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## Review Article

# Molecular mechanisms of anthracycline cardiovascular toxicity

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Anthracyclines are effective chemotherapeutic agents, commonly used in the treatment of a variety of hematologic malignancies and solid tumors. However, their use is associated with a significant risk of cardiovascular toxicities and may result in cardiomyopathy and heart failure. Cardiomyocyte toxicity occurs via multiple molecular mechanisms, including topoisomerase II-mediated DNA double-strand breaks and reactive oxygen species (ROS) formation via effects on the mitochondrial electron transport chain, NADPH oxidases (NOXs), and nitric oxide synthases (NOSs). Excess ROS may cause mitochondrial dysfunction, endoplasmic reticulum stress, calcium release, and DNA damage, which may result in cardiomyocyte dysfunction or cell death. These pathophysiologic mechanisms cause tissue-level manifestations, including characteristic histopathologic changes (myocyte vacuolization, myofibrillar loss, and cell death), atrophy and fibrosis, and organ-level manifestations including cardiac contractile dysfunction and vascular dysfunction. In addition, these mechanisms are relevant to current and emerging strategies to diagnose, prevent, and treat anthracycline-induced cardiomyopathy. This review details the established and emerging data regarding the molecular mechanisms of anthracycline-induced cardiovascular toxicity.

## Background

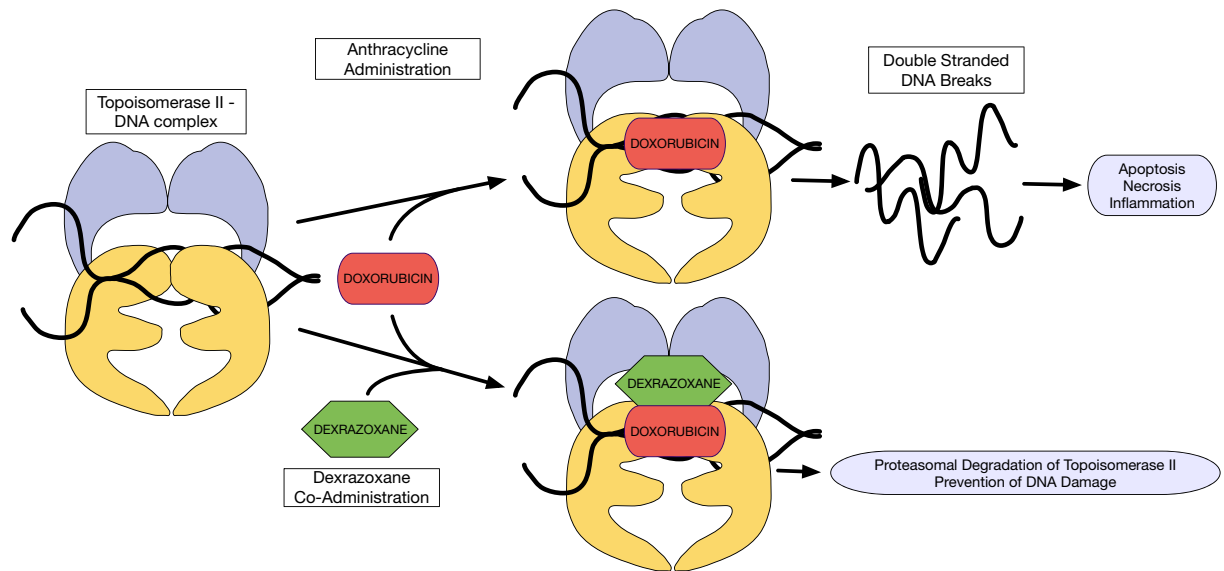
Anthracyclines are highly effective chemotherapeutic agents that remain a cornerstone of contemporary therapeutic regimens for a number of malignancies despite a remarkable number of recent advances in cancer therapeutics. Anthracyclines are used in up to 50–70% of adult lymphoma patients [1] and over half of childhood cancer patients [2], and remain the first-line therapeutic agent for soft tissue sarcomas [3]. Despite the increased and preferential use of taxane-based regimens, a significant proportion of breast cancer patients are still treated with anthracyclines [4].

Cardiovascular mortality is the leading non-cancer, health-related cause of death in childhood cancer survivors, and the prevalence of heart failure is not decreasing in this population despite improvements in cancer care and overall chronic disease burden [5,6]. Among long-term breast cancer survivors cardiovascular disease is the leading cause of death [7]. Cancer therapies, particularly in anthracyclines, contribute significantly to the higher incidence of cardiomyopathy and heart failure among cancer survivors. These effects are dose-dependent; there is a direct correlation between cumulative anthracycline dose and the incidence of cardiomyopathy [8]. In patients receiving 250 mg/m<sup>2</sup> of doxorubicin the odds of developing cardiomyopathy are 25-times greater than in cancer patients with no anthracycline exposure [8,9]. According to the data from Cardinale et al., clinically detectable cardiotoxicity occurs in 9% of patients with a median time of onset of 3.5 months, and within the first year after chemotherapy in 98% of cases [10].

Anthracycline-induced cardiotoxicity significantly impairs patient's quality of life and places a considerable financial burden on the healthcare system [11]. Congestive heart failure secondary to anthracyclines

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**Figure 1. Anthracycline effect on Top2 $\beta$ , and its inhibition by dexrazoxane**

Doxorubicin intercalates DNA and inhibits Top2 $\beta$ . Doxorubicin forms cleavable complexes with DNA-bound Top2 $\beta$ , which may lead to DNA breaks and induce cell death. Dexrazoxane binds to DNA-bound Top2 $\beta$ , and triggers proteasomal degradation of Top2 $\beta$ , thereby reducing formation of doxorubicin-poisoned Top2 $\beta$  cleavage complexes. Abbreviation: DNA, deoxyribonucleic acid.

has a very poor outcome: a large population-based series reported median survival of 1 year [12]. Moreover, the development of cardiac dysfunction during cancer treatment may lead to anthracycline treatment delays and reductions, potentially impacting cancer outcomes [13]. Anthracycline-induced cardiotoxicities therefore have significant public health relevance.

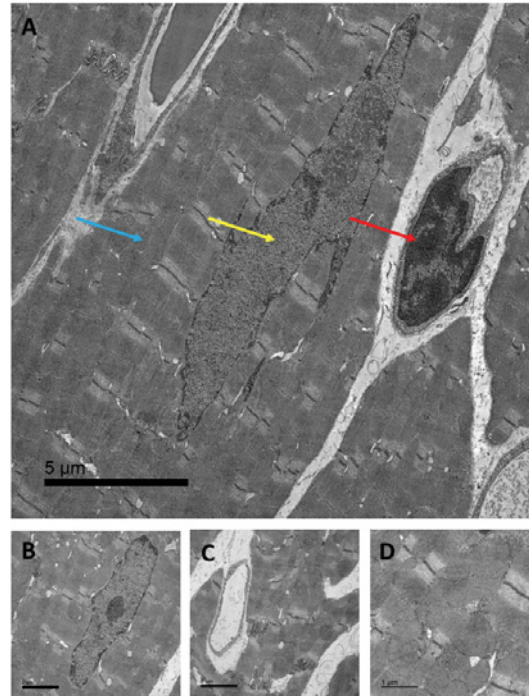
Anthracyclines cause cardiovascular toxicities via multiple molecular mechanisms. An understanding of these mechanisms is critical to inform diagnostic and therapeutic strategies in patients treated with these chemotherapeutic agents. In this review, we describe the pathophysiologic mechanisms associated with the cardiovascular toxicities of anthracycline therapy, their tissue- and organ-level effects, and the impact of these mechanisms on contemporary and emerging management strategies.

## Molecular mechanisms of anthracycline toxicity

### Anthracycline effects on DNA topoisomerase

One of the most important molecular mechanisms of cardiotoxicity is the effect of anthracycline therapy on topoisomerase II $\beta$  (Top2 $\beta$ ) [14]. The topological relationship between two DNA strands is critical for normal cellular function. Topoisomerases are DNA unwinding enzymes that are essential for cell replication and viability as they facilitate chromosome condensation, chromatid separation, and transient DNA strand breaks [15,16]. Two major topoisomerases are ubiquitously expressed: type I initiates cuts only in single-stranded DNA and type II enzyme can initiate double-stranded DNA breaks. In mammalian cells two topoisomerase II isoforms exist with distinct expression and localization of topoisomerase II $\alpha$  and topoisomerase II $\beta$  during the cell cycle [17,18]. Topoisomerase II $\alpha$  is thought to be the main isoform expressed during mitotic processes because it is expressed at higher levels in proliferating cells [19], and *in vitro* Top2 $\beta$  was not required for mitotic events [20,21]. Top2 $\beta$  is predominantly expressed in quiescent cells (such as cardiac myocytes) and localized to the nuclear matrix and the nucleolus [22,23].

Anthracyclines target and inhibit Top2 $\beta$ , produce persistent DNA cleavable complexes, inducing DNA breaks, and thereby leading to cancer cell death [24,25]. During the past five decades several anti-neoplastic topoisomerase II inhibitors were generated with the hope of identifying anti-cancer agents with less overall toxicity [26]. In addition to resulting in anticancer activity, Top2 $\beta$  inhibition also causes cardiotoxicity, and may result in cardiomyocyte injury and cell death (Figure 1). Highlighting the importance of Top2 $\beta$  inhibition in doxorubicin cardiotoxicity, a cardiomyocyte-specific deletion of Top2 $\beta$  was shown to protect cardiomyocytes from doxorubicin-induced DNA



**Figure 2. Myocardial cells and subcellular compartments affected by anthracycline-induced topoisomerase II inhibition**

Transmission electron microscopic scan of murine cardiac tissue\*. (A) Arrows indicate cardiomyocyte mitochondria (blue arrow, mitochondrial DNA), the cardiomyocyte nucleus (yellow arrow, nuclear DNA), and cardiac endothelial cells (red arrow, endothelial cell DNA, 2500 $\times$ , scale bar = 5  $\mu$ m). (B and C) Cardiomyocyte nucleus and cardiac capillary formed by a single endothelial cell, respectively (5000 $\times$ , scale bar = 2  $\mu$ m), and (D) cardiomyocyte inter-fibrillar mitochondria (10000 $\times$ , scale bar = 1  $\mu$ m). \*Animal care protocols and procedures are provided in supplemental material. Abbreviation: DNA = deoxyribonucleic acid.

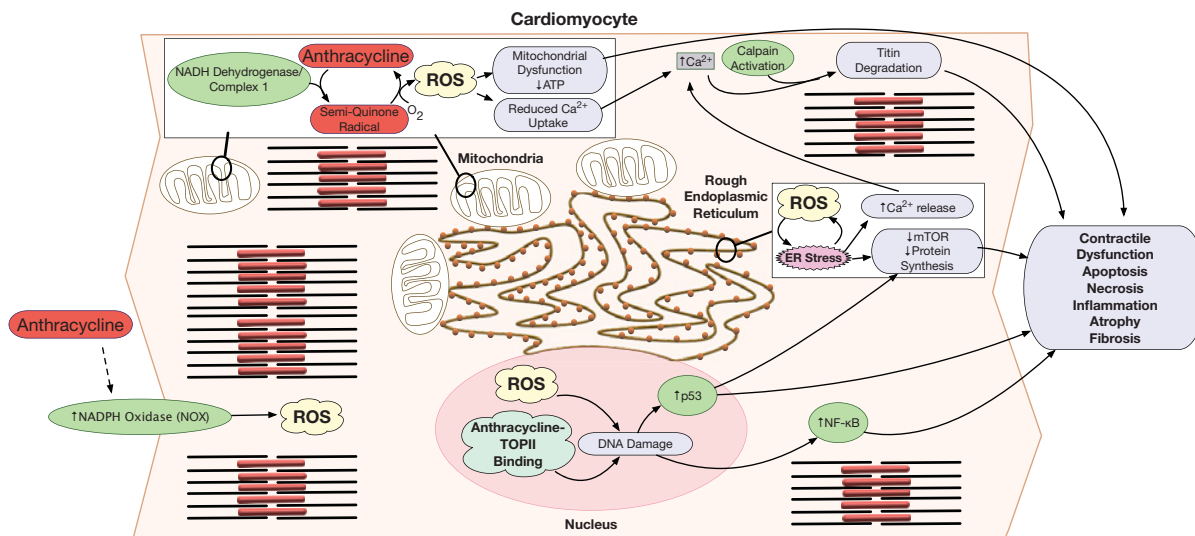
double-strand breaks, reduced reactive oxygen species (ROS) generation, inhibited transcriptional changes responsible for defective mitochondrial biogenesis and oxidative stress, and protected from doxorubicin-induced cardiomyopathy [14]. Recent studies describe an important role of mitochondrial Top2 $\beta$  in mitochondrial biogenesis [27,28]. Anthracyclines bind to mitochondrial DNA [29], and cause acute reduction in mitochondrial DNA synthesis [30]. Mitochondrial DNA lesions have been documented in patients after anthracycline therapy [31]. Therefore, anthracyclines affect nuclear and mitochondrial DNA simultaneously. Since several different cell types exist in the heart, anthracycline-induced cleavage of double-stranded DNA may occur not only in cardiomyocytes, but also in endothelial cells, fibroblasts, and progenitor cells (Figure 2). In summary, Top2 $\beta$ -mediated DNA damage is an important and well-described mechanism of anthracycline cardiotoxicity.

## Anthracycline-induced generation of ROS

Increased oxidative stress is another important mechanism of anthracycline cardiotoxicity [32]. ROS are natural byproducts of cellular metabolism and under physiologic conditions, a fine balance is maintained between ROS production and disposal. Oxidative stress occurs when ROS production overwhelms oxidative defense systems, or when antioxidant pathways are down-regulated. Massive production of reactive oxygen and nitrogen species triggered by anthracyclines leads to oxidative stress [14,33,34], and may ultimately activate cell death signaling [35]. Anthracyclines affect ROS production via multiple cellular mechanisms, including through effects on the mitochondrial electron transport chain (NADH/Complex-1), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), and dysfunctional nitric oxide synthases (NOs), as described here.

## Effects on the mitochondrial electron transport chain

The myocardium continuously consumes more energy per gram of tissue than any other organ. Cardiac metabolism is primarily aerobic with most of the energy supplied via an oxidative phosphorylation process in mitochondria. Electron carriers (complexes I–IV) embedded in the inner mitochondrial membrane export H<sup>+</sup> out of the mitochondrial



**Figure 3. Molecular pathways affected by anthracyclines in cardiac myocytes**

In myocytes, anthracyclines induce ROS generation by mitochondria and NOX, reduce mitochondrial respiration, induce ER stress, alter  $\text{Ca}^{2+}$  signaling, and induce DNA damage. NADH dehydrogenase of complex I of the mitochondrial electron transport chain reduces the quinone moiety of anthracyclines to a semiquinone radical. In the presence of molecular oxygen, the semiquinone auto-oxidizes to generate the parent anthracycline and a superoxide anion. The effects of anthracyclines on the electron transport chain and increased ROS generation cause mitochondrial dysfunction, decreased ATP synthesis, and reduced  $\text{Ca}^{++}$  uptake. Increased ROS production induces ER stress, further increasing cytoplasmic  $\text{Ca}^{++}$  concentrations due to ER  $\text{Ca}^{++}$  leak. ER stress also inhibits mTOR survival signaling and thereby reduces protein synthesis. ER stress and mTOR inhibition trigger inflammation, and cell death signaling. In addition, anthracyclines induce activation of the NOX transmembrane enzyme complex, further increasing production of ROS. Anthracycline binding to TopII in the nucleus, and the direct effect of ROS may cause irreversible DNA damage. Anthracycline-induced DNA damage and oxidative stress lead to p53 tumor suppressor activation, that modulates mTOR pathway. ROS and DNA damage result in NF- $\kappa$ B activation. These molecular pathways lead to contractile dysfunction, cardiac atrophy, fibrosis, apoptosis, necrosis, and inflammation. Abbreviations: DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; mTOR, mammalian target of rapamycin; TopII, topoisomerase II.

matrix, generating a  $\text{H}^+$  gradient across the membrane. Adenosine triphosphate (ATP) synthetase (also known as complex V) uses the energy released by  $\text{H}^+$  diffusion back into the mitochondrial matrix to synthesize ATP. To ensure high ATP production, the heart has the highest mitochondrial density among all the tissues [36,37]. The electron transport across the mitochondrial membrane is initiated in complex I (NADH dehydrogenase), when four protons ( $4\text{H}^+$ ) per molecule of oxidized NADH get translocated. In 1986, Davies and Doroshov showed that NADH dehydrogenase, associated with the mitochondrial complex I, is the site of anthracycline-induced free radical formation [38]. They demonstrated that in cardiomyocytes, NADH dehydrogenase reduces the quinone moiety of anthracyclines to a semiquinone radical (Figure 3). In the presence of molecular oxygen, the semiquinone auto-oxidizes to generate the parent anthracycline and a superoxide anion [1]. This self-perpetuating redox cycling of anthracycline in complex I results in the accumulation of superoxide anions and reduced ATP production. Superoxide may be spontaneously converted into  $\text{H}_2\text{O}_2$  by superoxide dismutase. Through this pathway, anthracycline-induced mitochondrial toxicity may result in cardiac dysfunction and cell death.

Catalase enzymes facilitate decomposition of  $\text{H}_2\text{O}_2$  to water and oxygen to protect from oxidative insults. However, catalase activity levels in the heart are only 2–4% of activity levels in the liver and kidneys [39], and this low cardiac catalase activity may predispose the myocardium to doxorubicin-induced toxicity. Additional studies comparing the levels of other antioxidant enzymes including superoxide dismutase and glutathione peroxidase in cardiac and hepatic tissues have found that the heart has a much lower concentration of enzymatic defenses against free radicals [40]. Moreover, exposure to doxorubicin leads to a prolonged depression in cardiac glutathione peroxidase activity [40].

## NOX

Besides mitochondria, key producers of cellular ROS are the NOXs. The NOX is a multisubunit transmembrane enzyme complex, and there are seven NOX family members (NOX 1–5 and dual oxidase 1–2) with different tissue distribution and activation mechanisms [41]. The primary NOX isoforms expressed in cardiac myocytes are the cell membrane-bound NOX2, and the organelle-bound NOX4. NOX2 is a mechanosensor in cardiac tissue [42]. NOX-dependent ROS production regulates several key components of cardiac remodeling, including myocyte hypertrophy, contractile dysfunction, apoptosis, and fibrosis [43].

Anthracyclines induce NOX activation resulting in the increased production of ROS (Figure 3) [44]. Genetic disruption of NOX2 protected against the development of cardiac contractile dysfunction, atrophic cardiomyocyte degeneration, apoptosis, and interstitial fibrosis after chronic doxorubicin treatment [43]. NOX2-deficient mice reportedly exhibit resistance to DOX-induced cardiac atrophy as well [45].

Transient receptor potential (TRP) channels are multimodal cation channels regulated by several environmental factors, including mechanical stress, several of which have been implicated in the development of pathological hypertrophy caused by neurohormonal factors and mechanical stress [46]. TRPC3 interaction with NOX2 protects it from proteasome-dependent degradation [47]. NOX2 also stabilizes TRPC3 proteins to enhance mechanical stress-induced background  $\text{Ca}^{2+}$  entry into cardiomyocytes, leading to maladaptive remodeling. Shimauchi et al. demonstrated that increased levels of the TRPC3–NOX2 complex correlate well with the severity of anthracycline-induced cardiac atrophy and that inhibition of TRPC3–NOX2 functional coupling suppresses DOX-induced myocardial atrophy in mice. By contrast, voluntary exercise, which induces physiological cardiac hypertrophy, reduced levels of the TRPC3–NOX2 complex [46]. Ibudilast, an inhibitor of phosphodiesterase 4 (PDE 4), destabilizes the TRPC3–NOX2 complex, attenuates doxorubicin-induced NOX activation and ROS production, and was found to be a potent inhibitor of doxorubicin-induced cytotoxicity in mice [48].

## Iron-dependent ROS production and ferroptosis

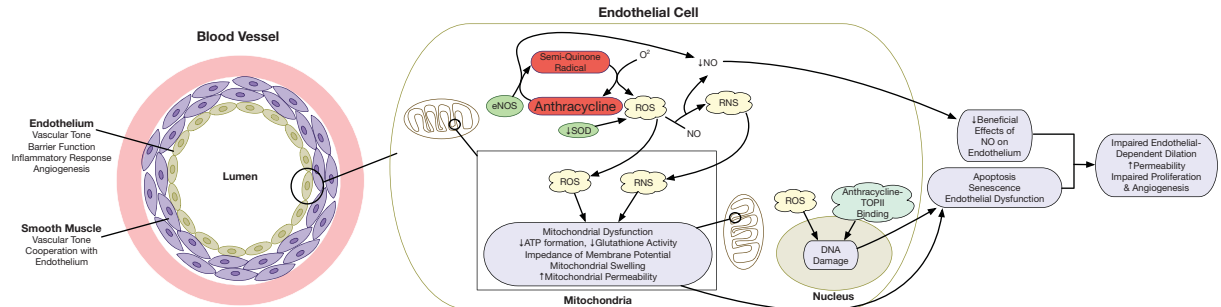
ROS generation with doxorubicin exposure is further increased by free cellular iron [1]. Doxorubicin leads to accumulation of iron in the mitochondria by inhibiting ABCB8, a mitochondrial protein that facilitates iron export [49].  $\text{H}_2\text{O}_2$  may be converted into highly toxic hydroxyl radicals in the presence of heavy metals, such as iron. Additionally, doxorubicin can form complexes with iron, resulting in iron cycling between Fe(II) and Fe(III) forms, and substantial ROS production [50].

Mutations of the *HFE* gene result in the development of hereditary hemochromatosis, a condition that causes iron overload. HFE-deficient mice were found to be more susceptible to doxorubicin toxicity than wildtype mice potentially through exaggerated ROS response to anthracycline exposure in the setting of iron excess [51].

Ferroptosis is iron-dependent regulated cell death that occurs as a consequence of lethal lipid oxidation, and could be prevented by iron chelation [52]. Glutathione peroxidase 4 (GPx4) is an endogenous scavenger for lipid peroxides and is a key regulator of ferroptosis. It was shown that doxorubicin down-regulates GPx4 and induced excessive lipid peroxidation through doxorubicin–Fe(II) complex in mitochondria, leading to mitochondria-dependent ferroptosis [53]. GPx4 overexpression protected against anthracycline-induced myocardial dysfunction in mice. Additionally, inhibition of ferroptosis by ferrostatin-1 significantly reduced anthracycline-induced cardiomyopathy [54]. Furthermore, concomitant inhibition of ferroptosis and apoptosis with ferrostatin-1 and zVAD-FMK fully prevented anthracycline-induced cardiomyocyte death [53].

## NOS

NOS expression in cardiomyocytes is induced following doxorubicin treatment, and leads to increased production of peroxynitrite (reactive nitrogen species; RNS) from nitric oxide (NO) and the superoxide anion [55]. RNS were shown to be a trigger of doxorubicin-induced cell death in cardiac myocytes [55]. In addition to its relevance to cardiomyocytes, endothelial NOS (eNOS) has particular importance in the pathophysiology of anthracycline-related endothelial toxicity. eNOS reduces doxorubicin to a semiquinone radical, which in turn leads to the generation of superoxide radicals and hydrogen peroxide, resulting in oxidative stress, and also leads to peroxynitrite formation and reduced NO levels, resulting in nitrosative stress (Figure 4) [56–60]. These effects appear to be most important in the endothelial mitochondria, where post-anthracycline changes include an increase in mitochondrial ROS and oxidative stress metabolites with a lack of compensatory stimulation of superoxide dismutases 1 and 3 (endogenous antioxidants) in addition to decreased phosphorylated eNOS and NO levels [61,62]. The generation of ROS may be related to reduced circulating vascular endothelial growth factor (VEGF) levels [61,63,64]. Human cell culture and animal studies have found that treatment with L-arginine, mitochondrial-specific antioxidants, restoration of VEGF-A levels and VEGF-B gene therapy can reverse these effects and restore endothelial function [61,63–65].



**Figure 4. Effects of anthracyclines on the vascular endothelium**

Within the vascular endothelium, anthracyclines result in the generation of ROS primarily via eNOS. Interactions of ROS with NO results in the formation of peroxynitrites (RNS) and result in reductions in NO. These reactive species result in mitochondrial dysfunction. DNA damage may be caused by ROS and TopII-mediated double-stranded DNA breaks. Through these mechanisms, anthracyclines may cause endothelial apoptosis, senescence, and dysfunction resulting in impaired endothelial-dependent vascular dilation, increased permeability, and impaired angiogenesis. Abbreviations: DNA, deoxyribonucleic acid; SOD, superoxide dismutase; TopII, topoisomerase II.

## Anthracycline-induced cell signaling and myocardial cell death pathways Endoplasmic reticulum stress, apoptosis, and autophagy

Increased ROS production leads to endoplasmic reticulum (ER) stress (Figure 3). Two kinds of ER stress exist. Beneficial ER stress inhibits mammalian target of rapamycin (mTOR) kinase signaling, that regulates cell growth, survival, and metabolism. mTOR inhibition suppresses protein translation to improve protein folding and quality control. When these compensatory mechanisms are overwhelmed, detrimental ER stress occurs and cell death signaling is triggered [66]. Because increased ROS production by stressed mitochondria and NOX activation induces ER stress, and ER stress ultimately can lead to increased ROS production a vicious cycle is initiated. ER stress signaling pathways intersect with oxidative stress signaling, and both pathways can trigger inflammatory processes [67,68].

It was confirmed experimentally that doxorubicin leads to ER stress in murine hearts. Doxorubicin disrupts expression of the ER chaperone, glucose-regulated protein 78 (GRP78, also called BiP), which plays a major role in adaptive responses to ER stress [69]. ER chaperones are involved in ensuring the quality control and post-translational modifications of newly synthesized proteins in addition to regulation of ER stress responses [70]. Doxorubicin activates caspase-12, an ER membrane-resident apoptotic molecule, which can lead to cardiomyocyte apoptosis and myocardial dysfunction. Cardiac-specific overexpression of GRP78/BiP chaperone attenuates caspase-12 cleavage, and alleviates cardiac apoptosis and dysfunction induced by doxorubicin [69].

CRTH2, a chemoattractant receptor-homologous molecule expressed on T helper type 2 cells, is highly expressed in the heart as well. CRTH2 activation facilitates ER stress-induced cardiomyocyte apoptosis via a caspase-12-dependent pathway. Doxorubicin significantly up-regulates CRTH2 gene expression in cardiomyocytes. Deletion of CRTH2 markedly prevented left ventricular (LV) dysfunction and improved the survival rate in mice after doxorubicin treatment by suppressing cardiomyocyte apoptosis [71].

ER stress activates autophagy, a cell defense mechanism for the removal of unfolded or damaged proteins [72]. Autophagosomes, vesicles surrounded by double membrane structures, encapsulate defective cellular components. Ultimately the outer membrane of autophagosomes fuses with the lysosomal membrane so that all cargo together with the inner membrane of the autophagosome will get digested. There are conflicting reports on the role of autophagy in anthracycline-induced cardiotoxicity. Most, but not all, of the studies conclude that doxorubicin up-regulates cardiac autophagy [73]. A number of studies indicated that anthracyclines inhibit autophagic flux, by inhibiting lysosomal biogenesis and function. The resulting accumulation of undegraded autolysosomes further stimulates ROS production [74,75]. Mitochondrial autophagy (mitophagy) degrades and recycles damaged mitochondria, and there is emerging evidence for defective mitophagy in doxorubicin-induced cardiotoxicity [74]. A recent study found that luteolin, a natural flavone found in vegetables, improved doxorubicin-induced cardiomyocyte contractile dysfunction in adult mouse cardiomyocytes, and attenuated doxorubicin-induced cardiotoxicity through promoting mitochondrial autophagy [76].

## Effects on calcium handling

In all cell types oxidative stress affects  $\text{Ca}^{2+}$  handling because mitochondria can uptake calcium and thereby buffer cytosolic  $\text{Ca}^{2+}$  concentrations [77]. Multiple studies have demonstrated that doxorubicin increases resting intracellular  $\text{Ca}^{2+}$  concentration (Figure 3) [78]. Excessive ROS production, combined with increased cytosolic calcium leads to opening of the mitochondrial permeability transition pore, and mitochondria lose their ability to develop and maintain electrochemical gradients across the inner membrane [79]. This could lead to the rupture of the mitochondrial outer membrane and release of proapoptotic factors from the mitochondrial intermembrane space, activating a mitochondrial-dependent apoptotic pathway [79].

Oxidative stress also activates calpains, a family of calcium-dependent cysteine proteases that play a role during myofibril remodeling. Since calcium-dependent calpain activation may trigger titin degradation, pathological remodeling of the sarcomere can be considered as a secondary effect of excessive ROS production. It was previously shown that the myofibrillar protein titin, which is the largest structural molecule in the cardiac sarcomere, was degraded after doxorubicin treatment and that pre-incubation with calpain inhibitors, but not caspase inhibitors, rescued titin degradation [80].

## p53 signaling

Anthracycline-induced DNA damage and oxidative stress lead to p53 tumor suppressor activation, which is a crucial protein for the induction of cell cycle arrest and apoptosis [81]. Numerous studies have shown that doxorubicin-induced cardiomyocyte apoptosis is associated with increased expression of the p53 protein [82,83]. Genetic deletion of p53 in mice protected animals against early anthracycline-induced cardiac dysfunction 2 weeks following exposure [83]. Zhu et al. found that acute anthracycline-induced cardiac dysfunction and cardiac mass loss occur via p53-dependent modulation of the mTOR pathway [82]. Anthracycline treatment significantly reduced level of activated mTOR in wildtype mice. Transgenic animals with constitutively elevated mTOR activity in the heart were protected against anthracycline-induced cardiomyopathy. However, this effect was independent of the degree of cardiomyocyte apoptosis, as apoptotic signaling in transgenic mTOR animals was not significantly different from wildtype animals [82].

Interestingly, although p53 inhibition offers cardioprotection early during anthracycline exposure, paradoxically, it predisposes to cardiotoxicity late after the exposure to the drug [84]. It was shown that p53 inhibition blocked activation of STAT3 (signal transducer and activator of transcription 3) transcription factor and led to late-stage cardiac dysfunction. STAT3 controls cell proliferation, growth and angiogenesis [85], but is also found in mitochondria, where it directly affects mitochondrial energetics [86]. Cardiac-specific overexpression of STAT3 in mice protected against doxorubicin-induced cardiomyopathy, and improved survival by preventing the reduction in contractile proteins [87]. Activation of STAT3 prevented doxorubicin-induced cardiomyopathy [88]. It was also shown that prostaglandin E<sub>2</sub>, a major cyclooxygenase-2 product, prevented doxorubicin-induced myocardial apoptosis and that this protection required the activation of STAT3 and ERK1/2 pathways [89].

Mice with homozygous knock-in of the p53 R172H (p53<sup>R172H/H</sup>) mutation lack the prototypical tumor suppressor activities similarly to the p53 knock-out state (p53<sup>-/-</sup>) [90]. However, p53 R172H retains its mitochondrial biogenesis capacity. Comparison of p53 R172H mutant with p53<sup>-/-</sup> mice revealed that p53 regulation of the mitochondrial genome following anthracycline exposure plays an important role in preventing cardiotoxicity [90]. Mutant p53 was protective against late-onset doxorubicin cardiotoxicity, however, this protective activity was associated with its regulation of the mitochondria and not with the loss of apoptosis or cell cycle arrest activity. Both p53 R172H mutant and p53<sup>-/-</sup> null showed loss of cell cycle inhibitor p21 induction, indicating that they are similarly defective in cell cycle regulation and apoptosis. However, unlike p53<sup>-/-</sup> null cardiomyocytes, p53<sup>R172H/H</sup> mutant cardiomyocytes show the increase in mtDNA transcripts in response to doxorubicin exposure [90].

## NF-κB signaling

ROS and RNS cause double-strand breaks in DNA, thereby activating the nuclear enzyme poly (ADP-ribose) polymerase (PARP) [55]. Overactivated PARP leads to depletion of intracellular ATP, slowing the rate of glycolysis and mitochondrial respiration, eventually resulting in cellular dysfunction and death, mostly by necrosis [55]. PARP-1 is also a known co-activator of a number of pro-inflammatory genes, including NF-κB.

The nuclear factor NF-κB superfamily of transcription factors regulates immune cell maturation, cell survival, and inflammation. During acute ischemic conditions, NF-κB has been shown to regulate cardiomyocyte survival through suppression of apoptotic cell death, but prolonged activation of NF-κB in the heart initiates inflammatory responses that lead to ER stress-induced cell death [91,92]. Increased inflammation further aggravates oxidative stress,



resulting in cardiovascular dysfunction [55]. Doxorubicin administration increases NF- $\kappa$ B binding activities [93], and activation of NF- $\kappa$ B in cardiomyocytes and endothelial cells may lead to apoptosis [94].

## Tissue- and organ-level manifestations of anthracycline toxicity

### Tissue-level manifestations

#### Histopathologic hallmarks of anthracycline cardiotoxicity

Histopathologic studies have demonstrated characteristic abnormalities resulting from anthracycline exposure [95,96]. Progressive sarcoplasmic reticular swelling and vacuolization may occur, resulting in distension and ballooning appearance of severely affected cells. The size and number of vacuoles may vary considerably within affected cells. Interstitial edema and perivascular fibrosis may accompany these findings. Myofibrils are displaced by vacuoles and degraded, leading to a reduced number of myofibrils in moderately to severely affected cells. In more severely affected cells, mitochondrial degradation occurs, and mitochondrial abnormalities may be observed such as prominent cristae, increased electron density of outer membranes, myelin figures, and mitochondrial dense bodies. Severely affected cells undergoing necrosis may have nuclear changes as well. Myocytes are not uniformly affected such that cells adjacent to affected cells may appear normal. In human autopsy studies of patients who had a long-term anthracycline cardiomyopathy, cell death may lead to a depleted number of cardiomyocytes, loss of normal myocardial fiber architecture, and interstitial and replacement fibrosis with a lack of hypertrophy of remaining cells may be observed [97,98]. In addition to these classically described findings, there has been a recent focus on anthracycline-induced development of cardiomyocyte atrophy and the development of cardiac fibrosis; we highlight recent research in these areas below.

#### Atrophy

Reduction in LV mass is observed both early after anthracycline therapy and during survivorship [99,100]. In addition to cell death, this finding may be related to cardiomyocyte atrophy. CMR imaging of breast cancer patients revealed that a reduction in LV mass index after anthracycline therapy was associated with an  $\approx$ 30% decrease in intracellular lifetime of water ( $T_{1\rho}$ ) [101], a noninvasive way for estimating the size of cardiomyocytes.  $T_{1\rho}$  measures the time it takes a water molecule to diffuse to the cell membrane, and in cardiomyocytes it is primarily affected by the cell diameter [102]. This finding suggests that LV mass reduction with anthracycline therapy is at least partly related to myocyte atrophy.

In a murine model of anthracycline-induced cardiomyopathy, Willis et al. showed that there was a 44% decrease in cardiomyocyte cross-sectional area, suggesting that cell atrophy was one of the causes of myocardial mass loss [103]. Expression of MuRF1, a striated muscle-specific ubiquitin ligase, and its upstream regulator, BNIP3 were increased in the myocardium of doxorubicin-treated animals. As typically seen in atrophy, expression of myosin heavy chain (MHC) switched from the  $\alpha$  to the  $\beta$  isoform. MuRF1 has been previously described as a mediator of cardiac atrophy in other mouse models [104]. Global MuRF1-deficient mice were resistant to doxorubicin-induced cardiomyopathy, they had preserved myocardial function and heart weight after anthracycline exposure.

Similarly, Xia et al. observed significant up-regulation of MuRF1 protein in myocardium of doxorubicin-treated mice, and proposed that the forkhead box family of transcription factors (FOXO) mediate myocardial atrophy through transcriptional activation of MuRF1 [105]. Administration of selective FOXO1 inhibitor AS1842856 to mice treated with doxorubicin prevented anthracycline-induced decline in ejection fraction and LV mass. Additionally, anthracycline-induced reduction in cardiomyocyte cross-sectional area was attenuated by AS1842856. Treatment with FOXO1 inhibitor suppressed doxorubicin-induced MuRF1 expression, suggesting that FOXO1-mediated transcription of MuRF1 is a potential mechanism of anthracycline-induced cardiac atrophy. Further studies are needed to confirm involvement of these molecular mechanisms of anthracycline toxicity in humans.

#### Fibrosis

Cardiac interstitial fibrosis has been reported in both animal and human studies of doxorubicin cardiotoxicity and may be related to oxidative stress. ROS generation is a driving force in transforming growth factor  $\beta$  (TGF- $\beta$ ) stimulation, that leads to activation of activin-linked kinase (ALK). ALK phosphorylates Smad2 and 3 signal transducers, which then bind Smad4 and induce a translocation to the nucleus where it activates transcriptional regulation [106]. TGF- $\beta$  up-regulation was described with physiological tissue repair as well as with pathological cardiac fibrosis [107,108]. NOX4 was shown to activate TGF- $\beta$ , thereby inducing the differentiation from fibroblasts to myofibroblasts [109]. Doxorubicin administration increased pathological remodeling through NOX2-dependent mechanisms

[43]. Doxorubicin up-regulated the expression of TGF- $\beta$ /Smad3, induced myofibroblast differentiation, and induced collagen-1 deposition [110].

Sirtuins belong to the family of nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases that promote DNA repair, and induce signaling that is pro-survival, stress-adaptive, and anti-aging. Sirt1 is highly expressed in cardiomyocytes, shuttles between the cytoplasm and the nucleus, and its activation by resveratrol was shown to reduce anthracycline-induced fibrosis, and protected from doxorubicin-induced cardiac dysfunction [110]. Sirt3, is localized to mitochondria, and adenovirus-mediated overexpression of Sirt3 restored mitochondrial respiratory chain defects thereby protecting from anthracycline-induced cell death [111]. Pillai et al. report that Sirt3 overexpression protected mitochondria from doxorubicin-induced DNA damage [112]. Because Sirt1 and Sirt3 are highly expressed in cardiac myocytes, Sirt1 and Sirt3 activation during anthracycline therapy may mitigate anthracycline-induced cardiotoxic effects [113]. Signaling involved in the regulation of pro-survival and anti-aging genes may be altered by anthracyclines, including Sirt [32].

Histopathologic animal studies have demonstrated the progression of fibrosis after anthracycline therapy and its correlation with CMR-derived T1 values and derived extracellular volume (ECV) measures [114]. A daunorubicin-induced rabbit heart failure model showed ultrastructural myocardial changes due to reduced mitochondrial size and number as well as increase caveolae (membrane lipid rafts) formation [115]. Fibrosis was unchanged after 3 weeks of daunorubicin administration but after daunorubicin-induced heart failure at 9 weeks post-treatment, increased fibrosis, activated  $\beta$ -1 integrin, and elevated membrane repair protein MG53 were found. A number of studies in anthracycline-exposed human cancer populations have demonstrated increased CMR-based measures of ECV, and that these parameters may have prognostic relevance [116–119]. However, it is possible that the increase in ECV fraction in human cancer survivors may be related to cardiomyocyte loss or atrophy rather than the progression of fibrosis [101,119,120]. As stated above, histopathologic findings in cancer survivors with significant heart failure have suggested that interstitial and replacement fibrosis may be an important pathologic finding in patients exposed to anthracyclines; however, it is unclear whether cardiac fibrosis is a late finding that manifests primarily after the development of end-stage heart failure [97,121,122].

## Organ-level manifestations

### Contractile dysfunction

Systolic cardiac dysfunction and heart failure is the most clinically important manifestation of anthracycline cardiotoxicity. Both acute and chronic systolic dysfunction may occur after anthracycline therapy [123–125]. Acutely, contractile dysfunction may result from multiple causes including mitochondrial dysfunction, altered calcium handling, and titin degradation. In the medium- to long-term, the reduced number of contractile units due to cardiomyocyte and myofibrillar loss contribute to contractile dysfunction. In addition, myocardial wall thinning resulting in increased systolic wall stress, and secondary stressors such as hypertension may further exacerbate systolic dysfunction [125,126]. Systolic dysfunction and heart failure with reduced ejection fraction may manifest during and early after anthracycline chemotherapy, and/or in long-term survivorship [125–129]. Rates of systolic dysfunction have been reported to be 9–17% during and shortly after therapy [10,130]. There may be some degree of improvement seen after cessation of anthracycline therapy, though systolic function may not recover to baseline levels [128]. In long-term survivors of childhood cancer, progressive incidence of heart failure over time leads to an average of ~9% of childhood cancer survivors developing heart failure by the age of 50 years [131]. Due to the dose dependence of anthracycline cardiotoxicity, heart failure rates are higher with higher dose exposure [132]. The development of heart failure after anthracycline therapy portends a poor prognosis [12,133].

### Vascular dysfunction

In addition to cardiomyocytes, anthracycline chemotherapy has direct toxic effects on the vasculature (Figure 4). The vascular wall contains both an intimal layer of endothelial cells and medial smooth muscle cells. Endothelial cells serve multiple functions including regulation of vascular tone, the passage of fluid and solutes, angiogenesis, and inflammatory responses, and smooth muscle cells cooperate with the endothelium to regulate vascular tone and blood pressure [134–136]. Although anthracyclines may cause vascular smooth muscle cell toxicity and autophagy, particularly at high doses and with repetitive and long-term treatment, the vascular endothelium is more sensitive to their effects [61,137,138]. The direct endotheliotoxic effects of anthracyclines result in reduced responsiveness to endothelium-dependent vasodilators such as acetylcholine and are distinct from the indirect vascular dysfunction caused by doxorubicin-induced nephrosis, which may cause impairment in bradykinin-mediated coronary artery dilation and enhanced vasoconstrictor response of the aorta to angiotensin II [139,140].

**Table 1** Cardioprotective strategies and their proposed mechanisms

Cardioprotective strategies	Proposed mechanisms
<i>Dexrazoxane</i>	<ul style="list-style-type: none"> <li>• Inhibits formation of anthracycline-DNA-Top2<math>\beta</math> cleavable complexes and prevents DNA damage</li> </ul>
Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs)	<ul style="list-style-type: none"> <li>• Afterload reduction</li> <li>• Improved endothelial function</li> <li>• Antioxidant properties</li> <li>• Improvement of intracellular Ca<sup>++</sup> handling</li> </ul>
$\beta$ -blockers	<ul style="list-style-type: none"> <li>• Normalization of <math>\beta_1</math> adrenergic receptor expression and sensitivity</li> <li>• Improvement of intracellular Ca<sup>++</sup> handling</li> <li>• Antioxidant properties (carvedilol)</li> </ul>
HMG-CoA reductase inhibitors ( <i>statins</i> )	<ul style="list-style-type: none"> <li>• Rho GTPase Rac1 inhibition</li> <li>• Antioxidant properties</li> <li>• Anti-inflammatory effect</li> </ul>
<i>Exercise</i>	<ul style="list-style-type: none"> <li>• Antioxidant properties</li> <li>• Improvement of intracellular Ca<sup>++</sup> handling</li> <li>• Inhibition of apoptotic signaling</li> <li>• Increased NO production</li> <li>• Improvement in mobilization and homing of cardiac progenitor cells</li> </ul>

Similar to their effects on cardiomyocytes, anthracyclines cause vascular endothelial toxicity through multiple mechanisms including topoisomerase II-related DNA damage, and ROS and RNS generation, with particular importance of eNOS and associated effects on endothelial mitochondria [141]. The oxidative and nitrosative stresses within the mitochondria cause reduced ATP formation and glutathione activity, mitochondrial dysfunction, impedance of the mitochondrial membrane potential, swelling, increased permeability, and release of cytochrome *c* into the cytosol, potentially resulting in caspase activation-mediated endothelial cell apoptosis, which is independent of Fas/FasL [58,62,142]. Cells that remain viable after anthracycline exposure show evidence of senescence, reduced proliferative ability, reduced metabolic activity, and impaired function [143]. These effects are associated with endothelial dysfunction, including reduced endothelial-dependent vascular dilation, increased permeability, impaired wound closure, reduced migratory capacity, altered angiogenic potential, and reduced vascular density [57,59,142,143].

Vascular disease is relevant to the clinical course of cancer survivors treated with anthracycline therapy. Several studies have shown that aortic stiffness increases shortly after anthracycline exposure, and there are both short- and longer term deficiencies in brachial artery flow-mediated dilation [59,144–149]. Moreover, ventricular–vascular interactions may contribute to the development of heart failure. Altered ventricular–arterial coupling has been demonstrated early after doxorubicin therapy in breast cancer patients and is predictive of subsequent cardiac dysfunction [128,150]. Arterial hypertension is highly prevalent among long-term childhood cancer survivors and is an important risk factor for anthracycline-related heart failure [126,151]. However, an association between anthracycline exposure and the development of hypertension in cancer survivors has not been established [151,152].

## Clinical implications

In this final section, we aim to link the described molecular mechanisms of anthracycline-induced cardiotoxicity to currently available clinical data on risk assessment, prevention, and treatment of toxicity (Table 1). Here we also seek to describe how underlying genetic polymorphisms affect these molecular mechanisms, and lead to alteration of individual susceptibility to anthracyclines.

### Top2 $\beta$

Top2 $\beta$  expression in peripheral blood was proposed as a biomarker of susceptibility to anthracycline-induced cardiotoxicity. Top2 $\beta$  levels in 21 patients who had evidence of cardiomyopathy with low doses of doxorubicin (<250 mg/m<sup>2</sup>) were significantly higher than among 15 patients who had preserved cardiac function despite exposure to high doses of doxorubicin (450 mg/m<sup>2</sup>) [153]. This finding suggested that patients with higher level of Top2 $\beta$  expression might be more susceptible to anthracycline cardiotoxicity.

Retinoic acid receptors bind to DNA regulatory sequences and regulate gene expression in response to their agonist all-*trans* retinoic acid (ATRA) [154]. Retinoic acid receptor  $\gamma$  (RARG) has been shown to bind to the Top2 $\beta$  promoter, and Top2 $\beta$  expression in cell culture was significantly decreased when human RARG was added, and this effect was further exacerbated with the addition of ATRA [155]. A genome-wide association study among children treated with anthracyclines revealed that an RARGS427L variant had a strong association with anthracycline-induced

cardiomyopathy [155]. RARGS427L variant did not repress Top2 $\beta$  expression as effectively as wildtype RARG, therefore, leading to higher levels of Top2 $\beta$  in cardiac cells, predisposing them to anthracycline-induced cardiotoxicity.

Dexrazoxane is a Top2 $\beta$  inhibitor that is approved for use for the prevention of anthracycline-induced cardiomyopathy in certain specific cancer populations. Initial interest in studying dexrazoxane and related substances was fueled by their *in vitro* antineoplastic effects [156]. But subsequent series of preclinical studies revealed that dexrazoxane has the potential to prevent anthracycline-related cardiotoxicity [157]. Its cardioprotective effect was later confirmed in a number of clinical trials [158,159]. Initially, the protective mechanism of dexrazoxane had been attributed to iron chelation. However, that hypothesis remains controversial, as other iron chelators (deferiasirox) do not offer protection against doxorubicin-induced cardiotoxicity [160]. It is more widely accepted that dexrazoxane prevents doxorubicin-induced DNA damage via two potential mechanisms (Figure 1) [160]. Dexrazoxane forms a complex with free Top2 $\beta$ , stabilizing its closed-clamp conformation, therefore, preventing Top2 $\beta$  binding to chromosomal DNA. As a result, doxorubicin is unable to bring Top2 $\beta$  into the DNA cleavage complexes. Additionally, dexrazoxane can bind to DNA-bound Top2 $\beta$  and trigger its proteasomal degradation [160]. This process results in fewer doxorubicin-Top2 $\beta$  DNA cleavage complexes. Meta-analysis of eight placebo-controlled trials of 1358 adult patients showed that dexrazoxane significantly reduced the risk of anthracycline-induced cardiotoxicity [161]. Most importantly, dexrazoxane use was associated with significantly reduced risk of clinical heart failure, with pooled odds ratio of 0.12 (95% confidence interval: 0.06–20.23) [161]. It is important to mention that a majority of clinical trials showing a benefit of dexrazoxane in adult patients focused on subjects receiving very high doses of anthracycline (over 400 mg/m<sup>2</sup> of doxorubicin). A meta-analysis of dexrazoxane randomized controlled trials in pediatric populations demonstrated a benefit in preventing subclinical cardiotoxicity, represented by asymptomatic LV function declines and adverse remodeling [162]. Although an initial signal suggesting that increased secondary malignancies may occur in pediatric patients receiving dexrazoxane, subsequent studies and a meta-analysis did not find this association [162]. Currently, the FDA restricts dexrazoxane use to metastatic breast cancer patients, who have received more than 300 mg/m<sup>2</sup> of doxorubicin, and require further therapy with doxorubicin [163]. Though the FDA has not approved its use in children, it has designated dexrazoxane as an orphan drug for the prevention of cardiomyopathy in children treated with anthracyclines, allowing its clinical use in pediatric patients within the United States [164]. The European Medicines Agency has also lifted its dexrazoxane contraindication to children under the age of 18 years who are intended to receive at least 300 mg/m<sup>2</sup> of doxorubicin equivalents [165].

## ROS production and mitochondrial dysfunction

Wang et al. identified a common single nucleotide polymorphism, rs2232228, in the hyaluronan synthase 3 (*HAS3*) gene that modifies the risk of anthracycline-induced cardiomyopathy [166]. *HAS3* gene encodes hyaluronan, which plays an active role in tissue remodeling, and reduces ROS-induced cardiac injury. Among individuals with rs2232228 GG genotype, cardiomyopathy was infrequent and not dose-dependent. However, in individuals exposed to high-dose (>250 mg/m<sup>2</sup>) anthracyclines, the rs2232228 AA genotype was associated with 8.9-times higher risk of cardiomyopathy compared with the GG genotype. Relative *HAS3* mRNA levels measured in healthy hearts tended to be lower among individuals with AA compared with GA genotype. The authors hypothesized that higher risk of cardiomyopathy associated with AA genotype could be due to inadequate protection of the myocardium from ROS-mediated injury and/or inadequate remodeling following high-dose anthracycline exposure [166].

## Iron-dependent ROS production

The hypothesis of iron-dependent ROS production as a mechanism of anthracycline-related cardiotoxicity has been challenged by a number of studies that did not prove effectiveness of iron chelators in preventing anthracycline-induced cardiomyopathy [167]. However, there is an association between mutations predisposing to iron overload, and susceptibility to doxorubicin damage supports this mechanism of toxicity [168]. The most common gene affected in humans with hereditary hemochromatosis is *HFE*: most affected individuals carry at least one copy of C282Y gene mutation. Genetic testing of pediatric patients treated with doxorubicin for acute lymphoblastic leukemia revealed that C282Y *HFE* carriers had higher incidence of myocardial injury during doxorubicin therapy, detected by troponin elevation [168]. Additionally, children with C282Y and/or H63D *HFE* variants had significantly lower LV function, mass, and wall thickness 2 years after diagnosis [168].

### **NOX-dependent ROS production**

Genotyping of adult non-Hodgkin lymphoma patients revealed that anthracycline-induced heart failure was associated with a variant of the NOX subunit NCF4, which is responsible for down-regulation of the enzyme [45]. This association between NOX variants and risk of cardiotoxicity was replicated in a number of other studies [169,170], suggesting that increased NOX activity could predispose to anthracycline-induced cardiomyopathy through increased ROS production. Cascales et al. correlated histopathological findings with NOX polymorphisms, and revealed that certain variants protected against focal myocardial necrosis, whereas others were strongly associated with cardiac fibrosis [171].

### **Mitochondrial dysfunction**

Enough evidence indicates that the effects on mitochondrial function and biogenesis are one of the key manifestations of doxorubicin toxicity [79]. Cardiac mitochondria are affected by anthracycline therapy via multiple mechanisms, including irreversible mtDNA breaks, anthracycline interaction with NADH, and increased ROS production. Ghrelin is a signaling peptide that prevented impairment of mitochondrial bioenergetics and reduced mitochondrial pathways of apoptosis in cultured cardiac myocytes and mice following doxorubicin exposure [172,173]. The mitochondria-specific antioxidant, MitoQ, was shown to be protective against vascular dysfunction in mice [61]. However, the translational value of these findings remains unclear, as it is recommended that cancer patients avoid antioxidant medications and supplements during cancer treatments due to their potential to interfere with cancer therapies. A recent study found that antioxidant supplementation among participants was associated with worse breast cancer outcomes [174]. These findings limit the potential for antioxidants to be beneficial in the prevention of anthracycline-induced vascular toxicity.

The role of mitochondrial dysfunction in the pathogenesis of anthracycline-induced cardiomyopathy is underlined by the finding of an association between a missense variant of the electron transfer flavoprotein  $\beta$  subunit (*ETFB*) gene and the risk of cardiomyopathy in anthracycline-treated patients [175]. *ETFB* is involved in mitochondrial  $\beta$ -oxidation and ATP production [175]. Deciphering how to protect solely cardiac mitochondria, but not mitochondria residing in cancer cells, would enable us to design novel preventive therapies to reduce anthracycline cardiotoxicity.

### **Neurohormonal antagonists**

Angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs) and  $\beta$ -blockers are the first-line therapies in the treatment of anthracycline-related cardiac dysfunction and heart failure, based on broader heart failure treatment guidelines, not specific to cardio-oncology populations [176]. Afterload reduction is the primary target of ACEI/ARBs, though preclinical studies also showed that ACEIs have an antioxidant effect [177,178], and protect against doxorubicin-induced abnormal calcium handling [179]. Blockade of adrenergic overstimulation and heart rate reduction are the primary targets of  $\beta$ -blockers, however they also have antioxidant properties and may reverse maladaptive effects on  $\text{Ca}^{2+}$  signaling [180]. Preclinical studies have demonstrated that cardiomyocyte  $\beta$ 1AR expression and responsiveness decrease due to doxorubicin-induced oxidative stress, and these effects may be prevented by carvedilol [181].

One large observational study found that most patients who received early ACEI and  $\beta$ -blocker therapy for anthracycline-induced cardiac dysfunction had successful recovery of LV ejection fraction (LVEF) [182]. However, these results have not been widely replicated. In survivorship, anthracycline-related heart failure is associated with a poor prognosis despite standard heart failure therapy [12,133]. ACEI/ARBs and carvedilol have all demonstrated some benefit in primary prevention trials [182–186]. However, these agents are not widely used for the primary prevention of cardiotoxicity during therapy with anthracyclines due to their relatively modest benefit in these trials, which is outweighed by potential side effects.

### **HMG-CoA reductase inhibitors (statins)**

Retrospective studies indicate that statin use is associated with reduced risk of anthracycline-induced heart failure [187,188]. A small prospective trial showed a modest effect on EF preservation in atorvastatin-treated cohort, when compared with a placebo group [189]. Statins exhibit pleiotropic anti-inflammatory and antioxidative effects linked to indirect inhibition of small Ras homologous (Rho) GTPases. Rho GTPase Rac1 has been shown to be a major factor in the regulation of the NOX and Top2 [190]. Therefore, Rac1 inhibition by statins is a proposed mechanism that could explain their cardioprotective effect with anthracycline use, however further studies are needed to confirm this hypothesis. PREVENT (Preventing Anthracycline Cardiovascular Toxicity With Statins) is a randomized controlled

clinical trial that is currently under way, and it aims to evaluate atorvastatin effect on LVEF preservation in 250 anthracycline-treated breast cancer patients [191].

## Cell transport and hepatic metabolism of anthracyclines

Besides the genes controlling Top2 $\beta$  activity, and redox pathways, there are also a number of genetic variants that affect cell transport and hepatic metabolism of anthracyclines that have been associated with anthracycline-induced cardiotoxicity. UDP-glucuronosyltransferase family 1A, isoform 6 (UGT1A6) is involved in hepatic anthracycline glucuronidation pathway [192,193]. UGT1A6 variants (rs17863783, V209V) with reduced enzymatic activity lead to accumulation of toxic anthracycline metabolites. They were found to be associated with an increased risk of anthracycline-induced cardiomyopathy in pediatric patient cohorts.

A number of studies identified an association between anthracycline-induced cardiotoxicity and polymorphisms in *ABCC* genes [194–196]. Human *ABCC* genes encode membrane-bound glycoprotein that is almost ubiquitously expressed in different cell types, and primarily involved in energy-dependent transport of cytotoxic agents out of the cell [197]. Genetic variants of the solute carrier (SLC) transporter *SLC28A3* (rs7853758, rs885004) were also associated with anthracycline-induced cardiotoxicity in three independent pediatric cohorts [192,198]. Canadian Pharmacogenomics Network for Drug Safety (CPNDS) recommendations suggest routine screening for *RARG* rs2229774, *SLC28A3* rs7853758, and *UGT1A6*\*4 rs17863783 variants in pediatric patients requiring anthracycline-based chemotherapy [199].

## Exercise training

Anthracycline chemotherapy is associated with decreased functional capacity measured as the peak oxygen uptake during exercise (peak  $\text{VO}_2$ ) [200]. Exercise training has been proposed as an effective and easily accessible cardio-protective strategy among patients undergoing anthracycline chemotherapy [201]. Meta-analysis of 48 randomized controlled trials of exercise training among cancer patients showed improvement in peak  $\text{VO}_2$  with exercise therapy [202]. Costello et al. found that there is an improvement in CMR-based global longitudinal strain among patients undergoing exercise training while receiving anthracycline chemotherapy [203]. Exercise training has been shown to affect many of the pathways implicated in anthracycline-induced cardiomyopathy (for review, see [200]). Exercise blunts ROS production from cardiac mitochondria in murine models, and also significantly increases expression of antioxidant enzymes such as glutathione peroxidase 1, catalase, and manganese superoxide dismutase in cardiac tissue [204]. Exercise reduced levels of the TRPC3–NOX2 complex, that was shown to correlate with the severity of anthracycline-induced cardiac atrophy [46]. Physical exercise in rodents reduces doxorubicin-induced p53 expression and prevents cardiomyocyte apoptosis [205], improves intracellular  $\text{Ca}^{2+}$  handling, and stimulates mobilization and homing of cardiac progenitor cells [200].

The growing body of evidence supporting the beneficial effects of exercise in preventing anthracycline-induced cardiotoxicity makes it an attractive potential modality for primary prevention. A recent statement from American Heart Association proposed the development of a comprehensive cardio-oncology rehabilitation model that will use the multimodality approach of cardiac rehabilitation for prevention of cardiovascular events in patients at high risk for chemotherapy-related cardiotoxicity [201].

## Future directions

As detailed in this review, there are a number of active areas of investigation to further elucidate the mechanisms of anthracycline cardiovascular toxicity and their relevance to clinical management. We seek to highlight a few interesting areas of future research.

## Dosing, time-course, and validation in human studies

A majority of the described studies investigating mechanisms of anthracycline cardiotoxicity utilized cell culture and small animal models. However, doses of doxorubicin used in these studies are frequently above the typical doses utilized in clinical practice.

Additionally, studies in human induced pluripotent stem cell derived cardiomyocytes indicate that mechanisms of toxicity are dose dependent, as different molecular pathways are involved with different anthracycline doses [206]. In addition, these studies have generally focused on acute effects of doxorubicin therapy, with findings that are not necessarily applicable to the cardiovascular health of long-term human survivors of cancer. This discrepancy highlights that basic science studies should attempt to examine dosing that is comparable with clinical use and should seek to utilize animal models of chronic effects of anthracycline therapy.

## Computational modeling

Multiple specific effects of anthracyclines at the biochemical, cellular, and tissue levels play roles in the development of cardiovascular toxicity. However, there is a limited understanding of the complex interplay between different mechanisms, and their relative significance. Computational modeling of anthracycline-induced toxicity could offer a unique *in silico* platform for exploring the complex interactions between multiple pathogenetic mechanisms [207].

## Primary prevention studies

Robust clinical studies in primary prevention seeking to alter the pharmacokinetics and cardiotoxic specificity of anthracyclines such as liposomal anthracycline formulations, dexrazoxane, and exercise training are needed. A notable limitation in prior primary prevention studies with neurohormonal antagonist therapy is that relatively low-risk patients were enrolled. The incidence of cardiomyopathy was low even in the placebo groups, making it challenging to demonstrate any cardioprotective effect in the treatment groups. In future primary prevention trials, targeted enrollment of individuals at higher risk for anthracycline-induced cardiomyopathy should be considered, focusing on individuals with pre-existing cardiovascular risk factors and cardiac disease. It is also unknown whether inherited forms of cardiomyopathies (dilated, hypertrophic cardiomyopathy) predispose to anthracycline-induced heart failure.

## Risk prediction and secondary prevention

Secondary prevention has been limited by late diagnosis of disease, at a time at which prognosis has been suboptimal. Improved risk prediction strategies are needed, which may include multimarker analyses of clinical factors, imaging, and biomarkers to better predict an individual's propensity to develop heart failure to guide therapy. In addition, a growing body of evidence indicates that genetic polymorphisms may determine individual susceptibility to anthracycline cardiotoxicities. However, studies of genetic predisposition have generally been derived from relatively small patient cohorts, often restricted to a certain geographic region. Therefore, additional studies among larger and more diverse patient populations are needed before these findings are generalized and applied in clinical practice. The ability to predict an individual's risk for anthracycline-induced cardiotoxicity is one of the ultimate goals in clinical practice; and incorporating genetic factors into the models for risk assessment could improve the accuracy of prediction [193].

## Conclusion

Anthracycline-related cardiovascular toxicities are an important public health concern relevant to a substantial number of cancer patients both during therapy and in survivorship. Anthracyclines cause cardiovascular toxicities through multiple molecular mechanisms, with important clinical implications. Through further basic and translational research a more comprehensive understanding of the molecular mechanisms of the cardiotoxic effects of anthracyclines is necessary to inform the development of innovative strategies for the prevention and treatment of anthracycline-induced cardiomyopathy.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Abbreviations

ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; ALK, activin-linked kinase; ATP, adenosine triphosphate; ATRA, all-*trans* retinoic acid; CMR, cardiac magnetic resonance imaging; DNA, deoxyribonucleic acid; ECV, extracellular volume; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ETFB, electron transfer flavoprotein  $\beta$  subunit; GPx4, glutathione peroxidase 4; GRP78/BiP, glucose-regulated protein 78; HAS3, hyaluronan synthase 3; LV, left ventricular; LVEF, left ventricular ejection fraction; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; PARP, poly (ADP-ribose) polymerase; peak  $\text{VO}_2$ , maximal oxygen consumption; RARG, retinoic acid receptor  $\gamma$ ; RNS, reactive nitrogen species; ROS, reactive oxygen

species; SLC, solute carrier; STAT3, signal transducer and activator of transcription 3; TGF- $\beta$ , transforming growth factor  $\beta$ ;  $\tau$ ic, intracellular lifetime of water; Top2 $\beta$ , topoisomerase II $\beta$ ; TRP, transient receptor potential; VEGF, vascular endothelial growth factor.

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