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Anticancer drugs and cardiotoxicity: the role of cardiomyocyte and non-cardiomyocyte cells

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Cardiotoxicity can be defined as “chemically induced heart disease”, which can occur with many different drug classes treating a range of diseases. It is the primary cause of drug attrition during pre-clinical development and withdrawal from the market. Drug induced cardiovascular toxicity can result from both functional effects with alteration of the contractile and electrical regulation in the heart and structural changes with morphological changes to cardiomyocytes and other cardiac cells. These adverse effects result in conditions such as arrhythmia or a more serious reduction in left ventricular ejection fraction (LVEF), which can lead to heart failure and death. Anticancer drugs can adversely affect cardiomyocyte function as well as cardiac fibroblasts and cardiac endothelial cells, interfering in autocrine and paracrine signalling between these cell types and ultimately altering cardiac cellular homeostasis. This review aims to highlight potential toxicity mechanisms involving cardiomyocytes and non-cardiomyocyte cells by first introducing the physiological roles of these cells within the myocardium and secondly, identifying the physiological pathways perturbed by anticancer drugs in these cells.

KEYWORDS

cardiotoxicity, cardio-oncology, cardiomyocytes, endothelial, fibroblast

1 Introduction

Cardiovascular toxicity can be defined as “chemically induced heart disease”. Drug discovery and development has improved significantly over recent years, particularly with respect to cancer therapeutics, altering the landscape for modern cancer therapy. The established chemotherapy drugs, developed in the 1950s–1970s, have been reinforced with the advent of molecular targeted cancer therapy and the recent development of immune checkpoint therapy providing an unprecedented arsenal of anti-cancer therapies. However, as cancer patients survive longer, often following treatment with multiple anticancer drugs for relatively longer periods of time, drug-induced organ toxicity, and in particular cardiotoxicity, is now becoming a major problem (1). Combined with an aging population, often with pre-existing cardiovascular problems, the cardiac safety of anticancer drugs is now necessitating the clinical

implementation of the new field of cardio-oncology to manage and moderate cardiovascular toxicity (2, 3).

Ultimately the problematic endpoint of drug-induced cardiotoxicity is the inability of the heart to efficiently pump blood around the body. The specific mechanisms of drug-induced cardiovascular toxicities are not well understood and can occur via direct interactions of the drug with cardiomyocytes or indirectly through interactions with other components of the cardiovascular system. Direct actions can be functional or structural changes to the cardiomyocytes. Functional changes include inducing arrhythmias or QT prolongation by dysregulating cardiomyocyte contractility (4), while structural changes, such as cardiomyocyte hypertrophy or loss of viability can occur, which lead to an eventual decline in cardiac function. Drugs may also directly affect non-cardiomyocyte cells such as fibroblasts, macrophages and vascular muscle cells, adversely affecting their physiology (5, 6). Evidence suggests interactions between these cell types are essential to the metabolism, growth, contractile performance and rhythmicity of the myocardium.

2 Cardiac development and cardiac cell physiology

The cardiovascular system is the first functional organ to develop during human embryonic development (7). During gastrulation, a single-layered blastula is re-organized into three germ layers: a dorsal ectoderm, a ventral endoderm and a mesoderm layer. From the mesoderm germ layer myocardial progenitor cells differentiate into cardiomyocytes and non-cardiomyocytes (endothelial cells, postnatal cardiac progenitor cells and vascular smooth muscle cells) (8). These myocardial progenitor cells migrate to form a bilateral cardiogenic plate composed of two endocardial tubes. These tubes fuse to form a primary heart tube (9). At the cellular level the endocardial tubes originate from the mesoderm germ layer, which during cardiac embryogenesis differentiates into mesothelium, endothelium and myocardium enabling the development of both cardiomyocytes and non-cardiomyocytes (Figure 1). The primary heart tube consists of an inner endocardial layer and an outer myocardial layer separated by an extracellular matrix (11). This tubular heart further differentiates via folding and looping into the truncus arteriosus, bulbus cordis, primitive ventricle, primitive atrium and the sinus venosus forming a primitive heart containing the origins of cardiomyocytes and non-cardiomyocytes within different morphological arrangements (12). This primitive heart continues to elongate and loop anatomically arranging the cell populations into chambers and major vessels. This results in the aorta and pulmonary trunk originating from the truncus arteriosus, the right ventricle from the bulbus cordis, the left ventricle from the primitive ventricle, the left and right atria from the primitive atrium and SA node and coronary sinus from the sinus venosus. Further septa and valves develop separating the left and right sides of the heart and the major vessels from the heart, respectively, in conjunction cells of cardiac neural crest and proepicardium origin migrate. This cell migration

enables the separation of outflow tracts and formation of the epicardium. The proepicardium cells are also the origin for coronary vasculature cells and cardiac fibroblasts (8). However, recent data have suggested that the coronary vasculature may be derived from multiple sources such as the proepicardium, the sinus venosus and ventricular endocardium and circulating endothelial progenitor cells (13). Ultimately, these developmental processes result in the formation of the myocardium consisting of the heart, coronary vascular and microvascular network. The developed heart is comprised of 30% cardiomyocytes and 70% non-cardiomyocyte cells including cardiac fibroblasts, endothelial cells and pericytes (14). Both endothelial cells and pericytes form a microvascular network surrounded by an extracellular matrix known as intramyocardial capillaries. This extensive microvascular network enables efficient perfusion of the heart delivering nutrients such as free-fatty acids and oxygen and facilitates cellular signaling (14).

Cardiomyocytes are the contractile competent muscle cells responsible for the coordinated contractile force required to eject blood from the left and right ventricles during systole (15). During pre-natal heart growth, cardiomyocytes show a high proliferation rate (16). After birth, the cardiomyocyte proliferation rate declines rapidly and remains at ~1% in adult humans (17). This decline in proliferation is caused by cells exiting the cell cycle before cytokinesis causing cardiomyocytes to become binucleated and polyploid (18). The subsequent postnatal increase in cardiac volume occurs almost exclusively by hypertrophy of cardiomyocytes (19). Both binucleated and polyploid cardiomyocytes are generally believed to be terminally differentiated and unable to contribute to cardiomyocyte renewal during cardiac homeostasis and injury, which has profound implications for drug-induced cardiotoxicity. In contrast, the small remaining population of diploid, mononucleated cardiomyocytes is thought to still have the capacity to proliferate (20–22).

The cardiac microvascular endothelial cells are present both within the endocardium that forms the inner lining of the heart chambers and in intramyocardial capillaries. Their primary function is to supply cardiomyocytes with free-fatty acids and oxygen to meet their high metabolic demands. Other roles of cardiac endothelial cells include aiding the adherence of immune cells, allowing inter-cellular signalling factors to be communicated and to impede thrombus formation. Recent evidence suggests that cardiac microvascular endothelial cells are present at a higher abundance than previously reported, and have been estimated to be the most prominent cell type in the myocardium accounting for 40% of the cells (23). Cardiomyocyte-endothelial cell cross talk results in paracrine signalling to coordinate cellular responses within the myocardium. Cardiomyocytes secrete VEGF-A and angiopoetin-1 (Ang-1) which support angiogenesis and vessel maturation respectively (24). Cardiac endothelial cells are known to secrete proteins such as neuregulin-1 (NRG-1), which binds to and activates the receptor tyrosine kinase ErbB4 (HER4) on cardiomyocytes, allowing dimerization with ErbB2 (HER2) (25). This activates an intracellular signalling cascade in cardiomyocytes that suppresses apoptosis and stimulates a cardioprotective effect (24, 26).

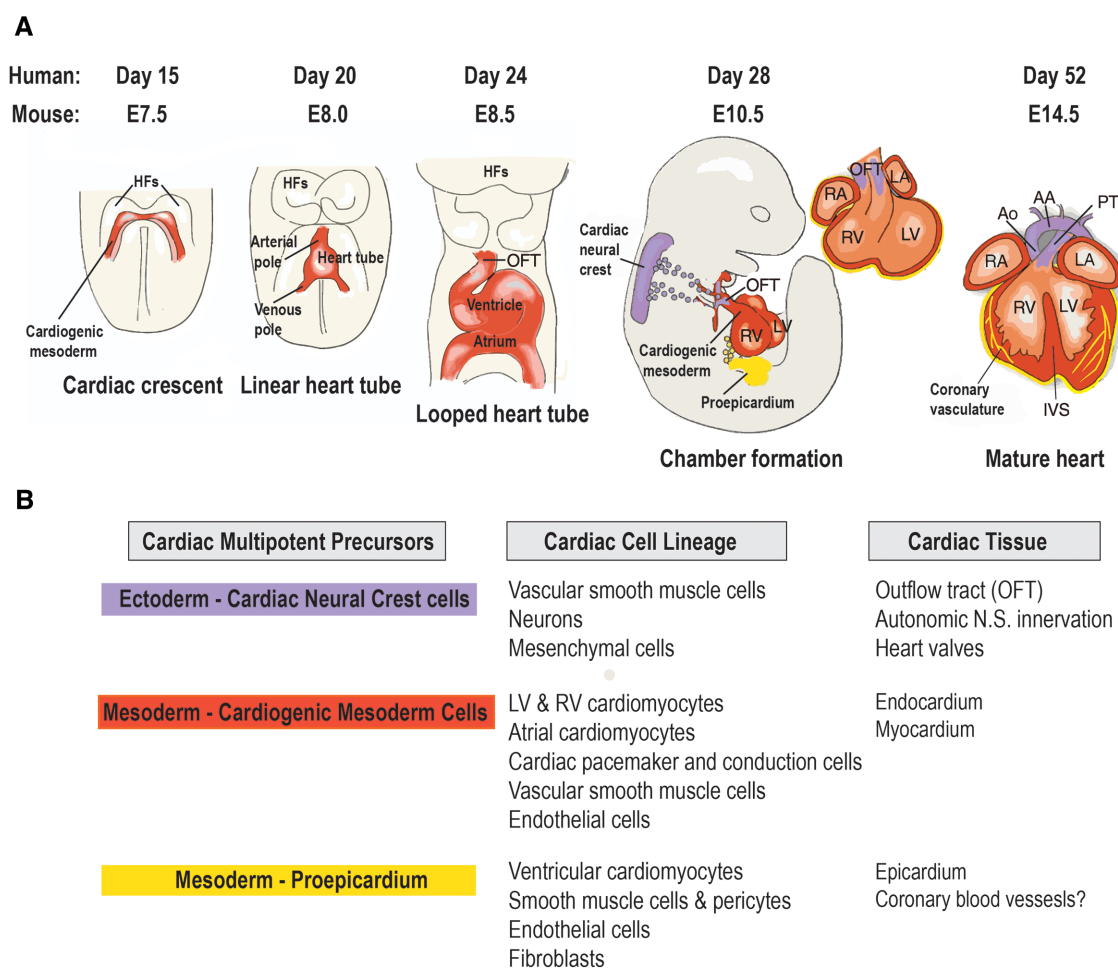


FIGURE 1

Origin and lineage relationship of cardiac cell types. (A) Contribution of three embryonic heart progenitor populations—cardiogenic mesoderm (red), cardiac neural crest (purple), and proepicardial organ (yellow)—to various heart compartments during cardiac morphogenesis. Cardiogenic mesoderm progenitors first appear beneath the head folds (HFfs) at embryonic day 7.5 (E7.5) in the mouse embryo, then migrate ventrally to the midline (ML), forming the linear heart tube, which subsequently develops into the four heart chambers. Following heart tube looping (E8.5), cardiac neural crest progenitors migrate from the dorsal neural tube to the aortic arch arteries, differentiating into vascular smooth muscle cells of the outflow tract (OFT) by E10.5. Concurrently, proepicardial organ precursors contact the heart's surface, forming the epicardial mantle and later contributing to the coronary vasculature. By foetal stage E14, the heart chambers undergo septation and establish connections to the pulmonary trunk (PT) and aorta (Ao). (B) Cardiac cell types arising from the lineage diversification of the three embryonic precursor pools in the mouse heart. AA, aortic arch; IVS, interventricular septum; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Adapted with permission from (10).

Pericytes are also present in these cardiac capillaries, on average each endothelial cell is associated with two to three pericytes (27). These pericytes are contractile cells which wrap themselves around the microvasculature and support the normal functioning of the endothelial cells. They play many physiological roles from barrier function, regulation of homeostasis, facilitation of angiogenesis to initiation of coagulation (28).

Cardiac fibroblasts (CFs) are spindle shaped connective tissue cells that do not form a basement membrane but are responsible for the majority of the extracellular matrix (ECM) proteins found in the myocardium. During embryonic development, CFs arise from multiple sources. The epicardium is considered the major source of CFs found in the ventricular myocardium (29), with epicardial cells undergoing an epithelial-to-mesenchymal transition requiring expression of Tcf21 to generate CFs (30).

Another significant population of CFs is derived from the endocardium at the time of endocardial cushion formation by an endothelial-to-mesenchymal transition (29, 31) and endocardial cells are generated in part from the second heart field progenitors (SHFPs). Lastly, a small fraction of fibroblasts are derived from neural crest lineages (32). Situated between cardiomyocytes, their close proximity teamed with the capacity to influence ECM composition provides fibroblasts with the ability to influence the phenotype of cardiomyocytes (33). Cardiac fibroblasts are essential for proper electrical conduction in the heart as they regulate calcium homeostasis through myocyte: fibroblast coupling (34, 35). In addition, cardiac fibroblasts help maintain tissue structure by regulating the deposition and remodelling of collagen and other ECM components (36, 37). Beyond their important role in maintaining normal myocardial function, CFs also

contribute to adverse cardiac remodelling during pathological conditions, such as hypertension, myocardial infarction, and heart failure (38) and are involved in arrhythmia initiation and maintenance by affecting electrical propagation (39).

3 Cardiotoxicity

Drug-induced cardiovascular toxicity is defined as a severe and potentially fatal adverse reaction to certain drugs. Cardiotoxicity is a major cause of attrition in preclinical and clinical drug development (40–42). Drug-induced cardiovascular toxicity can affect all components and functions of the cardiovascular system and can be functional, causing alteration of the mechanical function of the heart and vasculature and/or structural, causing morphological changes to cardiomyocytes, endothelial cells, cardiac fibroblasts, ultimately adversely affecting heart and vascular function and culminating in the development of conditions such as arrhythmia, LV systolic dysfunction, cardiomyopathy, myocardial infarction and heart failure (43) (Figure 2). Historically, pre-clinical *in vitro* strategies for the detection of drug-induced cardiovascular toxicity have primarily focused on detecting functional cardiotoxicity with a particular

focus on electrophysiology and electrocardiogram (ECG) abnormalities detected by high-throughput screening of key ion channels, for example, hERG, implicated in functional cardiotoxicity (44, 45). However, cardiovascular functional abnormalities can also arise through impaired left ventricular function characterized by changes in cardiomyocyte contractility. Structural cardiotoxicity can occur with a wide range of drugs and is a concern with several classes of anti-cancer agents ranging from classical chemotherapy agents such as anthracyclines, to the more modern molecular targeted therapy aimed at inhibiting specific protein kinases that play a role in cell proliferation in cancer cells (46). These kinases are often expressed in a range of normal cell types in addition to cancer cells, meaning that inadvertent effects in cardiac cells can result in structural changes to specific cardiac cell types over time, which can ultimately render the patient sensitive to the development of cardiac problems including: heart muscle injury with cardiomyopathy and heart failure, complications of coronary artery disease leading to myocardial ischaemia, arrhythmias, hypertension and thromboembolism (47, 48). Whilst doxorubicin remains one of the most widely studied cardiotoxic drugs, adverse drug effects can occur across a range of different anti-cancer drugs (Table 1) and affect multiple

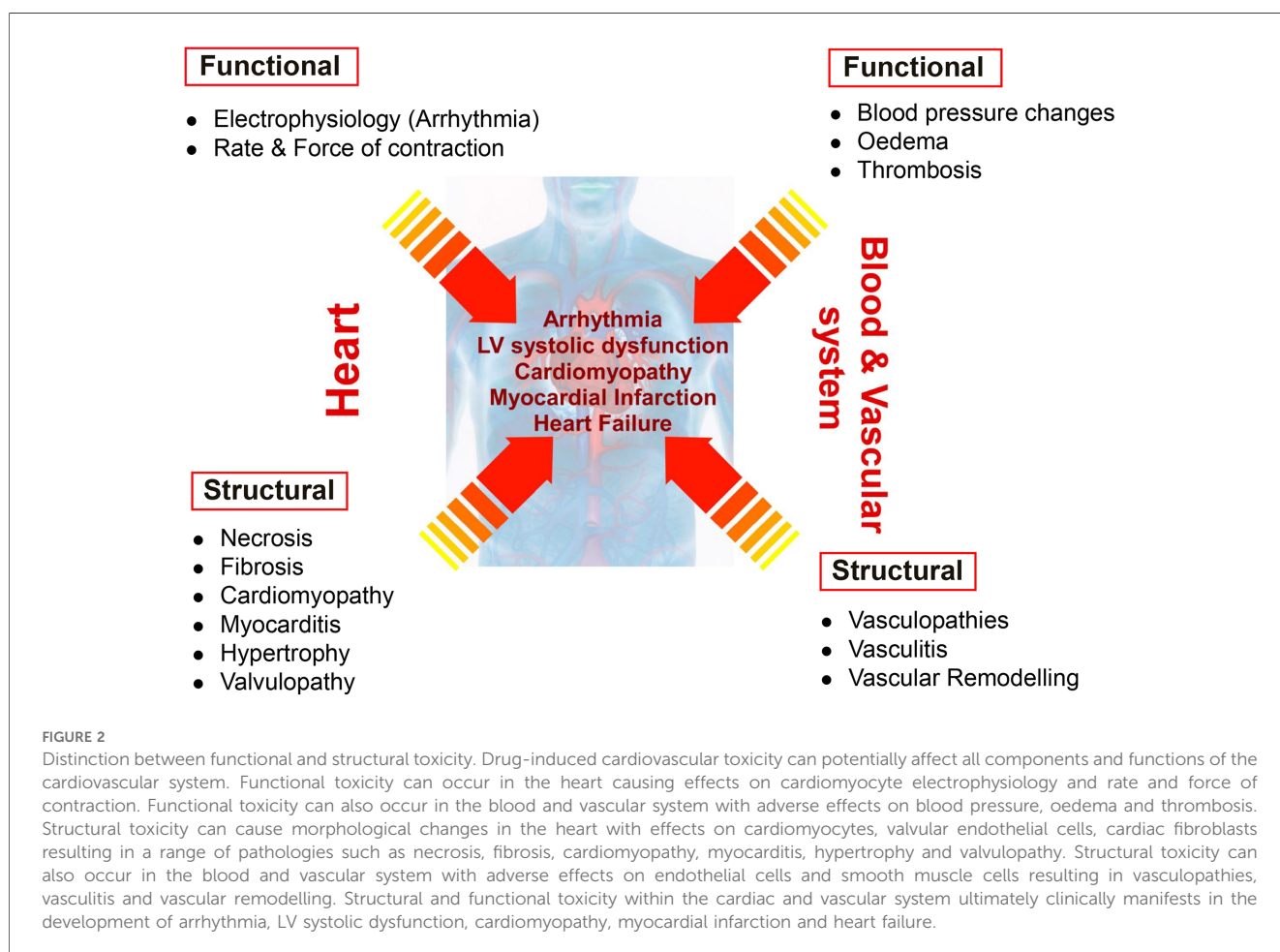


TABLE 1 Cardiovascular toxicities associated with anticancer drugs.

| Drug Class | Adverse cardiovascular event | Incidence of LVD & HF (%) | Incidence of general ADRs (%) | Reference |
|--|--|---------------------------|-------------------------------|-----------|
| Chemotherapy | | | | |
| Anthracyclines | | | | |
| Doxorubicin (Adriamycin®) | Arrhythmia, LVD, HF | 7–26 | | (49–51) |
| Alkylating agents | | | | |
| Cyclophosphamide (Cytosan®) | Arrhythmia, LVD, HF, | 7–28 | | (52) |
| Anti-metabolites | | | | |
| 5-Fluorouracil (Aducril®) | Coronary vasospasm, Cardiomyopathy & LVD, Arrhythmia | 4–12 | | (53) |
| Platinum based therapy | | | | |
| Cisplatin, Carboplatin (Paraplatin®) | Arterial vascular disease, hypertension | | | (54) |
| Microtubule binding drugs | | | | |
| Docetaxel (Taxotere®), Paclitaxel (Taxol®) Vincristine (Oncovin®) | Arrhythmias, LVD, HF | 2–8 | | (52) |
| Molecular Targeted Therapy | | | | |
| Her2 inhibitors | | | | |
| Trastuzumab (Herceptin®) | LVD, HF | 2–28 | | (51) |
| Lapatinib (Tyverb®) | LVD, HF | 1.6 | | (55) |
| VEGF pathway inhibitors | | | | |
| Bevacizumab (Avastin®) | Hypertension, LVD, HF | 2–4 | | |
| Sunitinib (Sutent®) | Hypertension, LVD, HF | 3–8 | | (56) |
| Bcr-Abl inhibitors | | | | |
| Imatinib (Glivec®) | LVD, HF | 0.7–1.1 | | (51, 57) |
| Dasatinib (Sprycel®) | Hypertension, Arrhythmia LVD, HF | 2–4 | | |
| BRaf inhibitors | | | | |
| Vemurafenib (Zelboraf®) | Hypertension, QT prolongation, LVD | 9 | | (58) |
| MEK inhibitors | | | | |
| Trametinib (Mekinist®) | Hypertension, LVD | 5.2 | | (59, 60) |
| Immune Checkpoint Inhibitors (ICIs) | | | | |
| CTLA4 inhibitors | | | | |
| Ipilimumab (Yervoy®) | General cardiac ADRs: pericarditis, myocarditis, conduction abnormalities | | 1–1.8 | (61) |
| PDI inhibitors | | | | |
| Pembrolizumab (Keytruda®) | General cardiac ADRs: myocarditis, pericardial disease, conduction abnormalities | | 1.99 | (62) |
| Nivolumab (Opdivo®) | | | 2.23 | |
| PDL1 inhibitors | | | | |
| Atezolizumab (Tecentriq®) | General cardiac ADRs: myocarditis, pericardial disease, conduction abnormalities | | 2.59 | (62) |

ADRs, adverse drug reactions; HF, heart failure, LVD, left ventricular dysfunction.

cardiac cell types leading to pathological changes that ultimately reduce cardiac function in patients (Figure 3).

3.1 Adverse effects on endothelial cells

Endothelial cells line the blood vessels of the coronary microcirculation, arteries and veins regulating the permeability and tone (63). Furthermore, endothelial cells also regulate immune cell adhesion and suppress thrombosis. Endothelial cells are in intimate contact with the blood and xenobiotics, and can be adversely affected by cancer drugs in a number of ways (Figure 3).

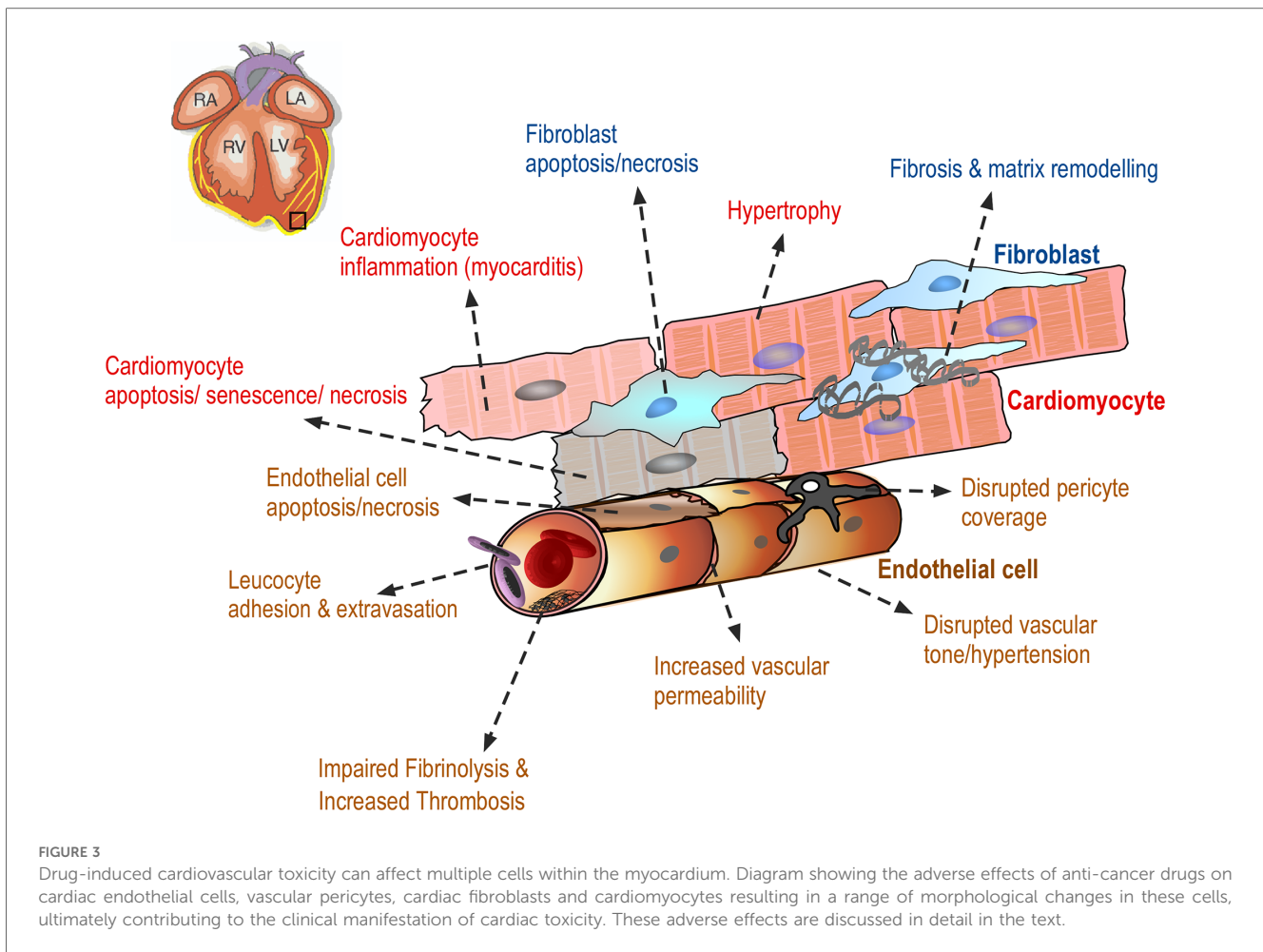
3.1.1 Increased vascular permeability

Vascular toxicity in response to chemotherapy often reflects endothelial dysfunction with loss of vasorelaxant effects and suppressed anti-inflammatory and vascular reparative functions (64). These effects can exacerbate underlying conditions of hypertension, thrombosis and atherosclerosis. Furthermore, chemotherapy can also promote coagulation of platelets due to

reduced nitric oxide bioavailability (65). Vascular permeability regulates the movement of solutes, xenobiotics and immune cells from the circulation to the underlying tissues and is critical for normal vascular homeostasis. Vascular permeability is regulated by endothelial tight junctions and adherens junctions (66). *In vivo* studies in a rat model of doxorubicin injury have shown increased cardiac capillary permeability following doxorubicin treatment (67). *In vitro* studies using bovine pulmonary artery endothelial cells have shown that doxorubicin can increase endothelial permeability (68). Recent *in vitro* data have shown that the anti-cancer drugs doxorubicin and trastuzumab (Herceptin) can adversely affect human cardiac microvascular endothelial cell barrier function by reducing expression of the tight junction protein ZO-1, leading to increased drug permeability (69, 70).

3.1.2 Apoptosis

Apoptosis is a form of programmed cell death that occurs in multicellular organisms (71). Whilst the exact mechanism of doxorubicin induced cardiotoxicity is still unknown, studies in rats have shown that doxorubicin is able to induce both



cardiomyocyte and endothelial cell apoptosis (72). Organ culture of rabbit mesenteric arteries treated with doxorubicin revealed endothelial apoptosis and impairment of endothelium dependent relaxation (73). Recent data from a mouse model of doxorubicin-induced cardiotoxicity has shown that doxorubicin-induced endothelial cell apoptosis and cardiotoxicity are alleviated by treatment with VEGF-B, which acts primarily on its cognate receptor, VEGFR-1, expressed on vascular endothelial cells (74). This novel study shows that specifically targeting the endothelium could be a potential therapeutic strategy to protect against doxorubicin induced cardiotoxicity. Clinical data from children receiving doxorubicin, in addition to other chemotherapy, has shown reduced brachial artery vasomotor reactivity months after treatment ended (75). Primary endothelial damage is the mechanism of cardiotoxicity of tubulin-binding drugs, such as vinblastine and vincristine, which have been shown to increase mitotic arrest and endothelial cell apoptosis (76).

3.1.3 Dysregulated angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing vasculature and is important for efficient cardiac vascularization (77). *In vitro* experiments using rat cardiac microvascular endothelial cells have shown that doxorubicin

stabilises hypoxia inducible factor 1- α (HIF-1 α) levels resulting in the increased transcription of VEGF mRNA and release of VEGF-A and down-regulation of VEGFR-2 levels (78). Recent data has also shown that doxorubicin is also able to down-regulate VEGFR-2 levels and cause cellular senescence in endothelial cells (79).

3.1.4 Disrupted vascular tone/hypertension

Vascular tone refers to the degree of constriction experienced by a blood vessel relative to its maximally dilated state. All arterial and venous vessels under basal conditions exhibit some degree of smooth muscle contraction that determines the diameter, and hence tone, of the vessel. Vascular tone is determined by many different competing vasoconstrictor and vasodilator influences acting on the blood vessel. These influences can be separated into *extrinsic* factors such as the sympathetic and parasympathetic nervous system, and *intrinsic* factors that originate from the vessel itself or the surrounding tissue such as adenosine and nitric oxide (NO) (80, 81). NO is a natural vasodilator, acting on smooth muscle cells to promote vasodilation. Angiogenesis inhibitors targeting the vascular endothelial growth factor (VEGF) signaling pathway have been important additions in the therapy of various cancers, especially renal cell carcinoma and colorectal cancer. Bevacizumab

(Avastin) targets the VEGF-A ligand whereas TKIs such as sunitinib, sorafenib, pazopanib and regorafenib target VEGFR-2 and other RTKs. All of these anti-angiogenic drugs lead to hypertension, which is thought to occur by loss of VEGF-mediated NO synthase activity, reducing the level of NO production in the walls of arterioles, ultimately increasing vascular tone (56). A marked increase in systemic hypertension results in significant increases in the afterload of left ventricle and myocardial oxygen demand leading to myocardial injury/infarction and LV systolic dysfunction in vulnerable patients. The incidence of hypertension has been estimated at 15%–47% with sunitinib and 17%–42% in patients treated with sorafenib (82).

Cyclophosphamide is thought to suppress NO production via a different mechanism to anti-angiogenic drugs, by generating free radicals which react with NO to produce peroxynitrite (ONOO⁻), thus reducing the level of free NO (83). Vasospasm refers to an acute condition in which an arterial spasm leads to vasoconstriction. Coronary artery vasospasm can lead to reduced blood flow and ischaemia. 5-FU can worsen or aggravate vascular spasm, resulting in acute coronary syndrome, peripheral arterial disease, cerebrovascular disease, or stress-induced cardiomyopathy (84).

3.1.5 Leukocyte adhesion and transmigration

Leukocyte migration into the vessel wall is an early pathological event in the progression of a number of cardiovascular pathologies such as atherosclerosis, diabetic cardiomyopathy and heart failure (85, 86). In response to cytokines and pro-inflammatory mediators, the endothelial lining of the microvasculature will increase expression of intracellular adhesion molecule 1 (ICAM-1) and/or vascular cell adhesion molecule (VCAM-1) that interact with leukocyte-expressed integrins allowing adhesion to the luminal surface of endothelial cells and transmigration through endothelial junctions to the underlying myocardium. Immune checkpoint inhibitors (ICIs) are a recently developed class of anti-cancer drugs, consisting of humanised monoclonal antibodies that target T lymphocytes to prevent down-regulation of the immune response. Iplimumab (Yervoy[®]) targets CTLA4, nivolumab (Opdivo[®]) and pembrolizumab (Keytruda) target PD-1, whilst atezolizumab (Tecentriq[®]) targets PDL-1. There is a growing awareness that these molecules can cause adverse cardiac effects such as atrial fibrillation, ventricular arrhythmia and most seriously, inflammation of the myocardium, termed myocarditis, occurring in up to 1% of patients taking immune checkpoint inhibitors (87, 88). The exact mechanism of myocarditis is not fully understood, however, as in viral myocarditis, patients with ICI-related myocarditis have been shown to have T-cell infiltration of the myocardium consistent with inflammation (89).

3.1.6 Impaired fibrinolysis/increased thrombosis

Haemostasis is a complex biochemical response to injury allowing the formation of a blood clot and repair of damaged endothelium. The maintenance of the equilibrium between coagulation and fibrinolysis is vital, as imbalance can lead to abnormal bleeding or increased risk of thrombosis (90).

VEGF inhibitors (bevacizumab, sunitinib, sorafenib) can cause endothelial dysfunction and platelet function inhibition, resulting in arterial thrombosis in approximately 4% of patients in addition to venous thrombosis. Alkylating agents such as cisplatin can trigger platelet aggregation (91). Recent data has shown that cyclophosphamide can potentially induce a prothrombotic state in endothelial cells by upregulating the release of prostacyclin (PGI₂) and thromboxane A₂ (TXA₂) (92).

3.1.7 Disrupted pericyte coverage

Pericytes are mural cells which stabilise capillary blood vessels through direct contact with endothelial cells on the abluminal surface of the blood vessel (27). The absence of pericytes can lead to vascular leakage and haemorrhaging (93). Sunitinib, a TKI used to treat metastatic renal cancer, has been shown to deplete cardiac microvascular pericytes resulting in changes in the cardiac microvasculature coverage in a mouse study of cardiotoxicity (94). Interestingly, coverage of skeletal muscle pericytes was not affected by sunitinib. This effect of sunitinib on cardiac pericytes was via inhibition of PDGFR- β signalling and was not reproduced by doxorubicin.

3.2 Adverse effects on cardiomyocytes

Cardiac muscle contraction is a strictly regulated process which coordinates a series of electrophysiological, biochemical and mechanical events, resulting in the ejection of blood from the right ventricle into the pulmonary circulation and left ventricle into the systemic circulation. LV dysfunction induced by cardiotoxic chemotherapies is defined by a decrease in left ventricular ejection fraction (LVEF) of >10 percentage points to a value <53% and is usually monitored by echocardiography (95). To detect early myocardial damage before a change in LVEF is evident, levels of biomarkers such as cardiac troponin-I are monitored to detect any increase in plasma concentration. Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are cardiomyocyte specific proteins that form the troponin complex with thick filaments in the contractile sarcomeres of cardiomyocytes. Both cTnI and cTnT are released into the bloodstream on acute myocardial injury, possibly due to necrosis of cardiomyocytes (96). Human adult cardiomyocytes have a restricted replicative and regenerative capacity (18, 97, 98), so irreversible damage from xenobiotics can have an accumulative effect over time in reducing cardiac contractile function. Damage to the myocardial cells can occur due to reduced blood supply in ischaemia or via direct drug-induced toxicity, leading to changes in myocardial morphology, physiology and biochemistry.

3.2.1 Apoptosis

Cardiomyocyte cell death is thought to be regulated by a number of mechanisms such as apoptosis and necrosis. Exposure to doxorubicin is known to lead to cardiomyocyte cell death via a number of different mechanisms: generation of reactive oxygen species (ROS) leading to damage to lipids, DNA and proteins

and topoisomerase 2 beta (TOP2B) inhibition leading to DNA strand breaks in cardiomyocytes and mitochondrial damage (99). Analysis of doxorubicin's effects in studies using rat cardiomyocytes and *in vivo* analysis has shown caspase 3 activation (100) and cytochrome c release (101). *In vitro* analysis using human pluripotent stem cell-derived cardiomyocytes (hiPSCMs) has shown that doxorubicin induces apoptosis through upregulation of death receptors (102). Other forms of regulated cell death such as autophagy, ferroptosis and necroptosis have also been implicated in doxorubicin injury (103), suggesting that doxorubicin has the potential to activate multiple pathways leading to cardiomyocyte death.

Classical chemotherapy drugs have also been shown to induce apoptosis in cardiomyocytes. Cisplatin and cyclophosphamide can induce ROS and mitochondrial dysfunction in rat cardiomyocytes (104, 105).

Trastuzumab (Herceptin) binds to the HER2 protein, which is upregulated on certain breast cancer cells, but can also bind to HER2 expressed on cardiomyocytes, interfering with the normal NRG-1/ErbB2 signalling axis resulting in cardiomyocyte apoptosis (106). Trastuzumab (Herceptin) induces cardiomyocyte toxicity through a mitochondrial pathway depending on ROS production and oxidative stress and is reversed by the antioxidant N-acetyl cysteine (NAC) (107). HER2 inhibition by trastuzumab is associated with an increase in expression of the proapoptotic Bcl-xS and decreased levels of antiapoptotic Bcl-xL. These alterations induce mitochondrial dysfunctions such as loss of mitochondrial membrane potential ($\Delta\Psi$), and ATP depletion with the disruption of cardiomyocyte cellular energetic and reversible contractile impairment (108). Trastuzumab-mediated blockade of ErbB2 signalling increases anthracycline-induced cardiotoxicity, most probably due to the suppression of the protective NRG-1/ErbB2 signalling axis resulting in the augmentation of anthracycline toxicity (109).

Molecular targeting drugs inhibit RTKs present on cardiomyocytes affecting cell survival. Sunitinib, which inhibits a broad range of RTKs such as PDGFR, VEGFR, c-Kit, has been shown to adversely affect mitochondrial function and cause cardiomyocyte apoptosis in animal studies (110). Furthermore, studies utilizing endomyocardial biopsies from two patients with gastrointestinal stromal tumours (GIST), who had developed severe left ventricular dysfunction during sunitinib treatment, revealed mitochondrial structure abnormalities following TEM analysis of cardiac sections (82).

3.2.2 Hypertrophy

Cardiac hypertrophy is an abnormal enlargement or thickening of the heart muscle resulting from an increase in the size of cardiomyocytes. It is an adaptive response to pressure or volume stress as a result of hypertension or valvular disease, and can also occur from mutations of sarcomeric proteins, or loss of contractile mass from prior infarction (111). Anti-cancer drugs have the potential to indirectly cause cardiac hypertrophy by reducing cardiac performance. Sunitinib has been reported to induce cardiac hypertrophy in mice by inducing cardiac

remodeling and increasing cardiac glycolytic metabolism resulting in fibrosis, increased LV mass and increased EF (112).

3.2.3 Myocarditis and immune cell infiltration

Myocarditis is an inflammatory disease of the myocardium that can be caused by a viral or bacterial infection or an autoimmune disease (113). The advent of immune checkpoint inhibitor (ICI) therapy has heralded a new era in cancer therapy with drugs targeting CTLA4 (ipilimumab) and PD-1 (nivolumab, pembrolizumab) on activated T-cells, and PD-L1 (atezolizumab) on epithelial and antigen presenting cells (APC). However, ICI therapy is also associated with a spectrum of immune-related adverse events (irAEs) such as colitis, hepatitis and myocarditis. ICI-associated myocarditis occurs in approximately 1% of patients and can be severe and life threatening (89). It is characterized by CD4+ve T-cell and macrophage infiltration into the myocardium, ultimately resulting in myocyte damage and cardiac dysfunction. Previous genetic knockout studies in mice have shown that loss of immune checkpoint protein function can cause cardiac problems; deficiency of CTLA-4 in mice is associated with a severe autoimmune myocarditis (114) whilst PD-1 or PD-L1 deficient mice are susceptible to autoimmune myocarditis (115–117). Immune cell infiltration is thought to contribute to cardiotoxicity observed with other anti-cancer drugs. Studies in mouse models of doxorubicin-induced acute cardiotoxicity have shown a significant infiltration of neutrophils into hearts 24 h after doxorubicin treatment, which was accompanied by an acute and late decrease in cardiac function, disruption of the vascular endothelium, and an increase in collagen deposition, leading to fibrosis. The depletion of neutrophils prevented doxorubicin-induced cardiotoxicity with the preservation of vascular structures and prevention of excess collagen deposition (118, 119). Another mouse study has shown that doxorubicin treatment enhanced the pro-inflammatory M1 macrophage derived from monocytes and suppressed the reparative M2 macrophage population present within the heart (120).

3.2.4 Senescence

Cellular senescence is a phenomenon characterized by stable cell cycle arrest, mitochondrial dysfunction and cessation of cell division (121). Senescence is a hallmark of aging and accumulation of senescent cells over time is associated with the declining function of the aged cardiovascular system and many age-related CVDs, including atherosclerosis and heart failure (122). *In vivo* studies in rodents have shown that doxorubicin can induce biomarkers of senescence in the myocardium of mice (123) and rats (124). Analysis of LV human heart tissue from patients with doxorubicin-induced cardiotoxicity has revealed increased expression of a range of markers of senescence in cardiomyocytes compared to LV tissue from healthy donors (125). In addition to cardiomyocytes, doxorubicin-induced senescence can potentially occur in cardiac fibroblasts and cardiac endothelial cells (126).

3.3 Adverse effects on cardiac fibroblasts

In response to cardiac injury, both cytokine and neurohumoral factors are released and profound changes occur in the mechanical strain relationships within the ventricular wall (or septum), which together are thought to underlie fibroblast activation. In response to various stressors, cardiac fibroblasts trans-differentiate into myofibroblasts, which synthesize larger amounts of ECM and gain contractile activity due to expression of α -smooth muscle actin (α -SMA) (127).

3.3.1 Apoptosis and necrosis

The potential for cardiac fibroblasts to undergo apoptosis in response to anti-cancer drugs has not been widely studied. However, a recent study has shown that doxorubicin caused both apoptosis of cardiac fibroblasts and secretion of Fas ligand, which in turn promoted cardiomyocyte death in a paracrine manner (128). Furthermore, conditional deletion of ataxia telangiectasia mutated kinase (ATM) in mouse cardiac fibroblasts attenuated cardiac cell apoptosis, Left Ventricular Dysfunction (LVD), and mortality in response to doxorubicin, suggesting that fibroblasts are central in the pathogenesis of doxorubicin cardiotoxicity through ATM.

3.3.2 Cardiac fibrosis and matrix remodelling

Accumulation of fibrotic tissue is one of the underlying features of doxorubicin-induced cardiomyopathy triggered by inflammation and free radical ROS generation. Doxorubicin has been shown to induce ROS and TGF- β and transformation of cardiac fibroblasts to myofibroblasts in a rat model of cardiotoxicity (129). The myofibroblasts express contractile proteins such as α -smooth muscle actin (α -SMA) and secrete pro-fibrotic factors such as collagen type 1 and fibronectin. Another study in rats has shown that doxorubicin acts on cardiac fibroblasts, independent of cardiomyocyte injury, to produce excess collagen (130). *In vitro* studies have also shown that doxorubicin induces α -SMA and trans-differentiation in human cardiac fibroblasts. Furthermore, induction of matrix metalloproteinase-1 (MMP-1) and IL-6 was also reported indicating the potential for matrix remodelling and inflammatory cell recruitment (131). Together, this data suggests that adverse effects of doxorubicin on cardiac fibroblasts can result in long-term changes in matrix remodelling, potentially disrupting the stromal environment around cardiomyocytes leading to long-term changes in cardiac physiology.

combination of therapies which can increase therapeutic efficacy with a concomitant increase in toxicity. Research over the last two decades has started to reveal the complexity of cardiovascular toxicity induced by anti-cancer drugs and highlighted the potential to adversely affect multiple cardiac cell types. This has profound implications for current and future approaches to pre-clinical cardiovascular safety assessment (132). Historically, drug safety assessment has focused on analysing electrophysiological effects in cardiomyocytes, or ion channel expressing cell lines, with pathological assessment of organ toxicity in rodents and dogs (133, 134). There is a clear need for more advanced *in vitro* cardiac cell models that mirror human cardiac physiology.

The advent of iPSC-derived human cardiomyocytes has allowed the development of contractile-competent 3D cardiac microtissues that combine cardiomyocytes, endothelial cells and fibroblasts (135–139). These multi-cellular cardiac cell models are amenable to plate based high content imaging and other technologies to assess effects on contractility and cell morphology (140, 141), allowing simultaneous assessment of both functional and structural cardiotoxicity.

Progression in the development of suitable cardiac endothelial and fibroblast iPSC differentiation protocols, alongside iPSC derived cardiomyocytes containing disease relevant mutations, will enable the formation of cardiac microtissue derived entirely from iPSC sources. Advantages include provision of cells at scale suitable for high volume toxicity profiling in wild type and disease models, and the potential to derive cells from multiple gender and ethnic backgrounds (20). This may yield cardiac models that better translate to patients reflecting clinically observed differences in cardiotoxicity typically revealed only during later stage clinical trials or post marketing.

Going forward, the challenges with *in vitro* cardiac cell models are to try and recapitulate haemodynamic flow, where endothelial cells form lumen-containing vessels surrounded by cardiac pericytes and stromal cardiac fibroblasts and cardiomyocytes. These models will allow us to recapitulate the physiological environment in which xenobiotics are delivered to the heart and allow more physiologically relevant analysis of drug-induced changes in cardiac physiology, this level of complexity is a real challenge for heart-on-a-chip approaches. As our understanding of the multi-cellular nature of cancer drug induced cardiotoxicity increases, so does the potential to exploit this knowledge to identify new biomarkers of cardiac cell injury as well as therapeutic interventions to try and ameliorate the adverse effects of drugs on cardiac cells.

4 Conclusions and future perspectives Author contributions

Cancer therapies have evolved remarkably from classical chemotherapy, first developed in the 1950s–1970s to target rapidly dividing cells, through molecular targeted therapies developed in the 1990s to target specific signalling pathways and more recently to immunotherapies harnessing the potential of the immune system to target cancer cells. As patients with cancer are living longer, the potential for drug-induced cardiovascular toxicity is also increasing. Furthermore, patients may be treated with a

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Conflict of interest

Author AP was employed by company AstraZeneca.

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