflozin (EMPA) was found to be cardioprotective regardless of heart failure phenotype, whether it is HFrEF or heart failure with a preserved ejection fraction (HFpEF).[161,162] So far, EMPA has the most clinical evidence regarding its efficacy and safety of all SGLT2 inhibitors, and has the highest selectivity to bind to SGLT2 over SGLT1 (2,500-fold), as compared to other approved SGLT2 inhibitors in Canada, namely dapagliflozin (1,200-fold) and canagliflozin (413-fold).[168]

The benefits of SGLT2 inhibitors for the cardiovascular system can be explained by two broad main pathways, which have downstream tissue, molecular, and cellular effects. Hemodynamic mechanisms include reductions in extracellular volume due to natriuresis, which lead to a decrease in systemic BP and preload.[184,191] Metabolically speaking, in the failing heart, glycolysis is abnormally upregulated and fatty acid oxidation is downregulated, resulting in diminished ATP production. SGLT2 inhibitors lower blood glucose levels, leading to increased reliance on fat breakdown for energy, which also leads to creation of ketone bodies, which are the most energetically efficient fuel source, due to their ability to produce many ATP molecules while having the lowest oxygen requirements. SGLT2 inhibitors were found to target glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) receptors, inhibiting glucose uptake by the cardiomyocytes. This shift towards oxidative phosphorylation leads to greater ATP production and improved cardiac function.[184,190,192] Overall, the favourable effects of SGLT2 inhibitors on the

cardiovascular system warrant their use in the clinical setting, and ongoing research is elucidating the mechanisms by which they exert their actions, in both diabetic and non-diabetic patients.

1.3.4 SGLT2 Inhibitors in the Prevention of Chemotherapy Mediated Cardiotoxicity

Although the current heart failure guidelines recommend the use of SGLT2 inhibitors in the setting of HFrEF, the latest CCS guidelines for the management of CTRCD from 2016 do not mention their use in the cardio-oncology setting.[119,165] Despite this, preclinical *in vitro* and *in vivo* studies showed improvements in cardiac function and in various biomarkers when SGLT2 inhibitor therapy was added to the anti-cancer treatment.[169-172] At present, four pre-clinical studies have investigated the possible cardioprotective effect of SGLT2 inhibitors in murine models of DOX-mediated cardiotoxicity. Oh et al. found that administering EMPA in both acute and chronic *in vivo* models of DOX-mediated cardiotoxicity improved LV systolic function on CMR imaging, while also reducing perivascular and interstitial fibrosis.[170] Sabatino et al. found that preventive therapy with EMPA attenuated LV remodelling and significantly improved myofibril integrity in a chronic *in vivo* model of DOX-mediated cardiotoxicity.[169] Chang et al. found that prophylactic administration of dapagliflozin in a chronic *in vivo* model of DOX-mediated cardiotoxicity prevented LV remodelling and myocardial fibrosis by attenuating ER stress.[171] Finally, Quagliariello et al. showed that in an acute *in vivo* model of DOX-mediated cardiotoxicity, prophylactic use of EMPA reduced inflammation, preventing adverse cardiovascular remodelling.[172] Although these basic science studies have shown evidence of preventative cardioprotection in the setting of DOX treatment, none of the models to date have assessed the role of SGLT2 inhibitors in the prevention of **both** DOX+TRZ mediated cardiotoxicity, which is more clinically relevant.

To date, research has shown that SGLT2 inhibitors administered concurrently with anti-cancer agents have beneficial cardiovascular properties as they inhibit inflammation, attenuate markers of ER stress and apoptosis, and down-regulate oxidative stress (Figure 1).[166,167,169-173,176-179] SGLT2 inhibitors have potent anti-inflammatory activity by decreasing the expression of inflammatory biomarkers, including NLRP3, NF-κB, TNF-α, and downstream interleukins.[166,172,176-178] SGLT2 inhibitors also attenuate markers of ER stress by decreasing GRP78, PERK, TRAF2, and apoptosis.[68,171] Finally, SGLT2 inhibitors inhibit oxidative stress by decreasing MAPK, Caspase, and Bax/Bcl-xL.[170,171,179] As DOX+TRZ mediated cardiotoxicity involves similar mechanisms, including up-regulation of inflammatory biomarkers, increased ER stress/ apoptosis, and increased oxidative stress (Figure 1),[169-179] further research is warranted to elucidate the potential cardioprotective role of SGLT2 inhibitors in blocking these pathways.

In addition, the use of SGLT2 inhibition in cancer patients undergoing cardiotoxic therapies has been supported by a number of recent retrospective observational clinical studies. A 2022 study evaluated patients with diabetes mellitus and cancer receiving an anthracycline treatment regimen. Patients on SGLT2 inhibitors had a lower rate of cardiac events including heart failure and lower rate of cardiac dysfunction, compared to those who were not treated with SGLT2 inhibitors.[193] A 2023 study demonstrated that in elderly patients treated with SGLT2 inhibitors for diabetes, and who received anthracyclines, the rate of heart failure hospitalization was reduced.[194] Finally, a recent study published in 2024 revealed that patients treated with SGLT2 inhibitors for diabetes while receiving anthracyclines had lower rates of hospitalization for heart failure.[195] The data obtained by these studies warrant exploration of the benefits of SGLT2 inhibitors in the Cardio-Oncology setting via randomized controlled trials. Furthermore, the effects of EMPA, or any other

SGLT2 inhibitor for that matter, have not yet been explored in the setting of combination therapy using DOX+TRZ, supporting investigation into the role of EMPA in preventing the development of heart failure from this cardiotoxic treatment regimen.

Chapter 2: Rationale, Hypothesis, & Objective

2.1 Rationale

Although the combination of DOX+TRZ is a highly effective treatment modality for breast cancer, its benefits are greatly reduced by the associated cardiotoxic side effects. Approximately 25% of patients are susceptible to experiencing cardiotoxicity caused by the combination of DOX and TRZ.[83,84] Consequently, it is crucial to prioritize the development of methods to prevent chemotherapy-induced cardiotoxicity in the field of Cardio-Oncology.

At present, once CTRCD is evident, the anti-cancer therapy is either adjusted or stopped, and patients are prescribed an ACEi, a β-blocker, and/or a MRA to counter the existing damage and prevent further damage to the heart, based on CCS recommendations, consensus statements, and clinical evidence.[54,57,119] This poses a significant hurdle in the treatment of breast cancer, as cardiotoxicity may restrict the further use of chemotherapy and targeted therapy agents, potentially affecting cancer treatment outcomes, and possibly leading the patient to experience side effects associated with heart failure medications. Therefore, we focus our attention on preventing chemotherapy-mediated cardiotoxicity which may allow breast cancer patients to receive tumor-suppressing treatment while minimizing damage to the heart.

Currently, SGLT2 inhibitors are a well-established and well-tolerated class of medications for the management of diabetes and heart failure. In addition, growing preclinical and retrospective clinical evidence shows that these agents benefit heart health when used alongside anti-cancer treatment.[169-172,193-195] However, it is still unknown if prophylactic treatment with SGLT2 inhibitors can be beneficial in preventing cardiotoxicity from occurring in the first place, which is

a promising research avenue given the implications for cancer treatment and heart health. Further, the effects of these agents have not been explored yet in the setting of combination therapy using DOX and TRZ, which exert their cardiotoxicity via different mechanisms of action. Therefore, we seek to explore whether EMPA is equivalent and/or synergistic with PER in the prevention of chemotherapy induced cardiotoxicity.

2.2 Hypothesis

The prophylactic use of SGLT2 inhibition with EMPA will be comparable and/or synergistic with PER in reducing the cardiotoxic side effects of DOX+TRZ. We propose that the underlying cardioprotective mechanisms include down regulation of the inflammatory, ER stress/apoptosis, and oxidative stress pathways, which collectively preserve overall cardiac function.

2.3 Objective

The specific aim is to evaluate whether the prophylactic use of SGLT2 inhibition with EMPA will be comparable and/or synergistic to standard pharmacological therapy using the ACEi PER in preventing DOX+TRZ mediated cardiotoxicity in a chronic *in vivo* female murine model.

Chapter 3: Methods

3.1 Animal Model

The guidelines of the Canadian Council on Animal Care were strictly followed for all animal procedures, including drug administrations and longitudinal echocardiographic studies, as approved by the Animal Protocol Review Committee at the University of Manitoba [REB: 20-004/1 (AC11548)].

A total of 160 wild-type C57Bl/6 female mice (12-15 weeks old; Jackson Laboratories, Bar Harbor, ME, US) were used for this study, randomized into pre-specified groups (Figure 2). They had *ad libitum* access to water and regular chow diet, maintained on a 12-hour day/night cycle while being housed in cages of 2 to 3 mice in the animal holding facility. Mice received prophylactic treatment with control [(0.5% hydroxyethyl-cellulose (HEC)],[177] EMPA (10 mg/kg/day),[169,172,177,192] PER (3 mg/kg/day),[143,149,196,197], or EMPA+PER via oral gavage for a total of 3 weeks as a run-in period prior to administration of saline or DOX+TRZ and continued their initial treatment for an additional 3 weeks (total of 6 weeks). At the end of weeks 3, 4, and 5, mice received weekly treatment with DOX (8 mg/kg) + TRZ (3 mg/kg) intraperitoneally (i.p.) to create a chronic *in vivo* murine model of chemotherapy induced cardiotoxicity, as previously validated by our group and others.[70,137,138,140,143,145,149,169,172,196,198-203] HEC was used as the agent in which EMPA and PER was dissolved and thus the control group received vehicle only. All mice were subject to weekly echocardiograms as shown in Figure 3. Non-invasive hemodynamic parameters and random glucose levels were measured at baseline, week 3, and week 6 of the study.

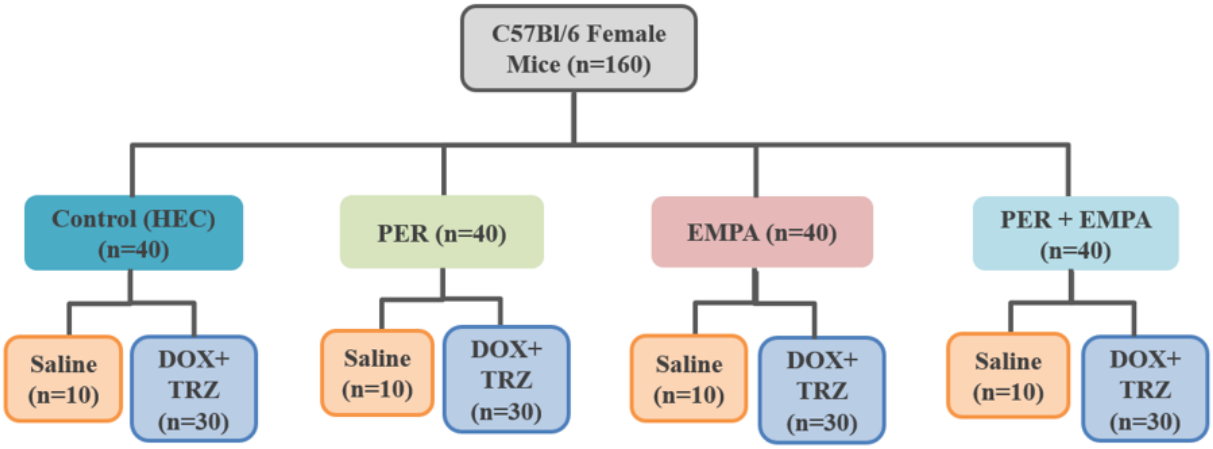


Figure 2. Experimental randomization. A total of 160 WT C57Bl/6 female mice (12-15 weeks old; Jackson Laboratories, ME, US) were randomized to the various groups. Mice received prophylactic treatment with 0.5% HEC (n=40), PER (3 mg/kg/day) (n=40), EMPA (10 mg/kg/day) (n=40), or EMPA+PER (n=40) for a total of 3 weeks as a run-in period prior to administration of 0.9% saline (n=40) or DOX (8 mg/kg) + TRZ (3 mg/kg) (n=120) and continued their initial treatment for an additional 3 weeks (total of 6 weeks). At the end of weeks 3, 4, and 5, mice received weekly treatment with DOX + TRZ via i.p. injection to create a chronic *in vivo* murine model of chemotherapy induced cardiotoxicity.

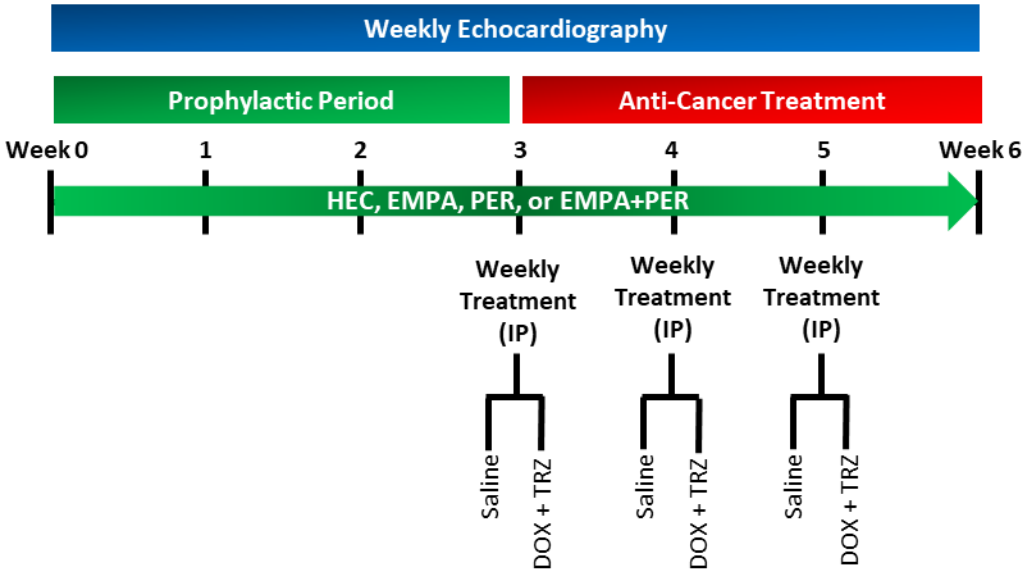


Figure 3. Experimental timeline. Mice were randomized to: i) 0.5% HEC (n=40); ii) PER (3 mg/kg/day) (n=40); iii) EMPA (10 mg/kg/day) (n=40); iv) or EMPA+PER (n=40) prophylactic treatment groups, receiving the respective treatment via oral gavage daily for the entire 6 weeks of the study. After the prophylactic period (3 weeks), mice received 0.9% saline (n=40) or DOX (8 mg/kg) + TRZ (3 mg/kg) (n=94) as anti-cancer treatment for the next 3 weeks. Cardiac function was assessed weekly using non-invasive TTE.

3.2 Murine Echocardiography

Serial non-invasive TTEs were performed weekly on awake mice at baseline and during the six experimental weeks to evaluate cardiovascular remodelling and function.[137,140,143,149,169,199-203] Images were captured using a 13-MHz linear array intraoperative epicardial ultrasound probe (GE Medical Systems, model i13L, Milwaukee, WI, US) on a GE Vivid 7 Ultrasound system and then processed using the EchoPAC PC software (GE Medical Systems, Version 112, Milwaukee, WI, US). Images from the parasternal long axis (PLAX) view were analyzed to calculate LVEF based on the endocardial borders and application of the Teicholtz formula (Figure 4). Images acquired in the parasternal short axis (PSAX) M-mode view were analyzed to calculate heart rate (HR), interventricular septal wall thickness (IVS), posterior wall thickness (PWT), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), and LVEF using the Simpson formula (Figure 5).

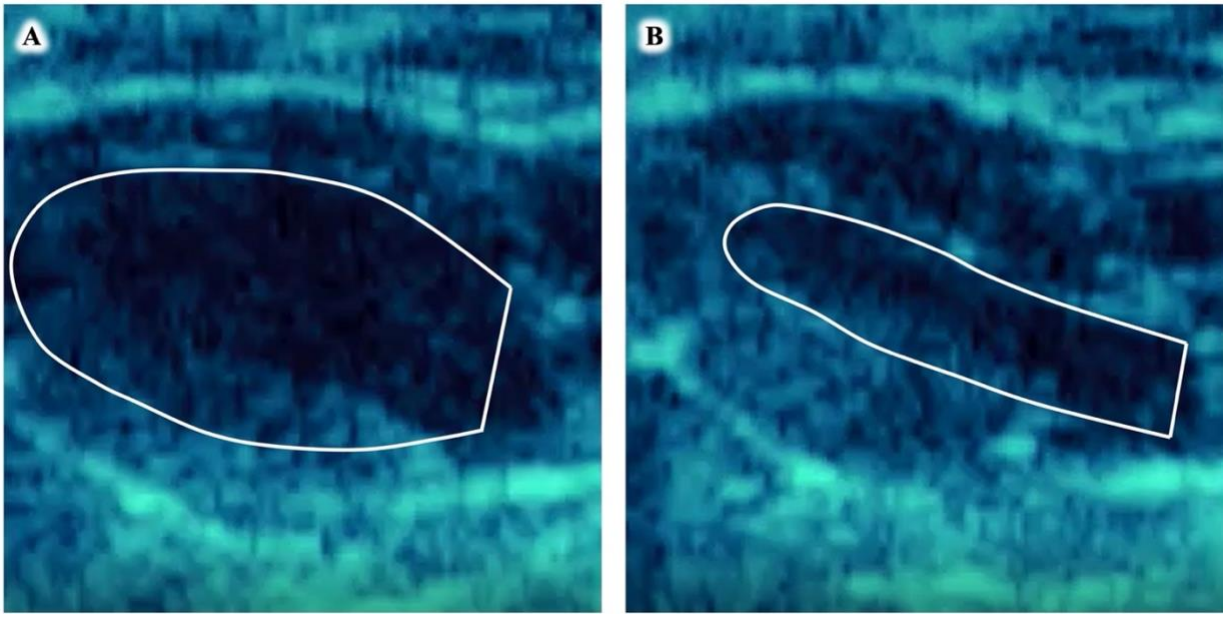


Figure 4. Parasternal long axis view on 2D transthoracic murine echocardiography. LV endocardial border delineation on EchoPAC PC software (GE Medical Systems, Version 112, Milwaukee, WI, US) for calculation of LVEF using the Teicholtz formula. **Panel A:** Endocardial border tracing at end diastole. **Panel B:** Endocardial border tracing at end systole.

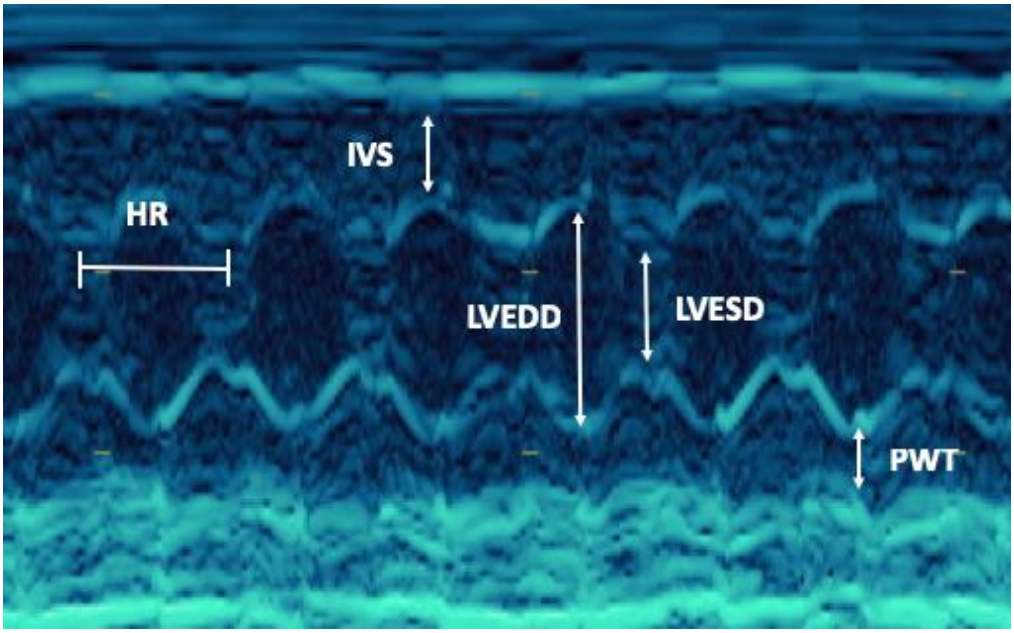


Figure 5. M-mode parasternal short axis view on 2D transthoracic murine echocardiography. LV cavity dimensions and HR as measured using M-mode on EchoPAC PC software (GE Medical Systems, Version 112, Milwaukee, WI, US).

HR, Heart rate; IVS, Interventricular septum; LVEDD, Left ventricular end-diastolic diameter; LVESD, Left ventricular end-systolic diameter; PWT, Posterior wall thickness.

3.3 Hemodynamics

BP was assessed at baseline, week 3, and week 6 in restrained, non-sedated mice using the non-invasive tail cuff method (CODA system, Kent Scientific, Torrington, CT, US) on a platform heated to 30°C. Prior to measurement, the mice were subjected to three days of BP training to familiarize them with the tail cuff apparatus. Measurements included SBP, diastolic blood pressure (DBP), pulse pressure (PP), and mean arterial pressure (MAP). A total of 20 consecutive readings were recorded at 1-minute intervals, and the mean scores of a minimum of 9 true readings were utilized for data analysis.

SBP and DBP were measured directly from the tail cuff system. PP was calculated from the systolic and diastolic readings using Equation 1. MAP was calculated from the calculated PP and diastolic readings using Equation 2.

Equation 1. Pulse pressure.

$$\text{Pulse pressure} = \text{Systolic blood pressure} - \text{Diastolic blood pressure}$$

Equation 2. Mean arterial pressure.

$$\text{Mean arterial pressure} = (\text{Pulse pressure} / 3) + \text{Diastolic pressure}$$

3.4 Histology

LV tissue was prepared in accordance with lab-established protocols and utilized for histological examination.[143,201,204] Tissue for electron microscopy was sectioned, cut into 0.5 mm^2 pieces, fixed in a 1:1 ratio of 0.2M PO_4 buffer and 5% glutaraldehyde for 3 hours, rinsed and stored overnight at 4°C in a 5% sucrose in PO_4 buffer, followed by post-fixation with 1% osmium tetroxide in 0.1M phosphate buffer for 2 hours at room temperature. Tissues were then dehydrated in increasing ethanol concentrations and embedded in Epon 812. Finally, tissue sections were stained with uranyl acetate and lead citrate. To eliminate observer bias, grids were coded without prior knowledge of their origin. The degree of cellular integrity was then determined using digital pictures captured with a Philips CM12 electron microscope.[143,201,204]

Mann-Whitney and Kruskal-Wallis tests were used for non-parametric comparisons of scores between groups during histological examination. The values ranged from 0 to 5, with 0 denoting no tissue damage and 5 indicating severe damage.

3.5 Western Blot Analyses

Western blot analysis was performed to quantify markers of oxidative stress-induced apoptosis (Bax/Bcl-xL) using a specific antibody for each protein. LV tissue samples were flash frozen in liquid nitrogen before being crushed and homogenized in a radioimmunoprecipitation assay (RIPA) lysis buffer containing phosphatase inhibitor (product #: A32957, Thermo Scientific) and protease inhibitor (product #: A32965, Thermo Scientific) to isolate total cellular protein while preventing protein degradation. After a 30-minute incubation period on ice, the samples were centrifuged to remove cellular debris before the supernatants were collected. A Bradford protein assay was then used to quantify protein concentrations, followed by comparison to bovine serum albumin (BSA) standards (product #: 23209, Thermo Scientific). The Bradford protein assay findings were used to create samples containing 30μg of protein, lamelli sample buffer (product #: 1610747, BioRad), dye diluted with 2-mercaptoethanol (product #: M6250-100ML, Aldrich Chemistry), and autoclaved water for western blot analysis.

Samples were stacked at 5% and resolved on a 12% sodium dodecyl sulphate (SDS) polyacrylamide gel. Proteins in each sample were separated using electrophoresis for 90 minutes at 55mA and 15°C. Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane with 0.2 μm pore size (product #: 88520, Thermo Scientific) for 60 minutes at 100V and 7°C. After transferring the proteins, the membranes were blocked with 5% skim milk powder (SMP) in 1x Tris Buffered Saline with 0.1% Tween 20 (product #: 0777-1L, VWR) for 60 minutes at 20°C. The membranes were then probed with rabbit-source target-specific primary antibodies for 16 hours at 4°C (Table 2), followed by 1/5000 horseradish peroxidase-conjugated goat anti-rabbit

secondary antibodies (product #: 1706515, BioRad) for 60 minutes at 20°C to visualize the protein bands.

Antibody	Product Number	Dilution	Gel Percentage	Probing Solution
Bcl-2 associated X protein (Bax)	2772S, New England Biolab	1/500	12%	2.5% SMP
B-cell lymphoma extra-large (Bcl-XL)	2762S, New England Biolab	1/500	12%	2.5% SMP

Table 2. Western gel percentage and probing conditions for each target protein. Target-specific antibodies, antibody product numbers, antibody dilutions, SDS polyacrylamide electrophoresis gel concentrations, and probing solution concentrations.

Protein samples were standardized to 30 μg using the Bradford protein assay, and results were also standardized with a loading control and a matched sample between gels. For the loading control, each membrane was probed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primary antibody (2118L, Cell Signaling) for 2 hours at 20°C, followed by 1/5000 horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies for 45 minutes at 20°C to enable visualization. Before analysis, the protein band intensity was standardized to the GAPDH loading control. As there were more samples being studied than could be loaded into a single gel, an additional matched sample was added into each gel, allowing different gels with the same target proteins to be normalized prior to comparison.

Protein detection was performed after each incubation with secondary antibodies with enhanced chemiluminescence (ECL) western blotting substrate (product #: 32106, Thermo Scientific). The chemiluminescence was captured using a BioRad ChemiDoc Imaging System (Image Lab Touch Software, Version 2.4.0.03). Protein band intensity was then measured using Densitometric analysis with Image Lab 5.2.1 software (BioRad).

3.6 Oxidized Phospholipids

Heart tissues were stored in an Eppendorf tube containing a solution of phosphate buffered saline (PBS, pH 7.4) and ethylenediaminetetraacetic acid (EDTA). The tubes were infused with gaseous nitrogen, then flash frozen in liquid nitrogen and stored at -80ºC. To extract phospholipids, thawed heart tissues were ground into a fine powder using a cold mortar, pestle and liquid nitrogen.[205] The powdered heart tissue was then transferred to a zeroed glass centrifuge tube to determine tissue weight, followed by the addition of 6 mL of ice-cold chloroform:methanol (2:1 CM, vol/vol) containing 0.01% butylated hydroxytoluene (BHT).[206] After mixing, 100 µL of a global internal standard mixture was added to the tubes followed by 1.5 mL of ice-cold PBS. After vortexing three times, the tubes were centrifuged at 3500 rpm for 5 minutes at 4ºC. While the residual aqueous phase was combined with 4.5 mL of ice-cold CM-PBS (86:14:1) and centrifuged for 5 minutes at 4ºC, the lower lipid phase was removed and transferred to a new glass tube. After the lower lipid phase was moved to the first organic phase, a nitrogen evaporator was used to evaporate the combined organic phase solvents. The lipid extracts were dissolved by adding 500 µL of CM (2:1). Following that, they were moved to autosampler vials, nitrogen flushed, and kept at -80ºC.

The samples were reconstituted in solvent A, the mobile phase, for oxolipidomics analysis (detailed below).[206] A Prominence UFLC system (Shimadzu Corporation, Canby, OR, US) was used to inject 30 µL of each sample onto an Ascentis Express C18 reversed-phase high performance liquid chromatography (HPLC) column (15 cm x 2.1 mm, 2.7 µm; Supelco Analytical, Bellefonte, PA, US).[206] A binary solvent system comprising solvents A (60:40 acetonitrile:water, vol/vol) and B (90:10 isopropanol:water, vol/vol) was used to separate the analytes. Both solvent solutions included 0.1% formic acid and 10 mM ammonium formate. The

program for mobile phase composition was set at 0.01 min, 32% B; 1.50 min, 32% B; 4.00 min, 45% B; 5.00 min, 52% B; 8.00 min, 58% B; 11.00 min, 66% B; 14.00 min, 70% B; 18.00 min, 75% B; 21.00 min, 97% B; 25.00 min, 97% B; 25.10 min, 32% B; and 30.00 min, 32% B.[216] At 30.10 minutes, the elution was halted. The column and sample trays were kept at 45ºC and 4ºC, respectively, and the flow rate employed for chromatographic separation was 260 µL/min.

A 4000 QTRAP triple quadrupole mass spectrometer system with a Turbo V electrospray ion source (AB Sciex, Framingham, MA, US) was connected to the HPLC system. Mass spectral and chromatographic data were collected with AB Sciex's Analyst Software 1.6. MultiQuant Software 2.1 was used to analyze the data (AB Sciex).[206]

3.7 Statistical Analysis

The statistical analyses were performed using the software packages SPSS version 24 (IBM, NY, US) and Graphpad Prism 5 (MA, US). Data is presented as mean ± standard deviation (SD) unless otherwise specified. Echocardiographic analyses were carried out using analysis of variance (ANOVA) and Dunnet's post-hoc test. Mann-Whitney and Kruskal-Wallis tests were used for non-parametric score comparisons between groups during histological examination. The values ranged from 0 to 5, with 0 denoting no tissue injury and 5 indicating severe damage. ANOVA was used for hemodynamic analyses, followed by Dunnet's post-hoc analysis. Western analytical data is reported as mean ± standard error of the mean (SEM). For post-hoc analysis, repeated measures of one-way ANOVA were employed to determine the significance of independent variables. Results with $p<0.05$ were considered significant.

Chapter 4: Results

4.1 Murine Echocardiography

At baseline and week 6, the HR and LV wall thickness measurements (IVS and PWT) were comparable for each study group. In DOX+TRZ treated mice, the LVEDD increased from 2.8±0.1mm at baseline to 4.5±0.2mm at week 6 ($p<0.05$), indicating adverse LV structural changes. LV structure was improved by prophylactic treatment with PER, EMPA, or EMPA+PER; at study end point, the LVEDD values were 3.8±0.2mm, 3.2±0.3mm, and 3.2±0.2mm, respectively (Table 3). In mice treated with DOX+TRZ, prophylactic treatment with EMPA or EMPA+PER was superior to PER alone in preventing adverse LV remodeling (Figure 6).

In mice treated with DOX+TRZ, the LVEF declined from 75±2% at baseline to 40±4% at week 6. Prophylactic treatment with either PER, EMPA, or EMPA+PER was cardioprotective with LVEF values of 58±3%, 66±3%, and 67±4%, respectively ($p<0.05$) (Table 3). Prophylactic treatment with EMPA or EMPA+PER was superior to PER alone in preventing LV systolic dysfunction in mice treated with DOX+TRZ (Figure 7).

Parameter	Group	Baseline	Week 6	p-value
HR (bpm)	Control (n=15)	697±5	692±7	0.91
	DOX+TRZ (n=15)	691±4	690±5	0.88
	PER+DOX+TRZ (n=15)	688±5	682±7	0.84
	EMPA+DOX+TRZ (n=15)	690±7	694±6	0.82
	EMPA+PER+DOX+TRZ (n=15)	682±4	688±4	0.86
IVS (mm)	Control (n=15)	0.81±0.02	0.81±0.01	0.98
	DOX+TRZ (n=15)	0.81±0.02	0.81±0.01	0.98
	PER+DOX+TRZ (n=15)	0.82±0.01	0.82±0.02	0.96
	EMPA+DOX+TRZ (n=15)	0.81±0.02	0.81±0.01	0.98
	EMPA+PER+DOX+TRZ (n=15)	0.82±0.01	0.82±0.02	0.97
PWT (mm)	Control (n=15)	0.81±0.01	0.82±0.01	0.97
	DOX+TRZ (n=15)	0.82±0.02	0.81±0.01	0.96
	PER+DOX+TRZ (n=15)	0.81±0.01	0.82±0.02	0.96
	EMPA+DOX+TRZ (n=15)	0.82±0.02	0.81±0.01	0.97
	EMPA+PER+DOX+TRZ (n=15)	0.82±0.01	0.82±0.02	0.97
LVEDD (mm)	Control (n=15)	2.8±0.1	2.9±0.1	0.78
	DOX+TRZ (n=15)	2.8±0.1	4.5±0.2*	<0.05
	PER+DOX+TRZ (n=15)	2.8±0.2	3.8±0.2$^{*\#}$	<0.05
	EMPA+DOX+TRZ (n=15)	2.8±0.1	3.2±0.3$^{\#\delta}$	<0.05
	EMPA+PER+DOX+TRZ (n=15)	2.8±0.2	3.2±0.2$^{\#\delta}$	<0.05
LVEF (%)	Control (n=15)	73±4	74±4	0.94
	DOX+TRZ (n=15)	75±2	40±4*	<0.05
	PER+DOX+TRZ (n=15)	74±4	58±3$^{*\#}$	<0.05
	EMPA+DOX+TRZ (n=15)	74±3	66±3$^{*\#\delta}$	<0.05
	EMPA+PER+DOX+TRZ (n=15)	74±3	67±4$^{*\#\delta}$	<0.05

Table 3. Echocardiographic parameters of C57Bl/6 mice receiving prophylactic treatment with EMPA, PER, or EMPA+PER followed by Saline or DOX+TRZ. Baseline and week 6 measures with p-values.

DOX, Doxorubicin; EMPA, Empagliflozin; HR, Heart rate; IVS, Interventricular septum; LVEDD, Left ventricular end-diastolic diameter; LVEF, Left ventricular ejection fraction; PER, Perindopril; PWT, Posterior wall thickness; TRZ, Trastuzumab.

The values are presented as mean ± SD. *p<0.05 comparing DOX+TRZ, PER+DOX+TRZ, EMPA+DOX+TRZ, and EMPA+PER+DOX+TRZ vs. Control at week 6.
#p<0.05 comparing PER+DOX+TRZ, EMPA+DOX+TRZ, and EMPA+PER+DOX+TRZ vs. DOX+TRZ at week 6.
δp<0.05 comparing EMPA+DOX+TRZ and EMPA+PER+DOX+TRZ vs. PER+DOX+TRZ at week 6.

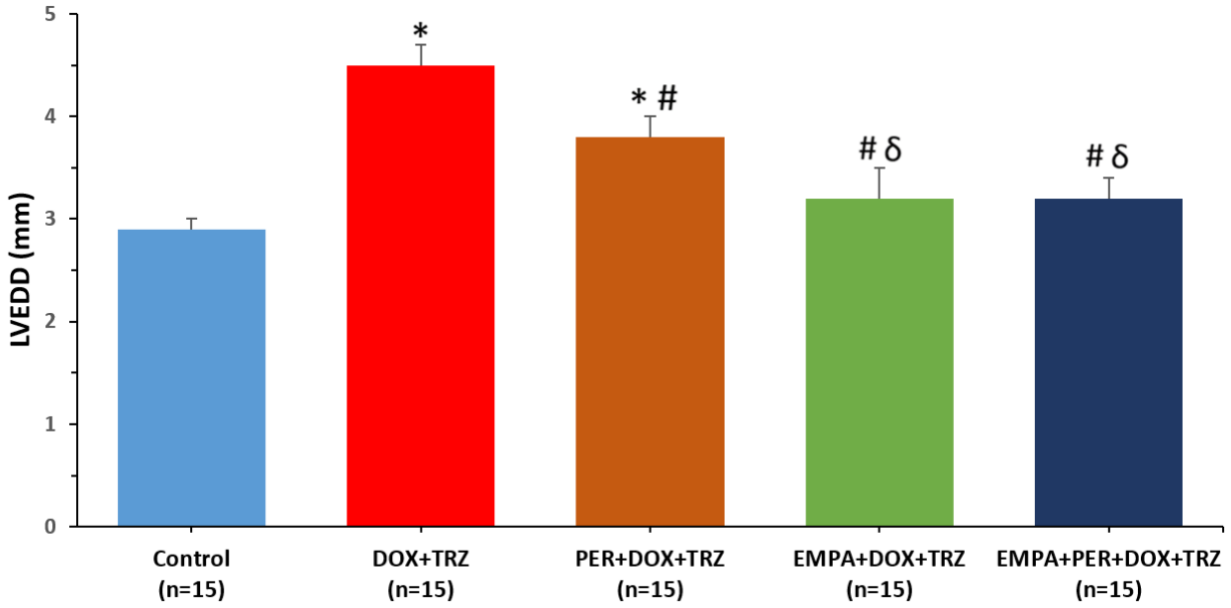


Figure 6. Changes in LVEDD in mice prophylactically administered with PER, EMPA, or EMPA+PER prior to treatment with DOX+TRZ. *p<0.05 DOX+TRZ or PER+DOX+TRZ vs. Control. #p<0.05 PER+DOX+TRZ or EMPA+DOX+TRZ or EMPA+PER+DOX+TRZ vs. DOX+TRZ. δp<0.05 EMPA+DOX+TRZ or EMPA+PER+DOX+TRZ vs. PER+DOX+TRZ.

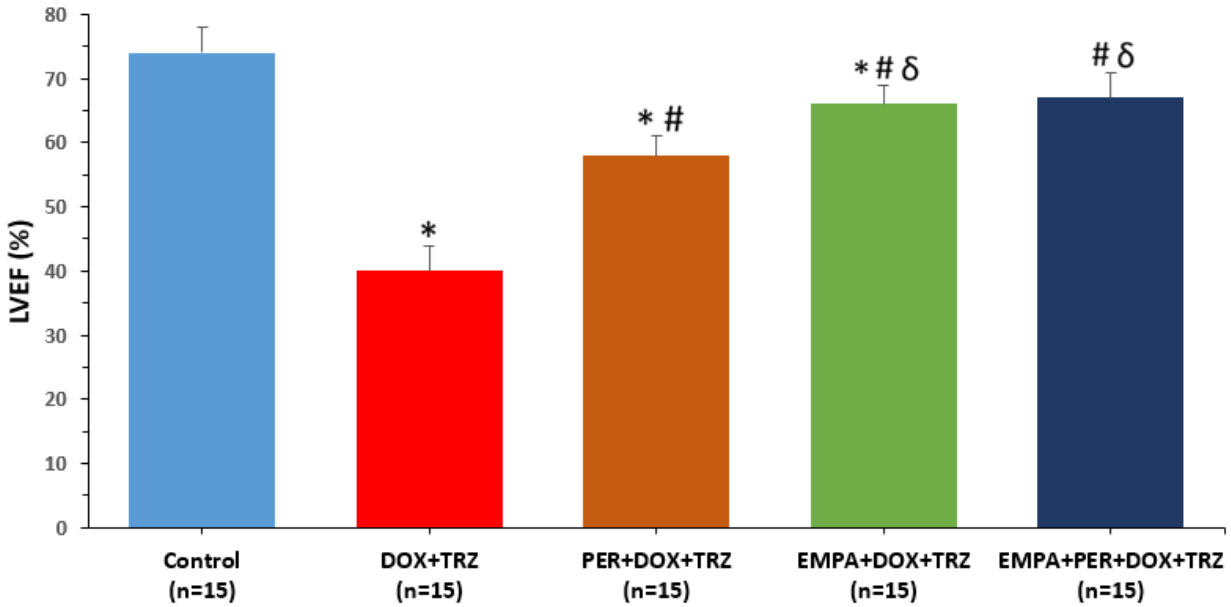


Figure 7. Changes in LVEF in mice prophylactically administered with PER, EMPA, or EMPA+PER prior to treatment with DOX+TRZ. *p<0.05 DOX+TRZ or PER+DOX+TRZ or EMPA+DOX+TRZ vs. Control. #p<0.05 PER+DOX+TRZ or EMPA+DOX+TRZ or EMPA+PER+DOX+TRZ vs. DOX+TRZ. δp<0.05 EMPA+DOX+TRZ or EMPA+PER+DOX+TRZ vs. PER+DOX+TRZ.

4.2 Histology

Electron microscopy was used to analyze LV tissue samples. Normal histological morphology of the cardiomyocytes was observed in the control group (Figure 8A). Histological analyses confirmed significant disruption of myofibrils, vacuolization, and loss of sarcomere integrity in the DOX+TRZ treated mice (Figure 8B). Prophylactic treatment with PER, EMPA, or EMPA+PER, however, improved myofibril integrity at week 6 in mice receiving DOX+TRZ (Figure 8C-8E). The prophylactic administration of EMPA+PER was superior to PER ($p<0.0001$) or EMPA alone ($p<0.001$) in preventing adverse cardiovascular remodeling (Figure 8C-8E).

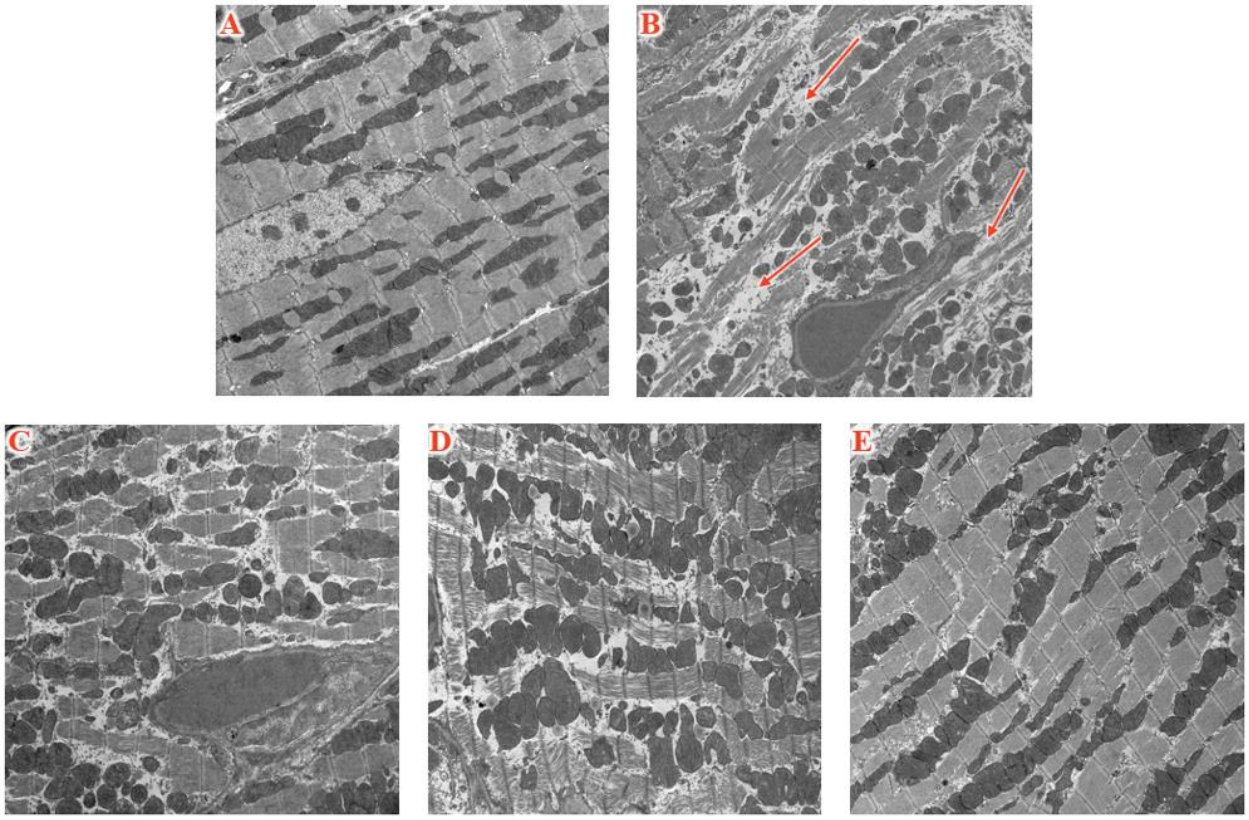


Figure 8. Electron microscopy slides representative of the cell morphology changes for each treatment group. (A) Control; (B) DOX+TRZ; (C) PER+DOX+TRZ; (D) EMPA+DOX+TRZ; and (E) EMPA+PER+DOX+TRZ.

Control	DOX+TRZ	PER+DOX+TRZ	EMPA+DOX+TRZ	EMPA+PER+DOX+TRZ
0.5	1	1.5	2	0.5
0	0.5	2	2	1
0	1.5	2.5	2	0.5
0	3	1.5	1.5	0.5
0	3	1	2	1.5
0.5	1.5	1	2	1.5
0.5	3	2	3	0.5
0	3	2	2	0.5
0	3.5	2.5	0.5	0.5
0	1	1	0.5	0
0.5	2	0.5	0	0
0	2	2	0.5	0.5
0	3.5	0.5	2	0.5
0.5	0	2	1	0.5
0	1.5	1	1	0
0	0	0.5	0.5	0
0	1.5	1	0	0
0.5	0.5	1	0.5	0.5

Table 4. Scoring quantification of LV myocardial tissue samples of C57Bl/6 mice receiving prophylactic treatment with EMPA, PER, or EMPA+PER followed by Saline or DOX+TRZ.

A score of 0 denotes no tissue damage and 5 indicates severe damage.

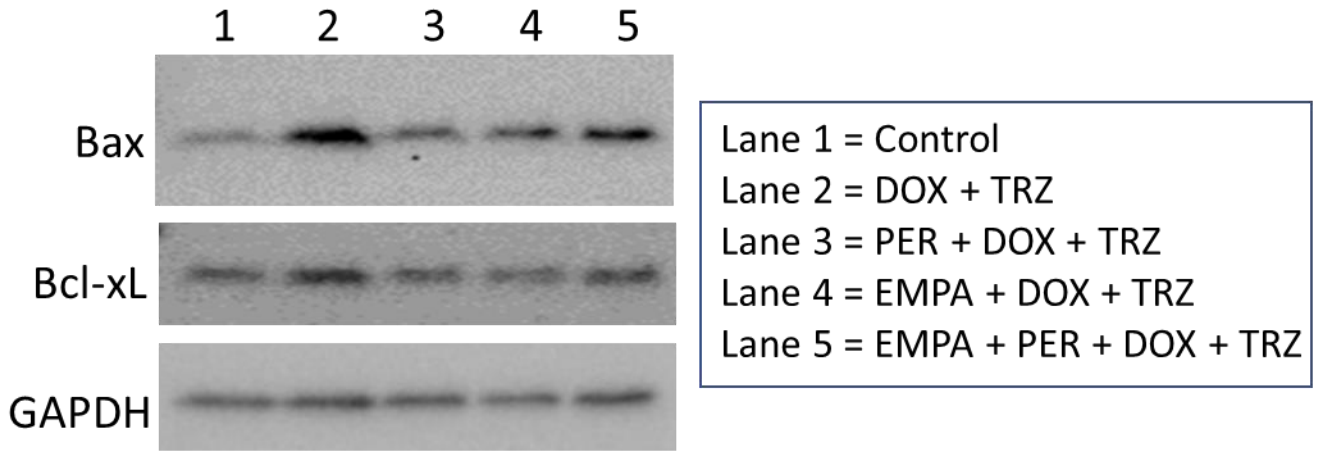
4.3 Hemodynamics

There was no statistically significant difference in SBP, DBP, or MAP at week 6 compared to baseline in all study animals (p = non-significant). Additionally, administration of PER, EMPA, or EMPA+PER did not significantly alter SBP, DBP, or MAP at week 6 (data not shown).

4.4 Western Blot Analyses

In mice treated with DOX+TRZ, there was a 1.5-fold increase in Bax/Bcl-xL expression as compared to healthy control mice ($p<0.05$) (Figure 9). Elevations in this oxidative stress biomarker were significantly downregulated in mice prophylactically treated with PER, EMPA, or EMPA+PER ($p<0.05$).

A



B

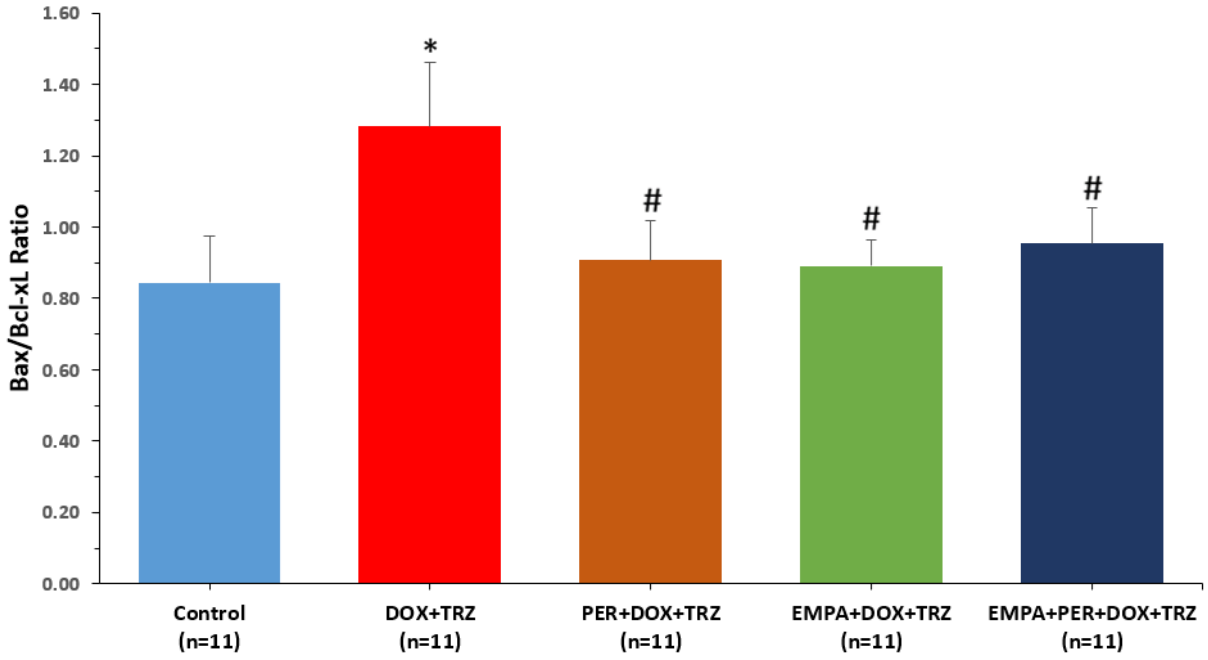


Figure 9. Western blot Bax/Bcl-xL expression. (A) Representative western blot. (B) Changes in Bax/Bcl-xL expression in mice from our 5 treatment groups at the end of the study. *p<0.05 DOX+TRZ vs. Control. #p<0.05 PER+DOX+TRZ or EMPA+DOX+TRZ or EMPA+PER+DOX+TRZ vs. DOX+TRZ.

4.4 Oxidized Phospholipids

In mice treated with DOX+TRZ, there was an increase in oxidized phosphatidylethanolamine species, including PE16:0, C7H11O3; and PE18:0, C11H19O3, as compared to healthy control mice (p<0.05) (Figures 10 and 11). Elevations in these oxidized phospholipids were significantly downregulated in mice prophylactically treated with EMPA+PER (p<0.05), while there were no significant changes in the PER and EMPA groups (p = non-significant).

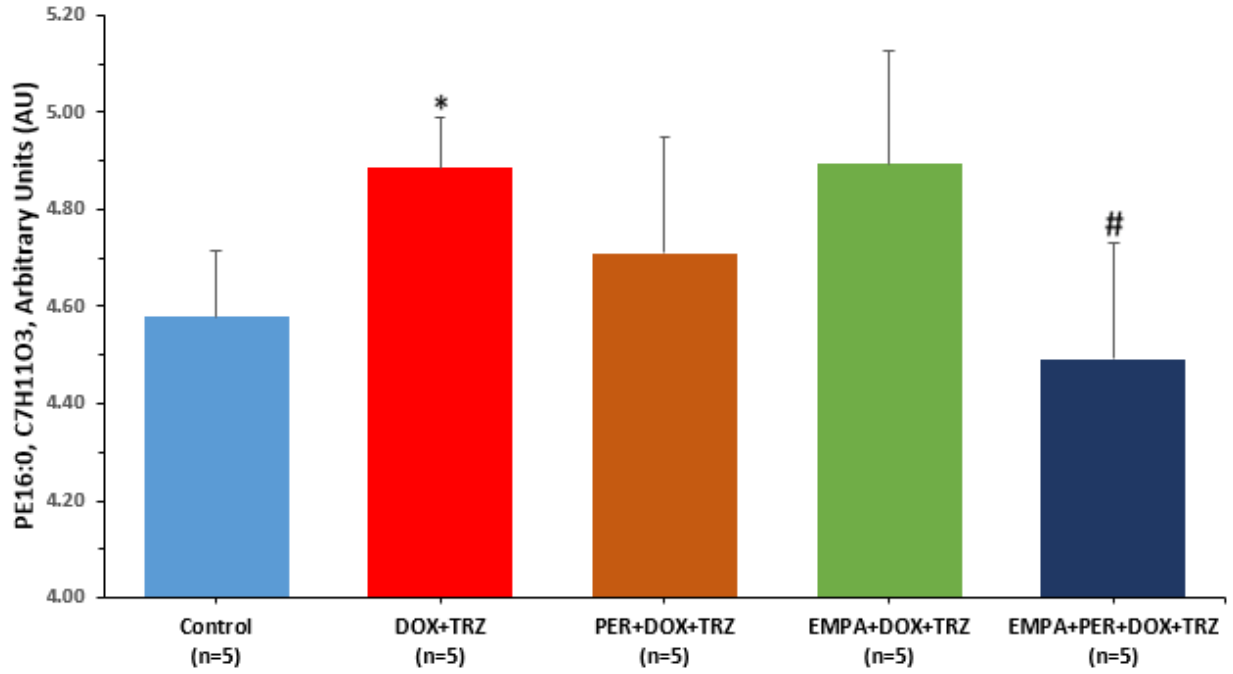


Figure 10. Changes in PE16:0, C7H11O3 oxidized phosphatidylethanolamine expression in mice from our 5 treatment groups at the end of the study. *p<0.05 DOX+TRZ vs. Control. #p<0.05 EMPA+PER+DOX+TRZ vs. DOX+TRZ.

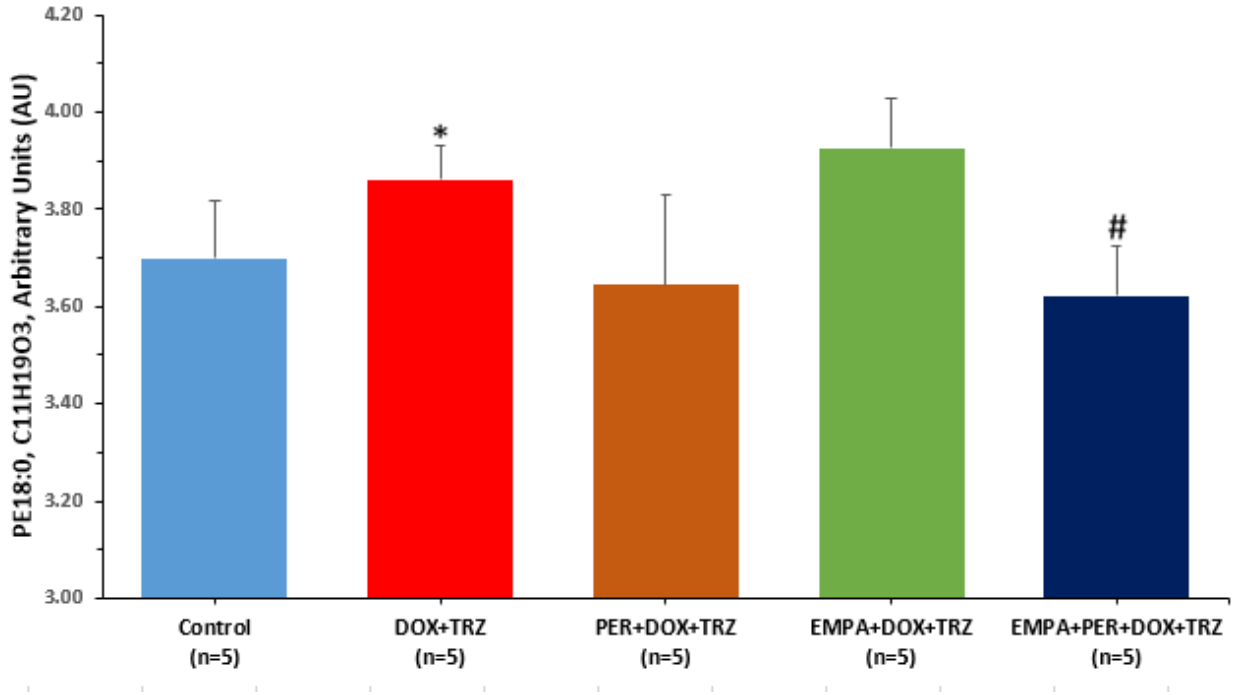


Figure 11. Changes in PE18:0, C11H19O3 oxidized phosphatidylethanolamine expression in mice from our 5 treatment groups at the end of the study. *p<0.05 DOX+TRZ vs. Control. #p<0.05 EMPA+PER+DOX+TRZ vs. DOX+TRZ.

Chapter 5: Discussion

Over the past several decades, the prevalence of cardiovascular disease among women with breast cancer has risen, driven in part by the cardiotoxic side effects of anti-cancer therapies and improved cardiac surveillance. Despite advances in surgery, radiation, chemotherapy, and targeted biological therapies that have significantly improved overall survival, cardiotoxicity remains a major complication and a leading cause of morbidity and mortality in the breast cancer population. Prevention of chemotherapy mediated cardiotoxicity is a growing focus in the evolving field of Cardio-Oncology. In our chronic *in vivo* murine model of DOX+TRZ induced cardiotoxicity, the prophylactic administration of EMPA or EMPA+PER was superior to PER alone in preventing adverse cardiovascular remodeling. Our study demonstrated that the prophylactic administration of EMPA and EMPA+PER: i) prevented adverse LV cavity remodelling; ii) decreased cardiomyocyte disruption of myofibrils, vacuolization, and loss of sarcomere integrity; and iii) attenuated oxidative stress and apoptosis in a chronic *in vivo* female murine model.

5.1 Prevention of Adverse Cardiovascular Remodelling

In the field of Cardio-Oncology, combined treatment with DOX and TRZ leads to detrimental cardiovascular remodeling in preclinical murine models.[149,196,207.208] Furthermore, these models have demonstrated the effectiveness of RAS antagonists in preventing structural and functional changes in the hearts of animals affected by chemotherapy-induced cardiotoxicity.[149,196,207.208] A previous study by Akolkar et al. explored the potential cardioprotective effects of prophylactic RAS antagonist therapy in preventing chemotherapy-induced cardiotoxicity.[149] In this study, 240 mice were prophylactically treated with oral aliskiren (50 mg/kg/day), PER (3 mg/kg/day), or valsartan (10 mg/kg/day) for 13 weeks. Additionally, mice were further randomized to receive i.p.

injections of DOX (4 mg/kg weekly) and TRZ (4 mg/kg weekly), administered weekly for five weeks. Serial echocardiographic measurements demonstrated that DOX+TRZ treatment resulted in a significant increase in LV cavity dimensions.[149] In mice treated with DOX+TRZ, LVEDD increased by 48% from baseline to week 13. Prophylactic administration of aliskiren, PER, or valsartan mitigated this LV cavity dilation, with LVEDD increases of 26%, 32%, and 35% at week 13, respectively.[149] In mice receiving DOX+TRZ, LVEF values significantly decreased from baseline to week 13. Prophylactic administration of RAS antagonists, however, preserved LVEF in all three groups as compared to placebo.[149]

More recently, Eekhoudt et al. demonstrated that prophylactic treatment with PER (3 mg/kg/day) was cardioprotective in a chronic 6-week *in vivo* murine model of DOX+TRZ-induced cardiotoxicity (n=81). This was reflected by a partial attenuation of LVEDD dilatation, with the PER-treated group exhibiting a 22% improvement relative to the untreated cohort at the study endpoint. Furthermore, mice that received prophylactic PER treatment maintained a LVEF of 62% at week 6, whereas mice treated with DOX+TRZ without preventative intervention exhibited a marked decline in LVEF to 34% by the study endpoint, indicative of severe cardiotoxicity.[196]

Additionally, a study by Lódi et al. investigated the cardioprotective benefits of early intervention compared to conventional treatment schedules in a rat model of DOX-induced cardiomyopathy.[207] The researchers compared the efficacy of prophylactic treatment with oral PER (2 mg/kg), bisoprolol (2.5 mg/kg), or eplerenone (6.25 mg/kg) initiated one week before DOX exposure to a delayed treatment regimen introduced one month after DOX administration. The rats were divided into four groups: (i) a control group receiving saline, (ii) a DOX-treated group (six cycles of 1.5

mg/kg intravenously), (iii) a group receiving DOX with early bisoprolol, PER, and eplerenone treatment, and (iv) a group receiving DOX with delayed bisoprolol, PER, and eplerenone treatment. Echocardiographic studies were conducted at baseline, as well as on days 51 and 80. The results demonstrated a significant reduction in LV systolic function in DOX-treated rats compared to controls. Interestingly, LVEF decreased from 83% to 72% in rats receiving heart failure medications using the conventional schedule, whereas in the prophylactically treated group, LVEF declined only from 84% to 81%.[207] These findings underscore the benefits of early cardioprotective intervention over delayed treatment in DOX-induced cardiotoxicity. These results highlight the critical role of preventative strategies, such as those employed in our study, in mitigating chemotherapy-induced cardiotoxicity.

Finally, Hiona et al. conducted a chronic 10-week rat model study (n=24), where they found that administering the ACEi enalapril (10 mg/kg/day) prior to chemotherapy treatment significantly preserved systolic function in animals exposed to DOX (25 mg/kg), improving contractility of the LV.[208]

Several preclinical studies have demonstrated the cardioprotective effects of SGLT2 inhibitors in models of DOX-induced cardiotoxicity, and our findings extend these results in both magnitude and clinical relevance.[169-172] Sabatino et al. used echocardiography to show that EMPA improved LVEF from 49% in DOX-treated mice to 61%, while LV cavity dimensions were equally dilated in both groups.[169] Oh et al. used CMR as their imaging modality, and did not observe a significant improvement in LVEF or LVEDD values from EMPA treatment in DOX-treated mice, but showed a decrease in LVESD, indicating a degree of cardioprotection.[170] Chang et al. observed a small but

significant LVEF improvement with no changes in LV cavity dimensions as measured via echocardiography in rats pretreated with dapagliflozin.[171] Finally, Quagliariello et al. found that EMPA prevented reductions in GLS in DOX-treated mice, but did not assess other echocardiographic parameters.[172] While these studies demonstrated variable efficacy in DOX-only models, our study is the first to evaluate EMPA in a clinically relevant DOX+TRZ model. Furthermore, we uniquely included an ACE inhibitor, PER, as a standard-of-care comparator, whereas prior studies used either vehicle,[170-172] or a non-cardioprotective agent like furosemide.[169] In our model, prophylactic administration of PER resulted in improvements in both LVEDD and LVEF, with EMPA yielding even more pronounced cardioprotective effects. Notably, combination therapy with EMPA+PER restored cardiac function to near-control levels. These results not only reinforce the cardioprotective effects of EMPA seen in prior DOX-only studies, but also highlight the superior efficacy and potential synergy of SGLT2 and ACE inhibition in mitigating cardiotoxicity from modern oncologic regimens.[169-172]

In the current chronic *in vivo* study, we evaluated whether EMPA, PER, or the combination can be used in the prevention of DOX+TRZ mediated cardiotoxicity. Both structurally and functionally, prophylactic administration of EMPA, PER, and EMPA+PER prevented adverse LV remodeling as compared to the DOX+TRZ group alone. While prophylactic treatment with PER alone improved LVEDD by ~17%, the EMPA and EMPA+PER groups showed an even greater improvement of ~35%, comparable with control animals (Figure 6). Prophylactic PER treatment alone improved LVEF by ~37%, while EMPA treatment showed an LVEF increase of ~49% compared to untreated mice. Notably, the LVEF of mice treated with both EMPA and PER was preserved in a similar way to control animals (Figure 7). This is the first study to demonstrate the

superiority of the SGLT2 inhibitor EMPA as compared to the ACEi PER in the prevention of DOX+TRZ mediated cardiotoxicity in a murine model. These findings merit further clinical exploration of preventative treatment with EMPA for patients undergoing chemotherapy treatment, and their possible inclusion in the CCS guidelines for the management of CTRCD. These guidelines currently include ACEi's but not SGLT2 inhibitors, despite their classification as standard pharmacological therapy in heart failure patients outside of the Cardio-Oncology setting.[119,165]

5.2 Histological Overview of Cardiotoxicity

The administration of DOX and TRZ alone, as well a combination of these two agents have been associated with histopathological changes in cardiac tissue. Their concurrent administration has been shown to cause loss of cardiomyocyte integrity, a reduced number of functional mitochondria, mitochondrial swelling, and cytoplasmic vacuolization.[140,143,196,198,201,204] Additional structural abnormalities include perinuclear cisternae and sarcotubular system dilation, lysosomal body formation, myofibril disarray, increased cardiomyocyte diameter, interstitial and perivascular infiltration of lymphocytes and macrophages, as well as elevated apoptosis, necrosis, and fibrosis.[140,143,196,198,201,204] Notably, these pathological changes may be prevented with the prophylactic administration of ACEi's and SGLT2 inhibitors.

Previous murine studies examining the role of RAS antagonists and SGLT2 inhibitors in preventing chemotherapy-mediated cardiotoxicity have demonstrated a reduction in histopathological alterations in cardiac tissue.[169,196,171] Eekhoudt et al. investigated the prophylactic effects of PER and flaxseed in an *in vivo* murine model (n=81).[196] Electron

microscopy analysis revealed that mice treated with DOX (24 mg/kg) + TRZ (9 mg/kg) experienced significant cardiomyocyte damage, including myofibril disarray, cytoplasmic vacuolization, and loss of sarcoplasmic reticulum. However, 3 weeks of prophylactic administration of a 10% flaxseed-enriched diet or PER (3 mg/kg/day) significantly mitigated these structural alterations, preserving cardiomyocyte integrity.[196] Additionally, Sabatino et al. conducted a 5-week study investigating the prophylactic effects of EMPA (5 mg/kg/day) and furosemide (20 mg/kg/day) in DOX (25 mg/kg) treated mice (n=42).[169] Histological analysis using hematoxylin and eosin staining as well as Masson's trichrome staining revealed that DOX treatment induced significant myocardial fibrosis, myofibrillar disarray, and vacuolization. However, mice pretreated with EMPA exhibited a 50% reduction in myocardial fibrosis compared to the untreated group, along with improved preservation of myocardial structure. EMPA also mitigated the loss of cardiac fibers and reduced myocardial disarray. In contrast, furosemide did not significantly prevent fibrosis or structural damage.[169] Finally, Chang et al. conducted a study to investigate the cardioprotective effects of the SGLT2 inhibitor dapagliflozin against DOX-induced cardiotoxicity in diabetic rats.[171] The animals were pretreated with dapagliflozin (10 mg/kg/day) for 6 weeks, followed by DOX (5 mg/kg/week) for 4 weeks. Histological examination using Masson's trichrome staining confirmed that dapagliflozin reduced myocardial fibrosis, while significantly alleviating the number of apoptotic cardiomyocytes as compared to the no-pretreatment DOX group.[171]

In the current study, we performed histological analysis of tissue samples using electron microscopy. As compared to controls, mice treated with DOX+TRZ demonstrated significant myofibril disruption, vacuolization, and loss of sarcomere integrity (Figure 8). As a novel finding,

the current study revealed that all three experimental groups using PER, EMPA, or EMPA+PER, prevented DOX+TRZ induced myocyte damage to a certain extent. The intermediate levels of cellular damage in the three experimental groups suggests that EMPA and PER are both effective at partially attenuating DOX+TRZ mediated cardiotoxicity. Combination therapy using EMPA+PER showed less damage as compared to PER or EMPA alone, revealing to be most similar to the cardiac muscle tissue of our healthy control group. This finding suggests a synergistic effect of EMPA and PER, which was not supported by the echocardiographic results, which showed that EMPA alone was just as effective in preserving LVEDD and LVEF (Figures 6 and 7). It is possible that structural damage on the cellular level precedes functional alterations in the LV, and therefore we hypothesize that if the study was extended to a longer timeframe than 6 weeks, we would observe a more significant reduction in LVEF values in the EMPA only group, as compared to EMPA+PER treated mice that did not develop the same degree of myocardial damage.

5.3 Mechanistic Pathways of the Cardioprotective Effects of SGLT2 Inhibitors

In our study, mechanistic pathways elucidated through protein and lipid analyses demonstrate: i) reduced expression of pro-apoptotic proteins linked to oxidative stress in cardiomyocytes; and ii) lower levels of oxidative stress-related lipid markers in heart tissue of mice pre-treated with EMPA+PER as compared to DOX+TRZ alone. DOX+TRZ mediated cardiotoxicity is characterized by randomly distributed cardiomyocyte death leading to functional decline.[21] Apoptosis is one of the main mechanisms that contributes to this cell death pattern. Our current mechanistic results suggest that proteomic and lipidomic markers of oxidative stress are partially mitigated by pre-treatment with the combination of EMPA+PER. However, it is important to

recognize that other mechanistic pathways may be involved in the dysfunction and death of cardiomyocytes in DOX+TRZ treated mice. These pathways include including inflammation and ER stress, with their associated proteomic markers.[60-71]

5.3.1 Oxidative Stress Induced Apoptosis

DOX and TRZ exert their cardiotoxic effects through a variety of mechanisms, ultimately triggering several distinct pathways of cellular death (Figure 1). A prominent factor in DOX+TRZ-induced heart damage is the degree of oxidative stress imposed on cardiomyocytes, a process that can readily pave the way for programmed cell death, or apoptosis.[72-79,87,88,97,98] The execution of apoptosis is carefully controlled, with proteins from the Bcl-2 family serving as central regulators. The balance between pro-apoptotic members, such as Bax, and anti-apoptotic members, like Bcl-xL, critically dictates a cell's susceptibility to apoptosis, especially via the intrinsic mitochondrial pathway.[77-79] When the Bax/Bcl-xL ratio shifts upwards, it facilitates the permeabilization of the mitochondrial outer membrane. This event allows for the escape of cytochrome c and other molecules from the mitochondria into the cell's cytoplasm, thereby initiating a cascade of caspase enzyme activation that trigger apoptosis.[77-79]

Pre-clinical investigations have provided evidence that SGLT2 inhibitors can attenuate chemotherapy-induced cardiotoxicity by modulating apoptotic pathways, specifically impacting the balance of Bcl-2 family proteins.[171,209] A study by Yang et al. created a rat model of cardiorenal syndrome (CRS) by inducing dilated cardiomyopathy via DOX treatment (cumulative dose of 28 mg/kg given over 20 days) and chronic kidney disease (via subtotal nephrectomy).[209] The CRS+EMPA treatment received EMPA at a dosage of 20 mg/kg/day intraperitonially,

administered from day 24 after CRS induction. Western blot analysis of LV tissues harvested on day 42 after CRS induction demonstrated that EMPA treatment significantly decreased the protein expression of mitochondrial Bax compared to the CRS group treated with DOX alone, indicating a reduction in this pro-apoptotic marker.[209] In another study by Chang et al., streptozotocin-induced diabetic rats were pretreated with oral dapagliflozin (10 mg/kg/day) for 6 weeks, followed by DOX (5 mg/kg/week, i.p.) for 4 weeks.[171] Western blot analysis of cardiac tissue revealed that dapagliflozin treatment decreased the expression of the pro-apoptotic protein Bax and increased the expression of the anti-apoptotic protein Bcl-2 (which functions analogously to Bcl-xL in regulating apoptosis) compared to diabetic rats receiving DOX alone. This modulation resulted in a more favorable anti-apoptotic protein balance in the dapagliflozin-treated, DOX-exposed group.[171]

While the aforementioned studies demonstrate a favorable effect of SGLT2 inhibition on regulating the Bax/Bcl-xL ratio, evidence supporting the cardioprotective role of ACEi's in this pathway is lacking. A 10-week study by Hiona et al. showed that pretreatment with the ACEi enalapril (10 mg/kg/day starting 1 week before DOX administration and for the entire duration of the study) improved markers of oxidative stress in DOX-treated rats (25 mg/kg administered i.p. over 6 weeks).[210] While the study did not measure Bax/Bcl-xL levels, they demonstrated that DOX treatment significantly increased both caspase-3 and caspase-9, which are downstream of Bax/Bcl-xL in the oxidative stress pathway leading to apoptosis (Figure 1). Although enalapril did not mitigate an increase in caspase levels, it did improve other markers related to oxidative stress and mitochondrial function, warranting further investigation into the protective effects of ACEi's on

the Bax/Bcl-xL pathway and its downstream molecules in DOX-induced cardiotoxicity pre-clinical models.[210]

Our investigation revealed that the Bax/Bcl-xL ratio, a key indicator of apoptosis, was markedly increased in mice subjected to DOX+TRZ treatment. Specifically, these mice exhibited a 1.5-fold increase in Bax/Bcl-xL expression level as compared to their healthy counterparts (Figure 9), signaling a heightened susceptibility to apoptosis within this group. Conversely, this rise in the pro-apoptotic Bax/Bcl-xL ratio was substantially mitigated in animals that received prophylactic administration of PER, EMPA, or the EMPA+PER combination. The data suggests that DOX+TRZ likely inflicts stress and damage upon mitochondria, disrupting the equilibrium of Bcl-2 family proteins in favor of apoptosis. The cardioprotective effects observed with PER, EMPA, and their combined use may stem from their capacity to counteract oxidative stress or directly shield mitochondria, thereby tempering this apoptotic signaling and enhancing cardiomyocyte survival. In fact, our study is the first to demonstrate the cardioprotective effects of an ACEi on preventing increases in the Bax/Bcl-xL ratio, which were equivalent to SGLT2 inhibition in this chronic murine model of chemotherapy mediated cardiotoxicity. These findings are distinct from the cardiovascular remodeling results in which EMPA and EMPA+PER were superior to PER alone in improving LVEDD and LVEF parameters, but this can be explained by the fact that the Bax/Bcl-xL ratio is just one of many markers that may be upregulated by DOX+TRZ treatment (Figure 1), and the functional activity of the LV is impacted by a multitude of cellular processes rather than oxidate stress induced apoptosis alone.

5.3.2 Oxidative Lipid Damage

DOX promotes oxidative stress in cardiomyocytes through mitochondrial dysfunction and iron accumulation, leading to lipid peroxidation and the generation of oxidized phospholipids such as oxidized phosphatidylethanolamine (OxPE).[211] This particular class of oxidized lipids is a critical executor of ferroptosis, a regulated form of cell death driven by iron and lipid peroxidation. OxPE accumulation impairs mitochondrial function and amplifies cardiomyocyte death.[211] TRZ exacerbates these effects by inhibiting HER2-mediated survival pathways in cardiomyocytes, compounding oxidative stress.[87-105] The combined use of DOX and TRZ significantly increases ROS production and potentiates lipid oxidation, as supported by mechanistic reviews and *in vivo* studies.[212,213]

As of now, there is no published preclinical evidence directly demonstrating that SGLT2 inhibitors or ACEi's improve or reduce oxidized phospholipid levels in DOX and/or TRZ mediated cardiotoxicity. Our study is the first to demonstrate that although prophylactic treatment with PER or EMPA alone was not cardioprotective against accumulation of OxPEs in DOX+TRZ treated mice, the combination of both EMPA and PER significantly reduced two OxPE species, specifically PE16:0, C7H11O3 and PE18:0, C11H19O3 (Figures 10 and 11). The observed reduction in OxPE species with the combined treatment of EMPA and PER likely results from synergistic attenuation of ferroptosis and oxidative stress pathways, both of which are central to DOX+TRZ-induced cardiotoxicity. EMPA has been shown to protect cardiomyocytes by restoring mitochondrial respiratory chain function, improving cardiolipin stability, and reducing ROS generation, a key driver of lipid peroxidation.[214] Additionally, EMPA activates the adenosine monophosphate-activated protein kinase (AMPK) / nuclear factor erythroid 2-related factor 2

(NRF2) antioxidant pathway, which upregulates glutathione peroxidase 4 (GPX4) and cystine transporter solute carrier family 7 member 11 (SLC7A11), critical enzymes that detoxify lipid hydroperoxides and suppress ferroptosis.[215] In a mouse model of acute myocardial infarction, PER significantly inhibited myocardial apoptosis and improved cardiac function.[216] The protective effects were mediated through downregulation of toll-like receptor 4 (TLR4) / NF-κB signaling, a pathway tightly linked to inflammatory oxidative stress and lipid peroxidation.[216] Although the study did not quantify lipid peroxidation directly, TLR4/NF-κB suppression is known to reduce ROS and downstream lipid oxidation, suggesting an indirect suppression of oxidized phospholipids.[216] Thus, our results suggest that combined prophylactic treatment with EMPA and PER reduces oxidative stress and prevents lipid oxidation through various molecular pathways that complement one another to prevent overexpression of cytotoxic OxPE species implicated in ferroptosis and mitochondrial dysfunction.

5.4 Limitations

Our study presents certain limitations that should be acknowledged. Firstly, our model used only female mice, and although the vast majority of breast cancer patients are female, breast cancer does occur in males as well, and therefore the effects of preventative treatment with EMPA should be assessed in male models.[1] Another limitation is the fact that DOX and TRZ were administered concurrently, while in the clinical setting, these anti-cancer drugs are given sequentially.[35-45] In order to reproduce a model where DOX and TRZ are administered sequentially, we would need to extend the basic science study up to at least 12 weeks. Finally, our murine model involved healthy, cancer-free mice that received DOX+TRZ to induce cardiotoxicity. While we demonstrated that EMPA protects cardiac structure and function, we did not assess whether our SGLT2 inhibitor

affects DOX+TRZ's anti-tumor effects. Before EMPA is incorporated as standard pharmacological therapy in the prevention of cardiotoxicity in cancer patients, it is important to ensure that this drug is not an obstacle in patients' cancer treatment.

5.5 Future Directions & Clinical Implications

As DOX+TRZ are used in the breast cancer setting, it is important to assess whether EMPA promotes or inhibits the anti-cancer effects of these two chemotherapeutic agents. This remains to be studied in a *in vivo* human xenograft HER2 overexpressing tumor mouse model, via implantation of BT474 breast cancer cells in the mice.[217] In this model, echocardiography would be used to assess cardiac function, while monitoring tumor volumes and metastases using positron emission tomography-magnetic resonance imaging (PET-MRI).[217] Furthermore, while our 6-week study successfully created a model of HFrEF, a more clinically-relevant approach would require extending the study to a longer duration of at least 12 weeks, where DOX would be given first, followed by TRZ, emulating the approach taken by oncologists in treating patients with breast cancer.[35-45] After assessing the cardioprotective mechanisms of EMPA in a mouse model, the next step is to translate these basic science findings to the clinical setting by leading a multi-centre, double-blind, placebo-controlled, randomized clinical trial. In this trial, women diagnosed with HER2 positive breast cancer would be randomized to receive placebo, PER, EMPA, or the combination of both drugs, beginning at least 24 hours before initiation of chemotherapy treatment with DOX, followed by TRZ.[152,153] Cardiac function would be assessed throughout the study using echocardiography, MUGA, and/or CMR. The outcomes of this clinical trial will inform researchers and clinicians whether prophylactic treatment with EMPA would attenuate or prevent cardiac dysfunction in DOX+TRZ treated patients.

The findings of this study have the potential to preserve women's heart health in the setting of chemotherapy and targeted biological therapy treatment by preventing the development of heart failure and its devastating consequences. Our study sets the stage for a clinical trial assessing the role of EMPA in preventing heart failure in breast cancer patients. While SGLT2 inhibitors are incorporated in the current guidelines for management of HFrEF, they are not presently part of the Cardio-Oncology recommendations for the management of CTRCD. [119,165] Our study serves as a stepping stone towards the incorporation of these cost-effective, clinically tolerable medications in women who are about to undergo chemotherapy treatment, and we hope that SGLT2 inhibitors will help prevent cases of heart failure in this patient population.[180-182]

Chapter 6: Conclusions

Our innovative study demonstrated that while prophylactic treatment with PER alone was cardioprotective, preventative treatment with EMPA or combination therapy with EMPA+PER worked even better than PER alone in preventing chemotherapy-mediated cardiotoxicity caused by DOX+TRZ in a chronic *in vivo* female murine model.

According to the results of our study, EMPA alone or EMPA+PER were equally beneficial in preventing adverse LV cavity remodelling as measured by LVEDD and LVEF parameters, as well as in decreasing the expression of the pro-apoptotic Bax/Bcl-xL ratio. EMPA and PER worked synergistically to attenuate disruption of myofibrils, vacuolization, and loss of sarcomere integrity in the cardiomyocytes, and this combination therapy also prevented deleterious increases in two species of OxPEs, which serve as a marker for oxidative lipid damage.

These findings showcase the potential of SGLT2 inhibition as a cardioprotective strategy in the setting of chemotherapy-induced heart failure, while supporting the exploration of combinatorial therapies to optimize cardiac outcomes in at-risk cancer patients.

Chapter 7: References

1. Canadian Cancer Society. Breast Cancer Statistics. Available at: https://www.cancer.ca/en/cancer-information/cancer-type/breast/statistics/

2. Zakaria D, Shaw A. Trends in mammography, hormone replacement therapy, and breast cancer incidence and mortality in Canadian women. Cancer Causes Control. 2019 Feb;30(2):137-47. doi:10.1007/s10552-019-1127-3.

3. Heer E, Ruan Y, Mealey N, Quan ML, Brenner DR. The incidence of breast cancer in Canada 1971-2015: trends in screening-eligible and young-onset age groups. Can J Public Health. 2020 Oct;111(5):787-93. doi:10.17269/s41997-020-00305-6.

4. Franklin M, Pollard D, Sah J, Rayner A, Sun Y, Dube F, Sutton A, Qin L. Direct and Indirect Costs of Breast Cancer and Associated Implications: A Systematic Review. Adv Ther. 2024 Jul;41(7):2700-2722. doi:10.1007/s12325-024-02893-y.

5. World Health Organization. Breast Cancer. Available at: https://www.who.int/news-room/fact-sheets/detail/breast-cancer.

6. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer. 2004 Sep;4(9):665-76. doi:10.1038/nrc1431.

7. Foulkes WD. BRCA1 and BRCA2: chemosensitivity, treatment outcomes and prognosis. Fam Cancer. 2006;5(2):135-42. doi:10.1007/s10689-005-2832-5.

8. Coughlin SS. Social determinants of breast cancer risk, stage, and survival. Breast Cancer Res Treat. 2019 Oct;177(3):537-548. doi:10.1007/s10549-019-05340-7. Epub 2019 Jul 3.

9. King MC, Marks JH, Mandell JB; New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003 Oct 24;302(5645):643-6. doi:10.1126/science.1088759.

10. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjäkoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003 May;72(5):1117-30. doi:10.1086/375033.

11. Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, Lee A, Subramanian D, De Leeneer K, Fostira F, Tomiak E, Neuhausen SL, Teo ZL, Khan S, Aittomäki K, Moilanen JS, Turnbull C, Seal S, Mannermaa A, Kallioniemi A, Lindeman GJ, Buys SS, Andrulis IL, Radice P, Tondini C, Manoukian S, Toland AE, Miron P, Weitzel JN, Domchek SM, Poppe B, Claes KB, Yannoukakos D, Concannon P, Bernstein

JL, James PA, Easton DF, Goldgar DE, Hopper JL, Rahman N, Peterlongo P, Nevanlinna H, King MC, Couch FJ, Southey MC, Winqvist R, Foulkes WD, Tischkowitz M. Breast-cancer risk in families with mutations in PALB2. N Engl J Med. 2014 Aug;371(6):497-506. doi:10.1056/NEJMoa1400382.

12. Yang X, Leslie G, Doroszuk A, Schneider S, Allen J, Decker B, Dunning AM, Redman J, Scarth J, Plaskocinska I, Luccarini C, Shah M, Pooley K, Dorling L, Lee A, Adank MA, Adlard J, Aittomäki K, Andrulis IL, Ang P, Barwell J, Bernstein JL, Bobolis K, Borg Å, Blomqvist C, Claes KBM, Concannon P, Cuggia A, Culver JO, Damiola F, de Pauw A, Diez O, Dolinsky JS, Domchek SM, Engel C, Evans DG, Fostira F, Garber J, Golmard L, Goode EL, Gruber SB, Hahnen E, Hake C, Heikkinen T, Hurley JE, Janavicius R, Kleibl Z, Kleiblova P, Konstantopoulou I, Kvist A, Laduca H, Lee ASG, Lesueur F, Maher ER, Mannermaa A, Manoukian S, McFarland R, McKinnon W, Meindl A, Metcalfe K, Mohd Taib NA, Moilanen J, Nathanson KL, Neuhausen S, Ng PS, Nguyen-Dumont T, Nielsen SM, Obermair F, Offit K, Olopade OI, Ottini L, Penkert J, Pylkäs K, Radice P, Ramus SJ, Rudaitis V, Side L, Silva-Smith R, Silvestri V, Skytte AB, Slavin T, Soukupova J, Tondini C, Trainer AH, Unzeitig G, Usha L, van Overeem Hansen T, Whitworth J, Wood M, Yip CH, Yoon SY, Yussuf A, Zogopoulos G, Goldgar D, Hopper JL, Chenevix-Trench G, Pharoah P, George SHL, Balmaña J, Houdayer C, James P, El-Haffaf Z, Ehrencrona H, Janatova M, Peterlongo P, Nevanlinna H, Schmutzler R, Teo SH, Robson M, Pal T, Couch F, Weitzel JN, Elliott A, Southey M, Winqvist R, Easton DF, Foulkes WD, Antoniou AC, Tischkowitz M. Cancer Risks Associated With Germline PALB2 Pathogenic Variants: An International Study of 524 Families. J Clin Oncol. 2020 Mar;38(7):674-685. doi:10.1200/JCO.19.01907.

13. Scoccianti C, Lauby-Secretan B, Bello PY, Chajes V, Romieu I. Female breast cancer and alcohol consumption: a review of the literature. Am J Prev Med. 2014 Mar;46(3 Suppl 1):S16-25. doi:10.1016/j.amepre.2013.10.031.

14. Chen WY, Rosner B, Hankinson SE, Colditz GA, Willett WC. Moderate alcohol consumption during adult life, drinking patterns, and breast cancer risk. JAMA. 2011 Nov 2;306(17):1884-90. doi:10.1001/jama.2011.1590.

15. Rock CL, Thomson C, Gansler T, Gapstur SM, McCullough ML, Patel AV, Andrews KS, Bandera EV, Spees CK, Robien K, Hartman S, Sullivan K, Grant BL, Hamilton KK, Kushi LH, Caan BJ, Kibbe D, Black JD, Wiedt TL, McMahon C, Sloan K, Doyle C. American Cancer Society guideline for diet and physical activity for cancer prevention. CA Cancer J Clin. 2020 Jul;70(4):245-271. doi:10.3322/caac.21591.

16. Bhushan A, Gonsalves A, Menon JU. Current State of Breast Cancer Diagnosis, Treatment, and Theranostics. Pharmaceutics. 2021 May;13(5):723. doi:10.3390/pharmaceutics13050723.

17. Matsen CB, Neumayer LA. Breast cancer: a review for the general surgeon. JAMA Surg. 2013 Oct;148(10):971-9. doi:10.1001/jamasurg.2013.3393.

18. Rahman GA. Breast Conserving Therapy: A surgical Technique where Little can Mean More. J Surg Tech Case Rep. 2011 Jan;3(1):1-4. doi:10.4103/2006-8808.78459.

19. Corradini S, Reitz D, Pazos M, Schönecker S, Braun M, Harbeck N, Matuschek C, Bölke E, Ganswindt U, Alongi F, Niyazi M, Belka C. Mastectomy or Breast-Conserving Therapy for Early Breast Cancer in Real-Life Clinical Practice: Outcome Comparison of 7565 Cases. Cancers (Basel). 2019 Jan 31;11(2):160. doi:10.3390/cancers11020160.

20. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, Cutter D, Davies C, Ewertz M, Godwin J, Gray R, Pierce L, Whelan T, Wang Y, Peto R. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet. 2011 Nov 12;378(9804):1707-16. doi:10.1016/S0140-6736(11)61629-2.

21. Poleszczuk J, Luddy K, Chen L, Lee JK, Harrison LB, Czerniecki BJ, Soliman H, Enderling H. Neoadjuvant radiotherapy of early-stage breast cancer and long-term disease-free survival. Breast Cancer Res. 2017 Jun 30;19(1):75. doi:10.1186/s13058-017-0870-1.

22. Roth SL, Audretsch W, Bojar H, Lang I, Willers R, Budach W. Retrospective study of neoadjuvant versus adjuvant radiochemotherapy in locally advanced noninflammatory breast cancer: survival advantage in cT2 category by neoadjuvant radiochemotherapy. Strahlenther Onkol. 2010 Jun;186(6):299-306. doi:10.1007/s00066-010-2143-0.

23. Anampa J, Makower D, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: an overview. BMC Med. 2015 Aug 17;13:195. doi:10.1186/s12916-015-0439-8.

24. AC-PACL(DD) Patient Information – Cancer Care Ontario. Available at: https://www.cancercareontario.ca/en/drugformulary/regimens/regimen-info/ac-pacl-dd-patient-info

25. Altena R, Perik PJ, van Veldhuisen DJ, de Vries EG, Gietema JA. Cardiovascular toxicity caused by cancer treatment: strategies for early detection. Lancet Oncol. 2009 Apr;10(4):391-9. doi:10.1016/S1470-2045(09)70042-7.

26. Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Engl J Med. 1998 Sep 24;339(13):900-5. doi:10.1056/NEJM199809243391307.

27. den Hollander P, Savage MI, Brown PH. Targeted therapy for breast cancer prevention. Front Oncol. 2013 Sep 23;3:250. doi:10.3389/fonc.2013.00250.

28. American Cancer Society. Breast Cancer Hormone Receptor Status. Available at: https://www.cancer.org/cancer/types/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-hormone-receptor-status.html

29. Wardell SE, Norris JD, McDonnell DP. Targeting mutant estrogen receptors. Elife. 2019 Jan 16;8:e44181. doi:10.7554/eLife.44181.

30. Meisel JL, Venur VA, Gnant M, Carey L. Evolution of Targeted Therapy in Breast Cancer: Where Precision Medicine Began. Am Soc Clin Oncol Educ Book. 2018 May 23;38:78-86. doi:10.1200/EDBK_201037.

31. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001 Mar 15;344(11):783-92. doi:10.1056/NEJM200103153441101.

32. Plosker GL, Keam SJ. Spotlight on Trastuzumab in the management of HER2-positive metastatic and early-stage breast cancer. BioDrugs. 2006;20(4):259-62. doi:10.2165/00063030-200620040-00007.

33. Plosker GL, Keam SJ. Trastuzumab: a review of its use in the management of HER2-positive metastatic and early-stage breast cancer. Drugs. 2006;66(4):449-75. doi:10.2165/00003495-200666040-00005.

34. Vogel C, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ; Genentech H0650 study investigators. First-line, single-agent Herceptin(trastuzumab) in metastatic breast cancer: a preliminary report. Eur J Cancer. 2001 Jan;37 Suppl 1:S25-9. doi: 10.1016/S0959-8049(00)00405-6

35. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005 Oct 20;353(16):1673-84. doi:10.1056/NEJMoa052122.

36. Baselga J, Carbonell X, Castañeda-Soto NJ, Clemens M, Green M, Harvey V, Morales S, Barton C, Ghahramani P. Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. J Clin Oncol. 2005 Apr 1;23(10):2162-71. doi:10.1200/JCO.2005.01.014.

37. Perez EA, Suman VJ, Davidson NE, Sledge GW, Kaufman PA, Hudis CA, Martino S, Gralow JR, Dakhil SR, Ingle JN, Winer EP, Gelmon KA, Gersh BJ, Jaffe AS, Rodeheffer RJ. Cardiac safety analysis of doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab in the North Central Cancer Treatment Group N9831 adjuvant breast cancer trial. J Clin Oncol. 2008 Mar 10;26(8):1231-8. doi:10.1200/JCO.2007.13.5467.

38. Suter TM, Procter M, van Veldhuisen DJ, Muscholl M, Bergh J, Carlomagno C, Perren T, Passalacqua R, Bighin C, Klijn JG, Ageev FT, Hitre E, Groetz J, Iwata H, Knap M, Gnant M, Muehlbauer S, Spence A, Gelber RD, Piccart-Gebhart MJ. Trastuzumab-associated cardiac adverse effects in the herceptin adjuvant trial. J Clin Oncol. 2007 Sep 1;25(25):3859-65. doi:10.1200/JCO.2006.09.1611.

39. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Láng I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Rüschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD; Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005 Oct 20;353(16):1659-72. doi:10.1056/NEJMoa052306.

40. Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro G Jr, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, McFadden E, Leyland-Jones B, Bell R, Dowsett M, Jackisch C; Herceptin Adjuvant (HERA) Trial Study Team. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. Lancet. 2017 Mar 25;389(10075):1195-1205. doi:10.1016/S0140-6736(16)32616-2.

41. Pogue-Geile KL, Kim C, Jeong JH, Tanaka N, Bandos H, Gavin PG, Fumagalli D, Goldstein LC, Sneige N, Burandt E, Taniyama Y, Bohn OL, Lee A, Kim SI, Reilly ML, Remillard MY, Blackmon NL, Kim SR, Horne ZD, Rastogi P, Fehrenbacher L, Romond EH, Swain SM, Mamounas EP, Wickerham DL, Geyer CE Jr, Costantino JP, Wolmark N, Paik S. Predicting degree of benefit from adjuvant trastuzumab in NSABP trial B-31. J Natl Cancer Inst. 2013 Dec 4;105(23):1782-8. doi:10.1093/jnci/djt321.

42. Baselga J, Perez EA, Pienkowski T, Bell R. Adjuvant trastuzumab: a milestone in the treatment of HER-2-positive early breast cancer. Oncologist. 2006;11 Suppl 1:4-12. doi:10.1634/theoncologist.11-90001-4.

43. Perez EA, Suman VJ, Davidson NE, Martino S, Kaufman PA, Lingle WL, Flynn PJ, Ingle JN, Visscher D, Jenkins RB. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol. 2006 Jul 1;24(19):3032-8. doi:10.1200/JCO.2005.03.4744.

44. Nabholtz JM, Reese DM, Lindsay MA, Riva A. HER2-positive breast cancer: update on Breast Cancer International Research Group trials. Clin Breast Cancer. 2002 Oct;3 Suppl 2:S75-9. doi:10.3816/cbc.2002.s.016.

45. Gianni L, Dafni U, Gelber RD, Azambuja E, Muehlbauer S, Goldhirsch A, Untch M, Smith I, Baselga J, Jackisch C, Cameron D, Mano M, Pedrini JL, Veronesi A, Mendiola C, Pluzanska A, Semiglazov V, Vrdoljak E, Eckart MJ, Shen Z, Skiadopoulos G, Procter M, Pritchard KI, Piccart-Gebhart MJ, Bell R; Herceptin Adjuvant (HERA) Trial Study Team. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol. 2011 Mar;12(3):236-44. doi:10.1016/S1470-2045(11)70033-X.

46. Balduzzi S, Mantarro S, Guarneri V, Tagliabue L, Pistotti V, Moja L, D'Amico R. Trastuzumab-containing regimens for metastatic breast cancer. Cochrane Database Syst Rev. 2014 Jun 12;2014(6):CD006242. doi:10.1002/14651858.CD006242.pub2.

47. British Columbia Cancer. Metastatic Breast Cancer. Available at: http://www.bccancer.bc.ca/books/breast/management/metastatic-breast-cancer

48. Cleveland Clinic. HER2-Positive Breast Cancer. Available at: https://my.clevelandclinic.org/health/diseases/25213-her2-positive-breast-cancer

49. Anampa J, Sparano JA. New agents for the management of resistant metastatic breast cancer. Expert Opin Pharmacother. 2017 Dec;18(17):1815-1831. doi:10.1080/14656566.2017.1409206.

50. Olin JJ, Muss HB. New strategies for managing metastatic breast cancer. Oncology (Williston Park). 2000 May;14(5):629-41; discussion 642-4, 647-8.

51. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol. 2002 Feb 1;20(3):719-26. doi:10.1200/JCO.2002.20.3.719.

52. Mackey JR, Clemons M, Côté MA, Delgado D, Dent S, Paterson A, Provencher L, Sawyer MB, Verma S. Cardiac management during adjuvant trastuzumab therapy: recommendations of the Canadian Trastuzumab Working Group. Curr Oncol. 2008 Jan;15(1):24-35. doi:10.3747/co.2008.199.

53. McArthur HL, Chia S. Cardiotoxicity of trastuzumab in clinical practice. N Engl J Med. 2007 Jul 5;357(1):94-5. doi:10.1056/NEJMc070065.

54. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, Ganame J, Sebag IA, Agler DA, Badano LP, Banchs J, Cardinale D, Carver J, Cerqueira M, DeCara JM, Edvardsen T, Flamm SD, Force T, Griffin BP, Jerusalem G, Liu JE, Magalhães A, Marwick T, Sanchez LY, Sicari R, Villarraga HR, Lancellotti P. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2014 Oct;15(10):1063-93. doi:10.1093/ehjci/jeu192.

55. Simões R, Silva LM, Cruz ALVM, Fraga VG, de Paula Sabino A, Gomes KB. Troponin as a cardiotoxicity marker in breast cancer patients receiving anthracycline-based chemotherapy: A narrative review. Biomed Pharmacother. 2018 Nov;107:989-996. doi:10.1016/j.biopha.2018.08.035.

56. Xiao H, Wang X, Li S, Liu Y, Cui Y, Deng X. Advances in Biomarkers for Detecting Early Cancer Treatment-Related Cardiac Dysfunction. Front Cardiovasc Med. 2021 Nov 10;8:753313. doi: 10.3389/fcvm.2021.753313.

57. Hamo CE, Bloom MW, Cardinale D, Ky B, Nohria A, Baer L, Skopicki H, Lenihan DJ, Gheorghiade M, Lyon AR, Butler J. Cancer Therapy-Related Cardiac Dysfunction and Heart Failure: Part 2: Prevention, Treatment, Guidelines, and Future Directions. Circ Heart Fail. 2016 Feb;9(2):e002843. doi:10.1161/CIRCHEARTFAILURE.115.002843.

58. Cancer Care Ontario. Doxorubicin. Available at: https://www.cancercareontario.ca/en/system/files_force/doxorubicin.pdf

59. Cancer Care Ontario. Epirubicin. Available at: https://www.cancercareontario.ca/en/system/files_force/epirubicin.pdf

60. Abd El-Aziz TA, Mohamed RH, Pasha HF, Abdel-Aziz HR. Catechin protects against oxidative stress and inflammatory-mediated cardiotoxicity in adriamycin-treated rats. Clin Exp Med. 2012 Dec;12(4):233-40. doi:10.1007/s10238-011-0165-2.

61. Bozkurt B, Kribbs SB, Clubb FJ Jr, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation. 1998 Apr 14;97(14):1382-91. doi:10.1161/01.cir.97.14.1382.

62. Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK. Protection against acute adriamycin-induced cardiotoxicity by garlic: role of endogenous antioxidants and inhibition of TNF-alpha expression. BMC Pharmacol. 2003 Dec 20;3:16. doi:10.1186/1471-2210-3-16.

63. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 1998;16:225-60. doi:10.1146/annurev.immunol.16.1.225.

64. Kaur K, Sharma AK, Singal PK. Significance of changes in TNF-alpha and IL-10 levels in the progression of heart failure subsequent to myocardial infarction. Am J Physiol Heart Circ Physiol. 2006 Jul;291(1):H106-13. doi:10.1152/ajpheart.01327.2005.

65. Bews HJ, Mackic L, Jassal DS. Preventing broken hearts in women with breast cancer: a concise review on chemotherapy-mediated cardiotoxicity. Can J Physiol Pharmacol. 2024 Sep 1;102(9):487-497. doi:10.1139/cjpp-2023-0358.

66. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, Cohen V, Banchs J, Carver JR, Wiegers SE, Martin RP, Picard MH, Gerszten RE, Halpern EF, Passeri J, Kuter I, Scherrer-Crosbie M. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. Circ Cardiovasc Imaging. 2012 Sep 1;5(5):596-603. doi:10.1161/CIRCIMAGING.112.973321.

67. Matsuzawa A, Nishitoh H, Tobiume K, Takeda K, Ichijo H. Physiological roles of ASK1-mediated signal transduction in oxidative stress- and endoplasmic reticulum stress-induced apoptosis: advanced findings from ASK1 knockout mice. Antioxid Redox Signal. 2002 Jun;4(3):415-25. doi:10.1089/15230860260196218.

68. Malik A, Bagchi AK, Jassal DS, Singal PK. Doxorubicin-induced cardiomyopathy is mitigated by empagliflozin via the modulation of endoplasmic reticulum stress pathways. Mol Med Rep. 2024 May;29(5):74. doi:10.3892/mmr.2024.13198.

69. Tong Q, Wu L, Jiang T, Ou Z, Zhang Y, Zhu D. Inhibition of endoplasmic reticulum stress-activated IRE1α-TRAF2-caspase-12 apoptotic pathway is involved in the neuroprotective effects of telmisartan in the rotenone rat model of Parkinson's disease. Eur J Pharmacol. 2016;776:106-115. doi:10.1016/j.ejphar.2016.02.042.

70. Bagchi AK, Malik A, Akolkar G, Zimmer A, Belló-Klein A, De Angelis K, Jassal DS, Fini MA, Stenmark KR, Singal PK. Study of ER stress and apoptotic proteins in the heart and tumor exposed to doxorubicin. Biochim Biophys Acta Mol Cell Res. 2021;1868(7):119039. doi:10.1016/j.bbamcr.2021.119039.

71. Belmadani S, Matrougui K. Broken heart: A matter of the endoplasmic reticulum stress bad management? World J Cardiol. 2019 Jun 26;11(6):159-170. doi:10.4330/wjc.v11.i6.159.

72. Singal PK, Khaper N, Palace V, Kumar D. The role of oxidative stress in the genesis of heart disease. Cardiovasc Res. 1998 Dec;40(3):426-32. doi:10.1016/s0008-6363(98)00244-2.

73. Kirshenbaum LA, Thomas TP, Randhawa AK, Singal PK. Time-course of cardiac myocyte injury due to oxidative stress. Mol Cell Biochem. 1992 Apr;111(1-2):25-31. doi:10.1007/BF00229570.

74. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science. 1997 Jan 3;275(5296):90-4. doi:10.1126/science.275.5296.90.

75. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001 Apr;22(2):153-83. doi:10.1210/edrv.22.2.0428.

76. Habiro A, Tanno S, Koizumi K, Izawa T, Nakano Y, Osanai M, Mizukami Y, Okumura T, Kohgo Y. Involvement of p38 mitogen-activated protein kinase in gemcitabine-induced apoptosis in human pancreatic cancer cells. Biochem Biophys Res Commun. 2004 Mar 26;316(1):71-7. doi:10.1016/j.bbrc.2004.02.017.

77. Lou H, Danelisen I, Singal PK. Involvement of mitogen-activated protein kinases in adriamycin-induced cardiomyopathy. Am J Physiol Heart Circ Physiol. 2005 Apr;288(4):H1925-30. doi:10.1152/ajpheart.01054.2004.

78. Cuenda A, Rousseau S. p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim Biophys Acta. 2007 Aug;1773(8):1358-75. doi:10.1016/j.bbamcr.2007.03.010.

79. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev. 2004 Jun;56(2):185-229. doi:10.1124/pr.56.2.6.

80. Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol. 1999 Apr 1;57(7):727-41. doi:10.1016/s0006-2952(98)00307-4.

81. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer. 2003 Jun 1;97(11):2869-79. doi:10.1002/cncr.11407.

82. Kirkham AA, Pituskin E, Thompson RB, Mackey JR, Koshman SL, Jassal D, Pitz M, Haykowsky MJ, Pagano JJ, Chow K, Tsui AK, Ezekowitz JA, Oudit GY, Paterson DI. Cardiac and cardiometabolic phenotyping of trastuzumab-mediated cardiotoxicity: a secondary analysis of the MANTICORE trial. Eur Heart J Cardiovasc Pharmacother. 2022 Feb 16;8(2):130-139. doi:10.1093/ehjcvp/pvab016.

83. Guglin M, Hartlage G, Reynolds C, Chen R, Patel V. Trastuzumab-induced cardiomyopathy: not as benign as it looks? A retrospective study. J Card Fail. 2009 Oct;15(8):651-7. doi:10.1016/j.cardfail.2009.04.011.

84. Wadhwa D, Fallah-Rad N, Grenier D, Krahn M, Fang T, Ahmadie R, Walker JR, Lister D, Arora RC, Barac I, Morris A, Jassal DS. Trastuzumab mediated cardiotoxicity in the setting of adjuvant chemotherapy for breast cancer: a retrospective study. Breast Cancer Res Treat. 2009 Sep;117(2):357-64. doi:10.1007/s10549-008-0260-6.

85. Zheng H, Mahmood SS, Khalique OK, Zhan H. Trastuzumab-Induced Cardiotoxicity: When and How Much Should We Worry? JCO Oncol Pract. 2024 Aug;20(8):1055-1063. doi:10.1200/OP.23.00816.

86. Pituskin E, Mackey JR, Koshman S, Jassal D, Pitz M, Haykowsky MJ, Pagano JJ, Chow K, Thompson RB, Vos LJ, Ghosh S, Oudit GY, Ezekowitz JA, Paterson DI. Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research (MANTICORE 101-Breast): A Randomized Trial for the Prevention of Trastuzumab-Associated Cardiotoxicity. J Clin Oncol. 2017 Mar 10;35(8):870-877. doi:10.1200/JCO.2016.68.7830.

87. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 1999 Aug 1;13(15):1899-911. doi:10.1101/gad.13.15.1899.

88. Grazette LP, Boecker W, Matsui T, Semigran M, Force TL, Hajjar RJ, Rosenzweig A. Inhibition of ErbB2 causes mitochondrial dysfunction in cardiomyocytes: implications for herceptin-induced cardiomyopathy. J Am Coll Cardiol. 2004 Dec 7;44(11):2231-8. doi:10.1016/j.jacc.2004.08.066.

89. Sandoo A, Kitas GD, Carmichael AR. Breast cancer therapy and cardiovascular risk: focus on trastuzumab. Vasc Health Risk Manag. 2015 Apr 7;11:223-8. doi:10.2147/VHRM.S69641.

90. Konecny GE. Emerging strategies for the dual inhibition of HER2-positive breast cancer. Curr Opin Obstet Gynecol. 2013 Feb;25(1):55-65. doi:10.1097/GCO.0b013e32835c5e90.

91. Mitri Z, Constantine T, O'Regan R. The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. Chemother Res Pract. 2012;2012:743193. doi:10.1155/2012/743193.

92. Nakagami H, Takemoto M, Liao JK. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced cardiac hypertrophy. J Mol Cell Cardiol. 2003 Jul;35(7):851-9. doi:10.1016/s0022-2828(03)00145-7.

93. Zhao W, Ahokas RA, Weber KT, Sun Y. ANG II-induced cardiac molecular and cellular events: role of aldosterone. Am J Physiol Heart Circ Physiol. 2006 Jul;291(1):H336-43. doi:10.1152/ajpheart.01307.2005.

94. Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Lett. 2008 Jul 18;266(1):37-52. doi:10.1016/j.canlet.2008.02.044.

95. Touyz RM. Reactive oxygen species and angiotensin II signaling in vascular cells -- implications in cardiovascular disease. Braz J Med Biol Res. 2004 Aug;37(8):1263-73. doi:10.1590/s0100-879x2004000800018.

96. Hao J, Wang B, Jones SC, Jassal DS, Dixon IM. Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and in vitro. Am J Physiol Heart Circ Physiol. 2000 Dec;279(6):H3020-30. doi:10.1152/ajpheart.2000.279.6.H3020.

97. Dhingra S, Sharma AK, Arora RC, Slezak J, Singal PK. IL-10 attenuates TNF-alpha-induced NF kappaB pathway activation and cardiomyocyte apoptosis. Cardiovasc Res. 2009 Apr 1;82(1):59-66. doi:10.1093/cvr/cvp040.

98. Lemarié CA, Paradis P, Schiffrin EL. New insights on signaling cascades induced by cross-talk between angiotensin II and aldosterone. J Mol Med (Berl). 2008 Jun;86(6):673-8. doi:10.1007/s00109-008-0323-5.

99. Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney DW Jr, Leahy DJ. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature. 2003 Feb 13;421(6924):756-60. doi:10.1038/nature01392.

100. Zhang Y, Opresko L, Shankaran H, Chrisler WB, Wiley HS, Resat H. HER/ErbB receptor interactions and signaling patterns in human mammary epithelial cells. BMC Cell Biol. 2009 Oct 31;10:78. doi:10.1186/1471-2121-10-78.

101. Burstein HJ, Keshaviah A, Baron AD, Hart RD, Lambert-Falls R, Marcom PK, Gelman R, Winer EP. Trastuzumab plus vinorelbine or taxane chemotherapy for HER2-overexpressing metastatic breast cancer: the trastuzumab and vinorelbine or taxane study. Cancer. 2007 Sep 1;110(5):965-72. doi:10.1002/cncr.22885.

102. Gordon LI, Burke MA, Singh AT, Prachand S, Lieberman ED, Sun L, Naik TJ, Prasad SV, Ardehali H. Blockade of the erbB2 receptor induces cardiomyocyte death through

mitochondrial and reactive oxygen species-dependent pathways. J Biol Chem. 2009 Jan 23;284(4):2080-7. doi:10.1074/jbc.M804570200.

103. Rochette L, Guenancia C, Gudjoncik A, Hachet O, Zeller M, Cottin Y, Vergely C. Anthracyclines/trastuzumab: new aspects of cardiotoxicity and molecular mechanisms. Trends Pharmacol Sci. 2015 Jun;36(6):326-48. doi:10.1016/j.tips.2015.03.005.

104. Arpino G, Gutierrez C, Weiss H, Rimawi M, Massarweh S, Bharwani L, De Placido S, Osborne CK, Schiff R. Treatment of human epidermal growth factor receptor 2-overexpressing breast cancer xenografts with multiagent HER-targeted therapy. J Natl Cancer Inst. 2007 May 2;99(9):694-705. doi:10.1093/jnci/djk151.

105. Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y, Peterson KL, Chen J, Kahn R, Condorelli G, Ross J Jr, Chien KR, Lee KF. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med. 2002 May;8(5):459-65. doi:10.1038/nm0502-459.

106. Pentassuglia L, Sawyer DB. The role of Neuregulin-1beta/ErbB signaling in the heart. Exp Cell Res. 2009 Feb 15;315(4):627-37. doi:10.1016/j.yexcr.2008.08.015.

107. Kuramochi Y, Guo X, Sawyer DB. Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes. J Mol Cell Cardiol. 2006 Aug;41(2):228-35. doi:10.1016/j.yjmcc.2006.04.007.

108. Sobczuk P, Czerwińska M, Kleibert M, Cudnoch-Jędrzejewska A. Anthracycline-induced cardiotoxicity and renin-angiotensin-aldosterone system-from molecular mechanisms to therapeutic applications. Heart Fail Rev. 2022 Jan;27(1):295-319. doi:10.1007/s10741-020-09977-1.

109. Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. Int J Cardiol Heart Vasc. 2016 Mar;10:17-24. doi:10.1016/j.ijcha.2015.11.004.

110. Jiji RS, Kramer CM, Salerno M. Non-invasive imaging and monitoring cardiotoxicity of cancer therapeutic drugs. J Nucl Cardiol. 2012 Apr;19(2):377-88. doi:10.1007/s12350-012-9512-2.

111. Teske AJ, Linschoten M, Kamphuis JAM, Naaktgeboren WR, Leiner T, van der Wall E, Kuball J, van Rhenen A, Doevendans PA, Cramer MJ, Asselbergs FW. Cardio-oncology: an overview on outpatient management and future developments. Neth Heart J. 2018 Nov;26(11):521-532. doi:10.1007/s12471-018-1148-7.

112. Armstrong GT, Plana JC, Zhang N, Srivastava D, Green DM, Ness KK, Daniel Donovan F, Metzger ML, Arevalo A, Durand JB, Joshi V, Hudson MM, Robison LL, Flamm SD. Screening adult survivors of childhood cancer for cardiomyopathy: comparison of echocardiography and cardiac magnetic resonance imaging. J Clin Oncol. 2012 Aug 10;30(23):2876-84. doi:10.1200/JCO.2011.40.3584.

113. Thavendiranathan P, Wintersperger BJ, Flamm SD, Marwick TH. Cardiac MRI in the assessment of cardiac injury and toxicity from cancer chemotherapy: a systematic review. Circ Cardiovasc Imaging. 2013 Nov;6(6):1080-91. doi:10.1161/CIRCIMAGING.113.000899.

114. Cheitlin MD, Armstrong WF, Aurigemma GP, Beller GA, Bierman FZ, Davis JL, Douglas PS, Faxon DP, Gillam LD, Kimball TR, Kussmaul WG, Pearlman AS, Philbrick JT, Rakowski H, Thys DM, Antman EM, Smith SC Jr, Alpert JS, Gregoratos G, Anderson JL, Hiratzka LF, Hunt SA, Fuster V, Jacobs AK, Gibbons RJ, Russell RO; American College of Cardiology; American Heart Association; American Society of Echocardiography. ACC/AHA/ASE 2003 guideline update for the clinical application of echocardiography: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for the Clinical Application of Echocardiography). Circulation. 2003 Sep 2;108(9):1146-62. doi:10.1161/01.CIR.0000073597.57414.A9.

115. Thavendiranathan P, Grant AD, Negishi T, Plana JC, Popović ZB, Marwick TH. Reproducibility of echocardiographic techniques for sequential assessment of left ventricular ejection fraction and volumes: application to patients undergoing cancer chemotherapy. J Am Coll Cardiol. 2013 Jan 8;61(1):77-84. doi:10.1016/j.jacc.2012.09.035.

116. Sławiński G, Hawryszko M, Liżewska-Springer A, Nabiałek-Trojanowska I, Lewicka E. Global Longitudinal Strain in Cardio-Oncology: A Review. Cancers (Basel). 2023 Feb 3;15(3):986. doi:10.3390/cancers15030986.

117. Yang H, Wright L, Negishi T, Negishi K, Liu J, Marwick TH. Research to Practice: Assessment of Left Ventricular Global Longitudinal Strain for Surveillance of Cancer Chemotherapeutic-Related Cardiac Dysfunction. JACC Cardiovasc Imaging. 2018 Aug;11(8):1196-1201. doi:10.1016/j.jcmg.2018.07.005.

118. Perez IE, Taveras Alam S, Hernandez GA, Sancassani R. Cancer Therapy-Related Cardiac Dysfunction: An Overview for the Clinician. Clin Med Insights Cardiol. 2019 Jul 29;13:1179546819866445. doi:10.1177/1179546819866445.

119. Virani SA, Dent S, Brezden-Masley C, Clarke B, Davis MK, Jassal DS, Johnson C, Lemieux J, Paterson I, Sebag IA, Simmons C, Sulpher J, Thain K, Thavendiranathan P, Wentzell JR, Wurtele N, Côté MA, Fine NM, Haddad H, Hayley BD, Hopkins S, Joy AA, Rayson D, Stadnick E, Straatman L. Canadian Cardiovascular Society Guidelines for Evaluation and Management of Cardiovascular Complications of Cancer Therapy. Can J Cardiol. 2016 Jul;32(7):831-41. doi:10.1016/j.cjca.2016.02.078.

120. Saeed MF, Premecz S, Goyal V, Singal PK, Jassal DS. Catching broken hearts: pre-clinical detection of doxorubicin and trastuzumab mediated cardiac dysfunction in the breast cancer setting. Can J Physiol Pharmacol. 2014 Jul;92(7):546-50. doi:10.1139/cjpp-2013-0470.

121. Fallah-Rad N, Walker JR, Wassef A, Lytwyn M, Bohonis S, Fang T, Tian G, Kirkpatrick ID, Singal PK, Krahn M, Grenier D, Jassal DS. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early

left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. J Am Coll Cardiol. 2011 May 31;57(22):2263-70. doi:10.1016/j.jacc.2010.11.063.

122. Cardinale D, Biasillo G, Salvatici M, Sandri MT, Cipolla CM. Using biomarkers to predict and to prevent cardiotoxicity of cancer therapy. Expert Rev Mol Diagn. 2017 Mar;17(3):245-256. doi:10.1080/14737159.2017.1283219.

123. Kilickap S, Barista I, Akgul E, Aytemir K, Aksoyek S, Aksoy S, Celik I, Kes S, Tekuzman G. cTnT can be a useful marker for early detection of anthracycline cardiotoxicity. Ann Oncol. 2005 May;16(5):798-804. doi:10.1093/annonc/mdi152.

124. Herman EH, Zhang J, Lipshultz SE, Rifai N, Chadwick D, Takeda K, Yu ZX, Ferrans VJ. Correlation between serum levels of cardiac troponin-T and the severity of the chronic cardiomyopathy induced by doxorubicin. J Clin Oncol. 1999 Jul;17(7):2237-43. doi:10.1200/JCO.1999.17.7.2237.

125. Adamcová M, Gersl V, Hrdina R, Melka M, Mazurová Y, Vávrová J, Palicka V, Kokstein Z. Cardiac troponin T as a marker of myocardial damage caused by antineoplastic drugs in rabbits. J Cancer Res Clin Oncol. 1999;125(5):268-74. doi:10.1007/s004320050273.

126. Auner HW, Tinchon C, Linkesch W, Tiran A, Quehenberger F, Link H, Sill H. Prolonged monitoring of troponin T for the detection of anthracycline cardiotoxicity in adults with hematological malignancies. Ann Hematol. 2003 Apr;82(4):218-22. doi:10.1007/s00277-003-0615-3.

127. Herman EH, Lipshultz SE, Rifai N, Zhang J, Papoian T, Yu ZX, Takeda K, Ferrans VJ. Use of cardiac troponin T levels as an indicator of doxorubicin-induced cardiotoxicity. Cancer Res. 1998 Jan 15;58(2):195-7.

128. Cardinale D, Colombo A, Torrisi R, Sandri MT, Civelli M, Salvatici M, Lamantia G, Colombo N, Cortinovis S, Dessanai MA, Nolè F, Veglia F, Cipolla CM. Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. J Clin Oncol. 2010 Sep 1;28(25):3910-6. doi:10.1200/JCO.2009.27.3615.

129. Arnold JM, Liu P, Demers C, Dorian P, Giannetti N, Haddad H, Heckman GA, Howlett JG, Ignaszewski A, Johnstone DE, Jong P, McKelvie RS, Moe GW, Parker JD, Rao V, Ross HJ, Sequeira EJ, Svendsen AM, Teo K, Tsuyuki RT, White M; Canadian Cardiovascular Society. Canadian Cardiovascular Society consensus conference recommendations on heart failure 2006: diagnosis and management. Can J Cardiol. 2006 Jan;22(1):23-45. doi: 10.1016/s0828-282x(06)70237-9. Erratum in: Can J Cardiol. 2006 Mar 1;22(3):271.

130. Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, Colan SD, Asselin BL, Barr RD, Clavell LA, Hurwitz CA, Moghrabi A, Samson Y, Schorin MA, Gelber RD, Sallan SE. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. N Engl J Med. 2004 Jul 8;351(2):145-53. doi:10.1056/NEJMoa035153.

131. Riddell E, Lenihan D. The role of cardiac biomarkers in cardio-oncology. Curr Probl Cancer. 2018 Jul;42(4):375-385. doi:10.1016/j.currproblcancer.2018.06.012.

132. Suzuki T, Hayashi D, Yamazaki T, Mizuno T, Kanda Y, Komuro I, Kurabayashi M, Yamaoki K, Mitani K, Hirai H, Nagai R, Yazaki Y. Elevated B-type natriuretic peptide levels after anthracycline administration. Am Heart J. 1998 Aug;136(2):362-3. doi:10.1053/hj.1998.v136.89908.

133. Sandri MT, Salvatici M, Cardinale D, Zorzino L, Passerini R, Lentati P, Leon M, Civelli M, Martinelli G, Cipolla CM. N-terminal pro-B-type natriuretic peptide after high-dose chemotherapy: a marker predictive of cardiac dysfunction? Clin Chem. 2005 Aug;51(8):1405-10. doi:10.1373/clinchem.2005.050153.

134. Skovgaard D, Hasbak P, Kjaer A. BNP predicts chemotherapy-related cardiotoxicity and death: comparison with gated equilibrium radionuclide ventriculography. PLoS One. 2014 May 6;9(5):e96736. doi:10.1371/journal.pone.0096736.

135. Gulati G, Heck SL, Røsjø H, Ree AH, Hoffmann P, Hagve TA, Norseth J, Gravdehaug B, Steine K, Geisler J, Omland T. Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results From the PRADA (Prevention of Cardiac Dysfunction During Adjuvant Breast Cancer Therapy) Study. J Am Heart Assoc. 2017 Nov 8;6(11):e006513. doi:10.1161/JAHA.117.006513.

136. Onitilo AA, Engel JM, Stankowski RV, Liang H, Berg RL, Doi SA. 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 Jul;134(1):291-8. doi:10.1007/s10549-012-2039-z.

137. Akolkar G, da Silva Dias D, Ayyappan P, Bagchi AK, Jassal DS, Salemi VMC, Irigoyen MC, De Angelis K, Singal PK. Vitamin C mitigates oxidative/nitrosative stress and inflammation in doxorubicin-induced cardiomyopathy. Am J Physiol Heart Circ Physiol. 2017 Oct 1;313(4):H795-H809. doi:10.1152/ajpheart.00253.2017.

138. Kalantary-Charvadeh A, Sanajou D, Hemmati-Dinarvand M, Marandi Y, Khojastehfard M, Hajipour H, Mesgari-Abbasi M, Roshangar L, Nazari Soltan Ahmad S. Micheliolide Protects Against Doxorubicin-Induced Cardiotoxicity in Mice by Regulating PI3K/Akt/NF-kB Signaling Pathway. Cardiovasc Toxicol. 2019 Aug;19(4):297-305. doi:10.1007/s12012-019-09511-2.

139. Li J, Wu Y, Wang D, Zou L, Fu C, Zhang J, Leung GP. Oridonin synergistically enhances the anti-tumor efficacy of doxorubicin against aggressive breast cancer via pro-apoptotic and anti-angiogenic effects. Pharmacol Res. 2019 Aug;146:104313. doi:10.1016/j.phrs.2019.104313.

140. Walker JR, Sharma A, Lytwyn M, Bohonis S, Thliveris J, Singal PK, Jassal DS. The cardioprotective role of probucol against anthracycline and trastuzumab-mediated cardiotoxicity. J Am Soc Echocardiogr. 2011 Jun;24(6):699-705. doi:10.1016/j.echo.2011.01.018.

141. Firoozbakhsh P, Ghaffarinejad Z, Arbabi M, Dokhani N, Alizadehasl A. Cardioprotective potential of botanical agents against anthracycline-induced cardiotoxicity. Phytomed Plus. 2024 Apr;4:100575. doi:10.1016/j.phyplu.2024.100575.

142. Ludke AR, Al-Shudiefat AA, Dhingra S, Jassal DS, Singal PK. A concise description of cardioprotective strategies in doxorubicin-induced cardiotoxicity. Can J Physiol Pharmacol. 2009 Oct;87(10):756-63. doi:10.1139/Y09-059.

143. Telles-Langdon SM, Arya V, Haasbeek PR, Cheung DYC, Eekhoudt CR, Mackic L, Bryson AN, Varghese SS, Austria JA, Thliveris JA, Aukema HM, Ravandi A, Singal PK, Jassal DS. Efficacy of Flaxseed Compared to ACE Inhibition in Treating Anthracycline- and Trastuzumab-Induced Cardiotoxicity. CJC Open. 2024 Mar 25;6(7):925-937. doi:10.1016/j.cjco.2024.03.009.

144. Block KI, Koch AC, Mead MN, Tothy PK, Newman RA, Gyllenhaal C. Impact of antioxidant supplementation on chemotherapeutic efficacy: a systematic review of the evidence from randomized controlled trials. Cancer Treat Rev. 2007 Aug;33(5):407-18. doi:10.1016/j.ctrv.2007.01.005. Epub 2007 Mar 23.

145. Henninger C, Huelsenbeck S, Wenzel P, Brand M, Huelsenbeck J, Schad A, Fritz G. Chronic heart damage following doxorubicin treatment is alleviated by lovastatin. Pharmacol Res. 2015 Jan;91:47-56. doi:10.1016/j.phrs.2014.11.003. Epub 2014 Nov 21.

146. Calvillo-Argüelles O, Abdel-Qadir H, Michalowska M, Billia F, Suntheralingam S, Amir E, Thavendiranathan P. Cardioprotective Effect of Statins in Patients With HER2-Positive Breast Cancer Receiving Trastuzumab Therapy. Can J Cardiol. 2019 Feb;35(2):153-159. doi:10.1016/j.cjca.2018.11.028.

147. Nabati M, Janbabai G, Esmailian J, Yazdani J. Effect of Rosuvastatin in Preventing Chemotherapy-Induced Cardiotoxicity in Women With Breast Cancer: A Randomized, Single-Blind, Placebo-Controlled Trial. J Cardiovasc Pharmacol Ther. 2019 May;24(3):233-241. doi:10.1177/1074248418821721. Epub 2019 Jan 2.

148. Telles-Langdon SM, Arya V, Jassal DS. Chapter 21: Renin angiotensin system (RAS): The common thread between cancer and heart failure. In: Bhullar SK, Tappia PS, Dhalla NS, eds. *The Renin Angiotensin System in Cancer, Lung, Liver and Infectious Diseases*. Advances in Biochemistry in Health and Disease (vol. 25): Springer; 2023:429-449. doi:10.1007/978-3-031-23621-1_21.

149. Akolkar G, Bhullar N, Bews H, Shaikh B, Premecz S, Bordun KA, Cheung DY, Goyal V, Sharma AK, Garber P, Singal PK, Jassal DS. The role of renin angiotensin system antagonists in the prevention of doxorubicin and trastuzumab induced cardiotoxicity. Cardiovasc Ultrasound. 2015 Apr 3;13:18. doi: 10.1186/s12947-015-0011-x.

150. Mentz RJ, Bakris GL, Waeber B, McMurray JJ, Gheorghiade M, Ruilope LM, Maggioni AP, Swedberg K, Piña IL, Fiuzat M, O'Connor CM, Zannad F, Pitt B. The past, present and future of renin-angiotensin aldosterone system inhibition. Int J Cardiol. 2013 Sep 1;167(5):1677-87. doi:10.1016/j.ijcard.2012.10.007.

151. Blaes AH, Gaillard P, Peterson BA, Yee D, Virnig B. Angiotensin converting enzyme inhibitors may be protective against cardiac complications following anthracycline chemotherapy. Breast Cancer Res Treat. 2010 Jul;122(2):585-90. doi:10.1007/s10549-009-0730-5.

152. Janbabai G, Nabati M, Faghihinia M, Azizi S, Borhani S, Yazdani J. Effect of Enalapril on Preventing Anthracycline-Induced Cardiomyopathy. Cardiovasc Toxicol. 2017 Apr;17(2):130-139. doi:10.1007/s12012-016-9365-z.

153. Bosch X, Rovira M, Sitges M, Domènech A, Ortiz-Pérez JT, de Caralt TM, Morales-Ruiz M, Perea RJ, Monzó M, Esteve J. Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hEmopathies). J Am Coll Cardiol. 2013 Jun 11;61(23):2355-62. doi:10.1016/j.jacc.2013.02.072.

154. Guglin M, Krischer J, Tamura R, Fink A, Bello-Matricaria L, McCaskill-Stevens W, Munster PN. Randomized Trial of Lisinopril Versus Carvedilol to Prevent Trastuzumab Cardiotoxicity in Patients With Breast Cancer. J Am Coll Cardiol. 2019 Jun 11;73(22):2859-2868. doi:10.1016/j.jacc.2019.03.495.

155. Heck SL, Gulati G, Hoffmann P, et al. Effect of candesartan and metoprolol on myocardial tissue composition during anthracycline treatment: The PRADA trial. Eur Heart J Cardiovasc Imaging. 2018;19(5):544-552. doi:10.1093/ehjci/jex159.

156. Dessì M, Madeddu C, Piras A, Cadeddu C, Antoni G, Mercuro G, Mantovani G. Long-term, up to 18 months, protective effects of the angiotensin II receptor blocker telmisartan on Epirubin-induced inflammation and oxidative stress assessed by serial strain rate. Springerplus. 2013 Apr 30;2(1):198. doi:10.1186/2193-1801-2-198.

157. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, Gravdehaug B, von Knobelsdorff-Brenkenhoff F, Bratland Å, Storås TH, Hagve TA, Røsjø H, Steine K, Geisler J, Omland T. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. Eur Heart J. 2016 Jun 1;37(21):1671-80. doi:10.1093/eurheartj/ehw022.

158. Avila MS, Ayub-Ferreira SM, de Barros Wanderley MR Jr, das Dores Cruz F, Gonçalves Brandão SM, Rigaud VOC, Higuchi-Dos-Santos MH, Hajjar LA, Kalil Filho R, Hoff PM, Sahade M, Ferrari MSM, de Paula Costa RL, Mano MS, Bittencourt Viana Cruz CB, Abduch MC, Lofrano Alves MS, Guimaraes GV, Issa VS, Bittencourt MS, Bocchi EA. Carvedilol for Prevention of Chemotherapy-Related Cardiotoxicity: The CECCY Trial. J Am Coll Cardiol. 2018 May 22;71(20):2281-2290. doi:10.1016/j.jacc.2018.02.049.

159. Lange-Chenier H. US approves “new class” of diabetes drug, under review in Canada. CMAJ. 2013;185(10):E470. doi:10.1503/cmaj.109-4483.

160. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. New England Journal of Medicine. 2015 Nov 26;373(22):2117–28. doi:10.1056/NEJMoa1504720.

161. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, Januzzi J, Verma S, Tsutsui H, Brueckmann M, Jamal W, Kimura K, Schnee J, Zeller C, Cotton D, Bocchi E, Böhm M, Choi DJ, Chopra V, Chuquiure E, Giannetti N, Janssens S, Zhang J, Gonzalez Juanatey JR, Kaul S, Brunner-La Rocca HP, Merkely B, Nicholls SJ, Perrone S, Pina I, Ponikowski P, Sattar N, Senni M, Seronde MF, Spinar J, Squire I, Taddei S, Wanner C, Zannad F. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. New England Journal of Medicine. 2020 Oct 8;383(15):1413–24. doi:10.1056/NEJMoa2022190.

162. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Rocca HPB, Choi DJ, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. New England Journal of Medicine. 2021 Oct 13;385(16):1451–61. doi:10.1056/NEJMoa2107038.

163. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Bělohlávek J, Böhm M, Chiang CE, Chopra VK, de Boer RA, Desai AS, Diez M, Drozdz J, Dukát A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjöstrand M, Langkilde AM, DAPA-HF Trial Committees and Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. N Engl J Med. 2019 Nov 21;381(21):1995–2008. doi:10.1056/NEJMoa1911303.

164. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR, CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017 Aug 17;377(7):644–57. doi:10.1056/NEJMoa1611925.

165. McDonald M, Virani S, Chan M, Ducharme A, Ezekowitz JA, Giannetti N, Heckman GA, Howlett JG, Koshman SL, Lepage S, Mielniczuk L, Moe GW, O'Meara E, Swiggum E, Toma M, Zieroth S, Anderson K, Bray SA, Clarke B, Cohen-Solal A, D'Astous M, Davis M, De S, Grant ADM, Grzeslo A, Heshka J, Keen S, Kouz S, Lee D, Masoudi FA, McKelvie R, Parent MC, Poon S, Rajda M, Sharma A, Siatecki K, Storm K, Sussex B, Van Spall H, Yip AMC. CCS/CHFS Heart Failure Guidelines Update: Defining a New Pharmacologic Standard of Care for Heart Failure With Reduced Ejection Fraction. Can J Cardiol. 2021 Apr;37(4):531–46. doi:10.1016/j.cjca.2021.01.017.

166. Dabour MS, George MY, Daniel MR, Blaes AH, Zordoky BN. The Cardioprotective and Anticancer Effects of SGLT2 Inhibitors. JACC: CardioOncology. 2024 Apr;6(2):159–82. doi:10.1016/j.jaccao.2024.01.007.

167. Omland T, Heck SL, Gulati G. The Role of Cardioprotection in Cancer Therapy Cardiotoxicity: JACC: CardioOncology State-of-the-Art Review. JACC CardioOncol. 2022 Mar 15;4(1):19-37. doi: 10.1016/j.jaccao.2022.01.101.

168. Li N, Zhou H. SGLT2 Inhibitors: A Novel Player in the Treatment and Prevention of Diabetic Cardiomyopathy. Drug Des Devel Ther. 2020 Nov 6;14:4775-4788. doi:10.2147/DDDT.S269514.

169. Sabatino J, De Rosa S, Tammè L, Iaconetti C, Sorrentino S, Polimeni A, Mignogna C, Amorosi A, Spaccarotella C, Yasuda M, Indolfi C. Empagliflozin prevents doxorubicin-induced myocardial dysfunction. Cardiovasc Diabetol. 2020;19(1):66. doi:10.1186/s12933-020-01040-5.

170. Oh CM, Cho S, Jang JY, Kim H, Chun S, Choi M, Park S, Ko YG. Cardioprotective Potential of an SGLT2 Inhibitor Against Doxorubicin-Induced Heart Failure. Korean Circ J. 2019 Dec;49(12):1183-1195. doi:10.4070/kcj.2019.0180.

171. Chang WT, Lin YW, Ho CH, Chen ZC, Liu PY, Shih JY. Dapagliflozin suppresses ER stress and protects doxorubicin-induced cardiotoxicity in breast cancer patients. Arch Toxicol. 2021 Feb;95(2):659-671. doi:10.1007/s00204-020-02951-8.

172. Quagliariello V, Coppola C, Rea D, Maurea C, Barbieri G, Botti G, Maurea N. Cardioprotective and anti-inflammatory effects of empagliflozin during treatment with Doxorubicin: a cellular and preclinical study. Annals of Oncology. 2019;30(5):v768. doi: 10.1093/annonc/mdz268.021.

173. Osataphan N, Abdel-Qadir H, Zebrowska AM, Borowiec A. Sodium-Glucose Cotransporter 2 Inhibitors During Cancer Therapy: Benefits, Risks, and Ongoing Clinical Trials. Curr Oncol Rep. 2024 Jul 11. doi:10.1007/s11912-024-01577-8.

174. Uthman L, Baartscheer A, Schumacher CA, Fiolet JWT, Kuschma MC, Hollmann MW, Coronel R, Weber NC, Zuurbier CJ. Direct Cardiac Actions of Sodium Glucose Cotransporter 2 Inhibitors Target Pathogenic Mechanisms Underlying Heart Failure in Diabetic Patients. Front Physiol. 2018;9:1575. doi:10.3389/fphys.2018.01575.

175. Lahnwong S, Chattipakorn SC, Chattipakorn N. Potential mechanisms responsible for cardioprotective effects of sodium-glucose co-transporter 2 inhibitors. Cardiovasc Diabetol. 2018;17(1):101. doi:10.1186/s12933-018-0745-5.

176. Ye Y, Bajaj M, Yang HC, Perez-Polo JR, Birnbaum Y. SGLT-2 Inhibition with Dapagliflozin Reduces the Activation of the Nlrp3/ASC Inflammasome and Attenuates the Development of Diabetic Cardiomyopathy in Mice with Type 2 Diabetes. Further Augmentation of the Effects with Saxagliptin, a DPP4 Inhibitor. Cardiovasc Drugs Ther. 2017;31(2):119-132. doi:10.1007/s10557-017-6725-2.

177. Byrne NJ, Matsumura N, Maayah ZH, Ferdaoussi M, Takahara S, Darwesh AM, Levasseur JL, Jahng JWS, Vos D, Parajuli N, El-Kadi AOS, Braam B, Young ME, Verma S, Light PE, Sweeney G, Seubert JM, Dyck JRB. Empagliflozin Blunts Worsening Cardiac Dysfunction Associated With Reduced NLRP3 (Nucleotide-Binding Domain-Like Receptor Protein 3) Inflammasome Activation in Heart Failure. Circ Heart Fail. 2020;13(1):e006277. doi: 10.1161/CIRCHEARTFAILURE.119.006277.

178. Shi X, Verma S, Yun J, Brand-Arzamendi K, Singh KK, Liu X, Garg A, Quan A, Wen XY. Effect of empagliflozin on cardiac biomarkers in a zebrafish model of heart failure: clues to the EMPA-REG OUTCOME trial?. Mol Cell Biochem. 2017;433(1-2):97-102. doi:10.1007/s11010-017-3018-9.

179. Tanajak P, Sa-Nguanmoo P, Sivasinprasasn S, Thummasorn S, Siri-Angkul N, Chattipakorn SC, Chattipakorn N. Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. J Endocrinol. 2018;236(2):69-84. doi:10.1530/JOE-17-0457.

180. Correale M, Petroni R, Coiro S, Antohi EL, Monitillo F, Leone M, Triggiani M, Ishihara S, Dungen HD, Sarwar CMS, Memo M, Sabbah HN, Metra M, Butler J, Nodari S. Paradigm shift in heart failure treatment: are cardiologists ready to use gliflozins? Heart Fail Rev. 2022 Jul;27(4):1147-1163. doi:10.1007/s10741-021-10107-8.

181. Correale M, Tricarico L, Iacoviello M, Brunetti ND. SGLT2 Inhibitors: Statins or ACE-Inhibitors of the 21st Century? J Clin Med. 2023 Apr 4;12(7):2695. doi:10.3390/jcm12072695.

182. Pistelli L, Parisi F, Correale M, Cocuzza F, Campanella F, de Ferrari T, Crea P, De Sarro R, La Cognata O, Ceratti S, Recupero T, Ruocco G, Palazzuoli A, Imbalzano E, Dattilo G. Gliflozins: From Antidiabetic Drugs to Cornerstone in Heart Failure Therapy-A Boost to Their Utilization and Multidisciplinary Approach in the Management of Heart Failure. J Clin Med. 2023 Jan 3;12(1):379. doi:10.3390/jcm12010379.

183. Public Health Agency of Canada. Diabetes: Overview. Available at: https://www.canada.ca/en/public-health/services/chronic-diseases/diabetes.html

184. Ferrannini E. Sodium-Glucose Co-transporters and Their Inhibition: Clinical Physiology. Cell Metab. 2017 Jul 5;26(1):27–38. doi:10.1016/j.cmet.2017.04.011.

185. Ferrannini E, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol. 2012 Feb 7;8(8):495-502. doi:10.1038/nrendo.2011.243.

186. Gallo LA, Wright EM, Vallon V. Probing SGLT2 as a therapeutic target for diabetes: basic physiology and consequences. Diab Vasc Dis Res. 2015 Mar;12(2):78-89. doi:10.1177/1479164114561992.

187. Mudaliar S, Polidori D, Zambrowicz B, Henry RR. Sodium-Glucose Cotransporter Inhibitors: Effects on Renal and Intestinal Glucose Transport: From Bench to Bedside. Diabetes Care. 2015 Dec;38(12):2344-53. doi:10.2337/dc15-0642.

188. Shyangdan DS, Uthman OA, Waugh N. SGLT-2 receptor inhibitors for treating patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. BMJ Open. 2016 Feb 24;6(2):e009417. doi:10.1136/bmjopen-2015-009417.

189. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia. 2017 Feb;60(2):215-225. doi:10.1007/s00125-016-4157-3.

190. DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. Nat Rev Nephrol. 2017 Jan;13(1):11-26. doi:10.1038/nrneph.2016.170.

191. Rajasekeran H, Lytvyn Y, Cherney DZ. Sodium-glucose cotransporter 2 inhibition and cardiovascular risk reduction in patients with type 2 diabetes: the emerging role of natriuresis. Kidney Int. 2016 Mar;89(3):524-6. doi:10.1016/j.kint.2015.12.038.

192. Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Ishikawa K, Watanabe S, Picatoste B, Flores E, Garcia-Ropero A, Sanz J, Hajjar RJ, Fuster V, Badimon JJ. Empagliflozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. Journal of the American College of Cardiology. 2019 Apr 23;73(15):1931–44. doi:10.1016/j.jacc.2019.01.056.

193. Gongora CA, Drobni ZD, Quinaglia Araujo Costa Silva T, Zafar A, Gong J, Zlotoff DA, Gilman HK, Hartmann SE, Sama S, Nikolaidou S, Suero-Abreu GA, Jacobsen E, Abramson JS, Hochberg E, Barnes J, Armand P, Thavendiranathan P, Nohria A, Neilan TG. Sodium-Glucose Co-Transporter-2 Inhibitors and Cardiac Outcomes Among Patients Treated With Anthracyclines. JACC Heart Fail. 2022 Aug;10(8):559-567. doi:10.1016/j.jchf.2022.03.006.

194. Abdel-Qadir H, Carrasco R, Austin PC, Chen Y, Zhou L, Fang J, Su HMH, Lega IC, Kaul P, Neilan TG, Thavendiranathan P. The Association of Sodium-Glucose Cotransporter 2 Inhibitors With Cardiovascular Outcomes in Anthracycline-Treated Patients With Cancer. JACC CardioOncol. 2023 May 2;5(3):318-328. doi:10.1016/j.jaccao.2023.03.011.

195. Avula V, Sharma G, Kosiborod MN, Vaduganathan M, Neilan TG, Lopez T, Dent S, Baldassarre L, Scherrer-Crosbie M, Barac A, Liu J, Deswal A, Khadke S, Yang EH, Ky B, Lenihan D, Nohria A, Dani SS, Ganatra S. SGLT2 Inhibitor Use and Risk of Clinical Events in Patients With Cancer Therapy-Related Cardiac Dysfunction. JACC Heart Fail. 2024 Jan;12(1):67-78. doi:10.1016/j.jchf.2023.08.026.

196. Eekhoudt CR, Bortoluzzi T, Varghese SS, Cheung DYC, Christie S, Eastman S, Mittal I, Austria JA, Aukema HM, Ravandi A, Thliveris J, Singal PK, Jassal DS. (2022). Comparing flaxseed and perindopril in the prevention of doxorubicin and trastuzumab-induced cardiotoxicity in C57Bl/6 Mice. Curr. Oncol. 2022 Apr 21;29(5):2941-2953. doi:10.3390/curroncol29050241.

197. Jensen BV, Nielsen SL, Skovsgaard T. Treatment with angiotensin-converting-enzyme inhibitor for epirubicin-induced dilated cardiomyopathy. Lancet. 1996;347(8997):297-299. doi:10.1016/s0140-6736(96)90469-9.

198. Zeglinski M, Premecz S, Lerner J, Wtorek P, Dasilva M, Hasanally D, Chaudhary R, Sharma A, Thliveris J, Ravandi A, Singal PK, Jassal DS. Congenital absence of nitric oxide synthase 3 potentiates cardiac dysfunction and reduces survival in doxorubicin- and trastuzumab-mediated cardiomyopathy. Can J Cardiol. 2014 Mar;30(3):359–67. doi:10.1016/j.cjca.2013.11.013.

199. Jassal DS, Han SY, Hans C, Sharma A, Fang T, Ahmadie R, Lytwyn M, Walker JR, Bhalla RS, Czarnecki A, Moussa T, Singal PK. Utility of tissue Doppler and strain rate imaging in the early detection of trastuzumab and anthracycline mediated cardiomyopathy. J Am Soc Echocardiogr. 2009 Apr;22(4):418–24. doi:10.1016/j.echo.2009.01.016.

200. Milano G, Raucci A, Scopece A, Daniele R, Guerrini U, Sironi L, Cardinale D, Capogrossi MC, Pompilio G. Doxorubicin and trastuzumab regimen induces biventricular failure in mice. J Am Soc Echocardiogr. 2014 May;27(5):568–79. doi:10.1016/j.echo.2014.01.014.

201. Asselin CY, Lam A, Cheung DYC, Eekhoudt CR, Zhu A, Mittal I, Mayba A, Solati Z, Edel A, Austria JA, Aukema HM, Ravandi A, Thliveris J, Singal PK, Pierce GN, Niraula S, Jassal DS. The Cardioprotective Role of Flaxseed in the Prevention of Doxorubicin- and Trastuzumab-Mediated Cardiotoxicity in C57BL/6 Mice. J Nutr. 2020;150(9):2353-2363. doi:10.1093/jn/nxaa144.

202. Neilan TG, Jassal DS, Perez-Sanz TM, Raher MJ, Pradhan AD, Buys ES, Ichinose F, Bayne DB, Halpern EF, Weyman AE, Derumeaux G, Bloch KD, Picard MH, Scherrer-Crosbie M. Tissue Doppler imaging predicts left ventricular dysfunction and mortality in a murine model of cardiac injury. Eur Heart J. 2006;27(15):1868-1875. doi:10.1093/eurheartj/ehl013.

203. Neilan TG, Blake SL, Ichinose F, Raher MJ, Buys ES, Jassal DS, Furutani E, Perez-Sanz TM, Graveline A, Janssens SP, Picard MH, Scherrer-Crosbie M, Bloch KD. Disruption of nitric oxide synthase 3 protects against the cardiac injury, dysfunction, and mortality induced by doxorubicin. Circulation. 2007;116(5):506-514. doi:10.1161/CIRCULATIONAHA.106.652339.

204. Goyal V, Bews H, Cheung DYC, Premecz S, Mandal S, Shaikh B, Best R, Bhindi R, Chaudhary R, Ravandi A, Thliveris J, Singal PK, Niraula S, Jassal DS. The Cardioprotective Role of N-Acetyl Cysteine Amide in the Prevention of Doxorubicin and Trastuzumab-Mediated Cardiac Dysfunction. Can J Cardiol. 2016 Dec;32(12):1513-1519. doi:10.1016/j.cjca.2016.06.002. Epub 2016 Jun 20.

205. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226:497-509.

206. Hasanally D, Edel A, Chaudhary R, Ravandi A. Identification of Oxidized Phosphatidylinositols Present in OxLDL and Human Atherosclerotic Plaque. Lipids. 2017;52:11-26. doi:10.1007/s11745-016-4217-y. Epub 2016 Dec 2.

207. Lódi M, Priksz D, Fülöp GÁ, et al. Advantages of prophylactic versus conventionally scheduled heart failure therapy in an experimental model of doxorubicin-induced cardiomyopathy. J Transl Med. 2019;17(1):229-236. doi:10.1186/s12967-019-1978-0.

208. Hiona A, Lee AS, Nagendran J, Xie X, Connolly AJ, Robbins RC, Wu JC. Pretreatment with angiotensin-converting enzyme inhibitor improves doxorubicin-induced cardiomyopathy via preservation of mitochondrial function. J Thorac Cardiovasc Surg. 2011 Aug;142(2):396-403.e3. doi:10.1016/j.jtcvs.2010.07.097.

209. Yang CC, Chen YT, Wallace CG, Chen KH, Cheng BC, Sung PH, Li YC, Ko SF, Chang HW, Yip HK. Early administration of empagliflozin preserved heart function in cardiorenal syndrome in rat. Biomed Pharmacother. 2019 Jan;109:658-670. doi:10.1016/j.biopha.2018.10.095.

210. Hiona A, Lee AS, Nagendran J, Xie X, Connolly AJ, Robbins RC, Wu JC. Pretreatment with angiotensin-converting enzyme inhibitor improves doxorubicin-induced cardiomyopathy via preservation of mitochondrial function. J Thorac Cardiovasc Surg. 2011 Aug;142(2):396-403.e3. doi:10.1016/j.jtcvs.2010.07.097.

211. Koleini N, Nickel BE, Edel AL, Fandrich RR, Ravandi A, Kardami E. Oxidized phospholipids in Doxorubicin-induced cardiotoxicity. Chem Biol Interact. 2019 Apr 25;303:35-39. doi:10.1016/j.cbi.2019.01.032.

212. Wei S, Ma W, Yang Y, Sun T, Jiang C, Liu J, Zhang B, Li W. Trastuzumab potentiates doxorubicin-induced cardiotoxicity via activating the NLRP3 inflammasome in vivo and in vitro. Biochem Pharmacol. 2023 Aug;214:115662. doi:10.1016/j.bcp.2023.115662.

213. Anjos M, Fontes-Oliveira M, Costa VM, Santos M, Ferreira R. An update of the molecular mechanisms underlying doxorubicin plus trastuzumab induced cardiotoxicity. Life Sci. 2021 Sep 1;280:119760. doi:10.1016/j.lfs.2021.119760.

214. Schauer A, Adams V, Kämmerer S, Langner E, Augstein A, Barthel P, Männel A, Fabig G, Alves PKN, Günscht M, El-Armouche A, Müller-Reichert T, Linke A, Winzer EB. Empagliflozin Improves Diastolic Function in HFpEF by Restabilizing the Mitochondrial Respiratory Chain. Circ Heart Fail. 2024 Jun;17(6):e011107. doi:10.1161/CIRCHEARTFAILURE.123.011107.

215. Lu Q, Yang L, Xiao JJ, Liu Q, Ni L, Hu JW, Yu H, Wu X, Zhang BF. Empagliflozin attenuates the renal tubular ferroptosis in diabetic kidney disease through AMPK/NRF2 pathway. Free Radic Biol Med. 2023 Feb 1;195:89-102. doi:10.1016/j.freeradbiomed.2022.12.088.

216. Wang XZ, Yu ZX, Nie B, Chen DM. Perindopril inhibits myocardial apoptosis in mice with acute myocardial infarction through TLR4/NF-κB pathway. Eur Rev Med Pharmacol Sci. 2019 Aug;23(15):6672-6682. doi:10.26355/eurrev_201908_18558.

217. Fiordelisi MF, Auletta L, Meomartino L, Basso L, Fatone G, Salvatore M, Mancini M, Greco A. Preclinical Molecular Imaging for Precision Medicine in Breast Cancer Mouse Models. Contrast Media Mol Imaging. 2019 Sep 22;2019:8946729. doi:10.1155/2019/8946729.