**ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ**

**ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ**

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# ***Cardiotoxicity criteria for classification of substances as Specific Target Organ Toxicity from Animal Data***

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**«Κριτήρια καρδιοτοξικότητας για ταξινόμηση ουσιών ως Ειδικής Τοξικότητας σε όργανα στόχους από δεδομένα σε ζώα»**


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# 1. Introduction

Cancer is a leading cause of death in both more and less economically developed countries; the burden is expected to grow worldwide due to the growth and aging of the population, particularly in less developed countries, in which about 82% of the world's population resides. The adoption of lifestyle behaviors that are known to increase cancer risk, such as smoking, poor diet, physical inactivity, and reproductive changes (including lower parity and later age at first birth), have further increased the cancer burden in less economically developed countries.(Torre et al, 2015) Advances in treatment have led to improved survival of patients with cancer, but have also increased morbidity and mortality due to treatment side effects.(DeSantis et al, 2014; Ferlay et al, 2013) Cardiovascular diseases (CVDs) are one of the most frequent of these side effects, and there is a growing concern that they may lead to premature morbidity and death among cancer survivors. This may be the result of cardiotoxicity, which involves direct effects of the cancer treatment on heart function and structure, or may be due to accelerated development of CVD, especially in the presence of traditional cardiovascular risk factors.(Armstrong et al, 2013) Patients with cancer can experience adverse cardiovascular events secondary to the malignant process itself or its treatment. They might also have underlying cardiovascular illness, the consequences of which are often exacerbated by the stress of the tumour growth or its treatment. With the advent of new treatments and subsequent prolonged survival time, late effects of cancer treatment can become clinically evident decades after completion of therapy. The heart's extensive energy reserve and its ability to compensate for reduced function add to the complexity of diagnosis and timely initiation of therapy. Additionally, modern oncological treatment regimens often incorporate multiple agents whose deleterious cardiac effects might be additive or synergistic. Treatment-related impairment of cardiac contractility can be either transient or irreversible. Furthermore, cancer treatment is associated with life-threatening arrhythmia, ischaemia, infarction, and damage to cardiac valves, the conduction system, or the pericardium. Awareness of these processes has gained prominence with the arrival of strategies to monitor and to prevent or to mitigate the effects of cardiovascular damage. A greater understanding of the mechanisms of injury can prolong the lives of those cured of their malignancy, but left with potentially devastating cardiac sequelae.(Ewer & Ewer, 2015)

Although the field of cardio-oncology has received increasing attention in recent years, many aspects of both radiation-induced and cancer drug–induced CVD are still to be fully elucidated. Furthermore, the inability to predict the long-term consequences of cancer treatment–associated cardiovascular side effects leads to under- or overdiagnosis of CVD, sometimes resulting in the failure to prevent adverse events and sometimes to inappropriate interruption of a potentially lifesaving cancer treatment.

In general, the cardiovascular complications of cancer therapy can be divided into nine main categories, which are discussed in this document:

1. myocardial dysfunction and heart failure (HF);
2. coronary artery disease (CAD);

3. valvular disease;
4. arrhythmias, especially those induced by QT-prolonging drugs;
5. arterial hypertension;
6. thromboembolic disease;
7. peripheral vascular disease and stroke;
8. pulmonary hypertension and
9. pericardial complications.

The challenge for the cardiovascular specialist is to balance the need for life-saving cancer treatment with the assessment of risk from cancer drug-associated cardiovascular side effects to prevent long-term damage. This review discusses concepts for timely diagnosis, intervention, and surveillance of cancer patients undergoing treatment, and provides approaches to clinical uncertainties.

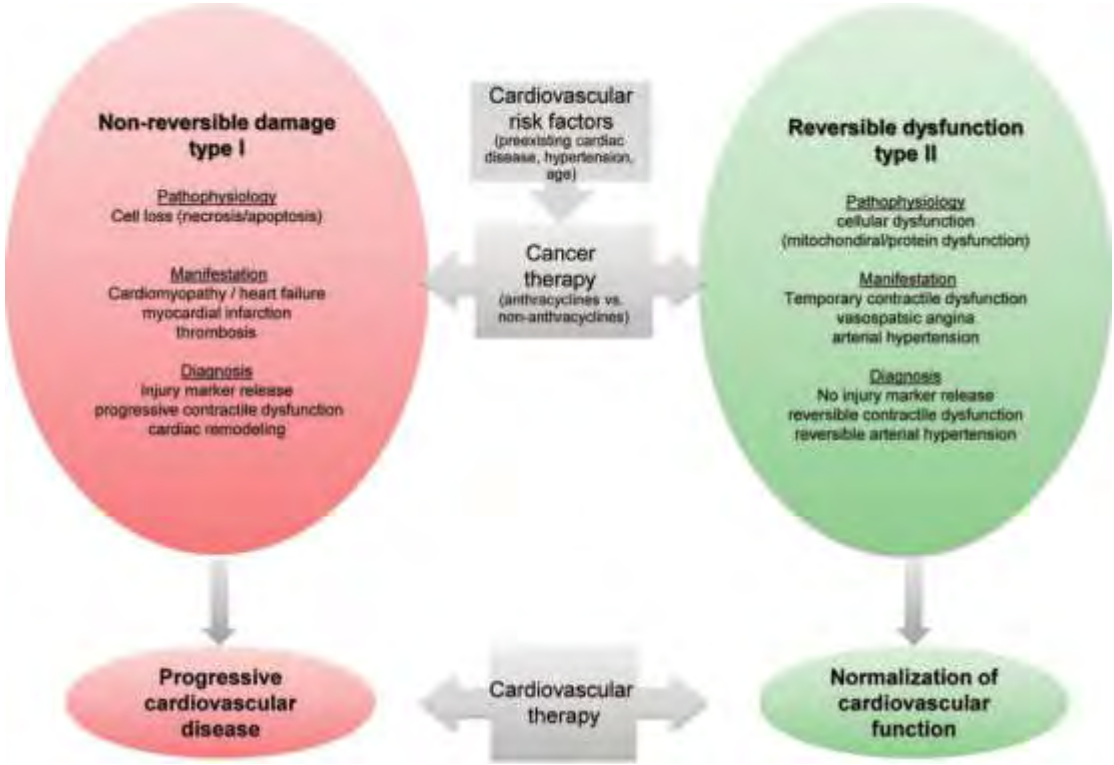



**Figure 1 Depiction of the fundamental differences between non-reversible damage (type I) and reversible dysfunction (type II) (Suter & Ewer, 2013)**

**Systemic cancer drugs with important cardiovascular side effects; selected indications**

| | Class/drug | Selected indications | Important CV side effects |
|---|---|---|---|
| Cytostatic chemotherapeutics | Anthracyclines/analogues | | |
| | Doxorubicin | Lymphoma | Cardiac dysfunction/heart failure |
| | Daunorubicin | Leukaemia | |
| | Epirubicin | Breast cancer | |
| | | Ovarian cancer | |
| | | Sarcoma | |
| | Mitoxantrone | Leukaemia | |
| | | Multiple sclerosis | |
| | Pyrimidine analogues | | |
| | Fluorouracil (5-FU) | Colorectal cancer | Coronary spasms/ischaemia |
| | Capecitabine | Breast cancer | |
| | Alkylating agents | | |
| | Cyclophosphamide | Breast cancer | Myocarditis (rare) |
| | Cisplatin | Genitourinary cancer | Thrombosis |
| | Antimicrotubule agents | | |
| | Paclitaxel | Breast cancer | Bradycardia |
| | | Colorectal cancer | |
| Signalling inhibitors | Anti-HER2 | | |
| | Trastuzumab | Breast cancer | Cardiac dysfunction |
| | Lapatinib | Gastric cancer | |
| | Angiogenesis inhibitors/anti-VEGF | | |
| | Bevacizumab | Gastrointestinal cancer | Hypertension |
| | Sunitinib | Renal cell carcinoma | Endovascular damage |
| | Sorafenib | Hepatocellular carcinoma | |
| | BCR-ABL inhibitors | | |
| | Imatinib | Leukaemia | Oedema, cardiac dysfunction (rare) |
| | Dasatinib | Gastric cancer | QTc prolongation |
| | Nilotinib | | |

(Ewer et al, 2011; Force et al, 2007)

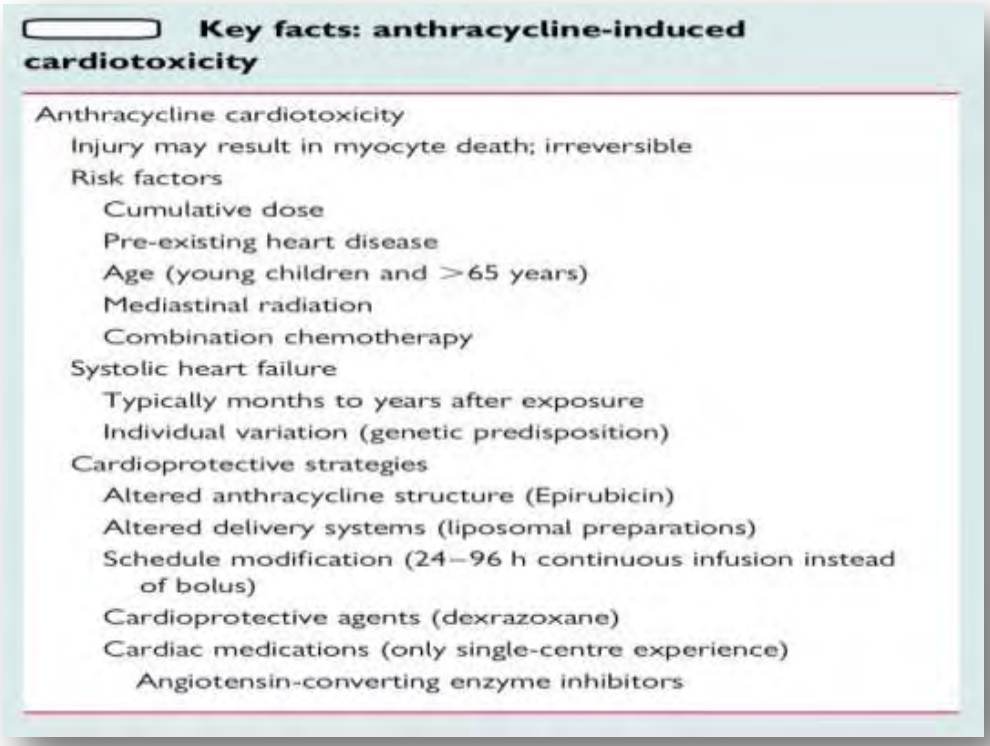
**Summary of cardiovascular side effects of selected cancer therapeutics**

| Cardiac response | Drug | Frequency | Mechanism | Reversibility |
|---|---|---|---|---|
| Contractile dysfunction/heart failure | Anthracyclines | Cumulative dose-related | Myocyte death | Minimal |
| | Cyclophosphamide | Rare | Myocarditis | Partial |
| | Cisplatin | Rare | Unknown | Unknown |
| | Trastuzumab | Variable* | Contractile protein dysfunction | High |
| | Lapatinib | | | Reported |
| | Bevacizumab | Low | Hypertension? | Reported |
| | Sunitinib | Low | Mitochondrial dysfunction | Partial |
| | Sorafenib | Rare | | Unknown |
| | Imatinib | Rare | Mitochondrial dysfunction | High |
| Arterial hypertension | All angiogenesis inhibitors | Moderate, dose-dependent | Endothelial dysfunction | Unknown |
| Myocardial ischaemia | Pyrimidine analogues | Moderate | Direct vasospasm | High, unless infarction |
| Thromboembolism | Cisplatin | Moderate | Endothelial dysfunction | Variable |
| | All angiogenesis inhibitors | Moderate | Endothelial dysfunction | Variable |
| Arrhythmia/QT prolongation | Arsenic trioxide | Moderate | HERG K+ blockage | High |
| | Lapatinib | Rare | HERG K+ blockage | Unknown |
| | Sunitinib | Rare | HERG K+ blockage | Unknown |
| | Nolitinib | Rare | HERG K+ blockage | Unknown |
| | Dasatinib | Rare | HERG K+ blockage | Unknown |

*Frequently in combination with anthracyclines.

## 2. Non-reversible or reversible: a cardinal distinction

Historically, non-reversible cardiovascular side effects that eventually led to progressive cardiac disease were the consequence of some oncologic therapies; a prime example being anthracycline induced cardiotoxicity leading to progressive systolic heart failure.(Gianni et al, 2008) With the introduction of new cancer drugs, such as signaling inhibitors, a new phenomenon has been observed; cardiac dysfunction that resolves for most patients over time. In an effort to classify cardiotoxicity of cancer drugs,(Ewer et al, 2005) proposed a system to identify drugs that have the potential to cause irreversible damage (Type I) vs. drugs that predominantly induce reversible dysfunction (Type II) (figure 1).



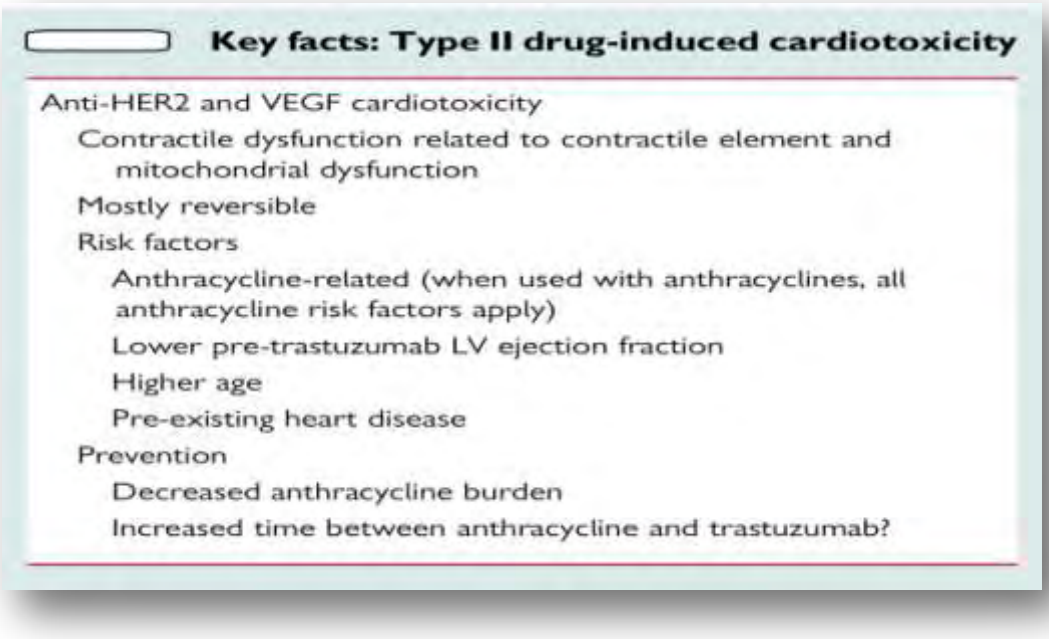
**Key facts: anthracycline-induced cardiotoxicity**

- Anthracycline cardiotoxicity
  - Injury may result in myocyte death; irreversible
  - Risk factors
    - Cumulative dose
    - Pre-existing heart disease
    - Age (young children and >65 years)
    - Mediastinal radiation
    - Combination chemotherapy
  - Systolic heart failure
    - Typically months to years after exposure
    - Individual variation (genetic predisposition)
  - Cardioprotective strategies
    - Altered anthracycline structure (Epirubicin)
    - Altered delivery systems (liposomal preparations)
    - Schedule modification (24–96 h continuous infusion instead of bolus)
    - Cardioprotective agents (dexrazoxane)
    - Cardiac medications (only single-centre experience)
      - Angiotensin-converting enzyme inhibitors

(Suter & Ewer, 2013)

However, this classification system does have limitations; for example, trastuzumab, a Type II drug, can trigger irreversible cardiac damage in patients with severe preexisting cardiac disease, or potentiate anthracycline Type I cardiotoxicity(Sawyer et al, 2002). For cardiovascular side effects from other modern cancer therapeutics, such as angiogenesis inhibitors-induced arterial hypertension and nephrotoxicity, the

reversibility remains unknown.




(de Azambuja et al, 2009; Slamon et al, 2001; Smith et al, 2010; Swain et al, 2003; Von Hoff et al, 1979)

# 3. Animal models on clinical trials

Animals have been used by humans for centuries to understand their own biology. In cardiovascular research, animal models have allowed the study of cardiovascular disease in the early stages, as well as the investigation of the mechanisms of the pathogenesis of cardiovascular disease and the effects of drug intervention. The aim of these studies is to provide clear concepts for selected investigations in humans. An ideal animal model for any cardiovascular disease in humans should have five characteristics: (i) mimic the human disease, (ii) allow studies in chronic, stable disease, (iii) produce symptoms which are predictable and controllable, (iv) satisfy economical, technical and animal welfare considerations, and (v) allow measurement of relevant cardiac, biochemical and haemodynamic parameters.

Animal species examined in detail as models of this cardiac toxicity include the rabbit, the normotensive and spontaneously hypertensive rat, the mouse, the pig, and the dog. The advantages and disadvantages of these animal models differ according to species: small animals can be used for comparative investigations, which may be available only in limited amounts, while large animals can be used for studies in which evaluation of cardiac function are to be made. Among the various animals examined, the spontaneously hypertensive rat and the beagle dog are considered the most suitable small and large animal models, respectively, because of the reproducibility of the lesions induced by anthracyclines in the two species. A variety of pharmacologic agents has been tested for cardioprotective activity. The most

successful of these agents are those that function as antioxidants, because they either scavenge reactive oxygen species or prevent their formation. The most clinically useful of these agents is ICRF-187 (dexrazoxane), which has been found to be cardioprotective in all animal models. These whole animal studies enable repeated administration of chemotherapeutic agents, mimicking chronic cardiotoxicity in clinical practice.

### 3.1 Mice as animal models

Cardiovascular diseases are common causes of morbidity and mortality in developed countries, and coronary heart disease is a relevant cause of congestive heart failure, which is frequently secondary to myocardial infarction (MI). Different species have been used to reproduce MI models, but in recent years mice have become the animals of choice for the analysis of several diseases, because of their short life cycle and the possibility of genetic manipulation(Acton et al, 2006). However, the dimensions of the mouse heart, with the left ventricular wall being thinner than 1 mm, represent a challenge for image acquisition and analysis that requires expensive equipment capable of obtaining high-resolution images and established expertise(Ratering et al, 2010). As a consequence, the procedures to measure myocardial tissue damage have some limitations. Measurements of the MI size with transmission microscopy and triphenyltetrazolium chloride staining can be performed only post mortem, and these techniques are not useful for longitudinal studies of the infarct area or to predict benefits of therapy (Saraste et al, 2009). There is therefore great interest in developing a non-invasive method for evaluation of infarct size in living mice, to improve our knowledge of metabolic and functional changes related to MI and the efficacy of interventional, pharmacological or molecular therapies. *In vivo* techniques currently used for infarct size delineation, such as echocardiography, X-ray computed tomography (CT), magnetic resonance (MR) and radionuclide imaging procedures, all have inherent advantages and limitations (Gargiulo et al, 2012). Echocardiography may be used serially to assess changes of cardiac structure, quantify ventricular function and detect non-perfused myocardium (Scherrer-Crosbie et al, 1999). However, this methodology is strongly operator dependent and does not provide information about glycolytic metabolism and myocardial viability (Ferro et al, 2007). Computed tomography allows an accurate evaluation of morphology (Nahrendorf et al, 2007). Disadvantages of CT include the use of ionizing radiation and the limited spatial resolution achievable *in vivo*, related to the radiation dose administered to the animals. Magnetic resonance imaging has the advantage of providing high tissue contrast and functional parameters, with the limitations of high cost and restricted availability (Vogel-Claussen et al, 2006).

### 3.2 Rats as animal modes

Most recent studies regarding central cardiac control have been conducted in the rat. A large amount of data already exists regarding neural mechanisms controlling heart rate in this species. Heart rate is a direct measure of autonomic outflow to the sino-atrial node. Since neural influences on this pacemaker region and on the ventricular myocardium may differ (Braga et al, 2007; Nalivaiko et al, 2004), it becomes evident that neurally induced chronotropic effects cannot be used as a general indicator of cardiac autonomic influences. The most commonly used ventricular indices are those reflecting cardiac inotropic state: the maximal left ventricular pressure ($LVP_{max}$) and the velocity of its rise (LVd*P*/d*t*). Methods of their assessment are invasive and technically demanding, especially in small mammals. The majority of studies of contractile function in the rat heart have been performed *in vitro*, in perfused hearts and in isolated papillary muscles or in fragments of myocardium (Piuhola et al, 2003; Vornanen, 1992).

Left ventricular d*P*/d*t* depends on many parameters, including intrinsic (heart rate, preload, afterload, coronary perfusion) and extrinsic parameters (sympathetic and vagal neural influences, and humoral effects). This complex dependence between variables, where alteration in one can lead to changes in several others, makes the study of ventricular contractility a challenging task. To overcome this difficulty, numerous attempts have been made to find contractility indices that are independent of heart rate, preload and afterload (Connelly et al, 2006).

### 3.2 Rabbits as animal models

There are several models of cardiac hypertrophy employed in experimental animals to generate the features of a failing heart, these have been reviewed elsewhere. A common approach is to simulate a myocardial infarction by ligation of a coronary artery via an operative procedure. The infarct is allowed to heal and the remaining myocardium hypertrophies to compensate for the loss. The myocardial infarction model in the rabbit has been extensively characterized. This model demonstrates many features of clinical heart failure, including impairment of left ventricular ejection fraction (Pye & Cobbe, 1996), compensatory hypertrophy (Ng et al, 1998) and neuroendocrine activation.

### 3.3 Pig as animal model

Transgenic technology has potentially solved many of the immunological difficulties of using pig organs to support life in the human recipient. Nevertheless, other problems still remain. Knowledge of cardiac anatomy of the pig (Sus scrofa) is limited despite the general acceptance in the literature that it is similar to that of man. A qualitative analysis of porcine and human cardiac anatomy was achieved by gross examination and dissection of hearts with macrophotography. The porcine organ had

a classic 'Valentine heart' shape, reflecting its location within the thorax and to the orientation of the pig's body (unguligrade stance). The human heart, in contrast, was trapezoidal in silhouette, reflecting man's orthograde posture (Sahni et al, 2008). The morphologically right atrium of the pig was characterised by the tubular shape of its appendage (a feature observed on the left in the human heart). The porcine superior and inferior caval veins opened into the atrium at right angles to one another, whereas in man the orifices were directly in line. A prominent left azygous vein (comparable to the much reduced left superior caval or oblique vein in man) entered on the left side of the pig heart and drained via the coronary sinus. The porcine left atrium received only 2 pulmonary veins, whereas 4 orifices were generally observed in man. The sweep between the inlet and outlet components of the porcine right ventricle was less marked than in man, and a prominent muscular moderator band was situated in a much higher position within the porcine right ventricle compared with that of man. The apical components of both porcine ventricles possessed very coarse trabeculations, much broader than those observed in the human ventricles. In general, aortic-mitral fibrous continuity was reduced in the outlet component of the porcine left ventricle, with approximately two-thirds of the aortic valve being supported by left ventricular musculature. Several potentially significant differences exist between porcine and human hearts. It is important that these differences are considered as the arguments continue concerning the use of transgenic pig hearts for xenotransplantation (Crick et al, 1998)

### 3.4 Dog as animal model

Dog and human hearts share many characteristics on both the organ and cellular levels. Dog heart rate, body weight, and heart weight are more comparable to humans than the heart rate, body weight, and heart weight of mice, rats, and rabbits. Due to their size, almost all in vivo techniques used to assess contractility of human hearts can be utilized in dogs. Housing and maintenance of dog models is considerably more expensive than small rodents and cats; therefore, this issue must be taken into consideration when long-term studies and disease models are being considered. One disadvantage, unique to canines, is obtaining the necessary approval for performing experiments in this species.

Furthermore, the human population is heterogeneous with slight genetic variations; a property which is not reflected in the inbred laboratory mouse, rat, or rabbit strains. In that regard, it is suggested that mixed dog breeds such as mongrels are better suited for addressing this issue. This is supported by reports that various cardiac functions, parameters, properties, and response to stress vary depending on the mouse strains. Due to their size, pretty much all in vivo techniques used to assess contractility of human hearts can be utilized in canines. Beyond these characteristics, the

myocardium of canines and humans share many properties and similarities. The action potential duration of canine is only a little bit shorter than that of humans, much more comparable than the animal models discussed previously (canine APD90: ~ 219 ms, human APD90: ~290 ms both measured in right ventricular papillary muscles at cycle length of 1000 ms and 37 °C). Furthermore, there is a high degree of resemblance between human and canine sino-atrial (SA) node activity and structure, purkinje fiber distribution, and activation sequence. Three separate surveys of the pharmaceutical industry revealed that canine was the most commonly used animal model for evaluation and determination of QT interval prolongation of novel compounds. For thoroughness purposes, we must point out that it is proposed that the "effective size" of the heart (third root of heart weight divided by spiral wave rotation period) determines ventricular fibrillation pattern. Based upon this analysis, it is argued that rabbits have a closer effective size to humans and hence more similar ventricular fibrillation patterns than either dogs or pigs. Nevertheless, the similarities of human and canine excitation processes and structure make canine a good animal model for studying cardiac electrophysiology as it relates to human health and disease.

Despite the high degree of similarity between human and dog myocardium, some differences have been reported between these two species. Such differences include resting and exercise heart rates , kinetics of Ito current inactivation, its recovery, and molecular size of its corresponding ion channels, sensitivity to flecanideand transmural epicardial:endocardial expression levels of HERG ion channel(Milani-Nejad & Janssen, 2014).

## 4. CARDIOVASCULAR DISEASES AS SIDE EFFECT OF CARDIOTOXICITY

### 4.1 Cardiac dysfunction and heart Failure

Cardiac dysfunction and heart failure are among the most serious cardiovascular side effects of systemic cancer treatment. Conventional chemotherapeutics, such as anthracyclines, anti-metabolites, and cyclophosphamide, can induce permanent myocardial cell injury—albeit by diverse mechanisms—and by cardiac remodeling (Mann & Bristow, 2005). Signalling inhibitors currently in use, like human epidermal growth factor receptor 2 (HER2/erbB2) and angiogenesis inhibitors, predominantly affect cardiac metabolism and contractile proteins, leading to transient contractile dysfunction. Understanding the mechanistic pathophysiology of cancer drug-associated cardiac dysfunction is important to predict, treat, and prevent these side effects, although it can be challenging to identify the proper mechanism in individual patients.

## 4.2 Cardiovascular side effects beyond cardiac dysfunction

### 4.2.1 Arterial hypertension

Cancer drug-induced arterial hypertension is now recognized as an entity primarily associated with the use of angiogenesis inhibitors (Maitland et al, 2010). On a dose-dependent basis, these drugs can worsen pre-existing hypertension, or can cause *de novo* hypertension to develop. It is difficult to determine the true incidence of anti-VEGF-induced hypertension since various methods of blood pressure measurement and definitions of hypertension have been used in trials. A recent meta-analysis of studies with bevacizumab reported an incidence of more than 23% for any grade hypertension, with almost 8% of patients experiencing severe hypertension(Ranpura et al, 2010). The incidence of arterial hypertension associated with sunitinib and sorafenib appear similar, and patients with pre-existing hypertension or renal cell cancer have a higher risk (Wu et al, 2008; Zhu et al, 2009).

Hypertension can occur at any time during treatment: acute complications include heart failure, proteinuria with renal thrombotic microangiopathy, intracerebral haemorrhage, and, infrequently, reversible posterior leukoencephalopathy (Maitland et al, 2010). It is unclear if patients will encounter hypertension-induced long-term consequences, such as cardiac and renal remodelling. For most patients, the condition improves when angiogenesis inhibitor treatment is held or stopped altogether, but in some instances profound hypertension may persist and become life-threatening. Interestingly, in the case of sunitinib, a correlation has been demonstrated between oncologic efficacy and hypertension, suggesting that blood pressure increases may be a marker for efficacy rather than, or in addition to, a simple cardiac adverse event. Treatment of sunitinib-associated hypertension has not been shown to impair oncologic response, but further studies are needed. Such interactions further support the need for careful monitoring and clinical correlations between efficacy and adversity, and the need for a broad onco-cardiologic perspective.

The mechanism of angiogenesis inhibitor-induced hypertension is not completely understood, but may be directly linked to the inhibition of VEGF-2 signalling (Chen & Cleck, 2009). Vascular endothelial growth factor signalling is important for proper endothelial function and nitric oxide synthesis; inhibition impairs vasodilation (Ku et al, 1993). Other effects of VEGF inhibition may include induction of endothelial cell death and rarefaction of resistance vessels (Maitland et al, 2010). Hypertension involves mechanisms similar to those of tumour destruction, and therefore may also be a marker for efficacy of angiogenesis inhibitors (Mir et al, 2009).

### 4.2.2 Vasospastic and thromboembolic ischaemia associated with anti-cancer treatment

Among agents associated with coronary artery spasm, the pyrimidine analogues 5-fluorouracil (5-FU) and its oral pre-drug capecitabine are the most common (Kleiman et al, 1987; Stewart et al, 2010). Underlying coronary artery disease has been associated with an increased incidence of coronary artery spasm, although there are patients with normal coronaries that have had spasms while being treated with these drugs. Rhythm disturbances accompanying ischaemic events have been reported as well. Ischaemia most typically occurs after the second or third administration of these antimetabolites. Nitroglycerin and calcium-channel blockers are often effective for the treatment and prevention of ischaemia. In rare instances, progression to myocardial infarction has been reported.

### 4.2.3 Thromboembolic events

Patients with malignancies are in a hypercoagulable state and conventional chemotherapeutics, signalling inhibitors, and endocrine cancer therapies can further increase a patient's risk of experiencing a venous or arterial thromboembolic event (VTE and ATE, respectively)(Ay et al, 2010). For example, cisplatin was found to cause VTE in up to 18% of patients; a direct endothelial-toxic effect and changes in the coagulation system are likely responsible for this side effect (Moore et al, 2011). Patients treated with bevacizumab also experienced a higher rate of thromboembolic events compared with patients treated with chemotherapy alone; elderly patients with cardiovascular risk factors were shown to have the highest rates of all(Nalluri et al, 2008; Scappaticci et al, 2007). Similar risks for ATEs have been reported for sunitinib and sorafenib (Choueiri et al, 2010). Increased incidences of thromboembolic events have also been found with hormonal therapy, such as tamoxifen, which is now an integral part of many breast cancer regimens (Freedman et al, 2011).

Prophylactic anticoagulation is only recommended in high-risk cancer patients who are hospitalized or undergo surgery and in selected patients with multiple myeloma (Lyman et al, 2007). Several trials are currently investigating the potential role of prophylactic low molecular heparin. There are no clear guidelines for the prevention of ATEs in the setting of cancer therapy; only evidence-based guidelines used for patients without cancer should be used until studies are conducted. It is unknown whether cancer patients have an altered risk of coronary thrombosis after stenting.

### 4.2.4 Dysrhythmia and QT prolongation

Rhythm disturbances associated with anti-cancer treatment are typically transient and not especially troubling. They occur most commonly as a consequence of metabolic changes and generally resolve after electrolyte homeostasis is re-established. Anthracyclines, for example, are associated with supraventricular arrhythmias and ventricular ectopy during and shortly after administration, a condition that usually resolves without sequelae after heart rate control. Similarly, taxanes can induce sinus bradycardia during treatment, but this is seldom severe and intervention is rarely warranted. Some rhythm disturbances are associated with structural changes within the heart, as may be seen with tumour invasion or as a manifestation of chronic anthracycline cardiomyopathy.

QT prolongation is associated with a number of anti-cancer drugs and may constitute a significant problem. Many cancer patients have multiple comorbidities, including diarrhea- and vomiting-induced electrolyte disturbances, and concomitant medications such as psychotropic medications and anti-emetics that may further prolong the QT interval. Among specific anti-cancer treatments, arsenic trioxide, typically used to treat leucemia, has received considerable attention in this regard since it may prolong the QT interval in up to 40% of treated patients and has a significant risk of Torsades de Pontes (Soignet et al, 2001). Several of the newer signalling inhibitors also prolong the QT interval, although Torsades is a relatively infrequent event. Vandetanib, an orally available multiple target TKI for the treatment of thyroid cancer, has been associated with a moderate risk of QT prolongation (Wells et al, 2010). Nilotinib and dasatinib can also prolong the QT interval, although symptomatic manifestations were recorded in only a few patients (Kantarjian et al, 2011). In patients treated with lapatinib or sunitinib, QT prolongation was rarely observed.

While cardiovascular side effects such as arterial hypertension, myocardial ischaemia, dysrhythmia, and thrombosis can be readily diagnosed, the assessment of cardiac dysfunction and its prognosis is more challenging. Most currently used methods assessing cardiac function cannot differentiate between irreversible (Type I) and reversible (Type II) cardiotoxicity, and may mislead physicians to stop potentially lifesaving cancer therapy unnecessarily.

Historically, patients with anthracycline-induced cardiotoxicity were evaluated with right-ventricular endomyocardial biopsies; morphologic changes correlated with the cumulative dose applied and (to a limited extent) with the onset of heart failure (Mackay et al, 1994). However, correlation of biopsy scores with non-invasively assessed LVEFs was poor (Ewer et al, 1984). More recently, the predictive value of serial LVEF evaluation by either echocardiography or multiple gated acquisition (MUGA) scans in adult cancer patients was assessed in several studies. While some

investigators found an early drop in LVEF to be predictive for later onset of heart failure, others did not (Jensen et al, 2002; Mitani et al, 2003; Swain et al, 2003)

## 5. EXPERIMENTAL DATA

Advances in early cancer diagnosis and therapy have improved the survival statistics of individuals with cancer in the last few decades. Current data reported by the American Cancer Society shows that the 5-year relative survival rate in cancer patients is 68%, or 19% higher than three decades prior.1 In children, mortality rates have dropped by more than 50% in the period between 1975 and 2010.1 These increased survival rates are bringing attention to the long-term effects of chemotherapy in children and adults, and to how chemotherapy affects organ systems, in our case the cardiovascular system.

**Drugs causes cardiotoxicity in animal models**

| | Animal specie | Class/Drug | Echocardiography results | Doses | Time of treatment | Citation |
|---|---|---|---|---|---|---|
| 1. | **Rats** | doxorubicin (DOX) - Anthracycline | **Franctional Shortening** -13,9% (average change in FS = 0.20) | 18 mg/kg | 12 days | (Fernandez-Fernandez et al, 2014) |
| 2. | Mice | Imatinib Mesylate | **LVPW** (mm) * Young 0.65± 0.02 Old 0,78± 0.12 | 200 mg/kg/day | 5 weeks | (Maharsy et al, 2014) |
| 3. | Rats | ramipril and darbepoetin (erythropoietin hormones) in doxorubicin-induced cardiotoxicity | **LVEF****, % DOX + RAM + DP - 63.43 ± 3.66b **LVFS*****, % DOX + RAM + DP - 30.71 ± 2.67b | 2.5 mg/kg i.v. DOX + 1 mg/kg p.o. ramipril + 10 μ/ kg i.p. darbepoetin alfa | 4 weeks | (Ozkanlar et al, 2014) |
| 4. | Wistar Rats | Doxorubicin and cardiac function improvement after | **1**.**EF** (%) – 60.8 (13), **FS** (%) – 33.4 (9.2) **2**. **EF** (%)-63.6 (4.1), **FS** (%) – 35.2 (3.5) | **1**.Dox 5mg/kg **2**.Dox 5mg/kg+ C.sinensis 100/kg | 4 weeks | (Oliveira et al, 2013) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | stem cell therapy and flavonoid extract treatment | **3**.**EF** (%) – 73.6(1.9), **FS** (%) – 43.6(1.6) | **3**.Dox 5mg/kg +MSC | | |
| **5**. | Wistar Rats | Pamidronate Attenuates Oxidative Stress in Acute Doxorubicin-Induced Cardiotoxicity | **PWRT ˣ**<br>**1**. 0,36<br>**2**. 0,51<br>**3**. 0,41<br><br>**LVDD+**<br>**1**. 7,14<br>**2**. 6,20<br>**3**. 6,77 | **1**.Pamidronate 3mg/kg<br>**2.** DOX 20mg/kg<br>**3**.DOX 20mg/kg + pamidronate 3mg/kg | 3 days | (Carvalho et al, 2016) |
| **6.** | Chinchilla rabbit | Daunorubicin | **LV fractional shortening** lower than 20 % | DAU 3mg/kg i.v | 10 weeks | (Lencova-Popelova et al, 2014) |
| **7.** | Wistar rats | (alpha) tocopherol (vitamin E) induces cardiotoxicity | **Ao (mm)** ₓ<br>Control 3.8 + 0.2<br>E 3.4 + 0.4<br>**PWT (mm)**<br>Control 1.64 + 0.26<br>E 1.42 + 0.14<br>**LVD (mm)**<br>Control 7.37 + 0.62<br>E 7.22 + 0.41 | 250 mg a-tocopherol/kg body wt/day orally | 7 weeks | (Nascimento et al, 2011) |
| **8.** | WT and GSTP null mice | Cyclophosphamide | **LVIDD : Null, 1h** 3.39±0.32<br>**EF : WT, 1h** 70.4±3.4<br>**Null, 1h** 72.2±3.4<br>**Vcf: Null, 4h** 11.91±1.00 | 300 mg/kg | 4 hours | (Conklin et al, 2015) |
| **9.** | wild-type (WT) mice and Mrp1 null ($Mrp1^{-/-}$) littermates | Doxorubicin | **FS** : 20.0%–24.3% Mrp1 versus 23.7%–29.5% WT Protocol A<br>**EF** : 41.5%–48.4% Mrp1 versus 47.7%–56.7% WT Protocol B | **protocol A** total dose: 18 mg/kg<br>**protocol B** total dose: 20 mg/kg | 5 weeks | (Zhang et al, 2015 b) |
| **10.** | Mice | N-Acetyl Cysteine Amide in the Prevention of Doxorubicin and Trastuzumab –Mediated Cardiac | **LVEF:**<br>**DOX** from 73±4% to 43±2%<br>**DOX+TRZ** from 72±3% to 32±2%<br>**NACA +/or DOX + TRZ** 62±3% and 55±3% | injection: (i) 0.9% saline; (ii) NACA (250mg/kg); (iii) DOX (20mg/kg); (iv) TRZ (10mg/kg); (v) DOX (20mg/kg) + TRZ (10mg/kg); (vi) NACA (250mg/kg) + DOX (20mg/kg); (vii) NACA (250mg/kg) + TRZ (10mg/kg); and | 10 days | (Goyal et al, 2016) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Dysfunction | | (viii) NACA (250mg/kg) + DOX (20mg/kg) + TRZ (10mg/kg) | | |
| 11. | Rats | Angiotensin Type I Receptor Antagonist, Fimasartan, Prevents Doxorubicin-induced Cardiotoxicity | **LV EF (%) :** **Control** 74.6 ± 3.7 **Dox-only** 54.6 ± 8.4 **Low-fima** 67.9 ± 5.3 **High-fima** 71.4 ± 6.3 | **DOX** : 500 mg per $m^2$ **Fimasartan (low - fima)** : 5 mg/kg **Fimasartan(high - fima) :** 10mg/kg | 8 weeks | (Chang et al, 2015) |
| 12. | Mice | Renin angiotensin system antagonists in the prevention of doxorubicin and trastuzumab induced cardiotoxicity | **LVFS ii :** from 51 ± 1% at baseline to 32 ± 2% **Bii :** 45 ± 1% **Cii** : 38 ± 1% **Dii** : 37 ± 2% **LVFS iii :** from 52 ± 2% at baseline to 26 ± 2% **Biii** : 40 ± 1% **Ciii** : 32 ± 1% **Diii** : 33 ± 2% | **(A) placebo** **(B)Aliskiren**: (50 mg/kg) **(C)Perindopril**: (3 mg/kg) **(D)Valsartan**:( 10 mg/kg) **+** **(i) TRZ** 4 mg/kg weekly (i.p.) **(ii) DOX** 4 mg/kg weekly, i.p **(iii) DOX+TRZ** | 13 weeks | (Akolkar et al, 2015) |
| 13. | Rats | Ginsenoside Rg3 antagonizes adriamycin-induced cardiotoxicity by improving endothelial dysfunction | **EF Rg3 40mg/kg :** 85,45% **FS Rg3 40mg/kg :** 56.04% | **Adriamycin** (doxorubicin hydrochloride) : 15mg/kg **Ginsenoside** Rg3 (ADM + 10/20/40 mg/kg daily) | 14 days | (Wang et al, 2015) |
| 14. | pregnant Wistar rats at | Cardiotoxicity in Response to Maternal Doxorubicin Treatment | **FS 20mg/kg** decreased by 5–10% 20-mg The umbilical systolic peak velocity and the ductus venosus Doppler measurements did not show significant differences between the groups | 10 or 20 mg/kg i.v. doxorubicin to pregnant Wistar rats at day 18 of pregnancy | 48 hours | (Gziri et al, 2013) |
| 15. | Wistar Rats | Bortezomib induced cardiotoxicity | **EF** 40% **FS** 34% | Bortezomib 0.2mg/kg three times/week | 3 weeks | (Nowis et al, 2010) |
| 16. | Sprag | Atorvastatin | **EF % :** | **E1** (10 | 6 | (Zhan |

| | | | | | | |
|---|---|---|---|---|---|---|
| | ue-Dawley rats | Ameliorates Radiation-Induced Cardiac Fibrosis | **Control** 80.13 ± 7.32<br>**Radiation only** 64.47 ± 11.56<br>**E1** 72.75 ± 6.91<br>**E2** 72.92 ± 10.88<br>**E3** 74.92 ± 10.88<br>**E4** 80.47 ± 4.02<br><br>**FS %**<br>**Control** 44.17 ± 5.99<br>**Radiation only** 33.13 ± 3.72<br>**E1** 38.24 ± 6.84<br>**E2** 39.10 ± 3.61<br>**E3** 39.93 ± 6.39<br>**E4** 41.93 ± 6.19 | mg/kg/day) and **E2** (20 mg/kg/day) atorvastatin and radiation **E3** (10 mg/kg/day) and **E4** (20 mg/kg/day) atorvastatin from 3 months before irradiation to week 12 after irradiation | months | g et al, 2015a) |
| **17.** | C57BL/6 Mice | NC-6300 reduces the cardiotoxicity of epirubicin | **EF %**<br>**EPI :** 48%<br>**NC-6300 :** 70%<br><br>**FS%**<br>**EPI** : 25%<br>**NC-6300** : 40% | **NC-6300** (10, 15 mg/kg) **Epirubicin** (10 mg/kg) | 12 weeks | (Takahashi et al, 2013) |
| **18.** | Beagle dogs | **cardiotoxicity** of minoxidil | **EF %**<br>**Control :** 65%<br>**0,5 minoxidil:** 84%<br>**2 minoxidil:** 89% | 0.5 or 2 mg/kg minoxidil | 48 hours | (Hanton et al, 2004) |
| **19.** | Sprague Dawley rats | testosterone and dehydroepiandrosterone induce cardiotoxicity | **Control EF% :** 67.80 ± 1.68, **FS%**: 33.00± 0.70<br>**DHEA EF%:** 64.00 ± 1.89, **FS%**: 32.40 ± 0.81<br>**T 10 mg/100 g EF%:** 76.33± 4.33, **FS%:** 42.00 ± 3.05<br><br>**T 30 mg/100 g EF%**: 70.60 ± 1.88, **FS%:** 36.20 ± 1.15<br>**T 100 mg/100 g EF% :** 72.25 ± 1.43, **FS% :** 36.00 ± 0.81 | **Testosterone** (10, 30, and 100 mg/100 g body weight) **DHEA** (10 mg/100 g body weight) | 3 months | (Emer et al, 2016) |
| **20.** | Rats | Cardioprotective effects of rosuvastatin in Dox induced cardiotoxicity | **FS%**<br>**I :** 50.0 ± 0.7<br>**II :** 39.7 ± 1.7<br>**III :** 40.5 ± 3.0<br>**IV :** 42.6 ± 2.6<br>**V :** 37.9 ± 2.2<br>**VI :** 34.9 ± 2.8<br><br>**LVESD (mm)**<br>**I :** 3.6 ± 0.09<br>**II :** 4.5 ± 0.09 | **Croup I** control **Group II**: doxorubicin 1.25 mg/kg **Group III**: doxorubicin + rosuvastatin 2 mg/kg/day, **Group IV**:doxorubicin + rosuvastatin 10 mg/kg/day, **GroupV**:doxorubi | 8 weeks | (Kim et al, 2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | **III :** 4.6 ± 0.22<br>**IV :** 4.2 ± 0.25<br>**V :** 4.6 ± 0.24<br>**VI :** 4.9 ± 0.23 | cin + carvedilol 5 mg/kg/day,<br>**Group VI**:doxorubicin + carvedilol 10 mg/kg/day | | |
| **21.** | Wistar rats | Oleuropein prevents doxorubicin-induced cardiomyopathy | **Control: FS%**69.6 (1.4), **PWTdmm** 2.8 (0.1)<br>**OLEU-1: FS%**67.4 (2.3), **PWTdmm** 2.7 (0.0)<br>**OLEU-2: FS%**69.1 (4.2), **PWTdmm** 2.7 (0.1)<br>**DXR: FS%**3.5 (2.5), **PWTdmm** 1.6 (0.1)<br>**OLEU-1-DXR: FS%**67.1 (1.6), **PWTdmm** 2.4 (0.2)<br>**OLEU-2-DXR: FS%**68.1 (2.0), **PWTdmm** 2.5 (0.4) | **Control group**: 2 ml of normal saline<br>**OLEU-1**: 1000mg/kg oleuropein<br>**OLEU-2**: 2000mg/kg oleuropein<br>**DXR group**: 8mg/kg DXR | 2 weeks | (Andreadou et al, 2014) |
| **22.** | Sprague–Dawley rats | telmisartan in limiting the cardiotoxic effect of daunorubicin | **Control EF%:** 83.4 ± 3.47, **FS%:** 47.5 ± 3.24<br>**DNR EF%:** 49.44± 7.02, **FS%:** 22.3± 3.84<br>**TELM EF%:** 64.87 ± 8.23, **FS%:** 32.63 ± 6.3 | **Control**<br>**DNR:** 3 mg/kg/day every other day<br>**Telm:** (10 mg/kg/day | 12 days | (Aroz al et al, 2010) |
| **23.** | Fisher 344 rats | metronomic treatment with cyclophosphamide | **DOX LVEF** 12% decrease<br>**CPA LVEF** 2% decrease | **Control**<br>**CPA** 150 mg/kg<br>**DOX** 12 mg/kg | 6 weeks | (Todorova et al, 2011) |
| **24.** | C57Bl/6 mice | Probucol against anthracycline and trastuzumab-mediated cardiotoxicity | **Control FS%:** 53 6 2, **LVEDDmm:** 3.2± 0.1<br>**DOX FS%:** 42 ± 2, **LVEDDmm**: 3.8± 0.2<br>**TRZ FS%:** 52± 3, **LVEDDmm**: 3.2± 0.1<br>**DOX + TRZ FS%:** 35 ± 3, **LVEDDmm**: 4.1 ± 0.2<br>**PROB + DOX FS%:** 47 ± 2, **LVEDDmm**: 3.5± 0.1<br>**PROB + DOX + TRZ FS%:**44±2, **LVEDDmm:** 3.6± 0.1 | **Control** 0.9% saline<br>**Prob** 15mg/kg<br>**Dox** 20 mg/kg<br>**Trz** 10 mg/kg | 24 days | (Walker et al, 2011) |
| **25.** | C57BL/6J mice | Anti-Fas Gene Therapy | **Control FS% :** 40%<br>**LacZ gene FS%:** | **DOX** 15 mg/kg<br>**sFas** gene or | 2 weeks | (Miyata et |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Prevents Doxorubicin-Induced Acute Cardiotoxicity | 28% | **LacZ** gene | | al, 2010) |
| **26.** | Wistar rats | Crocin treatment prevents doxorubicin-induced cardiotoxicity | **Control EF%:**70.19 ± 1.71, **FS%**:33.31 ± 1.24 **DOX EF%:**50.03 ± 5.10, **FS%**:21.16 ± 2.88 **CRO 20 EF%:**65.50 ± 1.50, **FS%**:29.94 ± 1.02 **CRO 40 EF%:**68.07 ± 3.01,**FS%**:31.98 ± 2.11 | **Group 1** :Control **Croup 2:**DOX 2mg/kg/48 h **Group 3:**DOX 2mg/kg+CRO 20 mg/kg/24 h **Group 4**: DOX 2mg/kg + CRO 40mg/kg | 20 days | (Razmarai et al, 2016) |
| **27.** | Wistar albino rats | Cardioprotective effect of metformin against doxorubicin cardiotoxicity | **Control EF%:** 77.76±4.95, **FS%:** 41.32±4.47 **DOX EF%:**60.92±9.25, **FS%:**28.76±6.16 **MET EF%:** 75.71±6.13, **FS%:**39.60±5.57 **DOX+MET EF%:**71.62±5.40, **FS%:**36.03±4.31 | **Control**: saline twice a week **DOX:** 4mg/kg twice a week **Met:** 250mg/kg/day | 2 weeks | (Argun et al, 2016) |
| **28.** | Sprague-Dawley rats | Anandamide in an acute doxorubicin cardiotoxicity | **CON+SAL FT(ms):** 58 + 3 **, ET(ms):** 63 + 3 **CON+DOX FT(ms):** 78 + 5, **ET(ms):** 60 + 3 **DOX+ANAN FT (ms):** 67 + 3, **ET(ms):** 63 + 3 | **Group1 :** ANAN + DOX (30 mg $kg^{-i}$ Anan to 10mg $kg^{-i}$) **Group2:** CON+DOX **Group3:** ANAN+Saline **Group4**: ANAN+DOX | 5 days | (Hydock et al, 2009) |
| **29** | Sprague-Dawley rats | Cardioprotective effect of dexrazoxane | **MI EF%:** 41.2± 11.5, **FS%:**17.9± 6.3 **MI+DZR EF%:** 52.0± 9.8**, FS%:**23.6± 5.7 **DZR EF%:** 77.0± 4.0, **FS%:**40.9± 3.6 | **DZR group:** 125mg/kg | 4 weeks | (Zhou et al, 2011) |
| **30.** | Wistar albino | Resveratrol treatment | **Group1 EF%:**63.8± | **Group1:** saline+doxorubi | 7 weeks | (Tatli |

| | | | | |
|---|---|---|---|---|
| rats | protects against doxorubicin-induced cardi otoxicity | 2.6, **FS%:** 23.6± 1.9 **Group2 EF%:**75.4± 1.5, **FS%:**37.2± 3.4 | cin (DOX; 20 mg/kg) **Group2:** RVT (10 mg/kg)+DOX (20mg/kg) | dede et al, 2009) |

*LVPW, left ventricular posterior wall (diastole)

** LVEF: Left ventricular ejection fraction

*** LVFS: Left ventricular fractional shortening

ˣ PWRT: left ventricular posterior wall relative thickness (2xposterior wall thickness/LVDD)

+ LVDD: left ventricular diastolic diameter

ₓ PWT: posterior wall thickness

~ Vcf, velocity circumferential fiber shortening (circ/s)

### 5.1 Doxorubicin (DOX) - Anthracycline induced cardiotoxicity

DOX-treated animals showed a significant ($p<0.001$) decrease between days 1 and 11, with an average FS change of −13.9%; whereas control animals did not undergo a significant change in fractional shortening (average change in FS = 0.20%)

### 5.2 Imatinib Mesylate induced cardiotoxicity

Mice treated with imatinib showed a reduced LV posterior wall thickness which was further confirmed by histological examination

### 5.3 Ramipril and darbepoetin (erythropoietin hormones) in doxorubicin-induced cardiotoxicity

Forty 6-month-old Sprague–Dawley male rats were divided into 5 groups. The Control group had no medication. The DOX group received 2.5 mg/kg i.v. DOX hydrochloride (Adriblastina, Pfizer Inc.) from tail veins weekly for 3w. The DOX + RAM group received 2.5 mg/kg i.v. DOX plus 1 mg/kg p.o. ramipril (Delix, Pharma Vision) via gavage daily for 4w. The DOX + DP group received 2.5 mg/kg i.v. DOX plus 10 μ/ kg i.p. darbepoetin alfa (Aranesp, Amgen Inc.) weekly for 3w. The DOX + RAM + DP group had 2.5 mg/kg i.v. DOX plus the same amount and duration of ramipril and darbepoetin. Sampling and measurement were obtained at the 4th week.

The animals, induced cardiotoxicity, and have shown anaemia, systolic dysfunction and repolarization abnormalities based on the results of haematology, echocardiography and ECG, respectively. It is concluded that anaemia and systolic dysfunction are the main problems of DOX cardiotoxicity and solving these problems should be the main goals of the therapy. Therefore, administration of weekly DP

injection and daily oral RAM together may have significant potentials to improve heart conduction abnormalities and anaemia but not the systolic dysfunction caused by DOX toxicity during same period of treatments.

### 5.4 Doxorubicin and cardiac function improvement after stem cell therapy and flavonoid extract treatment

Different therapy strategies, such as flavonoid plant extracts and stem cells, have been investigated to improve heart function in toxic cardiomyopathy. This work aimed to assess early cardiac function improvement after treatments with either flavonoid extract from *Camellia sinensis* or mesenchymal stem cells in Dox cardiotoxicity using strain echocardiography. Twenty Wistar rats were randomly assigned to four groups. They received water (control, Dox, Dox + stem cells) or 100 mg/kg *C. sinensis* extract (Dox + *C. sinensis*) via gavage, daily, for four weeks. Animals also received saline (control) or 5 mg/kg doxorubicin (Dox, Dox + *C. sinensis*, Dox + stem cells) via intraperitoneal injection, weekly, for four weeks. Stem cells were injected ($3 \times 10^6$ cells) through tail vein prior the beginning of the experiment (Dox + stem cells).

Animals were randomly categorized into four groups: control (distilled water orally (P.O.) daily; saline intraperitoneal (I.P.) weekly); Dox (distilled water P.O. daily; 5 mg/kg Dox I.P. weekly); Dox + *C. sinensis*(100 mg/kg *C. sinensis* extract P.O. daily; 5 mg/kg Dox I.P. weekly); Dox + MSC (distilled water P.O. daily; 5 mg/kg Dox IP weekly; MSC via intravenous injection). Distilled water and plant extract (1 ml) were given through gavage procedure, which was first performed three days prior, the beginning of the experiment (first Dox injection). The *C. sinensis* extract used contains polyphenols > 80%, catechins > 80%, epigallocatechin > 45%, and caffeine < 1% detected by high performance liquid chromatography analysis as informed by the manufacture. The MSCs were isolated from adipose tissue of Lewis LEW-Tg (EGFP) F455.5/Rrrc rats, which were obtained from the Rat Resource and Research Center (Missouri, USA).

#### Echocardiographic indices

Echocardiography examination showed left ventricular dysfunction in Dox group, compared to control ($p<0.05$), indicating that cardiotoxicity was effectively induced in the sample studied. Injection of MSC significantly promoted left ventricular function with respect to untreated Dox group. Radial velocity aand radial displacement parameters were significantly decreased in Dox and Dox + *C. sinensis* groups. On the other hand, animals from Dox + MSC group showed similar measurements to the control group.

### 5.5 Pamidronate Attenuates Oxidative Stress in Acute Doxorubicin-Induced Cardiotoxicity

Recent studies have shown that bisphosphonates can decrease oxidative stress. Therefore, the objective of this study was to evaluate the effect of pamidronate

on preventing acute doxorubicin-induced cardiotoxicity. The rats were allocated in four groups: the control group (C), the doxorubicin group (D), the pamidronate group (P) and the doxorubicin/pamidronate group (DP). Prior to the experiment, all animals were submitted to echocardiography. The rats allocated in the P and DP groups received a single dose of pamidronate (3 mg/kg, IP) and the C and D groups received sterile saline injections. Twenty-four hours after pamidronate injection, the rats allocated in the D and DP groups received a single dose of doxorubicin (20 mg/kg, IP), and the C and P groups received sterile saline injections.

### Echocardiographic indices

Doxorubicin treatment caused a decrease in the left atrium diameter and the left ventricular diastolic diameter and an increase in the left ventricular posterior wall relative thickness (PWRT). In the DP group we observed a decrease in the PWRT compared with the D group.

## 5.6 Daunorubicin induced cardiotoxicity

The cardiotoxicity was induced in a well-established schedule in male Chinchilla rabbits by repeated administration of daunorubicin (DAU, 3 mg/kg i.v., n=16, Daunoblastina, Pfizer, Rome, Italy) once weekly for ten weeks, whereas animals in the control group received saline (1 mL/kg i.v., n=16) in the same schedule. A week after the last administration (*i.e.*, at the end of the treatment period), the animals in control and DAU group were randomized to sacrifice (n=8 in each group) or to follow up for additional 10 weeks (follow up period, n=8 in each group). In the follow up period LV fractional shortening lower than 20% was taken as evidence for severe (decompensated) heart failure.

## 5.7 (alpha) tocopherol (vitamin E) induces cardiotoxicity

The present study was undertaken to evaluate the effect of pharmacological dose of a-tocopherol on heart from animals without previous cardiac disease. In addition, a-tocopherol uptake in the target tissue, heart, has been determined in the current study. a-Tocopherol dissolved in corn oil or corn oil (Mazola 1, Sa˜o Paulo, SP, Brazil) was administered daily by gavage every morning for the entire 7-wk period. The a-tocopherol supplement mixed with corn oil (250 mg a-tocopherol/[kg body wt/day]) was given to E group, while the C group received only corn oil.

### Echocardiographic indices

Echocardiographic examination allowed the evaluation of cardiac remodelling using morphological and functional variables, which are presented in Tables 2 and 3. Pharmacological dose of a-tocopherol was associated with significant decrease in left ventricular end-diastolic diameter (LVD) and aortic diameter (Ao), left ventricle fractional shortening (FS), ejection fraction (EF) and transmitral flow early peak velocity (E). There were no echocardiographic changes in the control group. The

current study indicates a cardiac harmful effect of pharmacological dose of a-tocopherol even under healthy conditions.

### 5.8 Cyclophosphamide induced cardiotoxicity in WT and GSTP null mice

Cyclophosphamide (CY; Cytoxan®) is a widely used anti-neoplastic drug for the treatment of lymphomas, brain cancer and leukemia, and as a preparatory treatment for bone marrow transplant. Because of its immunosuppressive activity, it is also frequently used for the treating autoimmune diseases, amyloidosis, idiopathic nephritis, severe rheumatoid arthritis and multiple sclerosis. Several of these treatment regimens require high doses of CY that are associated with significant side effects such as bone marrow suppression, and hemorrhagic cystitis that vary in incidence, manifestation and severity. However, some of the most serious side effects of high dose CY-containing regimens are associated with the acute cardiotoxic effects of the drug that usually manifest as endothelial damage followed by extravasation of toxic metabolites, and myocyte damage leading to diastolic contractile dysfunction. Frequent occurrences of acute fulminant congestive heart failure, hemorrhagic myopericarditis and sudden death have also been reported. Even though cardiac complications of CY therapy have declined due to recent adoption of multifractionated schedule of administration, the incidence of the cardiotoxic effects of CY treatment remains high. High dose CY infusion induces reversible stage 3 heart failure in 10% of metastatic breast cancer patients with a median decline in ejection fraction of 31% and nearly 40% of patients undergoing pediatric allogenic hematopoietic stem cell transplantation treated with high dose CY experience cardiac complications. In adult populations treated with CY, mortality rates up to 20% (breast cancer trial) have been reported Nevertheless, the mechanism of CY cardiotoxicity remains unclear, and there is an urgent need to better understand the acute cardiac effects of CY in order to treat or minimize cardiotoxicity of high dose CY chemotherapy.

#### Echocardiographic indices

To examine the selective effects of CY on cardiac injury, they measured cardiac function by echocardiography at 1h and 4h post-CY (300 mg/kg) in WT and GSTP-null mice. CY modestly, yet significantly, altered cardiac dimensions and function in both WT and GSTP-null mice with most changes occurring at 1h, and then diminishing or resolving by 4h post-CY. For example, CY significantly increased the velocity of circumferential fiber shortening (Vcf and Vcfc, i.e., the latter is corrected for heart rate) in GSTP-null, but not WT, hearts at 1h, an effect that was statistically insignificant ($p=0.087$) at 4h post-treatment. In contrast, CY-induced a significant increase in fractional change in area (FAC; %) in both WT and GSTP-null at 1h post-CY that was a function of decreased end systolic area (ESA, $mm^2$) in both WT and null mice. The effects of CY on ESA and FAC were absent in both WT and GSTP-null hearts at 4h post-treatment indicating this effect was reversible. This observation is consistent with clinical literature showing that high-dose CY associated cardiac

toxicity is potentially reversible. Surprisingly, ejection fraction (EF) was slightly increased in WT mice at both 1h and 4h post-CY yet unaffected in GSTP-null hearts (p=0.053; 1h only). Increased EF was likely due to decreased end systolic volume (ESV) at 1h (p=0.014) and 4h (p=0.062) in WT hearts, and ESV reflects more complete emptying of LV in WT hearts than in GSTP-null (p=0.10) mice. Ejection time (ET, a measure of aortic blood flow velocity) was significantly decreased in GSTP-null hearts only at 4h post-CY. These data indicate that GSTP-null hearts were more sensitive to acute cardiac injury due to a rapid-onset and reversible form of altered contractility than that observed in WT mice.

### 5.9 Doxorubicin induced cardiotoxicity in wild-type (WT) mice and Mrp1 null (Mrp1$^{-/-}$) littermates

Doxorubicin (DOX), an effective cancer chemotherapeutic agent, induces dose-dependent cardiotoxicity, in part due to its ability to cause oxidative stress. We investigated the role of multidrug resistance–associated protein 1 (Mrp1/Abcc1) in DOX-induced cardiotoxicity in C57BL wild-type (WT) mice and their Mrp1 null (Mrp1$^{-/-}$) littermates. Male mice were administered intraperitoneal DOX (3 or 2 mg/kg body weight) or saline twice a week for 3 weeks and examined 2 weeks after the last dose (protocol A total dose: 18 mg/kg) or for 5 weeks, and mice were examined 48 hours and 2 weeks after the last dose (protocol B total dose: 20 mg/kg). Chronic DOX induced body weight loss and hemotoxicity, adverse effects significantly exacerbated in Mrp1$^{-/-}$ versus WT mice. In the heart, significantly higher basal levels of glutathione (1.41-fold ± 0.27-fold) and glutathione disulfide (1.35-fold ± 0.16-fold) were detected in Mrp1$^{-/-}$ versus WT mice, and there were comparable decreases in the glutathione/glutathione disulfide ratio in WT and Mrp1$^{-/-}$ mice after DOX administration.

#### Echocardiographic indices

DOX induced comparable increases in 4-hydroxynonenal glutathione conjugate concentration in hearts from WT and Mrp1$^{-/-}$ mice. However, more DOX-induced apoptosis was detected in Mrp1$^{-/-}$ versus WT hearts ($P < 0.05$) (protocol A), and cardiac function, assessed by measurement of fractional shortening and ejection fraction with echocardiography, was significantly decreased by DOX in Mrp1$^{-/-}$ versus WT mice ($P < 0.05$; 95% confidence intervals of 20.0%–24.3% versus 23.7%–29.5% for fractional shortening, and 41.5%–48.4% versus 47.7%–56.7% for ejection fraction; protocol B). Together, these data indicate that Mrp1 protects the mouse heart against chronic DOX-induced cardiotoxicity.

### 5.10 N-Acetyl Cysteine Amide in the Prevention of Doxorubicin and Trastuzumab–Mediated Cardiac Dysfunction

N-acetyl cysteine amide (NACA), is a thiol structural analog of NAC, with increased lipophilicity and bioavailability, allowing it to traverse the cellular lipid bilayer. Previous investigations into the anti-oxidant properties of NACA have demonstrated that the compound is able to: i) scavenge free radicals and protect red blood cells from OS; ii) protect the blood brain barrier from OS in mice exposed to methamphetamine; and iii) reduce the levels of OS in embryonic rat cardiomyocytes exposed to DOX in vitro. The anti-oxidant properties of NACA from these previous studies provide a strong rationale to investigate whether it may provide cardioprotection against DOX+TRZ mediated cardiotoxicity in the in vivo setting.

#### Echocardiographic indices

In mice receiving DOX, left ventricular ejection fraction (LVEF) decreased from 73±4% to 43±2% at day 10. In mice receiving DOX+TRZ, LVEF decreased from 72±3% to 32±2% at day 10. Prophylactic administration of NACA to mice receiving DOX or DOX+TRZ was cardio-protective, with a LVEF of 62±3% and 55±3% at day 10, respectively. Histological and biochemical analyses demonstrated loss of cellular integrity, increased oxidative stress (OS), and increased cardiac apoptosis in mice treated with DOX+TRZ which was attenuated by the prophylactic administration of NACA. Conclusions: NACA attenuated the cardiotoxic side effects of DOX+TRZ in a murine model of chemotherapy induced cardiac dysfunction by decreasing OS and apoptosis.

### 5.11 Angiotensin Type I Receptor Antagonist, Fimasartan, Prevents Doxorubicin-induced Cardiotoxicity

Angiotensin receptor blockers (ARBs) have organ-protective effects in heart failure and may be also effective in doxorubicin-induced cardiomyopathy (DOX-CMP); however, the efficacy of ARBs on the prevention of DOX-CMP have not been investigated. We performed a preclinical experiment to evaluate the preventive effect of a novel ARB, fimasartan, in DOX-CMP. All animals underwent echocardiography and were randomly assigned into three groups: treated daily with vehicle (DOX-only group, n=22), 5 mg/kg of fimasartan (Low-fima group, n=22), and 10 mg/kg of fimasartan (High-fima group, n=19). DOX was injected once a week for six weeks. Echocardiography and hemodynamic assessment was performed at the 8th week using a miniaturized conductance catheter. Survival rate of the High-fima group was greater (100%) than that of the Low-fima (75%) and DOX-only groups (50%). Echocardiography showed preserved left ventricular (LV) ejection fraction in the High-fima group, but not in the DOX-only group ($P$=0.002). LV dimensions increased in the DOX-only group; however, remodeling was attenuated in the Low-fima and High-fima groups. Hemodynamic assessment showed higher dP/dt in the High-fima group compared with the DOX-only group. A novel ARB, fimasartan, may

prevent DOX-CMP and improve survival rate in a dose-dependent manner in a rat model of DOX-CMP and could be a treatment option for the prevention of DOX-CMP.

### Echocardiographic indices

Echocardiographic data showed progressive LV systolic dysfunction and dilation of the LV cavity in the DOX-only group. Specifically, LVESD at six weeks was maintained until end of the study in both the High-fima (4.94±1.08 mm) and Low-fima (4.95±0.52 mm) groups; however, LVESD in the DOX-only group was significantly increased compared with the fimasartan-treated groups (56±0.6 mm, $P$=0.009 by ANOVA). Furthermore, LVEF was preserved in the High-fima group (71.4%±6.3%) and slightly decreased in the Low-fima group (67.9%±5.3%) compared with the NC group (74.6%±3.7%). In contrast, LVEF was significantly decreased in the DOX-only group (54.6%±8.4%, $P$<0.001 by ANOVA).

## 5.12 Renin angiotensin system antagonists in the prevention of doxorubicin and trastuzumab induced cardiotoxicity.

The objective of the current study was to determine whether inhibition of the RAS pathway at three distinct levels, including direct renin inhibition (DRI; Aliskiren), angiotensin converting enzyme inhibition (ACEI; Perindopril), and angiotensin receptor blockade (ARB; Valsartan) can prevent cardiac dysfunction in a chronic *in vivo* model of DOX+TRZ mediated cardiotoxicity.

The pathogenesis of DOX+TRZ mediated cardiotoxicity is multifactorial, there is accumulating evidence to suggest the RAS as a key contributor. Angiotensin II plays an important role as a vasoconstrictor agent and a mitogenic factor by interacting with the $AT1_R$ in cardiovascular myocytes. Although DOX induces myofibrillar loss, apoptosis, and significantly impairs contractile function in WT mice, this drug induced cardiotoxicity was not observed in $AT1_R$-knockout mice or in animals treated with $AT1_R$ blockade. These findings suggest a contributory role for the RAS pathway in the development of DOX mediated cardiotoxicity. The administration of the ARB Telmisartan (10 mg/kg/day for 7 days), was cardioprotective in rats treated with a single dose of 20 mg/kg DOX, by decreasing lipid peroxidation, GSH depletion, and oxidative stress. Additionally, in a chronic rat model of DOX induced cardiotoxicity (DOX 25 mg/kg for a 6 week period), Enalapril served to preserve mitochondrial function, down-regulate free radical production, and preserve overall cardiac function. Finally, in a chronic rat model of DOX (15 mg/kg) mediated cardiac dysfunction, the prophylactic administration of either ACEI (Captopril 60 mg/kg) or ARB (Telmisartan 10 mg/kg) proved to be cardioprotective . This was evidenced by a significant reduction in the concentration of cardiac biomarkers and oxidative stress, and by the maintenance of characteristic cardiac histology in rats pre-treated with RAS antagonism. Our current study adds to the existing literature which supports the cardioprotective capabilities of RAS antagonism in a chronic murine model of chemotherapy induced cardiac dysfunction. Our study is the first to also demonstrate

the potential cardioprotective role of direct renin inhibition, specifically Aliskiren, in preventing DOX mediated cardiotoxicity. Additionally, although previous animal studies have focussed on a DOX only model, we have extended our important findings to a combined DOX+TRZ model, mimicking the clinical setting of breast cancer.

## Experimental protocol

A total of 240 C57Bl/6 male mice were randomized to one of the following prophylactic treatment arms: (A) placebo (saline; n = 60); (B) Aliskiren (50 mg/kg; n = 60); (C) Perindopril (3 mg/kg; n = 60); or (D) Valsartan (10 mg/kg; n = 60) (Figure 1). RAS antagonists were administered orally by gavage on a daily basis for the entire study period of 13 weeks. Furthermore, mice from each prophylactic treatment arm were randomized to one of the following chemotherapeutic regimens: (i) TRZ (4 mg/kg weekly, intraperitoneal (i.p.); n = 20); (ii) DOX (4 mg/kg weekly, i.p.; n = 20); or (iii) DOX+TRZ (n = 20) (Figure 1). TRZ, DOX, or DOX+TRZ injections were initiated at week 2, following 2 weeks of prophylactic treatment with a RAS antagonist or placebo, and continued for 5 weeks (Figure 2). The cumulative doses of DOX or TRZ achieved were the minimum concentration to induce a chemotherapy mediated cardiomyopathy, as previously validated by our group and others [26-28]. Cardiac function was evaluated over the course of the study via serial murine echocardiography. Mice were imaged at baseline and weekly until euthanization at week 13.

## Echocardiographic indices

Echocardiographic indices, including HR, PWT, LVID, LVFS, and LVEF were within normal physiologic limits for all mice at baseline. No significant change from baseline value was observed for HR or PWT at week 13 in any of the treatment arms. In C57Bl/6 mice treated with TRZ, there were no significant changes in LV cavity dimensions as compared to baseline. Mice treated with DOX alone demonstrated a significant increase in cavity dimensions, as the LVID increased from $3.2 \pm 0.1$ mm at baseline to $4.5 \pm 0.2$ mm at week 13 ($p < 0.05$). Mice receiving DOX+TRZ treatment demonstrated a similar increase in LVID from $3.1 \pm 0.2$ mm at baseline to $4.6 \pm 0.3$ mm at week 13 ($p < 0.05$). Pre-treatment with Aliskiren, Perindopril, and Valsartan significantly reduced LVID in mice administered DOX alone from $4.5 \pm 0.2$ mm to $3.6 \pm 0.2$ mm, $3.9 \pm 0.2$ mm, and $4.0 \pm 0.2$ mm, respectively, at week 13 ($p < 0.05$). Similarly, in mice treated with DOX+TRZ, the prophylactic administration of Aliskiren, Perindopril, and Valsartan significantly reduced LVID at week 13 from $4.6 \pm 0.3$ mm to $3.9 \pm 0.2$ mm, $4.1 \pm 0.2$ mm, and $4.2 \pm 0.1$ mm, respectively, ($p < 0.05$).

LVFS did not change from baseline values in mice administered TRZ over the course of the 13 week study. In mice treated with DOX alone, LVFS decreased from $51 \pm 1\%$ at baseline to $32 \pm 2\%$ at week 13 ($p < 0.05$). Pre-treatment with Aliskiren, Perindopril, and Valsartan attenuated DOX induced LV impairment, improving LVFS values to $45 \pm 1\%$, $38 \pm 1\%$, and $37 \pm 2\%$, respectively, at week 13 ($p < 0.05$). In mice treated with the combination of DOX+TRZ, LVFS further decreased from $52 \pm 2\%$ at

baseline to 26 ± 2% at week 13 ($p < 0.05$). Prophylactic treatment with Aliskiren, Perindopril, and Valsartan significantly improved LVFS to 40 ± 1%, 32 ± 1%, and 33 ± 2%, respectively, at week 13 ($p < 0.05$).

### 5.13 Ginsenoside Rg3 antagonizes adriamycin-induced cardiotoxicity by improving endothelial dysfunction from oxidative stress via upregulating the Nrf2-ARE pathway through the activation of akt

Ginseng, the root of Panax ginseng C.A. Meyer, is well known in herbal medicine as a tonic and restorative agent. The main ingredient responsible for the actions of ginseng is the ginsenoside. Ginsenoside Rg3 (Rg3), an active ingredient of Panax ginseng, is a commercially available ginsenoside (Shenyi Capsule) that is used to inhibit and prevent cancers. Rg3 has been shown to increase the efficacy of cancer chemotherapy with anti-carcinogenic and anti-metastatic processes. They also found that Rg3 could inhibit the cell viability and proliferation on human breast adenocarcinoma cells (MCF-7) with or without ADM (Supplementary files). Rg3 reportedly displays antioxidative and cardioprotective effects. These findings provide evidence that Rg3 might have potential therapeutic effects for cardiac damage caused by ADM-induced oxidative stress.

Adriamycin (doxorubicin hydrochloride, 99%, Solarbio, Beijing, China) was administered via a single intraperitoneal dose of 15 mg/kg in the ADM and ADM/Rg3 group. The control group received normal saline. One hour after the injection, ginsenoside Rg3 (10, 20, 40 mg/kg daily, > 98%, Mansite, Chengdu, China) was injected intraperitoneally in the ADM/Rg3 groups. Rats in the ADM group were treated with saline instead. The cardiac function was evaluated with echocardiography at baseline before the ADM treatment and 3, 7, and 14 days after the ADM injection. Rats were anesthetized using 3% inhalant isoflurane in 100% oxygen and maintained in 1.5–2% isoflurane during the echocardiogram testing. M-mode, B-mode and left ventricular outflow tract (LVOT) ultrasound images were obtained by a Vevo 2100 ultrasound system (Visualsonics, Toronto, Canada) using 8 rats for each observation (total of 40 rats).

#### Echocardiography indices

They examined the effect of the combination treatment with Rg3 using an in vivo rat model of ADM-induced cardiotoxicity. The rats were treated with ADM or a combination of ADM and Rg3 (10, 20, and 40 mg/kg) for 14 days and examined by M-mode and LVOT echocardiography. The ejection fraction (EF) and fractional shortening (FS) were significantly decreased in the rats given ADM treatment alone. Co-treatment with Rg3 significantly increased the EF and FS to maximums of 85.45% and 56.04% ($P = 0.000$ and 0.011 by 40 mg/kg Rg3 at the 14th day. ADM induced a significant increase in the left ventricle (LV) mass, and 40 mg/kg Rg3 could dramatically inhibit the LV mass increase ($P = 0.038$). On Fig. 2. Testing the endothelium function by an aortic ring assay. ADM or ADM/Rg3 (20 mg/kg) treated

S.D. rats were killed on the 14th day after the ADM-injection, and the thoracic aortas were rapidly dissected out for the following assay. The aorta rings were pre-treated with 1 μM NE to achieve a plateau phase, and then 10 μM Ach was added to induce vasodilation. Parallel experiments were performed on control rats with intact endothelium or mechanically denuded endothelium. (A) Vascular tone after NE and Ach treatment. The maximal constriction appeared at 6 min after the NE incubation, and then Ach was added to achieve vasodilation. (B) Maximal constriction and dilation by NE and Ach. The ADM and endothelium-denuded aortas showed increased contraction and decreased dilatation than the intact endothelium aortas. Cotreatment with Rg3 could partially recover the abnormal function of vasodilation and vasoconstriction. ∗P < 0.05 versus endothelium-intact, ∗∗P < 0.01 versus endotheliumintact; #P < 0.05 versus ADM-treated. The values are the mean ± S.D., n = 8. LVOT echocardiography, the peak aortic blood velocity (Vel) was significantly lower in the ADM-treat group (P = 0.047) but not in the co-treatment groups. Thoracic aortas were rapidly dissected out of ADM or ADM/Rg3 (20 mg/kg) treated rats on the 14th day after the ADM-injection. The aorta rings were pre-treated with 1 μM NE to achieve a plateau phase. In the control group, the vascular tone of the endotheliumintact aorta rings reached 3.27 ± 0.32 g from the baseline value of 2 g; the vascular tone of the endothelium-denuded aortas was 3.93 ± 0.27 g. Next, 10 μM Ach was added to induce vasodilation. The relaxation was more than 80% compared with the plateau phase in the endothelium-intact aortas and less than 30% in the endotheliumdenuded aortas. In the ADM treated rats, the maximal constriction by NE was close to level of constriction of the endothelium-denuded aortas, which was up to 4.07 ± 0.40 g. With the Ach administration, the vascular tone was reduced to 3.37 ± 0.21 g, and the maximal vasodilatation was 34%, showing significant endothelium dysfunction. Compared with the ADM group, the group co-treated with Rg3 showed less NE-induced constriction and more Ach-induced relaxation

### 5.14 Fetal Rat Hearts Do Not Display Acute Cardiotoxicity in Response to Maternal Doxorubicin Treatment

They analyzed the acute effect of doxorubicin, an anthracycline derivative, on fetal and maternal rat myocardium. They injected 10 or 20 mg/kg i.v. doxorubicin to pregnant Wistar rats at day 18 of pregnancy; age-matched pregnant rats injected with physiologic saline served as controls. Maternal echocardiography and fetal Doppler scanning were performed before the injection and before sacrifice. Cesarean operation was performed at day 19 or 20, and maternal and fetal blood samples and heart biopsies were collected to measure apoptosis, the impact on cell proliferation, and structural cardiac damage. Acute maternal cardiotoxicity is associated with loss of body weight, moderately deteriorated left ventricular function, induction of apoptosis, and a decrease in cell turnover. Despite a 30% lower fetal body weight and elevated plasma B-type natriuretic peptide concentrations after doxorubicin administration, the fetal hearts had intact microstructure, an unaltered number of apoptotic cells, and

preserved cell proliferation compared with controls. Our study suggests that acute treatment using anthracyclines during pregnancy impairs maternal cardiac function, whereas fetal hearts are protected.

After 1 week of acclimatization, 80-day-old female rats were mated in separate cages overnight. Day 0.5 (E0.5) of pregnancy was established when spermatozoa were found in vaginal smears. Doxorubicin was administered after E17 to avoid embryotoxicity, and the highest doses were used to induce an eventual cardiotoxicity (E9–12). At E18.5, rats were randomized into six groups ($n$ = 6 to 7 for each group) as follows: doxorubicin-treated (10 mg) and killed after 24 (group I) and 48 hours (group II); doxorubicin-treated (20 mg) and killed after 24 (group III) and 48 hours (group IV); and saline-treated controls killed after 24 (group V) and 48 hours (group VI). Finally, the effect of higher doxorubicin dose on cellular proliferation was tested in additional rats (saline or doxorubicin 20 mg for 48 hours, $n$ = 3, for each) using 5-bromo-2-deoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO) incorporation

### Echocardiographic indices

A decrease in heart rhythm is a phenomenon that occurs after doxorubicin administration. The greatest decrease (30 heartbeats per minute) in heart rhythm was observed with 10 mg of doxorubicin after 48 hours. Dimensions of the maternal heart, measured using echocardiography, were comparable between the different groups subjected to saline or doxorubicin administration, although there was a trend for reduced end-diastolic dimensions after 20 mg of doxorubicin, possibly related to impaired fluid intake. After administration of 20 mg of doxorubicin for 48 hours, the left posterior wall thickness in diastole was significantly decreased ($P < 0.05$), whereas the left ventricular internal diameter and interventricular septum in diastole did not change significantly ($P$ = 0.16 and 0.18). Systolic thickening of the septum was markedly reduced. Global myocardial function, measured as FS, was significantly decreased by 5–10% 24 and 48 hours after 20-mg bolus injection of doxorubicin. The umbilical systolic peak velocity and the ductus venosus Doppler measurements did not show significant differences between the groups.

## 5.15 Cardiotoxicity of the anticancer therapeutic agent bortezomib

Recent case reports provided alarming signals that treatment with bortezomib might be associated with cardiac events. In all reported cases, patients experiencing cardiac problems were previously or concomitantly treated with other chemotherapeutics including cardiotoxic anthracyclines. Therefore, it is difficult to distinguish which components of the therapeutic regimens contribute to cardiotoxicity. Here, we addressed the influence of bortezomib on cardiac function in rats that were not treated with other drugs. Rats were treated with bortezomib at a dose of 0.2 mg/kg thrice weekly. Echocardiography, histopathology, and electron microscopy were used to evaluate cardiac function and structural changes. Respiration of the rat heart

mitochondria was measured polarographically. bortezomib treatment leads to left ventricular contractile dysfunction manifested by a significant drop in left ventricle ejection fraction. Dramatic ultrastructural abnormalities of cardiomyocytes, especially within mitochondria, were accompanied by decreased ATP synthesis and decreased cardiomyocyte contractility. Monitoring of cardiac function in bortezomib-treated patients should be implemented to evaluate how frequently cardiotoxicity develops especially in patients with pre-existing cardiac conditions, as well as when using additional cardiotoxic drugs.

Male Wistar rats (250 to 350 g) were used in the experiments. Rats were injected i.p. with 0.2 mg/kg of bortezomib three times a week for 1 to 3 weeks. Wash-out indicates time after treatment termination.

### Echocardiographic indices

Echocardiographic analysis revealed left ventricular contractile dysfunction manifested by a significant drop in left ventricle ejection fraction which was detected already after second dose of bortezomib. This effect was accompanied by proportional decrease in LV fractional shortening and a rise in LV systolic area. There were no changes in LV diastolic area, heart rate, or blood pressure in bortezomib-treated rats.

## 5.16 Atorvastatin Ameliorates Radiation-Induced Cardiac Fibrosis in Rats

Radiation-induced heart injury is one of the major side effects of radiotherapy for thoracic malignancies. Previous studies have shown that radiotherapy induced myocardial fibrosis and intensified myocardial remodeling. In this study, we investigated whether atorvastatin could inhibit radiation-induced heart fibrosis in Sprague-Dawley rats, which were randomly divided into six groups: control; radiation only; and four treatment groups receiving atorvastatin plus radiation (E1, E2, E3 and E4). All rats, except the control group, received local heart irradiation in 7 daily fractions of 3 Gy for a total of 21 Gy. Rats in groups E1 (10 mg/kg/day) and E2 (20 mg/kg/day) received atorvastatin and radiation treatment until week 12 after exposure. Rats in groups E3 (10 mg/kg/day) and E4 (20 mg/kg/day) received atorvastatin treatment from 3 months before irradiation to week 12 after irradiation. The expressions of TGF-β1, Smad2, Smad3, fibronectin, ROCK I and p-Akt in heart tissues were evaluated using real-time PCR or Western blot analyses. Atorvastatin significantly reduced the expression of TGF-β1, Smad3/P-Smad3, ROCK I and p-Akt in rats of the E1-E4 groups and in a dose-dependent manner. Fibronectin exhibited a similar pattern of expression changes. In addition, echocardiography showed that atorvastatin treatment can inhibit the increase of left ventricular end-diastolic dimension, left ventricular end-systolic diameter and left ventricular posterior wall thickness, and prevent the decrease of ejection fraction and fraction shortening in E1-E4 groups compared with the radiation only group. This study demonstrated that

radiation exposure increased the expression of fibronectin in cardiac fibroblasts and induced cardiac fibrosis through activation of the TGF-β1/Smad3, RhoA/ROCK, and PI3K/AKT signaling pathways. Statins ameliorated radiation-induced cardiac fibrosis in Sprague-Dawley rats. Our results suggest that atorvastatin is effective for the treatment of radiation-induced cardiac fibrosis, especially with longer and higher dose atorvastatin treatment, as demonstrated in experimental group E4.

### Echocardiographic indices

Echocardiography was used to measure functional parameters (LVEDD, LVESD, LVPWT, EF and FS) to validate the effect of fibrosis reduction by statins. In the radiation only and E1–E3 groups, all of the parameters showed significant differences compared to pre-atorvastatin treatment. Comparison with the control group showed similar results. Significant differences were detected among the E1–E4 and radiation only groups, indicating that atorvastatin treatment can inhibit the increase of LVEDD, LVESD and LVPWT, and prevent the decrease of EF and FS. These effects were in a dose-dependent manner, which means the E4 group exhibited significantly improved cardiac-fibrotic status compared to the other groups

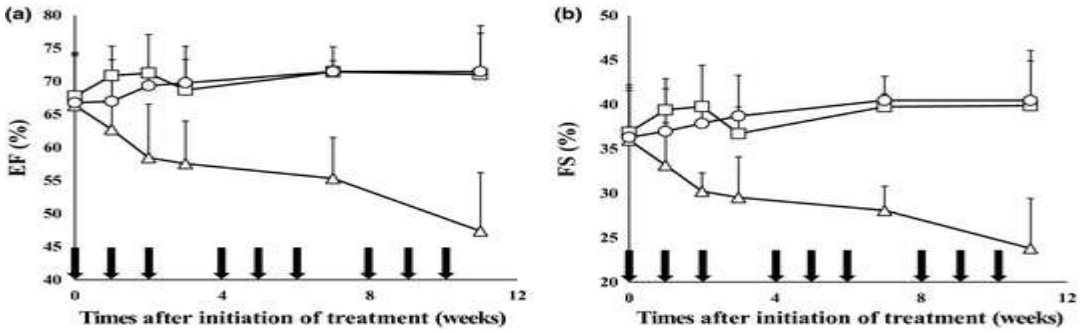
## 5.17 NC-6300, an epirubicin-incorporating micelle, extends the antitumor effect and reduces the cardiotoxicity of epirubicin

Epirubicin is widely used to treat various human tumors. However, it is difficult to achieve a sufficient antitumor effect because of dosage limitation to prevent cardiotoxicity. We hypothesized that epirubicin-incorporating micelle would reduce cardiotoxicity and improve the antitumor effect. NC-6300 comprises epirubicin covalently bound to PEG polyaspartate block copolymer through an acid–labile hydrazone bond. The conjugate forms a micellar structure of 40–80 nm in diameter in an aqueous milieu. NC-6300 (10, 15 mg/kg) and epirubicin (10 mg/kg) were given i.v. three times to mice bearing s.c. or liver xenograft of human hepatocellular carcinoma Hep3B cells. Cardiotoxicity was evaluated by echocardiography in C57BL/6 mice that were given NC-6300 (10 mg/kg) or epirubicin (10 mg/kg) in nine doses over 12 weeks. NC-6300 showed a significantly potent antitumor effect against Hep3B s.c. tumors compared with epirubicin. Moreover, NC-6300 also produced a significantly longer survival rate than epirubicin against the liver orthotopic tumor of Hep3B. With respect to cardiotoxicity, epirubicin-treated mice showed significant deteriorations in fractional shortening and ejection fraction. In contrast, cardiac functions of NC-6300 treated mice were no less well maintained than in control mice. This study warrants a clinical evaluation of NC-6300 in patients with hepatocellular carcinoma or other cancers.

### Echocardiographic indices

Left ventricular dimensions and wall thicknesses were determined from the echocardiography images, and ejection fraction (EF) and fractional shortening (FS)

were automatically calculated. (a) Changes in EF; ANOVA test between NC-6300 (10 mg/kg) and EPI (10 mg/kg), $P = 0.0081$. (b) Changes in FS; ANOVA test between NC-6300 (10 mg/kg) and EPI (10 mg/kg), $P = 0.0114$. Arrows, drug injections; bars, SD; points, mean.




### 5.18 Use of M-mode and Doppler echocardiography to investigate the cardiotoxicity of minoxidil in beagle dogs.

Doppler and M-mode echocardiography (EC) were used to investigate the effects of minoxidil on the cardiac function of the dog and potentially to clarify the pathogenesis of cardiac lesions, in particular the necrotic lesion in the left ventricle and the haemorrhagic lesion in the right atrium. Groups of three dogs were treated with a single oral dose of 0.5 or 2 mg/kg minoxidil or control vehicle, and M-mode and Doppler parameters were recorded at different time points before as well as 1, 3 and 24 h after treatment. The treatment produced a number of changes in M-mode parameters that indicate an increase in left ventricle contractility, in particular, increases in the percentage of thickening of the left ventricle wall during systole and in ejection fraction, and decrease of systolic volume. There was also a decrease in diastolic volume, which indicates a decrease in filling of the left ventricle probably due to the tachycardia and subsequent decrease in inter-systolic time. Doppler EC showed an increase in the velocity of the aortic flow, which indicates an increase in cardiac contractility. There was also a mild increase in stroke volume, which together with the tachycardia resulted in a marked increase in cardiac output. Together, Doppler and M-mode recordings gave evidence of an increase in the contractility of the left ventricle. This change is consistent with the generally accepted mechanism for the development of the left ventricle lesion induced by minoxidil. Minoxidil also produced changes in atrio-ventricular flows. The velocity and/or acceleration of E- and A-waves of the mitral and tricuspid flows increased, and the E/A ratio decreased. The changes in the E-wave indicate a faster diastole of the ventricle probably to compensate for the decrease in inter-systolic time. The changes in A wave are characteristic of an increased amplitude and velocity of the atrial contraction. This latter change is much more marked for tricuspid than for mitral flow. For both flows the E/A ratio decreased, which indicates that the contraction of the atria plays an

increased role in ventricle filling after minoxidil treatment. This stimulation of atrial contraction that we evaluate with Doppler EC may play a key role in the development of the atrial lesion produced by minoxidil. The fact that the change is more marked in the right than in the left atrium may explain why the lesion occurs only in the right atrium in dogs. This study showed, therefore, that Doppler EC associated with M-mode EC is a useful method for obtaining pertinent information on the pathogenesis of the left ventricle lesion induced by haemodynamic mechanisms. Moreover, Doppler EC allowed the assessment of changes in the function of the right atrium that may be involved in the development of the right atrial lesion.

### Echocardiographic indices

Changes in echocardiographic parameters were seen in the left ventricle and in the atria, and they can be associated with two different cardiac lesions produced by minoxidil. In addition to the marked tachycardia, both dose levels given in the current study produced changes in echocardiographic parameters consistent with stimulatory effects on the left ventricle. The increase in percentage thickening of the septum and of the posterior wall of the ventricle indicated an increase in the force of contraction of the left ventricle myocardium. The decrease in end systolic volume and the increase in ejection fraction showed an increase in the amplitude and efficiency of the left ventricle beat. These changes in the contractile function of the left ventricle seen in M-mode EC are associated with effects on the aortic flow that were assessed by Doppler measurements. In particular, the increase in maximal velocity, acceleration and time–velocity integral of the aortic wave are consequences of the increase in the amplitude and force of the left ventricle beat. In addition, changes in echocardiographic parameters reflected the increase in heart rate. The decrease in end diastolic volume is mainly due to the marked tachycardia and consequent decrease in diastolic time, which reduces ventricle filling. The decrease in ejection time is the Doppler correlate of a decrease in systolic time. The marked increase in the rate of circumferential fibre shortening reflected the increase both in the amplitude and in the velocity of the cardiac contraction. The end result of these changes in left ventricle function was a mild increase in the stroke volume determined by Doppler EC that, together with the increase in heart rate, produced a marked increase in cardiac output.

## 5.19 High-dose testosterone and dehydroepiandrosterone induce cardiotoxicity in rats: Assessment of echocardiographic, morphologic, and oxidative stress parameters

The aim of this study is to assess cardiotoxic effect of testosterone (TES) and dehydroepiandrosterone (DHEA) in Sprague Dawley rats. We compared the impact of subacute (14 days) and subchronic (90 days) administration of suprapharmacologic doses of TES and DHEA on body weight, locomotor activity, muscle strength, echocardiographic parameters, heart histopathology, and oxidative stress markers with the control group. Testosterone (10, 30, and 100 mg/100 g body weight) and DHEA

(10 mg/100 g body weight) administration decreased the body weights and locomotor activity ($p < 0.05$), and the combination of both increased muscle strength ($p < 0.05$) in rats. In our histopathological evaluation, misshapen cell nuclei, disorganized myocardial fibers, and leukocytic infiltrates were observed in high-dose TES (100 mg/100 g)-treated rats, especially on day 14. On day 90, mild changes such as misshapen cell nuclei, disorganized myocardial fibers, and leukocytic infiltrates were observed in TES and DHEA-treated groups. According to our echocardiographic study on day 14 and day 90, TES, especially at high doses, induced increase in left ventricular posterior wall diameter and ejection fraction ($p < 0.05$). In this study, blood oxidative stress marker malondialdehyde was increased slightly but not significantly in TES and DHEA groups. On the other hand, antioxidant enzymes such as SOD and glutathione peroxidase (GSH-Px) levels were slightly but not significantly increased in TES and DHEA groups. These data demonstrate that the potential risk to cardiac health due to exogenous androgen use may be related to oxidative stress in rats.

### Echocardiographic indices

On day 90, high doses of TES (30 mg/100 g and 100 mg/100 g) increased LWPWD significantly when compared with the control group ($p < 0.05$). Additionally, TES (30 mg/100 g and 100 mg/100 g) increased EF and FS slightly but not significantly. TES (10 mg/ 100 g) increased FS significantly when compared with the control group ($p < 0.01$). On the other hand, DHEA increased LWPWD, EF, and FS slightly but not significantly.

## 5.20 Cardioprotective effects of rosuvastatin and carvedilol on delayed cardiotoxicity of doxorubicin in rats.

The 3-hydroxy-3-methlyglutaryl coenzyme A reductase inhibitor (HMG CoA reductase inhibitor, statin) and carvedilol are the agents that were proved to have both of antioxidant properties and clinical benefits for heart failure. Of these, long-term cardioprotective effect of statin against delayed cardiotoxicity of doxorubicin was not clearly elucidated, while short-term cardioprotective effects of statin against acute doxorubicin cardiotoxicity were reported in a few animal studies. On the other hand, carvedilol is frequently used as a reference agent in the evaluation of antioxidant properties. Besides, a few animal studies reported antioxidant properties of carvedilol lead to the reduction of acute cardiotoxicity and nephrotoxicity from doxorubicin. In this study, low and high dose of rosuvastatin and carvedilol was co-administered with doxorubicin and long-term cardioprotective effect against delayed doxorubicin cardiotoxicity was evaluated. In addition, antioxidant effects of rosuvastatin and carvedilol were evaluated.

### Echocardiographic indices

At baseline, there was no significant difference in the LVEDD, LVESD and FS among the six groups. At eighth week, FS significantly decreased in all of the doxorubicin-treated groups (groups II–VI) compared with baseline. There was no significant difference in LVEDD among the groups I–VI at eighth week. All of the groups that were treated with doxorubicin showed an increase in LVESD and decrease in FS compared with group I. Although group VI showed marginally larger LVEDD ($p = 0.061$) and lower FS ($p = 0.072$) than group IV, there was no significant difference in LVESD and FS among the doxorubicin-treated groups (groups II–VI).

## 5.21 Oleuropein prevents doxorubicin-induced cardiomyopathy interfering with signaling molecules and cardiomyocyte metabolism

Anthracycline-associated cardiotoxicity has been the subject of considerable controversy. A variety of pathways and mechanisms have been proposed including impaired expression of cardiomyocyte proteins, disruption of cellular and mitochondrial Ca2+ homeostasis, disruption of mitochondrial bioenergetics, and interference with various pro-survival kinases leading to apoptosis. The most widely accepted mechanisms are the iron-mediated formation of reactive oxygen species (ROS), which promote myocardial oxidative stress. However, the ROS hypothesis has been tempered by a series of negative studies, while an alternative hypothesis is still pending. The natural phenolic compound oleuropein, which is present in high concentration in olives and olive tree leaves, has been shown to exert potential cardioprotective actions, and is capable of preventing acute DXR-induced cardiotoxicity. However, numerous antioxidants have been utilized for cardioprotection and, although efficient in cellular or acute animal experiments, they have failed to alleviate DXR cardiotoxicity in clinically relevant animal models or clinical trials. The evaluation of the potential cardioprotective effects of an antioxidant against DXR-induced cardiomyopathy with a parallel investigation of several possibly implicated pathways and use of holistic — omics techniques, is generally missing. As DXR is a commonly used drug in the treatment of a wide range of cancers, such a study would potentially provide important pathogenetic and therapeutic insights. The challenge for the future is to design protocols that are cardioprotective for both the short-term and long-term effects of doxorubicin, preferably without long-term administration and without hindering the antitumor activity of the drug.

### Echocardiographic indices

At the end of the interventions, there were no differences among groups in LV end-diastolic diameter. LV end-systolic diameter was marginally larger in the DXR group compared to controls ($p = 0.052$ in post-hoc analysis). However, animals in the DXR group had significantly lower fractional shortening compared to controls (p b 0.05)

and lower LV wall thickness (p b 0.05). All other groups did not differ from the control animals (p N 0.05).

### 5.22 Effect of telmisartan in limiting the cardiotoxic effect of daunorubicin in rats

The renin-angiotensin system (RAS) is a central component of the physiological and pathological responses of the cardiovascular system. The activity of angiotensin II (Ang II), the main effector of RAS, is initiated by its interaction with at least two pharmacologically distinct subtypes of cell-surface receptor, AT-1 and AT-2. The major functions of Ang II in the cardiovascular system are mediated by the AT-1 receptor (AT1R). Recent findings have suggested that Ang II activates intracellular signalling processes, which leads to events that include the generation of reactive oxygen species (ROS), myocardial apoptosis and fibrosisRecently, some investigators have shown that telmisartan, an ARB, is a partial agonist of the peroxisome proliferative activated receptor-gamma (PPAR-g), and it is reported to possess anti-inflammatory and antioxidant properties. To the best of our knowledge, no published study has investigated the cardioprotective effects of telmisartan in DNR-induced toxicity. In the present study, we have therefore investigated the mechanism of its protective effect against DNR-induced cardiotoxicity in rats, using changes in myocardial function, histopathology, myocardial apoptosis, and biochemical and oxidative-stress-related factors.

#### Echocardiographic indices

LVEDP was significantly higher and dP/dt was significantly lower in the DNR group than in group N, indicating LV dysfunction in the vehicle-treated DNR rats. Co-treatment with telmisartan reduced LVEDP (11 1.2 mmHg versus 7.8 0.4 mmHg, $P < 0.05$) and improved dP/dt (4722 295 mmHg/s versus 6635 90 mmHg/s, $P < 0.05$; and 4327 379 mmHg/s versus 6969 141 mmHg/s, $P < 0.05$) significantly in comparison with those in group DNR. Echocardiographic data revealed that both LVDd and LVDs were significantly increased in group DNR compared with those in group N. In addition, LV systolic function, as assessed by FS and EF, was also significantly reduced in group DNR compared with group N. The increases in both LVDd and LVDs were significantly attenuated in group Telm, since FS and EF were significantly increased.

## 5.23 Increased efficacy and reduced cardiotoxicity of metronomic treatment with cyclophosphamide in rat breast cancer

Cardiac toxicity, including acute lethal myocarditis resulting from high-dose CPA chemoradiotherapy, has been described in numerous reports. For CPA-containing regimens with doses ≥150 mg/kg, left ventricle dysfunction has been reported in 7% to 28% of patients (7). CPA-induced cardiomyopathy occurs within the initial two to three weeks after treatment. CPA is an inactive prodrug that undergoes a complex metabolic process. It is hydroxylated by the hepatic microsomal cytochrome P450 system to its active metabolite, 4-hydroxycyclophosphamide, and its tautomer, aldophosphamide. These intermediates undergo either conversion into acrolein and phosphoramide mustard, which are believed to be the toxic and active metabolites, respectively, or oxidation to the inactive compound carboxyphosphamide. CPA metabolites can react with carboxyl, mercapto, amino, phosphate and hydroxyl groups and can form cross-links with DNA and proteins.

Studies evaluating the cumulative probability of DOX-induced heart failure have found rates in the range of 3-5% at doses of 400 mg/m$^2$ and 7 to 26% at 550 mg/m$^2$. DOX-induced cardiomyopathy is reported to develop between 0 and 231 days following the final dose of DOX. However, delayed development of cardiotoxicity of up to 20 years following therapy has been reported, suggesting that cardiac myocytes are damaged during therapy. A number of different mechanisms for DOX action have been proposed, including free radical formation with glutathione depletion and DNA cross-linking.

The aim of this study was to examine the cardiotoxicity and efficacy of metronomic CPA and DOX treatment on experimental breast cancer of rats.

### Echocardiographic indices

Echocardiographic assessment of cardiac physiological alterations. Heart rate was similar among the groups and was not affected significantly by tumor presence or by CPA and DOX treatment (range, 260-325 beats per minute).
At two weeks after completion of the treatment, the average LVPW end-diastolic thickness was decreased in both DOX- and CPA-treated rats in comparison to the baseline, although the reduction was significant only for DOX-treated rats. LV Vol was increased in both CPA- and DOX-treated groups of rats in comparison to the baseline, but the increase was statistically significant only for the DOX-treated group. The average reduction of LVEF in the DOX-treated group was more than 10% (average 12% decrease), in comparison to the baseline and the same effect was detected for LVFS. The metronomic CPA also reduced LVEF but the reduction was only 2%.

### 5.24 The cardioprotective role of probucol against anthracycline and trastuzumab-mediated cardiotoxicity

Trastuzumab (Trz), a monoclonal antibody against HER2, is effective at reducing both progression and recurrence of breast cancer. Despite the therapeutic benefits of Trz, the incidence of cardiotoxicity is increased, particularly when administered after anthracyclinebased chemotherapy. Clinical trials in the adjuvant setting of breast cancer suggest that 5% to 10% of patients develop asymptomatic cardiac dysfunction, requiring discontinuation of Trz. Outside of clinical trials, recent studies suggest that up to 1 in 4 women undergoing treatment with doxorubicin (Dox) and adjuvant Trz may develop cardiotoxicity. Trz may directly block anti-apoptotic signaling, leading to premature cardiac dysfunction. In an in vivo model of acute chemotherapy-induced cardiac dysfunction, Dox+Trz synergistically increased the degree of myocardial apoptosis. In vitro blockade of HER2 is associated with increased oxidative stress, which is attenuated by the administration of the antioxidant N-acetylcysteine. In addition, the antioxidant drug probucol (Prob) is cardioprotective against Dox-induced cardiotoxicity by reducing apoptosis. Little is known, however, about the role of antioxidant therapy in the prevention of cardiotoxicity due to Dox+Trz. The aim of the current study was to determine whether prophylactic treatment with the antioxidant Prob is cardioprotective in an acute murine model of Dox and Trz-mediated cardiomyopathy

#### Echocardiographic indices

At baseline, the LV dimensions and systolic function, as determined by both conventional parameters (FS and EF) and TDI indices (Vendo and SR), were similar in all mice. Heart rates were within normal limits at baseline. There was no evidence of left ventricular (LV) hypertrophy comparing posterior end-diastolic wall thickness at baseline and day 10 of follow-up. The LVEDD was within normal limits in all groups at baseline. In mice receiving Dox alone, the LVEDD increased significantly from 3.2 6 0.1 mm at baseline to 3.8 6 0.2 mm by day 10 ($P < .05$). In mice receiving Dox+Trz, the LVEDD increased significantly from 3.1 6 0.1 mm at baseline to 4.1 6 0.2 mm by day 10 ($P < .05$). The administration of Prob, however, attenuated the increase in LVEDD to only 3.5 6 0.1 mm at day 10 in the Prob+Dox group and 3.6 6 0.1 mm at day 10 in the Prob+Dox+Trz group, respectively. LV FS was within normal limits in all groups at baseline. In mice receiving Dox alone, the FS decreased from 51% 6 2% at baseline to 42% 6 2% at day 10 ($P<.05$).

### 5.25 Anti-Fas gene therapy prevents doxorubicin-induced acute cardiotoxicity through mechanisms independent of apoptosis

Activation of Fas signaling is a key mediator of doxorubicin cardiotoxicity, which involves both cardiomyocyte apoptosis and myocardial inflammation. In this study, acute cardiotoxicity was induced in mice by doxorubicin, and some mice

simultaneously received an intramuscular injection of adenoviral vector encoding mouse soluble Fas (sFas) gene (Ad.CAG-sFas), an inhibitor of Fas/Fas ligand interaction. Two weeks later, left ventricular dilatation and dysfunction were apparent in the LacZ-treated control group, but both were significantly mitigated in the sFas-treated group. The ***in situ*** nick-end labeling-positive rate were similar in the two groups, and although electron microscopy revealed cardiomyocyte degeneration, no apoptotic structural features and no activation of caspases were detected, suggesting an insignificant role of apoptosis in this model. Instead, sFas treatment reversed doxorubicin-induced down-regulation of GATA-4 and attenuated ubiquitination of myosin heavy chain and troponin I to preserve these sarcomeric proteins. In addition, doxorubicin-induced significant leukocyte infiltration, fibrosis, and oxidative damage to the myocardium, all of which were largely reversed by sFas treatment. sFas treatment also suppressed doxorubicin-induced p53 overexpression, phosphorylation of c-Jun N-terminal kinase, c-Jun, and inhibitor of nuclear factor-κB, as well as production of cyclooxygenase-2 and monocyte chemoattractant protein-1, and it restored extracellular signal-regulated kinase activation. Therefore, sFas gene therapy prevents the progression of doxorubicin-induced acute cardiotoxicity, with accompanying attenuation of the cardiomyocyte degeneration, inflammation, fibrosis, and oxidative damage caused by Fas signaling.

### Echocardiographic indices

Echocardiography and cardiac catheterization performed at that time showed that mice receiving doxorubicin and LacZ gene had substantial deterioration of cardiac function characterized by enlargement of the LV cavity, increased LV diameter, reduced LV fractional shortening and reduced ±dP/dt, as compared with sham animals. Treatment with sFas gene significantly attenuated the doxorubicin-induced impairment of cardiac function but showed no influence on cardiac geometry and function in the sham-treated mice.

## 5.26 Crocin treatment prevents doxorubicin-induced cardiotoxicity in rats

CRO is well known as a unique water soluble carotenoids which is found in the stigmas of Crocus sativus Linne and in the fruits of Gardenia jasminoides Ellis. This natural compound has attracted research attention for its extensive pharmacological actions such as anti-inflammatory, antitumor, anti-hyperlipidemic, free radical scavenging, antioxidant and anti-atherosclerotic effects as well as protective against DNA damage. Several studies have also demonstrated that CRO has various neuroprotective activities in different animal models of brain disorders including cerebral ischemia, anxiety, depression, memory impairment, and Alzheimer's disease. This evidence has demonstrated the therapeutic potentials of CRO in the amelioration of different diseases; however, the efficacy of CRO for reducing DOX-induced cardiotoxicity has not yet been evaluated. Given that, the high antioxidant capacity of

CRO in vivo and in vitro has been shown to be the most interesting subject for research in recent years, in this study, we determined whether CRO could have cardioprotective effects in an animal model.

**Echocardiographic indices**

To assess the effect of the CRO and DOX on LV remodeling and function, a series of echocardiography studies were performed. As illustrated data analyses indicated that DOX treatment significantly decreased the FS (p b 0.01) and EF (p b 0.01), as compared with the Ctrl group. Moreover, the results showed that CRO 20 mg/kg and CRO 40 mg/kg treatments at both doses significantly increased the FS (p b 0.05 and p b 0.01) and EF (p b 0.05 and p b 0.01) in comparison with the DOX group.

### 5.27 Cardioprotective effect of metformin against doxorubicin cardiotoxicity in rats

Metformin is an oral antihyperglycemic drug that is used in type 2 diabetic patients. It has been reported to be cardioprotective in addition to decreasing basal and postprandial glucose levels, helping weight loss, and decreasing plasma lipid levels. Experimental animal models of isolated myocardial infarction and heart failure have shown that metformin increases the tolerance of the myocardium to ischemia-reperfusion injury, and decreases the development of heart failure after infarction. The aim of the present work was to investigate whether the metformin is able to reduce the cardiotoxic doxorubicin effects.

**Echocardiographic indices**

Regarding left ventricular functions, the decrease in IVSTs, EF, and FS values and the increase in LVESD were significant in the doxorubicin group compared with that in the control group ($p<0.05$). The cardioprotective effect of metformin against left ventricular systolic dysfunction was observed in the doxorubicin + metformin group.

### 5.28 Anandamide preserves cardiac function and geometry in an acute doxorubicin cardiotoxicity rat model

The endogenous cannabinoid system has been reported to be protective in the ischemic heart as there is evidence of nitric oxide preconditioning upregulating endocannabinoids. It is well known that during reperfusion of the ischemic heart ROS are generated, ultimately resulting in depressed cardiac function. Because of the similarity between reperfusion injury–induced and DOX-induced cardiac dysfunction (ie, ROS generation), we used the N-arachidonoyl-ethanolamide (anandamide, ANAN) compound as a pretreatment in DOX-treated rats to investigate its use as a cardioprotective agent. Anandamide has been shown to interact with the endogenous cannabinoid system via cannabinoid receptor 1 (CB1)27 and possibly cannabinoid

receptor 2 (CB2), and as such, we hypothesized that treatment with ANAN would have a cardioprotective effect on DOX-induced acute cardiotoxicity.

### Echocardiographic indices

This study shows promise in battling DOX cardiotoxicity due to the fact that ANAN treatment protected against the FS decrement and LV wall thinning observed in vivo and the LV developed pressure and þdP/dt decrements observed ex vivo. It is possible that ANAN's protective effect may involve p38 mitogen-activated protein kinase and PKC and/or reducing myocardial ROS damage, but further research needs to be done investigating the mechanisms involved in ANAN's protection against DOX cardiotoxicity. It may also be important to investigate ANAN's protective effects beyond 5 days post DOX administration as well as its use in combination with other cardioprotective therapies.

## 5.29 Cardioprotective effect of dexrazoxane in a rat model of myocardial infarction: anti-apoptosis and promoting angiogenesis

The heart when subjected to persistent ischemia or myocardial infarction (MI), may lose considerable function and normal shape due to ischemia/reperfusion (I/R) injury. A key process in this I/R injury is attributed to the formation of reactive oxygen species (ROS). ROS, produced by partial reduction of oxygen during mitochondrial respiration especially under oxidative stress, containing singlet oxygen, superoxide radical (O2 −), hydrogen peroxide (H2O2) and hydroxyl radical (OH), are extremely reactive and highly toxic for tissues. Mitochondrial ROS burst can in turn promote myocyte apoptosis and heart failure. In the mitochondrial apoptosis pathway, Bcl-2 family proteins are important mediators of pro-apoptotic (e.g. Bax and Bak) and anti-apoptotic (e.g. Bcl-2 and Bcl-XL) regulations. Hearts of Bax knockout mice had improved contractile function, reduced mitochondrial damage and decreased infarct size after I/R injury compared with their wild type counterparts, implicating that Bax plays a critical role in myocyte apoptosis induced by I/R. In recent years, activation of endothelial progenitor cells (EPCs) has been considered as vital for the repair of various cardiovascular insults. Endothelial progenitor cells have the ability to differentiate into mature endothelial cells for the formation of new blood vessels in the ischemic or damaged tissues. Bone marrow-derived EPCs can effectively migrate and home to target areas under some physiological and pathophysiological stimulations. Intramyocardial transplantation of human EPCs in the rat model of MI significantly improved cardiac function and reduced apoptosis. Notably, clinical trials using intracoronary bone marrow-derived progenitor cells for treatment of acute MI have been performed and the results appeared promising. Dexrazoxane (ICRF-187, Zinecard®, DZR), belonging to a class of bis (2,6-dioxopiperazines), has been proven effective both in patients and animal models for reduction of doxorubicin (DOX)-induced cardiotoxicity without affecting the antitumor activity. DZR was also used clinically as an antidote for alleviating tissue damage due to accidental anthracycline

extravasation. DOX-induced cardiotoxicity is cumulative, dose-dependent and essentially irreversible. The mechanism of DOX-induced cardiotoxicity is multifactorial and involves the induction of lipid peroxidation and generation of ROS. DZR could permeate the cell membrane and hydrolyze to its rings-opened metal-ion-binding metabolite (ADR-925) with a structure similar to EDTA. ADR-925 could remove iron from the iron–DOX complex or bind free iron to decrease ROS formation. Ramu reported that DZR significantly inhibited I/R injury, decreased protein carbonylation and improved hemodynamic recovery by decreasing free radical formation in an isolated perfused rat heart. However, the effect of DZR on MI has not been well explored. In the present study, we tested the hypothesis that DZR might exert a cardioprotective effect in a rat model of MI and attempted to elucidate its underlying mechanism.

### Echocardiographic indices

All echocardiographic parameters were similar among the 4 groups of animals at baseline. At 4 weeks post-MI, compromised heart function was observed in the MI animals compared with the SHAM group, as demonstrated in LVEDD (Pb0.001), LVESD (Pb0.001), FS (Pb0.001) and EF (Pb0.001). Treatment with DZR saw decreased LVEDD (P= 0.012) and LVESD (P= 0.009), and significant improvement in FS (P= 0.004) and EF (P= 0.004) compared with the MI group. MI+DZR animals also had a trend of increased CO (P= 0.062) compared with MI animals.

## 5.30 Resveratrol treatment protects against doxorubicin-induced cardiotoxicity by alleviating oxidative damage

Resveratrol is a polyphenol phytoalexin that is found in a number of edible materials, such as grape skins and seeds, peanuts, mulberries and red wine. Resveratrol has been reported to have a wide range of pharmacological effects that include cardiovascular protection, neuroprotection, modulation of lipid metabolism, anti-carcinogenesis and anti-inflammatory effects. Resveratrol, when added to cultured cardiomyocytes in low micromolar concentrations, was shown to induce a number of endogenous antioxidants and phase 2 enzymes, including superoxide dismutase (SOD), catalase, glutathione (GSH), glutathione reductase (GR), glutathione S-transferase (GST) and NAD(P)- H:quinone oxidoreductase 1 (NQO1). Animal studies have shown that resveratrol reduces biliary cirrhosis-induced oxidative damage of the kidney and liver, as well as ischemia/reperfusion-induced tissue damage in the ovaries. An immunohistochemical study has shown that resveratrol attenuated the expression of CD86 in the glomerular endothelium and peritubular vessels in rats with ischemiareperfusion injury. In accordance with these studies, we have previously demonstrated that resveratrol improves stomach, kidney, bladder and lung damage in rat models of ulcer, ischemia/reperfusion and sepsis. Furthermore, we have also reported that resveratrol attenuates acetaminopheninduced hepatotoxicity and ifosfamide-induced nephrotoxicity. Based on these above-mentioned findings, in the

present study they aimed to investigate the possible beneficial activities of resveratrol against doxorubicin induced oxidative damage in the cardiac tissues of rats.

### Echocardiographic indices

LV posterior wall thickness, LV end-diastolic and end-systolic dimensions, as well as relative wall thickness and percentage fractional shortening and ejection fraction were increased significantly (pB0.050.001) in the saline-treated rats with DOX cardiotoxicity. However, in the RVT-treated rats with DOX toxicity, these mesurements were significantly reduced (pB0.050.001).

## 6. SUMMARY

The recognition of cardiac problems related to the treatment of cancer is complex. The heterogeneity of the population makes comparison between and among groups difficult. Studies on sufficiently large populations are frequently not available, and the number of new agents used in the treatment of some diseases makes evaluation of large cohorts impossible. Some reported results are therefore fragmented, and prospective data on longterm survival, treatment strategies, and monitoring represents all too often expert opinion rather than firm and established dataderived certainty. Identifying patients who are at increased risk for cardiovascular problems associated with the cancer treatment, or who develop side effects following treatment is a major component of an evolving area often referred to cardio-oncology. Working together with oncologists, cardiologists can offer vital support to those who are the primarily clinicians treating cancer patients so that therapy can be optimized; the goal should be to maximize meaningful survival. Judicious scrutiny of the needs of these complex patients requires careful balance: excessive concern regarding potentially reversible cardiac issues may compromise the administration of highly beneficial anti-cancer therapies, while under-appreciation of cardiac risk may result in life-longcardiac concerns for a patient who has been cured of their cancer. Knowledge of the cardiac effects of anti-cancer agents balanced with knowledge regarding the natural history of the malignancy and the likelihood of tumour response offers such patients the greatest chance for long-term disease-free survival.

Through observation of side effects caused by newly developed cancer therapeutics, some cardiovascular signalling pathways have become more clearly understood. It is postulated that the Neuregulin/ erbB2/HER2 signalling pathway, the target of several anti-cancer therapies, plays an important role in cardiovascular homeostasis, and studies are being conducted to evaluate the stimulation of this pathway to treat heart failure patients.
Signalling inhibitors, chemotherapeutics, and combinations thereof are the subject of intense research and ongoing clinical trials in oncology. New cancer therapeutics will continue to target signalling cascades that may also be important for the survival and homeostasis of cardiovascular tissue. Cardiovascular side effects from these agents

should be expected because of their direct effect on signalling or the potential of additional nontargeted inhibitory effects.

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