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Anticancer drugs continue to cause significant reductions in left ventricular ejection fraction resulting in congestive heart failure. The best-known cardiotoxic agents are anthracyclines such as doxorubicin. For several decades, cardiotoxicity was almost exclusively associated with anthracyclines, for which cumulative dose-related cardiac damage was the use-limiting step. Human epidermal growth factor receptor 2 (HER2; ErbB2) has been identified as an important target for breast cancer. Trastuzumab (TRZ), a humanized anti-HER2 monoclonal antibody, is currently recommended as first-line treatment for patients with metastatic HER2+ tumours. The use of TRZ may be limited by the development of drug intolerance, such as cardiac dysfunction. Cardiotoxicity has been attributed to free-iron-based, radical-induced oxidative stress. Many approaches have been promoted to minimize these serious side effects, but they are still clinically problematic. A new approach to personalized medicine for cancer that involves molecular screening for clinically relevant genomic alterations and genotype-targeted treatments is emerging.

Keywords: cardiotoxicity, anthracyclines, trastuzumab, oxidative stress

Active anticancer drugs: anthracyclines and trastuzumab

In the study of cancer, interest has grown in topoisomerase II (TOP2) following the discovery that it is targeted by active anticancer drugs such as doxorubicin (DOX). DOX and derivatives are highly active anticancer agents in many different clinical settings, and the identification of a crucial target of these drugs was a major landmark in the pharmacology of anticancer drugs [1]. One approach that has frequently been used in clinical trials is to combine several different anticancer drugs. The rationale for this combination was originally based on the hypothesis that these drugs act on different pharmacological targets with additive actions. A new generation of antitumor drugs has been shown to be mediated by protein kinases and to act on a family of tyrosine kinase receptors. Various therapies that target these receptors have been approved for the treatment of several cancers (lung, breast, renal cell carcinoma and melanoma). Members of the epidermal growth factor family of transmembrane receptors (ErbB family) are potent mediators of normal cell growth and development. The ErbB family consists of four closely related type 1 transmembrane tyrosine kinase receptors: epidermal growth factor receptor (EGFR; also known as HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). Members of the EGFR family are frequently overexpressed in solid tumours. Many of the tumour-expressed targets for therapeutic antibodies are growth factor receptors. By blocking ligand binding and/or signalling through these receptors, monoclonal antibodies may serve to normalize growth rates, induce apoptosis and/or help sensitize tumours to chemotherapeutic agents. TRZ, pertuzumab, and ado-TRZ emtansine are monoclonal antibodies that target the extracellular domain and are used for the treatment of ErbB2-positive breast cancer [2]. The importance of ErbB2 signalling in cardiac physiology soon became evident by the discovery that some breast cancer patients treated with TRZ (Herceptin, anti-ErbB2), an inhibitor of HER2 signalling, develop synergistic cardiac dysfunction, particularly when TRZ is combined with DOXO. Some of these anticancer agents have associated cardiotoxicities and can, at least in some patients, cause symptomatic congestive heart failure (CHF) and, in others, asymptomatic left ventricular dysfunction; which is a greater risk in young cancer survivors treated with ANTH. Several mechanisms have been suggested associated with the pathogenesis of ANTH-induced cardiotoxicity. Oxidative stress, ion dysregulation and modifications of the cardiac-specific gene expression cooperate at inducing cardiomyopathy. Here, we review what is known about the basic mechanisms of cardiotoxicity of cancer therapies with ANTH and TRZ. The identification of targets that mediate cardiotoxicity can also help to guide future drug development.

Oxidative stress and redox signalling

Sources and metabolism of reactive nitrogen and oxygen species (RNOS): Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

Redox signalling is part of the normal physiology of all cells, and plays a significant role in pathophysiological responses. Cellular (reduction-oxidation) redox environment refers to the reduction potential or reducing capacity in cellular compartments. The redox state refers to the ratio of the inter-convertible oxidized and reduced forms of a specific redox couple. Cellular redox status is regulated by the balance between cellular oxidant and reductant levels. Oxidative and reductive stress can trigger redox cascades and the environment of the cell might determine if a cell will

proliferate, differentiate, or die [3]. Any imbalance between oxidants and reductants causes oxidative or reductive stress, which triggers cell damage or aberrant signalling, leading to dysregulation. Oxidative stress occurs during biological processes, including cardiovascular disease, atherosclerosis, diabetes, cancer, inflammation, and apoptosis [4].

Free radicals have emerged as important regulators of many physiological and pathological processes through the redox process. High levels of free radical production that overwhelm cellular antioxidant defence systems may damage biomolecules, and deregulate signalling pathways.

Free radicals can be defined as molecules or compounds containing one or more unpaired electrons, which confer a great degree of reactivity to free radicals. Radicals derived from oxygen (ROS) and nitrogen (RNS: derived from nitric oxide: NO) are the largest class of radical species generated in living systems; ROS and RNS corresponding to reactive nitrogen and oxygen species (RNOS). RNOS are products of cell metabolism and have either beneficial or deleterious effects, depending on the concentration reached in the area of the cells [5, 6]. RNOS includes superoxide ($O_2^{\bullet-}$), the hydroxyl radical ($^{\bullet}OH$), carboxyl radical ($CO_2^{\bullet-}$), nitric oxide ($^{\bullet}NO$), and ($^{\bullet}NO_2$) as well as the non-radical species hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and carbon monoxide (CO).

The major RNS is endothelium-derived nitric oxide: $^{\bullet}NO$. Under physiological conditions, in the presence of substrate (L-Arginine) and co-factors (tetrahydrobiopterin: BH_4) endothelial nitric oxide synthase (eNOS) produces $^{\bullet}NO$, which is a potent gaseous mediator widely accepted as a key determinant of endothelial function [7] . Of the ROS generated in cells, the major sources of $O_2^{\bullet-}$ include nicotinamide dinucleotide phosphate (NADPH) oxidases, xanthine oxidases and cyclooxygenases (COXs). In addition, the endothelial and neuronal nitric oxide (NO) synthases (eNOS and nNOS, respectively), both cytochrome p450 reductase-like enzymes, can produce large amounts of ROS when deprived of their critical cofactor tetrahydrobiopterin or their substrate L-arginine [7, 8]. Of the many ROS-generating enzymes, NADPH oxidase, of which there are 7 homologues (termed Nox 1-5, Duox1,2), appears to be particularly important in cardiovascular disease [9]. Activation of NADPH oxidases may result from the stimulation of a number of cell surface receptors, such as the angiotensin II receptor, which is particularly important in hypertension and heart failure. Upon stimulation by angiotensin II, the activity of NADPH oxidases is increased in endothelial and smooth muscle cells, suggesting that in the presence of an activated renin–angiotensin system (either local or circulating), dysfunction due to increased vascular production of superoxide anions is to be expected [10, 11].

NO has potent vasodilator, anti-inflammatory and anti-thrombotic properties [12, 13].The free radical $^{\bullet}NO$ has a half-life of only a few seconds in an aqueous environment. $^{\bullet}NO$ reacts with molecular oxygen and ROS to generate a range of oxidation products. One well-characterized RNS-forming reaction is that of $^{\bullet}NO$ with superoxide ($O_2^{\bullet-}$), which occurs at nearly diffusion-limited rates to produce peroxynitrite: ONOO−. The latter is itself strongly oxidizing, and when protonated, undergoes homolytic scission to produce $^{\bullet}OH$ and $^{\bullet}NO_2$. Additional reactive radicals derived from other endogenous molecules such as CO, and hydroperoxyl can be formed in living systems [14]. Under physiological conditions, scavengers or antioxidants can attenuate the toxic effects of ROS and RNS. An antioxidant can be defined as any substance that when present in very low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that

substrate. The non-enzymatic antioxidants can be classified further into directly acting antioxidants (e.g. scavengers and chain-breaking antioxidants) and indirectly acting antioxidants (e.g. chelating agents). Antioxidant vitamins (i.e., vitamins C, E, A and folic acid) are some of the main defence mechanisms of the body's non-enzymatic antioxidant systems. The functions of endogenous antioxidant vitamins are very important via the temporal and spatial monitoring of oxidative metabolic processes [15, 16]. Oxidative stress is associated with dysfunction of the mitochondria and endoplasmic reticulum, thus inducing apoptosis and protein misfolding. Mitochondria are endowed with an elaborate and well-defined multi-level antioxidant defence system of enzymes and non-enzymes to quench ROS. The scavenging system includes the matrix Mn superoxide dismutase (SOD), glutathione (GSH) and thioredoxin systems, peroxidases and catalases. Redox homeostasis is crucial for cell viability and normal cell function. Balance is maintained by two major cellular antioxidant systems, namely the glutathione system and the thioredoxin system [17, 18].

Several other antioxidants (N-acetylcysteine: NAC, lipoic acid) have been shown to reduce lipid peroxidation products [19-21]. Antioxidant defence enzymes such as heme oxygenase 1 (HO-1), catalases, superoxide dismutases: SODs, peroxiredoxins, and glutathione peroxydases: GPXs have been shown to significantly decrease lipid peroxidation products. Glutathione, an endogenous scavenger, exists in both reduced (GSH) and oxidized (GSSG) states. The balance between GSH/GSSG and pyridine nucleotides (NADH/NAD, NAD(P)H/NADP) determines the cellular redox status and the level of oxidative stress [22]. Thioredoxin-dependent peroxiredoxins are the first-line defence against ROS and peroxynitrite (ONOO-) and regulate H_2O_2-mediated signal transduction. H_2O_2 can act as either a destructive oxidant or as a second messenger [23].

Oxidative stress is able to induce deleterious modifications to a variety of cellular compounds: DNA, lipids and proteins. ROS can mediate an indirect attack on endogenous molecules by reacting with cellular membrane phospholipids, which results in the generation of secondary reactive intermediates [24]. Certain intermediary metabolites are electrophilic. The nature of electrophilic stress and its role is distinct from and can be functionally decoupled from oxidative stress. Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) constitute the major products of lipid peroxidation [25]. MDA is suggested to have both mutagenic and carcinogenic effects [26]. HNE is proposed to have an effect on cellular signal transduction pathways. Lipids are susceptible to oxidative degradation caused by radicals, and during autoxidation (peroxidation) the chain reaction is mediated by peroxyl radicals and leads to damaged membrane integrity.

Various transcription factors may be activated as a consequence of oxidative stress, leading to the expression of different genes. NF-κB proteins are a family of transcription factors that play a central role in inflammation, immunity, cell survival and cell proliferation. In cancer cells, the activity and/or expression of transcriptional factors are deregulated, and these modifications are associated with ROS generation. The Nrf2-Keap1 system is a key factor in cell protection from oxidative and electrophilic insults and contributes to maintaining the redox cellular microenvironment [27]. Nrf2 binding to antioxidant response element (ARE) not only triggers antioxidant programmes that scavenge intracellular ROS but also induces cell proliferation and tumorigenesis [28].

Transition metals and oxidative stress

Transition metals including iron, copper, manganese, zinc and selenium are at trace levels for normal cellular function. Iron plays an important role in cell metabolism. Its reactivity allows it to participate in the Fenton reaction as an electron donor to hydrogen peroxide with the subsequent production of •OH. The importance of iron in catalytic processes stems from its redox reactivity, which enables it to transition between a reduced ferrous and oxidative ferric state [29] (Figure 1). In the body, an excess or deficiency of metal ions can potentially inhibit protein function and promote oxidative stress [30]. Maintaining cellular iron content requires precise mechanisms for regulating its uptake, storage, and export (Figure 2). The amount of iron absorbed by enterocytes is adapted to the body's demands. Iron transporters from the SLC11 and SLC40 families are important in intestinal iron absorption as well as whole body iron homeostasis. SLC11(A2) and SLC40(A1) are regulated at transcriptional, post-transcriptional and post-translational levels. One mechanism involved in iron homeostasis is the post-transcriptional regulator system, which comprises iron responsive elements (IREs) to which iron regulatory proteins (IRPs: IRP1 and 2)) bind in an iron-dependent fashion. The IRE–IRP regulatory system is regulated by cellular iron status but also by ROS, cells eliciting defence mechanisms against iron toxicity and iron-catalysed oxidative stress [31]. Various cardiovascular disorders and tumorigenesis in multiple human cancer types are related to deregulated iron homeostasis [32-34].

RNOS and cellular organelles

In cardiomyocytes, the mitochondria are located near calcium-release sites on the sarcoplasmic reticulum (SR) and can capture a large quantity of the released calcium. Accumulating evidence supports a critical role of biochemical cross-talk between the SR and mitochondria in normal cardiomyocyte viability and excitation contraction (EC) coupling. Mitochondrial ROS production can modify excitation–contraction coupling. A number of Ca^{2+} channels or transporters, and also myofilaments are sensitive to redox modifications [35]. Furthermore, mitochondrial ion channels, such as the inner mitochondrial anion channel, the permeability transition pore or uncoupling proteins are activated by ROS, thus leading to redox regulation [36].

The endoplasmic reticulum (ER) and mitochondria are the main sources of free radicals (Figure 1). Mitochondria are key regulators of cellular energy and redox metabolism. A number of processes, including redox-dependent ATP synthesis by oxidative phosphorylation and ROS production, occur within mitochondria. In mitochondria, when ROS production exceeds local detoxification capacity, oxidative damage to proteins, to DNA (mtDNA) and to membrane lipids occurs [37]. Each mitochondrion has two specialized membranes dividing the organelle into a narrow intermembrane space (IMS) bordered by the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), which encloses the matrix. The OMM is a major source of ROS in particular at complexes I and III, and monoamine oxidases: MAO (mostly the A isoform). Under physiological conditions, the production of ROS is estimated to account for about ~2-5% of the total oxygen uptake by the organism. Oxidative stress accompanied by calcium overload and ATP depletion induces mitochondrial permeability transition (mPT) with the formation of pathological, non-specific mPT pores (mPTP) in the mitochondrial inner membrane [38]. A specific NOS (mtNOS) was localized in cardiac mitochondria [39]. The ER also plays an essential role in multiple cellular processes, such as the folding of secretory and membrane proteins, calcium homeostasis, and lipid biosynthesis. The ER

supports the biosynthesis of approximately one third of all cellular proteins in eukaryotes. The lumen of the ER provides a special environment to achieve the proper folding of proteins [40]. Redox imbalance caused by agents such as chemotherapeutic drugs or pathophysiological conditions leads to the accumulation of unfolded/misfolded proteins in the ER lumen. Various factors, such as oxidative stress and the disturbance of calcium compartmentalization, which interferes with ER function, lead to the accumulation of unfolded proteins. The resulting ER stress triggers the unfolded protein response [41]. The important role of maintaining Ca^{2+} homeostasis within the cell is consequently dependent upon the ER and its many Ca^{2+} binding chaperones, including heat shock proteins (HSP) and calreticulin (CRT). CRT, a central Ca^{2+} buffering protein that regulates Ca^{2+} storage and release within the ER, is integral to the quality control of protein folding and Ca^{2+} storage and release within the ER [42] (Figure 1).

Recently, a new concept of immunogenic cell death (ICD) implicating the ER has emerged. The immunogenic characteristics of ICD are mainly mediated by damage-associated molecular patterns (DAMPs), which include factors such as surface-exposed CRT, secreted ATP and released high mobility group protein B1 (HMGB1). The main function of CRT is in the CRT/calnexin cycle, where it interacts with calnexin and ER protein of 57-kDa (ERp57).

Changes in the redox state and the presence of ROS also affect Ca^{2+} homeostasis by modulating the functionality of ER-based channels and buffering chaperones. In addition, a close relationship exists between oxidative stress and ER stress, both of which may activate signalling events leading to a rebalance of folding capacity and folding demand or to cell death [43]. The physical association between the ER and mitochondria, which is known as the mitochondria-associated ER membrane (MAM), plays important roles in various cellular functions. It has clear that the MAM also enables highly efficient transmission of Ca^{2+} from the ER to mitochondria to stimulate oxidative metabolism [44]. An important group of MAM proteins are ER proteins folding chaperones and oxidoreductases and the past decade has seen the association of a number of MAM proteins with cancer.

RNOS and heat shock proteins

HSP play an important role in tissue protection. The expression of HSP is a basic and well conserved cellular response to an array of stresses. HSP protect organs against a number of lesions associated with the increased production of ROS and/or cytokines. In addition, HSP exert multiple protective effects in inflammation. HSP are the products of several distinct gene families that are required for cell survival during stress. These include HSP10, HSP20, HSP27, HSP40, HSP60, HSP70, HSP90 and HSP110. The primary factor in HSP transcription is heat shock transcription factor, such as HSF1. The cytoprotective properties of HSP are closely linked to their primary functions as molecular chaperones [45, 46]. Overexpression of HSP90 and HSP20 plays an important regulatory role in processes associated with oxidative stress and leads to a significant decrease in basal levels of ROS [47]. HSP90 plays a two-faced Janus-like role in that it is essential for both normal and cancer cells [48]. HSP are over-expressed in cancer and contribute to the malignant phenotype and to resistance to therapy [49]. Tumour cells overexpress HSP90 by a factor of 2 to 10 compared with normal cells of the related tissue. HSP90 are high-potential targets for cancer therapy and several HSP90 inhibitors are currently in clinical development. One of the most potent oncogenic dependent proteins of HSP90 is ErbB2.

Cardiomyocyte turnover, oxidative stress and immunity

The proliferation of mammalian cardiomyocytes stops during the first weeks after birth, thus preventing the heart from regenerating after injury. During the ensuing early post-natal period, cardiomyocyte replication in the mammalian myocardium becomes undetectable. The number of cardiomyocytes in the mature mammalian heart remains constant throughout life [50]. As a consequence, stress leads to remodelling in which cell death surpass cell renewal, resulting in progressive heart failure. The association between ANTH exposure in childhood, the development of cardiac dysfunction, and the underlying molecular mechanisms have not been fully elucidated. In the adult heart, mitotic division of cardiac myocytes is undetectable; cardiomyocytes become terminally differentiated. The majority of mature cardiomyocytes are growth arrested at the G0 or G1 phase. The population of cardiomyocytes was thought to remain stable in number and with a one-to-one ratio to the number of capillary microvessels providing oxygen and substrate delivery, not only during post-natal physiological growth of the heart, but also in the adult heart [51]. In these conditions, persistent stress leads to an ultrastructural remodelling in which cardiomyocyte death exceeds cardiomyocyte renewal, resulting in progressive heart failure. Recently, studies revealed that mammalian cardiomyocytes retain some capacity for division and identified endogenous cardiac progenitor cells in the heart. However, there is no consensus yet about the possibility for new cardiac myocyte generation [52]. The heart is particularly susceptible to oxidative damage because of the specificity of the biology of the cardiomyocyte. Important sources of ROS are present in the myocardium [53] and paradoxically, antioxidant defences are less abundant in this tissue than in other tissues such as liver. The activities of three enzymes (SODs, catalases and GPX) capable of detoxifying activated oxygen were determined in both the heart and liver (Figure 1). Cardiac muscle contains 150 times less catalase and nearly four times less SOD than liver. GPX activities are however similar in the two tissues [54, 55]. In the myocardium as in some other tissues, the reaction of radicals in the presence of O_2, or singlet oxygen, with some amino acids, peptides, and proteins yields hydroperoxides. These species are key intermediates in chain reactions and protein damage. They can be detected in cardiomyocytes and are poorly removed by enzymatic defences [56].

While the literature clearly indicates the effects of ROS on cardiac contractility, studies on their effects on cardiac excitability are limited. Cardiac excitability depends on the functions of various cardiac sarcolemal or mitochondrial ion channels, which carry various depolarizing or repolarizing currents that also maintain cellular ionic homeostasis. ROS alter the functions of these ion channels, thus affecting the cellular resting potential and the morphology of the cardiac action potential. Several inward and outward K^+ channels are affected by different ROS-generating systems. Thus, redox balance regulates cardiac excitability, and under pathological regulation, may alter action potential propagation to cause arrhythmia [57]. Exposure of proteins to radicals results in the formation of unstable protein-derived radicals. Protein hydroperoxides play a key role in the propagation of oxidative chain reactions within proteins. These species are capable of inducing strand breaks and mutagenic lesions in DNA, thus inhibiting key cellular enzymes, altering cellular redox status, and depleting antioxidants [58, 59]. Protein hydroperoxides are capable of initiating further radical chain reactions both intra- and inter-molecularly, in particular in cardiomyocytes, thus inducing heart failure [60]. The mechanisms of heart failure are complex and multiple, but mitochondrial dysfunction appears to be a critical factor in the development of this disease. Another

mechanism related to oxidative stress and concerning the myocardium is immunity. Although autoimmunity is a well-established pathogenic principle in several endocrine, rheumatic, and neurological disorders, the mechanism has only recently gained more attention in cardiac diseases. Recent studies suggest that the heart possesses an intrinsic system that is intended to delimit tissue injury. It is suggested that this intrinsic stress response is mediated, at least in part, by a family of pattern recognition receptors that belong to the innate immune system [61]. Depending on individual genetic predisposition, heart-directed autoimmune reactions are supposed to emerge as a consequence of cardiomyocyte injury induced by inflammation, ischemia, or exposure to cardiotoxic substances [62].

The discovery and characterization of the toll-like receptor (TLR) family has led to better understanding of the innate immune system and its function in the different organs and tissues. In addition to being expressed in immune cells, TLRs are expressed in numerous tissues such as those of the cardiovascular system. Many TLRs, including TLR 2, 4 and 9, are expressed in cardiomyocytes. Through these TLRs, cardiomyocytes respond to endogenous or exogenous signals, which may influence the pathophysiological responses to dilated cardiomyopathy (DCM) [63]. Moreover, heart failure of diverse aetiology is also now recognized as having an important immune component, with TLR signalling influencing the process of cardiac remodelling and prognosis. Hence, the inhibition of TLR signalling may be of great therapeutic benefit in coronary heart failure [64].

HSP20 may play a positive regulatory role in the treatment of DOX-induced cardiomyopathy. DOXO treatment was associated with the downregulation of HSP20 in the heart. Overexpression of HSP20 inhibits DOX-triggered cardiac injury, and these beneficial effects appear to be dependent on Akt activation. HSP20 interacts with p-Akt, preventing its dephosphorylation, which subsequently maintains BAD phosphorylation and inhibits activation of caspase-3, resulting in the attenuation of DOX-mediated cardiac injury. Thus, targeted therapy to increase HSP20 expression in the heart may hold promise in suppressing DOX-triggered cardiac toxicity [65].

Oxidative stress and cancer cells

Complex series of cellular and molecular changes that contribute to cancer development are mediated by a variety of endogenous and exogenous stimuli and important amongst these is the generation of ROS. High levels of ROS generate a chronic oxidative state in the tumour microenvironment, which promotes tumour aggressiveness and acquisition of the metastatic phenotype. ROS are generated by “aberrant activity” of malignant cells [66]. In normal cells, both iron and ROS are carefully managed by the cell to maintain homeostasis or to regulate their functions. However, in cancer cells, many of the regulatory processes that control iron and ROS are altered. The especially high iron demand of tumour cells indicates a vulnerable feature of these cells [67]. The persistent oxidative stress of cancer cells is caused by an imbalance between ROS generation and the cell's ability to scavenge these species. ROS and RNS affect the activity of proteins and genes that respond to stress and which act to regulate genes that are related to cell proliferation, differentiation and apoptosis. Chronic oxidative stress in tumour cells is influenced by numerous factors such as the deregulation of antioxidant enzymes, and mitochondrial dysfunction [66]. Oxidative post-translational modifications have been shown to contribute to cancer. It has been clearly demonstrated that the tumour microenvironment in vivo tends to be highly pro-oxidative.

The interdependent interaction between tumour and its environment is complex. The effect of oxidative stress at a certain stage of carcinogenesis is directly proportional to the type and the reactivity of the radicals involved. The initiation of cancer by ROS is supported by the presence of oxidative DNA modifications in cancer tissues [68].

NO is involved in various physiological functions and its role in tumorigenesis has been well studied. There are markedly conflicting findings in the literature regarding NO and its role in carcinogenesis and tumour progression [69]. NO is generated by isoforms of NOS that are widely expressed and sometimes upregulated in human tumours. NO is a cytotoxic or apoptotic molecule when produced at high concentrations by iNOS (NOS-2). The relationship between increased expression of NOS-2 and high angiogeneic activity (i.e. microvessel density or VEGF expression) in tumour tissues suggests that cancer-derived NO is associated with tumour angiogenesis [70, 71].

The development of the cell death concept is now reconsidered, with special attention to the aetiology of apoptosis and necrosis. The immunogenic characteristics of the cell death mode are mediated mainly by molecules such as DAMPs, most of which are recognized by pattern recognition receptors. Some DAMPs are actively emitted by cells undergoing ICD (e.g. CRT), whereas others are emitted passively (e.g. high-mobility group box 1 protein: HMGB1) [72]. NOX activation is initiated by the interaction of immunogens with specific membrane receptors. NOX is identified as a participant in the innate immune response in phagocytic cells, and NO activates specific signal transduction pathways in tumour cells [73, 74]. As reported above, the generation of $O_2^{\bullet -}$ and •NO may lead to the production of the harmful molecule ONOO-. It may result in S-nitrosylation and tyrosine nitration of proteins with a concomitant change in their function. ONOO- is a potent trigger of oxidative protein and DNA damage, including DNA strand breakage and base modification. It activates the nuclear enzyme poly-ADP ribose polymerase (PARP) resulting in energy depletion and apoptosis/necrosis of cells. Peroxynitrite has been associated with the regulation of tumour cell growth, and invasion [75, 76].

Inflammation associated with immune reactions or induced by viral infections and/or autoimmune antibodies, leads to cardiac remodelling processes with ventricular dilation and systolic dysfunction [77]. Cardiac inflammation is believed to be central during heart failure progression [78]. Fatty acids are ligands of nuclear receptors that affect gene expression, and lipid peroxidation results in the generation of specific structures that are recognized by pattern recognition receptors (PRR) of the innate immune system [79]. These include humoral responses, such as naturally occurring autoantibodies (NAbs), complement factor H, C reactive protein, as well as cellular receptors, such as scavenger receptor CD36 and TLR-4. Thus, oxidation-specific epitopes (OSEs) constitute a novel class of DAMPs targeted by both PRRs and soluble pattern recognition proteins [80]. Impaired NAb function may result in chronic inflammation. In the heart, NAb activation is a major mechanism of ischemia/reperfusion injury [81].

All NOX family members are transmembrane proteins that transport electrons across biological membranes to reduce oxygen to superoxide. In accordance with this preserved function, there are conserved structural properties of NOX enzymes that are common to all family members. Activation mechanisms and tissue distribution of the different members of the family are markedly different [82]. The physiological functions of NOX family enzymes include cellular signalling, regulation of gene expression, and cell differentiation; NOX enzymes contribute to a wide range of pathological

processes such as the regulation of immunomodulation and cellular proliferation [83]. In contrast to the other NOXs, the NADPH oxidase NOX4 exists in the immediate environment of the nucleus. There is accumulating evidence for the involvement of NOX4-derived ROS in genomic instability as well as in cancer and other inflammation-related diseases. Nuclear NOX4-derived ROS may help regulate interactions among nuclear components and enzymatic activities related to DNA damage signalling and repair [84]. NOX5 mRNA can be detected in all foetal tissues, whereas in adult tissues, its expression pattern appears more specific. NOX5 was found by immunohistology in endothelial and vascular smooth muscle cells. NOX5 expression is elevated in some cancers and in some cancer cell lines [85].

As reported above, many of the tumour-expressed targets for therapeutic antibodies are growth factors receptors. In this review, we summarize the neuregulins/erbB signalling pathway; this pathway emerging as an important therapeutic target for cancer growth and cardiac related diseases.

Neuregulins and ErbB signalling

The growth factor neuregulin and molecular mechanisms

In the early 1990s, a number of groups isolated the proteins encoded by the neuregulin (NRG) gene Neuregulins and their receptors [86]. These were named neu differentiation factor (NDF), heregulin, glial growth factor, acetylcholine receptor inducing activity, sensory and motor neuron-derived factor and neuregulin. NRGs are a subclass of transmembrane polypeptide growth factors belonging to the epidermal growth factor (EGF) family – expressed in the nervous system, the cardiovascular system, mammary glands, the intestine and kidneys. Four distinct genes (NRG-1 to NRG-4) code for different NRG proteins. The release of NRG-1 takes place at the plasma membrane when the N-terminal ectodomain of pro-NRG-1 undergoes proteolytic cleavage by specific proteases such as beta-secretase 1, a disintegrin, and metalloproteinase domain containing protein 10 (A Disintegrin And Metalloproteases: ADAM10). Four NRG genes are found in mammals. NRG isoforms are either Type I, II, III, IV, V, or VI. NRG-1, which is located on chromosome 8 in both humans and mice, is the most extensively studied gene [87].

NRGs are widely expressed signalling molecules (Figure 3). NRG-1 is the most extensively studied, particularly at the cardiovascular level. NRG-1 is expressed in the microvascular endothelium [88, 89]. NRG transfer their signals through interactions with membrane receptors of the ErbB (epidermal growth factor: EGF; receptor) family. In cancer, NRGs act by binding to the human epidermal growth factor (EGF) receptor (HER) family of receptor tyrosine kinases: HER/ErbB receptor tyrosine kinases: HER/ErbB receptor tyrosine kinase. Other names for NRGs include Neu differentiation factor, or glial growth factors, following some of their biological activities in breast and glial cells. Constitutively active forms of the HER/ErbB receptors have been reported in several tumours and certain types of human cancer. Studies in mice have shown that overexpression of NRGs in the mammary tissue results in the generation of adenocarcinomas [90].

It is now evident that ErbB family members play a prominent role in the initiation and maintenance of several solid tumours. This has led to the development of specific ErbB inhibitors as cancer

therapies. The therapeutic approaches for targeting ErbB family members in cancer concern HER2-amplified breast cancer and EGFR-mutant lung cancer. HER receptors include four HER1 receptors (also known as EGFR-epidermal growth factor receptor), HER2 (ErbB2/Neu), HER3 (ErbB3), and HER4 [91]. The structure of ErbB receptors is well-known. Figure 4 provides a summary of the NRG/ErbB signalling in the cardiomyocyte and endothelium. The human Epidermal Growth Factor Receptor (HER) activation is presented in the Figure 5; for HER2 function, receptor dimerization is required. Extracellular N-terminal domain contains four subdomains (L1:I, CR1:II, L2:III and CR2: IV) The leucine-rich subdomains L1 and L2 directly interact with ligand. The cysteine-rich subdomain CR1 contains the dimerization loop responsible for receptor-receptor interaction. A short transmembrane and juxtamembrane domain links the extracellular domain to the bilobed tyrosine kinase domain and the C-terminal tail. Receptor dimerization leads to C-terminal tyrosine phosphorylation. All of the ligands of the ErbB receptor family are expressed as single-pass integral membrane proteins. These ligand pre-cursors possess an extracellular component, a transmembrane segment, and a small intracellular portion. The growth factor pre-cursors occur in the extracellular segment and they are released by proteolysis by members of the ADAMs family. The binding of growth factors to ErbBs promotes dimerization of monomeric receptors and increases the tyrosyl kinase activity of the intracellular domains of ErbBs. The structure of ERBB2 is consistent with its role as a preferred dimerization partner of the other ErbB receptors.

Signalling in cardiomyocytes through the ErbB2–ErbB4 heterodimers is essential to cell proliferation during development and to contractile function in the adult. Various signalling pathways such as phosphatidylinositol-3-kinase (PI_3K)–Akt are activated in cardiomyocytes as in breast cancer cells. PI3K activation occurs at the cell membrane and initiates intracellular signalling cascades by generating phospholipids. The p110/p85 dimer receives regulatory stimuli from transmembrane receptors via tyrosine kinases. These tyrosine kinases are able to phosphorylate tyrosine residues within activation motifs, often located within the receptors themselves. Alteration in the p85-p110/ PI3K complex is one of the most frequent driver mutations in cancer [92, 93].

In growth factor signalling, multiple signalling pathways can be activated simultaneously. The two most frequently identified pathways are the PI_3K pathway and the MAPK pathway. The HER2-PI_3K pathway is the most frequently mutated or aberrantly amplified oncogenic pathway in cancer [94]. Inhibitors targeting HER receptors and kinases of the PI_3K pathway have been developed [95].

Neuregulins in the heart and vessels

Multiple isoforms of NRG-1 are expressed in microvascular endothelial cells and cardiomyocytes. There are many effects of NRG-1β on the biology of heart and vascular cells that could contribute to the beneficial effects of NRG-1β on cardiac function [96]. These peptides play an essential role in the development of the cardiovascular system, including angiogenesis and compensation of cardiac function. The importance of ErbB2 in normal cardiac development and physiology was demonstrated in mice by cardiac-specific knock-out of ErbB2 .The mice were initially normal, but developed cardiomyopathy as adults[96].

Cardiac microvascular endothelial cells (ECs) in rat culture express multiple Type I NRG-1 gene products, including both alpha and beta variants, but only beta-variants are biologically active on

cardiac myocytes [97]. Expression of NRG is up-regulated by hypoxia and inflammatory cytokines, such as inteleukin-6 and interferon-γ, in human ECs. NRG-β1 is thought to play various roles in the prevention of atherosclerosis. NRG -β1 has anti-oxidant and anti-apoptotic properties and activates eNOS in cardiomyocytes. Physiological stimuli implicated in the regulation of NRG-1 expression and activity, include angiotensin, phenylephrine, and endothelin [98]. An important point is that oxidative stress activates NRG-1 release and activity *in vitro* via a member of the matrix metalloproteinase family. In this field, it has been reported that H_2O_2 induced NRG-1β release from cardiac microvascular endothelial cells in a concentration-dependent manner. NRG-1β release occurred via proteolytic cleavage of 115-kDa transmembrane NRG-1β [99] (Figure 3). Upon ligand binding to the extracellular domain, HER proteins form dimers and mediate potent intracellular signalling. HER2-induced signalling is initiated by an increase in HER2 enzymatic (i.e. kinase) activity and elicits the expression of numerous genes. As a transmembrane protein, the HER2 receptor is a potential target for the physiological process of proteolysis mediated by alpha-proteases .This proteolysis event leads to the production of two receptor fragments, namely the p105 fragment of the extracellular domain, which is released in the extracellular compartment, and p95HER2, which is embedded in the plasma membrane. The short extracellular domain of the 100- to 115-kDa p95 HER2 fragment contains 5 cysteines. At least some of these cysteines establish intermolecular disulphide bonds. The membrane-anchored p95HER2 fragments were active and have been shown to interact with the full-length HER2 receptor. p95HER2 may be used as a biomarker of an aggressive subtype of HER2-positive breast cancer, and tumours expressing p95HER2 tend to be resistant to treatment with TRAZ but do respond to lapatinib [100, 101].

TRAZ acts by binding to domain IV of the extracellular structure of HER2 causing, among other effects, inhibition of HER2-elicited intracellular signalling and marking HER2-positive cells for antibody-dependent cellular cytotoxicity (ADCC) (Figure 4). The primary cleavage site of HER2 has been located at amino acid position 647–648 and a minor cleavage site has been located at amino acid position 644–645. TRAZ targets the ectodomain of HER2. Alternatively, another HER2-targeting drug, lapatinib (a tyrosine kinase inhibitor that competes with ATP for the tyrosine kinase domain of HER2 and induces stabilization and accumulation of inactive Her2), could be used [102].

Two key signalling pathways activated by the ErbB family dimers are the MAPK pathway, which stimulates proliferation, and the PI_3K–Akt pathway, which promotes tumour cell survival. Activation of the Akt family allows the activation through phosphorylation of many proteins, and this initiates processes to enable tumour cell survival, and the suppression of apoptosis and cell-cycle control. PI3K inhibitors, such as GDC-0941, could be used in combination with TRAZ-based therapies [103].

Blockade of PI_3K–AKT inhibits the phosphorylation of Forkhead box class O family member proteins. FOX proteins are possible therapeutic targets and putative biomarkers for specific cancers. FOXO factors are translocated to the nucleus, where they repress the transcription of survivin and IL-8, which are members of the inhibitor of apoptosis (IAP) family of proteins [104, 105].

NRG-1β rapidly enhances NO production in adult ventricular myocytes through the activation of the PI_3K–Akt pathway and thus protects them from cell death induced by oxidative stress. Akt is able to initiate a change in mitochondrial respiration, thereby decreasing the production of ROS and increasing cell survival. If Her2 signalling is blocked, cardiomyocytes are unable to activate cell survival pathways associated with excess ROS. Therefore, blockage of HER2 enables the

accumulation of ROS within cardiomyocytes, which leads to the development of cardiac dysfunction by triggering cardiomyocyte apoptosis.

In summary, several lines of evidence suggest that ErbB2 could be a potential target for anticancer therapy through two mechanisms: first the inhibition of 1) direct antibody binding 2) dimerization 3) tyrosine kinase activity 4) HSP90; the second mechanism concerns targeting for intracellular drug delivery or through the recruitment of cytotoxic effector cells [106].

Anthracyclines and cardiotoxicity: chemical properties and mechanisms of action

The potential mechanisms of doxorubicin-mediated cell death

A number of mechanisms have been proposed to explain DOXO-mediated cell death in clinically relevant doses (~ 40 to 60 mg/m^2). Several studies have reported that oxidative stress was a major mediator of DOX-induced cardiac gene dysregulation. However, it is also possible that DOXO inhibits cardiac gene expression by inhibiting DNA replication/transcription or by aggravating protein degradation. Many anticancer and antibacterial drugs such as DOXO target TOP: type I and type II to kill cells. Poisoning of DNA TOPI is the mechanism by which DOXO interferes with tumour growth. DOXO (also called adriamycin) belongs to a class of compounds with similar structures, called ANTH. Like daunorubicin, the first ANTH compound to be described, DOXO was isolated from Streptomyces peucetius, a soil bacterium [107].

DOXO has a high affinity for cell nuclei: as much as 60% of the total intracellular amount of DOXO is found in the nucleus. The binding of ANTH to DNA inhibits DNA polymerase and nucleic acid synthesis. In addition, ANTH stabilize the otherwise cleavable complex between DNA and homodimeric TOP subunits, resulting in the formation of protein-linked DNA double-strand breaks. DOXO and other ANTH (epirubicin: EPI, daunorubicin) possess a wide range of clinical indications, including the treatment of solid tumours of the ovary, breast, and gastrointestinal system, and leukaemia. DOXO is composed of a planar aromatic ring structure containing an anthraquinone chromophore, and a sugar group (daunosamine) (Figure 1). Once taken up by the tumour cell, the planar ring structure is intercalated between adjacent DNA base pairs. A correlation between the ability of DOXO to form a covalent adduct with DNA and its cytotoxicity has been found [108].

TOP belong to a family of highly conserved enzymes, which are ubiquitously found in all cells. They are essentially involved in the control of DNA topology. The human genome possesses seven Top genes and include nuclear TOP (TOP1), mitochondrial TOP1 (TOP1 mt), TOP 2 (α and β) and TOP 3 (α and β). Type I can be further subdivided into type IA and IB. Elevated TOP 2 expression is present in tumour tissue, this property has stimulated the development of anti-tumour agents that induce cytotoxicity through TOP2 inhibition and DNA damage [109]. The anti-cancer activity of DOXO is attributable to the killing of dividing cells, where TOP2α is the major form of the enzyme. An ANTH such as DOXO binds both DNA and TOP2 to form the ternary TOP2-DOXO -DNA cleavage complex, which triggers cell death. TOP2β is also a target for DOXO, and the TOP2β-DOXO-DNA ternary cleavage complex can induce DNA double-strand breaks (DSBs), leading to cell death. Heart muscle failure is a side effect that results from damage to non-dividing cells, where TOP2β is the major form.

In the presence of TOP2β, DOXO activates the DNA response and apoptosis pathways and triggers a marked alteration in the transcriptome that selectively affects oxidative phosphorylation and mitochondrial biogenesis in cardiomyocytes.

Cell cycle arrest or apoptosis in response to DNA damage is mediated primarily by the p53 transcription factor. At higher concentrations of DOXO, p53 is activated and induces apoptosis through transcriptional up-regulation of Bax. Mutations disturbing p53 function have been associated with resistance to ANTH-containing chemotherapy [110].

Mechanisms of cardiotoxicity by doxorubicin: the free radical hypothesis

In addition to causing direct DNA damage by free radical formation, the administration of low doses of DOXO can result in increased levels of oxidative metabolism. Increased oxidative stress due to DOXO metabolism has also been regarded as the classical mechanism of cardiotoxicity. The quinone structure of DOXO can be oxidized to a semiquinone radical through the addition of one electron, which is mediated by a number of NAD(P)H-oxidoreductases. Semiquinone radicals quickly react with oxygen to generate superoxide and hydrogen peroxide causing DNA damage. Cardiotoxicity has been attributed to iron-based free radical-induced oxidative stress. DOXO had a strong affinity for iron and the iron complex could cause lipid peroxidation through its interactions with the negatively-charged membranes. DOXO reduction in the presence of free iron also sets up a cycle for free radical generation (redox recycling) and the metabolite doxorubicinol (DOXOol) is known to interact with thiol groups on proteins, thus compounding the damage to the cell. Electron Paramagnetic Resonance spin-trapping studies have shown that among a set of ANTH–Fe(III) complexes only systems in which the drug contains an α-ketol group, DOXO–Fe(III) and EPI–Fe(III) were able to reduce Fe(III) and generate hydroxyl radicals under aerobic conditions and in the absence of added reductants. The α-ketol group reduces Fe(III) and induces an increase in hydroxyl radical production. Fe(III) oxidizes the α-ketol group leading to a semidione free radical intermediate, which after a second oxidation step becomes an α-ketoaldehyde. The iron (III) complexes of DOXO and EPI were observed to undergo a self-reduction (autoxidation) reaction in the absence of added reductants under aerobic conditions that resulted in the formation of ferrous ANTH complexes [111]. These reactions lead to oxidative stress that will cause cytotoxicity through multiple mechanisms. Mitochondrial dysfunction has been shown after DOXO treatment *in vitro*, with cytochrome c release leading to caspase-9-mediated caspase 3 activation and resulting in apoptosis.

The relationship between iron and ANTH cardiotoxicity may also be related to disruption of cardiac iron homeostasis, which occurs via the targeted interaction of DOXO with iron regulatory proteins such as IRP (Figure 2). Pathways linking interactions of IRPs with DOXO, DOXOol, and quinone-derived ROS have been characterized, and have been shown to play a role in cardiotoxicity. ANTH can interact with iron-responsive element (IRE) regions of mRNAs, thus affecting IRP-mediated regulation of several iron metabolism proteins [112]. It has been shown that ANTHs are able to interact with cellular iron in a more complex way than merely producing ROS. ANTHs decreased the binding of IRPs to the IREs of mRNA, thus modifying the expression of proteins that are critical for maintaining optimal intracellular iron levels, and iron chelators might also interfere with ANTHs and cellular iron in a more complex way than the Fenton reaction [112, 113].

We demonstrated that DOXO induced cardiotoxicity through redox cycling ROS generation, and lipid peroxidation [114]. It has also become apparent that DOXO can induce apoptosis via mechanisms that do not directly involve ROS production and oxidative stress, although this point is complicated by the fact that apoptosis itself also generates ROS and RNS. Multiple mechanisms are evidently involved in DOXO-induced cardiotoxicity. It is suggested that calcium dysregulation plays a major role in the pathogenesis of this cardiomyopathy. This cardiotoxicity is accompanied by an increase in intracellular calcium levels. Dysregulation of intracellular calcium concentrations is both a result and a cause of ROS-generation. DOXO-mediated ROS generation and apoptosis can be inhibited by using a Ca^{2+} antagonist [115]. Mechanisms that have been suggested include alterations in genes important for the structural integrity and enzymatic function of cardiac and vessel myocytes. These phenomena can lead to inadequate maintenance of contractile function in the heart and we demonstated that cardiac dysfunction has been associated with the up-regulation of foetal genes. DOXO-induced cardiotoxicity concerned mRNA modifications in the genes ANF, β-MHC, and SERCA2a in hearts collected 2 months after the end of the DOXO treatment. We observed a very significant increase in ANF and β-MHC expression associated with a significant decrease in SERCA2a. A significant positive correlation was found between SERCA2a expression and +dP/dt, whereas there was a significant negative correlation between ANF expression and +dP/dt in the hearts [116]. Concerning the role of mitochondria in DOXO-cardiotoxicity, data indicate that the major mechanism of the disease of the heart is via inhibition of the electron transport chain (Figure 1). There is a rapid response at the transcriptional and translational level of many of the genes coding for proteins of the electron transport chain complexes. Biochemical analysis showed that activity of complexes I to III was reduced while that of complexes IV and V was increased [117].

Other cytotoxic mechanisms have been explored in particular via pharmacological approaches using drugs with antioxidant properties [118]. In spite of abundant studies concerning the role of oxidative stress mediated by DOXO, it must be noted that the administration of an antioxidant such as vitamin E failed to protect the heart tissue against DOXO -induced cardiotoxicity. The preventive administration of sildenafil, a phosphodiesterase 5 inhibitor, can attenuate cardiomyocyte apoptosis and left ventricular dysfunction in a mouse model of DOXO-induced chronic cardiotoxicity [119]. In a rat model of DOXO cardiotoxicity, the administration of cannabinoid-1 receptor antagonists improved the degree of cardiac dysfunction [120]. Resveratrol pretreatment in acute DOXO treatment significantly decreased ROS generation and improved antioxidant enzyme activity. These effects were associated with cardiac protection [121]. The antioxidant efficacy of numerous compounds, such as ebselen: a glutathione peroxidase mimetic, probucol: a vitamin E mimetic, and ranolazine in the prevention of ANTH-induced cardiotoxicity has been demonstrated in various studies [122-124]. ANTH-induced cardiotoxicity is certainly a multifactorial process [125, 126]. Pathways of DOXO metabolism are also highly relevant in cardiotoxicity. DOXOol is more polar than DOXO and accumulates at higher levels and for longer times in the heart, and thus exacerbates cytotoxicity [127].

Anthracyclines, mitochondrial dynamics and the immune system

An important factor, which can mediate the toxic action of DOXO, especially in mitochondria, is the high affinity binding of DOXO to cardiolipin, an anionic phospholipid specific to the inner mitochondrial membrane, which has been recognized as an essential phospholipid in eukaryotic energy metabolism [128]. Cardiolipin, with its particular ability to interact with many proteins, is very important for mitochondrial structure and function. The toxicity of mitochondrial, mostly cardiolipin-bound, DOXO is mediated by oxidative stress. Oxidative and nitrosative stress interfere with many aspects of cardiac function, inducing among others energetic imbalance, mitochondrial permeability transition and apoptosis, as well as activation of various related signalling pathways. The functional and structural changes in mitochondria that occur after DOXO exposure suggest that ROS generation is a result of changes in the transcriptome rather than redox cycling of DOXO [129].

In addition to oxidative stress, DOXO may trigger other signalling cascades that may be related to the observed toxicity. HSF activation is one such consequence. HSPs are expressed in tissues by activation of the HSFs (HSF-1, HSF-2, HSF-3, and HSF-4), which are known to respond to various stresses. Upon oxidative stress, HSF-1 is trimerized and translocated to the nucleus to transcribe various HSPs. Thus HSP25 is an essential constitutive protein, but with either increased expression or decreased degradation due to the exerted stress, it causes a redox imbalance leading to tissue damage, especially in the heart [130]. Overexpression of HSP20 in the heart attenuates DOXO-induced cardiac injury. The mechanism underlying its protection depends on the maintenance of Akt signalling cascades (Akt/BAD/caspase-3); and the attenuation of DOXO-triggered oxidative stress, leading to inhibition of DOXO-induced cardiomyocyte death and apoptosis [65].

Anthracyclines and Immunogenic cell death

The immune system plays a critical role not only during oncogenesis and tumour progression, but also in the way established neoplastic lesions respond to therapy. Cytotoxic chemicals, such immunogenic chemotherapeutics including ANTH can indeed elicit ICD. In the concept of ICD, the analysis of surface proteome changes in ANTH-treated tumour cells shows that ICD is associated with the ectopic exposure of the ER chaperone CRT. The ability of DOXO to induce ICD was shown to depend on the induction of ER stress. The combined action of ROS and ER stress was shown to activate danger signalling pathways that help to traffic DAMPs to the extracellular space. ROS were proposed to be crucial because the immunogenicity of ICD was found to be diminished in the presence of antioxidants. Moreover, the simultaneous presence of ER stress and ROS production increased the number of different DAMPs emitted [131]. DAMPs include surface-exposed CRT, secreted ATP and high mobility group protein B1 (HMGB1) also called amphoterin. The extracellularly released HMGB1 induces intense inflammation, stimulating the production of pro-inflammatory cytokines such as TNF, IL-6 and IL-8 from neutrophils, macrophages and monocytes [132]. In response to ANTH, CRT exposure occurs before the first morphological signs of apoptosis and before the translocation of phosphatidylserine from the inner to the outer area of the plasma membrane [133]. Interestingly, HMGB1 is a redox-sensitive protein. Thus, the differential activity of HMGB1 in immunity, inflammation and cell death depends on the cellular redox status within tissues [134].

Treatment with anthracyclines, incidences and risk factors

ANTH such as DOXO, epirubicin, and daunorubicin appear to be among the most active anticancer agents for the treatment of a large variety of solid tumours and haematological malignancies. Many chemotherapeutic drugs have detrimental effects on cardiovascular functions [135-138]. These agents and their cardiovascular side effects are summarized in Table 1.

Cardiac toxicity

Depending on the dose, the pharmacokinetics, and the type of ANTH used, myocardial cell loss or functional damage can occur. Morphological changes to the myocardium following ANTH treatment include myocardial cell loss by necrosis or apoptosis, the loss of both myofibrils and sarcoplasmic reticulum, and mitochondrial swelling [116, 128]. DOXO-induced cardiotoxicity may be divided into acute, subacute and late forms. Increases in levels of troponins I are indicative of cardiomyocyte injury and brain natriuretic peptides (BNPs) and N-terminal prohormone of BNP might reflect increased myocardial stress. Acute DOXO-induced cardiotoxicity occurs in up to 30% of patients. This cardiotoxicity starts within 24 h of the infusion and includes ECG abnormalities such as atypical ST changes, reduced QRS voltages, tachycardia and supraventricular premature beats. Early toxicity develops months after the last chemotherapy dose and typically presents as new onset heart failure with left ventricular systolic dysfunction. The prevalence of left ventricular contractile dysfunction in patients with a cumulative DOXO dose of approximately 430–600 mg/m^2 is about 50–60%, in whom a significant incidence of cardiac diseases are observed. The incidence of heart failure is nearly 2% with a cumulative dose of 300 mg/m^2 but rapidly increases to 20% at cumulative doses in excess of 550 mg/m^2 [139]. A chronic side effect of DOXO is its dose-dependent cardiotoxicity. Sub-acute cardiotoxicity is rather rare, and appears several weeks or months (as late as 30 months) after the last dose of ANTH. The most frequent disease observed in patients is pericarditis; it is a condition in which the sac-like covering around the heart (pericardium) becomes inflamed. Moreover, patients treated at a younger age appear to be more vulnerable to ANTH-induced cardiotoxicity. An age of < 4 years at the time of exposure is associated with a significant risk of later cardiac dysfunction. The chronic form may not become evident until as many as 4 to 20 years after the last administration of DOXO, and is associated with progressive myocardial dysfunction (dilated cardiomyopathy and congestive heart failure). Late reactions are seen years after presentation as new-onset cardiomyopathy often in patients who were treated for childhood neoplasms. [140, 141]. A population-based study of breast cancer survivors showed that women aged 66 to 70 years who received ANTH and had a follow-up of more than 10 years experienced higher rates of CHF than did women who received neither ANTH nor chemotherapy [142]. It is important to remember that the degree and progression of ANTH-related toxicity differ among individuals, suggesting that the genetic predisposition and risk factors are involved [143].

Radiation therapy is frequently used in combination with chemotherapy and may worsen the cardiotoxic effects of ANTH. Experimentally, we demonstrated in rat hearts that the combination of DOXO and cardiac irradiation could precipitate the unexpected expression of congestive heart failure. Oxidative lesions induced by irradiation and DOXO could represent one of the pathogenic factors of myocardial dysfunction [144].

Iron chelators as chemotherapeutic agents

Tumours possess altered iron homeostasis, which is mediated by the perturbed expression of iron-related proteins. Cancer cells have a higher uptake and utilization of iron by virtue of possessing significantly higher levels of transferrin receptor 1 than healthy cells [145]. Iron chelators have also been studied as anticancer agents, because cancer cells have a higher requirement for iron than healthy cells due to their rapid rate of proliferation. Iron chelation has long been considered a promising strategy to limit cumulative, dose-dependent cardiac toxicity either by restoring cellular iron homeostasis or by removing redox-active iron, which may promote ANTH-induced oxidative stress. The development of several newer generations of cardioprotective iron chelators was a pharmacological intervention in response to the cardiotoxicity by DOXO, which is an iron chelator with TOP-inhibitory and DNA damaging activity [146].

One iron chelator that has consistently shown cardioprotective ability *in vitro* and *in vivo* test systems is dexrazoxane (ICRF-187) (Figure 1). Dexrazoxane remains the only approved drug for the prevention of ANTH-induced cardiomyopathy. Dexrazoxane is a bisdioxopiperazine that is orally active as a prodrug. The mechanism of action of this drug in cardioprotection is that the relatively non-polar compound is taken up by cardiomyocytes, where it is then converted to its ring-opened hydrolysis product ADR-925, which is a diacid–diamide analogue of EDTA. ICRF-187 (the ring closed form) is also a catalytic inhibitor of the nuclear enzyme TOP2. ADR-925 can rapidly displace iron from ANTH, suggesting that it has a stronger affinity for iron than does ANTH [147].

Several established and investigational iron chelators such as thiosemicarbazone derivatives inhibit TOPs. 3-Aminopyridine-2-carboxyaldehyde thiosemicarbazone (3-AP or triapine) reduces TOP1 activity. Among those known to chelate iron and target TOP2α are dexrazoxane, (E)-N,N-dimethyl-2-(quinolin-2-ylmethylene)hydrazinecarbothioamide (TSC-24), and Dp44mT [148]. TSC-24 is considered a catalytic TOP2α inhibitor due to a direct interaction with the ATPase domain of TOP2α, which leads to a blockade of ATP hydrolysis [149]. The two mechanisms: higher levels of iron metabolism and elevated expression of TOPs may work completely independently to elicit the convergent goal of growth arrest and cell death. Another possibility is that dual targeting may accentuate TOP inhibitory action [148]. Many chemotherapeutic drugs as well as targeted chemotherapeutic agents have been tested as single agents or in combinations. Cancer cells acquire drug resistance via various mechanisms. HO-1 is a key enzyme exerting potent cytoprotection; HO-1 plays critical roles in physiological iron homeostasis and antioxidant defence, and has anti-inflammatory and anti-apoptotic effects [14]. Recent studies show that inhibition of HO-1 induced the sensitization of human carcinoma cells to DOXO. Inhibition of HO-1 could be a strategy to enhance the response of carcinomia to chemotherapeutic drugs [150].

Anthracyclines, immunity and cardiotoxicity

Stimulation of TLRs to activate the innate immune system has been a therapeutic strategy for some years. TLRs 3, 4, 7, 8 and 9 are all validated targets for cancer. The products that have been developed specifically to target TLRs in cancer therapy are the imidazoquinolines, imiquimod and resiquimod, which target TLR7. The dominant antitumoural mode of action of these agents is TLR7-mediated activation of the central transcription of NF-κB, leading to the induction of proinflammatory cytokines and interferon (IFN)-α [151]. However, TLRR7 agonists are often cardiotoxic at or above therapeutically effective doses [152].

The objective of a recent study was to evaluate the therapeutic effect of TLR2 and TLR4 blockade on already established DOXO-induced cardiomyopathy. The most significant findings of this study were that the blockade of TLR2 attenuated left ventricular dysfunction and fibrosis in DOXO-triggered acute and chronic cardiomyopathy. This attenuation was strongly associated with reduced inflammation and reduced TLR2 endogenous agonist levels. In contrast, TLR4 inactivation aggravated DOXO-induced cardiac injury and dysfunction, which was related to increased inflammation and decreased autophagy. This study provided direct evidence of the differential effects of TLR2 or TLR4 inhibition on DOXO-induced acute and chronic cardiomyopathy [153].

Non-oligonucleotide small molecule inhibitors of TLR9, such as AT791 {3-[4-(6-(3-(dimethylamino)propoxy)benzo[d]oxazol-2-yl)phenoxy]-N,N-dimethylpropan-1-amine}- and E6446 {6-[3-(pyrrolidin-1-yl)propoxy)-2-(4-(3-(pyrrolidin-1-yl)propoxy)phenyl]benzo[d]oxazole}, are being developed. These compounds are orally bioavailable. AT791 and E6446 are typical of "lysosomotropic" compounds in that they are lipophilic and contain weak base amines. Interestingly, TLR9 has been shown to influence myocardial function, and the cardioprotective effects due to TLR9 deficiency were associated with suppression of the TLR9 downstream pathway [154]. The severity of DOXO-induced intestinal injury can also be reduced by using a TLR9 antagonist, which suggests a new therapeutic strategy for limiting DOXO-induced intestinal inflammation [155].

Obesity and chemotherapy

Obesity is associated with a poor outcome in breast cancer patients treated with DOXO-based chemotherapy [156, 157]. The mechanism by which obese organisms display enhanced sensitivity to DOXO-mediated toxicity is unknown. The dysregulation of adipocyte-derived hormones, adipocytokines, promotes the development of diverse obesity-linked diseases [158]. Plasma adiponectin levels are decreased in obese subjects. Adiponectin confers resistance to DOXO-induced myocardial damage through activation of Akt signalling within cardiomyocytes. Adiponectin-KO mice showed exacerbated left ventricle contractile dysfunction following DOXO injection, whereas exogenous adiponectin improved DOXO-induced left ventricular dysfunction in wild-type and adiponectin-KO mice [159]. It has also been demonstrated that metformin protects cardiomyocytes from DOXO-induced damage and that the cardiac adiponectin system plays an important role in this protective action [160].

Evidence clearly supports a concept in which cancer cells reprogram adipocytes to cancer-associated adipocytes. Reprogrammed adipocytes produce growth promoting cytokines and provide lipids and

other metabolites to cancer cells, thus promoting uncontrolled tumour growth [161]. DOXO treatment affects lipid and glucose metabolism. It increases serum total cholesterol, triglyceride and LDL cholesterol levels. DOXO inhibits adipogenesis in a dose-dependent manner and down-regulates the expression of peroxisome proliferator-activated receptor, PPARγ, leading to the prevention of bodyweight gain through the inhibition of adipogenesis. Activation of PPARγ using a PPARγ agonist may be useful in controlling bodyweight loss [162].

Trastuzumab and cardiotoxicity

Monoclonal antibody therapy

In the past 15 years, 12 therapeutic antibodies have obtained FDA approval for haematological malignancies as well as solid tumours [163]. Monoclonal antibodies (mAbs) are an important group of targeted therapies which are directed against transmembrane proteins with extracellular domains. Several mAbs have entered clinical practice. Notable examples include TRZ (Herceptin®), lapatinib (Tykerb®), pertuzumab (Omnitarg®), panitumumab (Vertibix®), rituximab (Mabthera®/Rituxan®) and cetuximab (Erbitux®) [164]. Four HER2-targeted therapies have been approved for HER2-positive breast cancer: two antibodies (TRZ and pertuzumab), an antibody-drug conjugate (ado-TRZ emtansine), and a small molecule kinase inhibitor (lapatinib). TRZ emtansine (Kadcyla™) is an antibody-drug conjugate consisting of TRZ covalently linked to the highly potent microtubule inhibitory agent DM1 (a cytotoxic derivative of maytansine) via a stable thioether linker [165].

TRZ complementary-determining region amino acids complement and bind to amino acids present on domain IV of the HER2 ectodomain. Its functions are divided into those mediated by Fab (fragment, antigen binding) or Fc (humanized fragment) regions. The Fab region contains the antigen-binding sites of the antibody, whereas the Fc region contains the binding sites for Fcy receptors (RIII) present on immune cells, platelets, hepatocytes, and endothelial cells [166]. The interaction between the Fab region of TRZ and a peptide fragment from HER2 was investigated with molecular dynamics simulations. The interaction energies of the mutated peptides indicated that TRZ binds to ligand through electrostatic and hydrophobic interactions [167]. The second HER2-targeted mAB, pertuzumab, binds to subdomain II of HER2 extracellular domain (See Fig.4 and 5).

Among the immune cells, natural killer (NK) cells constitute a group of normal lymphocytes that induce innate immune responses towards tumour and virus-infected cells. Treatment strategies to manipulate human NK cell functions have involved immunotherapies to treat cancer. Several approaches have been designed to enhance the NK cell–mediated ADCC activity through antibody engineering [168]. ADCC is considered a major mode of action of many therapeutic mAbs, including treatments for cancer [169]. Some of the clinically approved therapeutic antibodies to treat cancer, such as TRZ, rituximab (anti-CD20 mAb), cetuximab (anti-EGFR mAb), and mogamulizumab (anti-CCR4 mAb), are considered to function at least partially through triggering NK cell–mediated ADCC activity. TRZ, which can be used to treat HER2/neu-positive breast cancer patients, mediates abrogation of tumour cell signalling and ADCC [102]. HER2 is found to be overexpressed in 25% to 30% of breast cancer patients (breast and gastric cancers). TRZ is currently recommended as the first-line treatment for patients with metastatic HER2+ tumours, either as a single agent (limited group of

patients) or in combination with endocrine therapy or chemotherapy. The use of TRZ may be limited by the development of drug intolerance, manifesting as cardiac dysfunction, for example. [2, 170].

Trastuzumab: signalling, gene expression and cardiac dysfunction.

Clinical trials of TRZ have reported heart failure in 1.7% to 4.1% of subjects, and reduced left ventricular ejection fraction in 7.1% to 18.6% of subjects treated with adjuvant chemotherapy and TRZ [171]. Risk factors specific for TRZ -associated cardiotoxicity have not been clearly established, although the majority of patients had other risk factors for cardiac dysfunction. Analyses of the potential risk factors, including age, weight, hypertension, cumulative dose, and HER2 expression level have revealed that only age and concurrent DOXO therapy were significantly associated with an increased risk of cardiac disease [171]. Since the cardiotoxicity caused by this agent was reversible upon discontinuation of treatment or initiation of appropriate cardiovascular therapy, a mechanism not related to loss of the terminally differentiated cardiomyocytes was suggested. The clinical cardiotoxicity of TRZ, together with the dilated cardiomyopathy observed in NRG-1/ErbB-deficient mice, suggests a prominent role of NRG-1 in the pathogenesis of chronic heart failure [172]. Various strategies have been developed to reduce the cardiotoxicity of TRZ without significantly compromising its therapeutic efficacy. These include optimization of chemotherapeutic combinations, shortening of treatment duration and careful monitoring of patients. Different mechanisms are proposed for TRZ cardiotoxicity. It is likely that TRZ-induced cardiotoxicity results from interference with the action of NRG. NRG-1β activates phosphorylation of ErbB2 and ErbB4 receptors, which are expressed in cardiac myocytes of the adult rat. NRG-1/ErbB activity interacts with myocardial metabolism, thus improving mitochondrial function. Thus, the inhibition of ErbB2 signalling by TRZ in patients receiving DOXO may interfere with the protective effects of NRG on the ANTH-damaged myocardium. This may account for the increased clinical cardiotoxicity observed with concurrent and sequential administration of ANTH and TRZ [2]. Clearly, the most effective means to limit DOXO/TRZ-induced cardiotoxicity is modulating the dosages and delaying time between DOXO and TRZ treatment initiation.

Interestingly, recent results show that, in mice, TRZ treatment induces major effects on the expression of myocardial genes involved in myocardial functions, adaptation to stress, and DNA repair. These genetic changes are associated with increased myocardial oxidative and nitrosative stress and activate apoptotic pathways, leading to elevated serum troponin-I and cardiac myosin light chain-1 (cMLC1) levels [173]. A particularly important mechanism for TRZ-mediated cardiovascular disease appears to be via specific transcription factors such as Notch and NF-κB signalling.

The relationships between Notchs and ErbB2 are complex, and recently it has been demonstrated that Notch-1 was a novel target in TRZ-resistant breast cancer, suggesting that combined inhibition of Notch and ErbB2 signalling pathways may be beneficial in the treatment of recurrent TRZ-resistant disease [174]. Notch signalling regulates cardiovascular development and homeostasis and plays a role in regulating cardiac hypertrophy, cardiomyopathy and heart failure [175, 176]. TRZ treatment was able to effectively target tumour-initiating cells of ErbB2-positive breast cancer cell lines. However, Notch-1 has been implicated not only in the self-renewal of these tumour-initiating cells but also in TRZ resistance [177]. In this context, results from clinical studies of TRZ and animal studies

using transgenic mouse models or pharmacological approaches have demonstrated that major kinases of the HER2- PI_3K pathway are important for regulating cardiac physiological function [178]. Phosphorylation of Akt represents PI_3K pathway activation. This activation is regulated by different proteins such as survivin. Survivin modulates integrated cellular networks that are essential for tumour cell proliferation and viability. Survivin has been identified as a member of the IAPs family, which is undetectable in normal cells but overexpressed in several human cancers [179]. Survivin activity can be regulated by ErbB2 through the PI_3K-Akt pathway. The importance of the PI_3K/Akt/survivin pathway has been reported in lung and colon cancer [180]. HER2 therapy has been successful in many cases, but patients have a tendency to develop resistance to the inhibitory agents [181]. Increased ErbB2 expression has been associated with drug resistance in cancer cells [182]. In this field, the role of oxidative stress has been evoked. In the Calu-3 cell line, it has been demonstated that TRZ treatment was associated with an increase in cellular ROS production, glutathione depletion and a decrease in the activities of SOD and catalase enzymes. Also, impaired intracellular antioxidant/oxidant balance contributes to TRZ-mediated cell death [183]. There is cross-talk between the amount of HSPs, the antioxidant/oxidant balance and intracellular redox homeostasis. HSP90 was shown to possess reactive cysteines and was able to reduce cytochrome c, suggesting that this chaperone plays a role in modulating the redox status in resting and apoptotic cells [184]. HSP90 is able to buffer the effects of oxidative stress; HSP90 activity counteracts the effects of oxidative stress on enzyme functions [185]. In cancer, however, the chaperoning activity of HSP90 is often exploited by cancer cells to confer aberrant proliferative, survival, and/or metastatic potential. Pharmacologic blockade of HSP90 is an innovative approach in the development of new antineoplastic agents [186]. The combination of different HSP90 inhibitors with TRZ could also be effective, as is the case in HER2-positive breast cancer patients [187, 188].

Doxorubicin-Trastuzumab: synergic effects on cardiotoxicity

The addition of TRZ to adjuvant DOXO chemotherapy has reduced the risk for breast cancer recurrence by 50% and mortality by 30% in ErbB2–positive women. However, the major limitation of this therapeutic regimen is the onset of serious cardiac side effects. The combination of DOXO and TRZ therapy induced a synergistic detrimental cardiotoxic effect [189]. Subsequent TRZ adjuvant trials were designed to include prospective evaluations of cardiac effects: NSABP B-31 (National Surgical Adjuvant Breast and Bowel Project), NCCTG N9831 (North Central Cancer Treatment Group), BCIRG 006 (Breast Cancer International ResearchGroup) and FinHer (FinlandHerceptin trial) and HERA Herceptin Adjuvant trial (HERA). In the HERA trial, long term evaluation at 8-year median follow-up validates the low incidence of cardiac events for TRZ given sequentially after chemotherapy and radiotherapy. Importantly, cardiac events were reversible in the majority of patients [190]. Better understanding of the molecular mechanisms responsible for the synergistic TRZ-induced cardiac injury associated with ANTH-treatment may be important for novel protective or preventive therapeutic strategies. As we reported, TRZ cardiotoxicity is due to the deregulation of ErbB2/4–PI_3K signalling, which is essential to maintain cellular homeostasis. As developed previously, one of the mechanisms by which DOXO causes deleterious cardiac structural and functional changes involves oxidative stress. Recently, it has been demonstrated that, in mice, TRZ alone induced only a transient increase in myocardial 3-nitrotyrosine staining, a biomarker of nitrogen free radical species.

DOXO treatment induced sustained oxidative and nitrosative stress, which was exacerbated by DOXO and TRZ in combination [191] (Figure 6).

The precise mechanisms underlying DOXO/TRZ-induced cardiotoxicity remain incompletely understood. A significant number of adverse effects occur in patients without obvious risk factors. Some patients develop intense DOXO-induced cardiotoxicity at low doses, whereas other patients tolerate very high doses without any myocardial disease. Concerning treatment with TRZ, there is no relationship between the cumulative dose and the probability of developing cardiotoxicity. Recently, some studies investigated candidate-gene approaches to genotyping risk for anticancer drugs-induced cardiotoxicity. Variability in susceptibility to myocardial damage is incompletely explained by clinical factors, and evidence demonstrates a genetic predisposition [192]. Early changes in biomarkers may be useful in predicting adverse cardiovascular outcomes with DOXO and TRZ. Concerning TRZ treatment and its cardiotoxicity, pharmacogenetics is an essential approach. It determines whether there is a correlation between genetic polymorphism, such as in *HER2*, and the response to TRZ treatment or the development of TRZ-associated cardiotoxicity. Most reported polymorphisms affecting the efficacy of anticancer treatment are single nucleotide polymorphisms [193]. Crosstalk between the oestrogen receptor pathway and the PI_3K pathway is thought to be involved in the resistance to TRZ-containing chemotherapy [194].

When TRZ is used in association with DOXO, the development of heart failure is potentiated (Figure 6). This could be related to the inherent capacity of DOXO to increase oxidative stress. The use of angiotensin II type 1 (AT1)-inhibitors in patients with troponin elevation during chemotherapy may be an effective tool to prevent left ventricular ejection fraction reduction [195]. In experimental studies, we demonstrated that DOXO induced alterations in cardiac function, inflammation and plasma oxidative stress whereas tissue oxidative stress, and cardiac kinin receptor expression were not modified. AT1 inhibition did not improve cardiac performance, but it modulated kinin receptor expression and enhanced antioxidant defences [11]. In the clinical field, several studies have evaluated the efficacy of cardiac medications in preventing or reversing cancer therapy-induced cardiotoxicity, with angiotensin-converting enzyme inhibitors or angiotensin II receptor blocker. Studies with these drugs have shown their utility in preventing and reversing chemotherapy-induced left ventricular dysfunction [196].

It is well established that the signalling pathway mediated by (AT1) receptor plays an important role in DOXO- or TRZ-induced cardiac impairment, thus suggesting that an AT1 receptor blocker (ARB) might be used to prevent ANTH-induced cardiomoypathy. A large body of evidence has clarified the role of AT1 in mediating the development of cardiac hypertrophy and LV remodelling after myocardial infarction or acute ANTH exposure. DOXO induces myofibril loss, increases the number of apoptotic cells, and significantly impairs cardiac function in control mice, but not in AT1 knockout mice or in animals treated with an ARB. It appears that the AT1-mediated signalling pathway plays an important role in DOXO-induced cardiac failure [197].

More recently, an indirect relationship has been considered between the renin-angiotensin system and TRZ-induced cardiac dysfunction. TRZ binds to HER2 with high affinity, thereby eliminating its ability to dimerize with other HER receptors. By blocking HER2 signalling, cardiomyocytes are unable to activate the cell survival pathways associated with excess ROS. Therefore, blockage of HER2 allows the accumulation of ROS within cardiomyocytes, which leads to the development of cardiac

dysfunction associated with cells apoptosis. Combination therapy with DOXO and TRZ increases the formation of ROS, thus amplifying cardiac dysfunction. The increased stress on the heart leads to the up-regulation of circulating ANG II, which in turn contributes to the detrimental effects on the heart [170]. It has been also demonstrated that the pleiotropic biochemical and cellular effects of TRZ and DOXO induced ER stress in relationship with the oxidative stress [198, 199].

Concluding remarks

It is now clearly demonstrated that several chemotherapeutic agents, when combined or not with radiation therapy are associated with an increased risk of myocardial disease. The best treatment for chemotherapy-induced cardiotoxicity is prevention. Cardiospecific biomarkers have proved to be a valid diagnostic tool for the early identification and monitoring of cardiotoxicity. Our understanding of the mechanisms by which these therapies affect the heart is crucial for improving drug design and finding alternative therapies to protect patients predisposed to cardiovascular disease. Ideally, the goal of new therapies should be to improve the management of cancer through the specific targeting of malignant cells and fewer adverse cardiac side effects. New therapies include tyrosine kinase inhibitors, antibody–chemotherapy conjugates, HSP inhibitors and antibodies that interfere with the formation of ErbB2–ErbB3 dimers [106]. Recent bioinformatics and proteomic analyses have uncovered several previously unrecognized roles for specific proteins in regulating cell physiology under normal and stressed conditions. The optimal development and application of HSP90-targeted therapies will depend on synthesizing information gained from a careful genetic analysis of primary and metastatic tumours. BIIB021 is the first oral, synthetic, HSP90 inhibitor that showed activity when administered at nanomolar concentrations to subjects with advanced solid tumours [200, 201].

Major hypothesis for anticancer drug-induced cardiotoxicity has been attributed to a number of causes including the cellular basis for growth, hypertrophy and failure of the human heart. In this context, the future of pharmacological regeneration may lie in the local delivery of molecules targeting specific growth and differentiation pathways [202]. Chemopreventive strategies could represent another way to reduce cardiotoxicity. A detailed history of patients that focuses on cardiovascular risk factors (diabetes, obesity, pre-existing cardiovascular disorders) and mediastinal irradiation is required [203]. New approaches to personalized treatment for cancer that involve molecular screening for clinically relevant genomic alterations and genotype-targeted treatments are emerging.

In this arena of clinical oncology, the next decade should witness the development of novel therapeutic approaches in cardioprotection in patients treated with ANTH and TRZ [204]. Evidence in recent years has clearly established the beneficial action of cytokine leukaemia inhibitory factor (LIF) in preventing injury to the myocardium associated with various drugs (Figure 6). LIF is a member of the interleukin(IL)-6-type cytokine family, which includes IL-6, IL-11, IL-27,LIF, ciliary neurotrophic factor (CNTF), cardiotropin-1 (CT-1) , oncostatin M (OSM), and cardiotrophin-like cytokine-1/novel neurotrophin-1/B-cell stimulating factor-3 (CLC-1/NNT-1/ BSF-3). Current evidence suggests that CT-1, a 201 amino acid protein, plays an important role in the regulation of the cardiovascular system. CT-1 has a great number of functions that sometimes have opposite effects. In fact, it can promote cardiac cell survival but can also cause cardiac hypertrophy and ventricular remodelling [205]. LIF

signals through a shared gp130 receptor [206]. For LIF, signal induction occurs when this cytokine binds LIF receptor and evokes its dimerization with gp130. LIF activates different pathways in cardiac myocytes such as 1) JAK-STAT and MAPK; 2) PI_3K/AkT. The mechanism has been ascribed to inhibition of opening of the mPTP in response to increases in intracellular Ca^{2+} and ROS [207]. Evidence indicates that the gp130-mediated signalling networks play important roles in the progression of multiple types of cancer. Inhibition of gp130 activity offers a potential and promising approach to cancer therapy.

Acknowledgments

The authors wish to thank Martine Goiset for secretarial assistance and Philip Bastable for English assistance.

Funding

This work was supported by grants from the French Ministry of Research, the Institut National de la Santé et de la Recherche Médicale (INSERM), the Regional Council of Burgundy (Conseil Regional de Bourgogne), FEDER and Association de Cardiologie de Bourgogne.

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Figure Legends

Figure 1: Chemical structures of main anthracyclines (ANTH, A): doxorubicin: DOX (adriamycin), epirubicin (EPI) and the "iron" and free radical hypothesis of anthracycline-induced cardiotoxicity.

Metabolism: ANTH-generated free radicals by enzymatic and non-enzymatic mechanisms. One-electron addition to the quinone moiety in ring C of DOX induces the formation of a semiquinone that regenerates its origin quinone by reducing oxygen to reactive oxygen species (ROS) such as superoxide anion. This cycle is supported by oxidases and endothelial nitric oxide synthase (eNOS) (reductase domain). DOX in the quinone form accepts an electron. This generates a semiquinone free radical: $DOX^{\cdot}$. A superoxide radical is formed and then converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). The non-enzymatic generation of free radicals by DOX results of the formation of a complex associating DOX and ferric iron (DOX-Fe^{3+}) which undergoes redox-cycling to produce superoxide radical. The electron donor for this reaction is glutathione (GSH).
ANTH enters the mitochondria, causing the release of cytochrome C oxidase and selectively affects oxidative phosphorylation. These drugs cause death of cardiomyocytes through induction of cell apoptosis, reduction of adenosine triphosphate (ATP) production from the mitochondria, and generation of ROS. ANTHs also prolong the opening time of calcium channels in the sarcoplasmic reticulum (SR) and act on L-type Channel.
Chemistry of dexrazoxane: Hydrolysis of dexrazoxane to intermediate metabolite: iron-chelating metabolite ADR-925 and then a complex is formed: ADR-925 with Fe^{3+}.

ATP, adenosine triphosphate; CAT, catalase; DOX, doxorubicin; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione disulfide; H_2O_2, hydrogen peroxide; MnSOD, manganese superoxide dismutase; NOS, $^{\bullet}$NO synthase; NOX, NADPH oxidase; $O_2^{\bullet-}$, superoxide; $ONOO^{-}$, peroxynitrite; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; eNOS, endothelial nitric oxide synthase or NOS-3; $^{\bullet}$NO, nitric oxide; $^{\bullet}$OH, hydroxyl radical; RyR , ryanodine receptor; SR, sarcoplasmic reticulum.

Figure 2: Schema of the effect of anthracyclines (ANTH: A) on cellular iron metabolism.
Iron is scavenged by transferrin (Tf). $TfFe^{3+}$ binds to Tf receptor (TfR) on the cell surface and undergoes receptor-mediated endocytosis. The released Fe^{3+} is reduced to Fe^{2+} by the ferrireductase Steap3 within the endosome before export from the endosome by a divalent metal transporter 1 (DMT1). After Fe^{2+} is carried out of the endosome, it is integrated in a specific pool: labile iron pool (LIP).The major component of LIP is as glutathione-iron conjugate (Fe^{2+}/GS). Fe^{2+} that is not utilized or stored in ferritin is exported by ferroportin (FPN1). Copper-containing ferroxidase hephaestin assists by converting Fe^{2+} to Fe^{3+}, which is then bound by Tf.
ANTHs interact with oxidative phosphorylation and iron metabolism in the mitochondria. Inside the mitochondrial matrix, Fe can be directed to different pathways including iron-sulfur (Fe-S) cluster biogenesis. The clusters in the mitochondria are tightly regulated by a transporter: ATP-binding cassette (ABC) transporter (ABCB7). ANTH redox cycling at complex I of the electron transport chain results in the generation of superoxide. A-Fe^{3+} can reduce its chelated Fe through redox reaction, either by oxidation of the side chain on C9 or the hydroquinone moiety at rig C forming an A free radical A• .
Heme, the prosthetic group of hemoglobin, myoglobin, and the cytochromes, is generated in the mitochondrial matrix. The first step in mammalian heme biosynthesis is catalyzed by the enzyme 5-aminolevulinate synthase (ALA) synthase (ALAS). The terminal step of heme synthesis is the insertion of Fe^{2+} into the protoporphyrin to produce protoheme (heme). Heme oxygenases (HOs) catalyze the degradation of heme, producing biliverdin, iron and, carbon monoxide.
Iron-responsive elements (IREs) are present in the 5'-or 3'-untranslated regions of mRNAs. The iron-regulatory proteins 1 and 2 (IRP-1 and 2) are mRNA-binding molecules. The mRNA binding activity of

IRP1 is regulated by the presence of [4Fe-4S] cluster within the protein. In cells that are iron-, the depleted, the [4Fe-4S] is absent (apo-IRP1) and permits IRP1-IRE binding. In return, if iron levels are high, the cluster forms within the protein (Holo-IRP1) and blocks IRP1-IRE binding.
Ferritins and hepcidin are the major regulator proteins. The major target of hepcidin is the protein ferroportin. There is an equilibrium between ferritin-bound iron (Fe^{3+}) and the LIP in cells (Fe^{2+}) by which ferritin prevents the formation of ROS mediated by the Fenton reaction.

: site for ANTH redox cycling within the cardiomyocyte.


: Interaction of ANTH with various pathways in the cardiomyocyte.

Figure 3: Neuregulin structure.
Neuregulins (NRGs) are a subclass of transmembrane polypeptide growth factors among the epidermal growth factor (EGF). NRG1 generates six types of protein (I–VI) and at least 31 isoforms. The EGF-like domain is located in the membrane-proximal region of the extracellular domain that is necessary and sufficient for activation of the ErbB receptor tyrosine kinases. NRG1 isoforms are synthesized as transmembrane precursor polypeptides (pro-NRG1s). Once at the plasma membrane, the NRGs may remain at this location as anchored proteins, or may be solubilized. Release of soluble NRGs occurs by the action of membrane metalloproteinases proteases. 'A Disintegrin And Metalloproteinase' (ADAM) family are membrane-anchored proteases. Beta-secretase 1 (BACE1) is an aspartic-acid protease.

Figure 4: Neuregulins (NRG)/ErbB signaling in the cardiomyocyte and endothelium.
NRG-1 is expressed and released by the endocardial and microvascular endothelium. ErbBs are expressed in the ventricular cardiac myocytes. ErbB receptors are also co-localized with eNOS in the cavealae. NRG-1 exerts its effect in a paracrine manner via the ErbB receptors. Heterodimers are stimulators of downstream pathways such as PI_3-K/Akt and MAPK. Trastuzumab activity is associated with the inhibition of these ways, leading to an increase in cell cycle arrest and the suppression of cell proliferation, and survival.
A mechanism of trastuzumab is to attract immune cells to tumor sites that overexpress HER2 by the mechanism: antibody-dependent cellular cytotoxicity (ADCC).

Figure 5: Human Epidermal Growth Factor Receptor (HER) activation.
HER2 (ErbB2) is a member of the growth factor receptor family that includes four receptors: the epidermal growth factor receptor (EGFR) or ErbB1, HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). ErbBs consist of an extracellular ligang-binding domain (L1, CR1, L2, CR2), a transmembrane domain, and a cytoplasmic tyrosine kinase domain (N and C lobes), but not ErbB2.
For HER2 function, receptor dimerization is required. With the exception of HER2, ErbB family members are activated by ligand binding to the extracellular domain, which promotes conformational changes that enable the receptors to homodimerization and hetrodimerization.
Trastuzumab binds domain IV of the HER2 extracellular domain (ECD) and prevents dimerization between HER2 and HER1, 2, 3 or HER4.
HSP90, a chaperone protein, inhibits the proteasome-mediated degradation of HER2 and HER3.
Cleavage of the extracellular domain of HER2 generates a membrane-bound phosphorylated P95, which is able to activate signal-transduction pathways.

Figure 6: Molecular mechanisms and novel therapeutic approaches in cardioprotection in patients treated with anthracycline and trastuzumab.

Leukemia inhibitory factor (LIF) may either stimulate proliferation or induce differentiation depending upon the cell type and its stage of development. LIF is a member of the IL-6 family, which includes IL-6, type cytokine Family (6,11,27), cardiotrophin-1 (CT1) and oncostatin M (OSM). All IL-6-type cytokines assign the common signal transducer gp130. LIF evokes genomic and non-genomic events to protect cardiac myocytes. LIF induces dimerization of gp130 and LIFR, activating signalling pathways (PI_3-K/Akt, MAPK, JAK-STAT3). These actions are linked to cellular protection, cell regeneration and/or apoptosis.

Table 1. Anticancer therapy and cardiovascular toxicity [135-138]

	Arrhythmia	Long QTc	Myocardial ischemia	Thromboembolism	Systolic dysfunction	Hypertension
Anthracycline						
Doxorubicin	+++	NE	+	NE	+++	+
Doxorubicin (liposomal)	+	NE	++	NE	+	+
Epirubicin	+	NE	+	NE	+	+
Daunorubicin	++	NE	+	NE	+	+
Idarubicin	+++	NE	+	NE	++	+
Mitoxantrone	+++	NE	++	NE	++	++
Monoclonal antibody						
Trastuzumab	++	NE	+	++	+++	++
Bevacizumab	++	NE	++	+++	++	++
Cetuximab	++	NE	+	+	NE	++
Brentuzimab	+	NE	+	+	+	+
Ipilimumab	+	NE	+	+	NE	NE
Panitumumab	+	NE	++	++	NE	++
Pertuzumab	+	NE	+	+	++	+
Rituximab	+	NE	++	+++	+	++
Tyrosine kinase inhibitors						
Dasatinib	+++	++	++	++	++	++
Nilotinib	++	++	NE	+	++	+++
Vermurafenib	++	NE	++	++	+	++
Sorafenib	+	NE	++	++	++	+++
Sunitinib	+	+	++	++	+++	+++
Erlotinib	NE	NE	++	++	NE	NE
Gefitinib	NE	NE	++	++	NE	NE
Imatinib	NE	NE	+++	++	++	NE
Lapatinib	NE	+++	++	+	++	NE
Pazopanib	NE	NE	++	++	+	+++
Proteasome inhibitors						
Bortezomib	+	NE	+	+	+	+
Carfilzomib	++	NE	++	NE	+	+
Hormone therapy						
Tamoxifen	+	NE	++	++	++	++
Abiraterone	++	NE	++	NE	++	++
Anastrozole	NE	NE	++	++	NE	++
Exemestane	NE	NE	++	++	NE	++
Letrozole	NE	NE	+++	++	NE	++
Antimetabolite						
5-Fluorouracil	+++	NE	+++	NE	+	NE
Capecitabine	++	NE	++	++	NE	NE
Alkylating agent						
Cisplatin	NE	NE	NE	++	NE	NE
Cyclophosphamide	NE	NE	NE	+	NE	NE
Ifosfamide	NE	NE	NE	+	++	NE
Antimicrotubule agent						
Paclitaxel	++	NE	+	NE	+	+
Nab-paclitaxel	++	NE	NE	+	NE	+
Docetaxel	++	NE	++	NE	+	++

+++ represents > 10% ; **++** represents 1%-10% ; **+** represents < 1% ; **NE** : not well established